Lung injury persisted for 1 week. An initial brisk neutrophil response was followed by a 3-fold increase in alveolar macrophages 3 days postexposure. The numbers of bacteria cultured from lavage specimens paralleled the time course observed in whole lung homogenates. Immunofluorescence microscopy, transmission electron microscopy, and differential centrifugation studies suggested growth of *L pneumophila* within the cells of the alveolar inflammatory exudate.

**Discussion**

Guinea pigs and rats became infected following brief exposure to an aerosol of *L pneumophila* and developed pneumonia which closely simulates Legionnaires' disease in man. The experimental observations suggest a hypothetical sequence of events in which inhaled *L pneumophila* are phagocytosed and begin to grow immediately within alveolar macrophages4 and possibly PMN leukocytes. Despite prompt recruitment of inflammatory cells to the lung, bacterial growth proceeds exponentially for 1-3 days, and pneumonia consolidates extends for 3-5 days or longer. Fatality is maximal 5-6 days postexposure, and survivors show less extensive pneumonia and fewer bacteria than animals dying of infection.

Immunization protects rodents against *L pneumophila* infection.5 The appearance of immune responses 4-6 days after infection might modulate the course and outcome of disease. Guinea pigs developed severe illness and high mortality while rats were mildly ill and had low mortality, despite 100% infectivity in both species. These differences in severity and outcome between species cannot be explained by the effectiveness of resident lung defense mechanisms, since immediate exponential growth of bacteria occurred in both species. Susceptibility to a possible bacterial toxin,6 or differences in recruited and immune defenses might account for the difference in mortality between rats and guinea pigs.

These animal models may be studied and manipulated to permit clearer understanding of the pathogenesis, treatment, and prevention of human Legionnaires’ pneumonia.

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**Leukocytes in Lung Injury**

*Dale E. Hammerschmidt, M.D.*

The granulocyte is normally thought of as a friendly cell, important to the host's ability to defend against microbial invaders. Certainly, this is an important role, as evidenced by the infection propensity seen in patients with functional abnormalities of granulocytes or with extreme granulocytopenia. As early as 1957, Metchnikoff suggested that stimulated granulocytes might be also capable of doing harm. He postulated that "ferments" released from the cells at the site of inflammation might be capable of damaging host tissues, and might therefore be a part of the inflammatory reaction. In the last few decades, this idea has been refined as granulocyte function has become better understood; it survives as the "frustrated phagocytosis" model of immune tissue injury described by Henson (Fig 1).

In this most familiar model of granulocyte-mediated tissue injury, a host tissue becomes coated with immunoprotein, either an antibody directed against the tissue or an immune complex which becomes passively deposited. As a result, the complement system is activated, generating opsonin (C3b), chemotaxin (C5a) and at least three anaphylatoxins (C3a, C4a, C5a). The anaphylatoxins alter local vascular permeability; the chemotaxin attracts phagocytes to the scene, where they encounter a tissue which has been made to look good to eat. The phagocytes then attempt to engulf the host tissue; they fail in this attempt, since the host tissue is, in most cases, larger than a phagocyte (!). An incomplete phagocytic vacuole is formed, degranulation and production of toxic oxygen compounds occur, and the weaponry usually directed against invading micro-organisms is released to the milieu where it can damage the nearby tissues.2

This model of immune injury is probably important in inflammatory exudates, and in such diseases as immune glomerulitis and nephritis. More recently, interest has developed in the possibility that the intravascular behavior of granulocytes might also be of pathogenic importance. For example, studies of neutropenia and mild pulmonary dysfunction occurring during hemodialysis led to the observation that small blood vessels could become densely impacted with granulocytes when the complement system is activated.4 Because this leukostasis was associated with pulmonary edema in experimental animals and with mild lung dysfunction in patients, it was tempting to assume that the granulocytes were causing damage to the vessels in which they were sequestered. Mechanisms for both the leukostasis and for the presumed microvascular injury were suggested by subsequent experimental observations in vivo.

When granulocytes were exposed to activated complement, they were found to aggregate, in a manner similar to

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stimulated platelets (Fig 2), and thus to have the potential to embolize to microvascular sites as clumps of stimulated cells; moreover, the stimulated granulocyte was found to have increased adhesiveness with respect to a variety of substrata, including both foreign polymers and endothelial cells, allowing leukostasis to develop even if frank aggregation does not occur.

Other workers, including Drs. Charles Moldow and Thomas Sacks of our department, explored the possible contribution of stimulated granulocytes to microvascular injury. In tissue culture, endothelial cells were labeled with radio-chromium and were incubated with granulocytes and a variety of granulocyte stimuli, chromium release then being measured as an index of cell injury. The addition of endotoxin or of phorbol esters led to augmented chromium release, the former especially if a source of activatable complement was also present; this injury was largely inhibited by the pre-addition of superoxide dismutase and catalase, suggesting that it was largely the result of generation of toxic oxygen compounds. The release of proteolytic enzymes may also play a role in the development of microvascular leakage in animals, however, since Harlan, et al have noted delamination of endothelial cells from their substrata when they are exposed to granulocyte neutral proteases.

Each of these phenomena—aggregation, increased adhesion to endothelium and microvascular injury—has also been demonstrable in the small vascular beds of experimental animals using techniques of intravital microscopy.

![Figure 1](image1.png)  
*Figure 1.* The "frustrated phagocytosis" model of granulocyte-mediated immune tissue injury. A tissue may become coated with immunoprotein, either because antibodies are made against that tissue or because immune complexes are passively deposited upon it (panel a). When this occurs, the plasma complement system may be activated. As a result, the tissue becomes opsonized by C3b, the complement-derived anaphylatoxins C3a, C4a and C5a are generated, and the major complement chemotaxin, C5a, attracts granulocytes to the scene (panel b). The granulocyte, attracted to a tissue which has been opsonized, predictably attempts to engulf it (panel c). This attempt is unsuccessful; an enclosed phagosome cannot be formed, and such antimicrobial weaponry as toxic oxygen compounds and lysosomal enzymes are released into an incomplete phagosome, becoming free to damage the host tissue (panel d). Anthropomorphically, this may be thought of as the poor granulocyte trying and trying to engulf the enormous tissue, until she finally vomits! (ref 3, reused with permission.)

![Figure 2](image2.png)  
*Figure 2.* Granulocytes aggregate in response to a variety of stimuli, including C5a. When activated plasma complement is added to a suspension of granulocytes stirring in an aggregometer, a wave of increasing light transmission, reminiscent of a platelet aggregation wave, is seen (left panel). Microscopy of samples fixed during the wave (right panel) shows that aggregation has indeed occurred. The principal aggregant proved to be C5a (or C5a), part of the evidence is the inability to generate aggregating activity in zymosan-opsonized C5-deficient plasma (left panel). (From Greenberg et al, Trans AJP 1979; 92:130, used with permission.)
Granulocytes in the Lung

Extracorporeal Circulation

Hemodialysis neutropenia, as mentioned above, provided the context in which we first studied the possible contribution of stimulated granulocytes to pulmonary injury. Because gas tensions and pH change in consequence of gas and ion exchange in the dialyzer, some concern was voiced that the pulmonary function changes in that context might be unrelated to the stimulation of granulocytes. However, the same changes were seen when simple dialyzer-incubated plasma was infused into medical students or other suitable experimental animals, a context in which no gas or ion exchange occurs. Furthermore, similar changes occur with other extracorporeal circuits in which gas exchange does not occur, such as filtration leukopheresis. Therefore, although gas exchange and hypoventilation probably contribute to hypoxemia during dialysis, they appear not to be necessary for it. On the other hand, the magnitude of the pulmonary dysfunction during dialysis or leukopheresis should not be overstated; in most patients/donors, the process is not symptomatic, and will not be noted by the physician unless it is systematically sought.

Activation of the complement system and of granulocytes during extracorporeal circulation, therefore, seems more to be a clue that has led several workers down a productive path of work rather than a major clinical problem in its own right. However, it may prove of major import in patients with underlying cardiac or pulmonary disease, and it is tempting to speculate that it might play a role in long-range complications of hemodialysis or in the "post-perfusion syndrome" which occasionally follows cardiopulmonary bypass.

The Adult Respiratory Distress Syndrome

Our group became interested in the possible role of the stimulated granulocyte in ARDS, or "shock lung," because of several clinical and pathologic parallels, some growing from our work in extracorporeal circulations. First, the syndrome tended to occur in contexts which are characterized by the activation of complement—such contexts as acute pancreatitis, hypovolemic shock, and bacterial sepsis. Second, leukostasis in the lungs was a prominent morphologic finding in shock, and early in the transition from shock to ARDS. Ratliff and co-workers observed this in experimental animals subjected to shock; later, it was confirmed on lung biopsy in polytrauma patients at the Ludwig Boltzmann Traumatology Institute in Vienna (Fig 3). We found this particularly striking, since such leukostasis was mimicked on the intravenous infusion of soluble complement activators or of plasma in which the complement system had been deliberately activated ex vivo. Finally, in some experimental models of shock, the depletion of activatable complement or of granulocytes afforded some measure of protection against death or shock-associated pulmonary injury.

We postulated therefore that the granulocyte might be a part of the "trigger" mechanism initiating pulmonary injury in shock; complement activation (or production of other granulocyte activators) would lead to sequestration of stimulated PMNs in the pulmonary microvasculature, where release of toxic oxygen compounds and proteases would damage endothelium, leading to microvascular leak. Clearly, this was likely to be only a part of a complicated story, with other systems coming into play to render the damage more severe and lasting. In support of the concept, however, we found a correlation between evidence of complement activation—granulocyte-aggregating activity in the plasma—and the development of ARDS in patients at risk. In studies recently begun in collaboration with Drs. Heinz Redl and Gunther Schlag at the Boltzmann Institute, we are reproducing this finding in traumatized dogs: granulocyte aggregating activity appears in the plasma before evidence of pulmonary dysfunction, and leukostasis precedes the deposition of fibrin and/or platelets in the lungs. This parallels quite nicely those investigators' earlier ultrastructural observations in serial needle biopsy of the lungs of polytrauma patients, in whom the earliest morphologic finding was granulocytic leukostasis with degranulation and endothelial swelling; these findings were termed "the lung in shock" to distinguish them from "the shock lung syndrome," because they were felt to reflect important trigger mechanisms against which prophylactic interventions might be directed.

Emphysema

The recognition that patients with a hereditary deficiency of a major antiprotease, alpha, antitrypsin, were strongly predisposed to the development of pulmonary emphysema, led to the concept that proteases might cause much of the damage in that disease. This concept was strengthened by the experimental observation that neutrophil-derived elastase could induce destructive pulmonary lesions in normal (ie, anti-protease-replete) animals. Imbalance between protease "burden," then, and the anti-protease defense of the host would be expected to hasten the rate at which enzymatic digestion of the lung parenchyma occurred.

This concept leads one rather directly to consider a pathophysiologic role for the PMN, as that cell is one of the major and most mobile sources of proteases in the body. Were something to attract unusual numbers of granulocytes to the lung, especially in a way that led to the stimulation of those granulocytes, the protease burden in the lung would be increased, perhaps beyond that against which the lung tissue

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**Figure 3.** Pulmonary leukostasis, with degranulation of the neutrophils and endothelial swelling, was the earliest consistent finding in serial biopsy studies of the lungs of polytrauma patients at risk for development of the adult respiratory distress syndrome. A representative biopsy is shown here. (Electron micrograph courtesy of Dr. Heinz Redl, Ludwig Boltzmann Institut für experimentelle Traumatologie, Vienna.)
could defend itself. In the most obvious clinical context of increased risk of emphysema, tobacco smoking, this mechanism is quite feasible. That is, macrophages in the lung have been shown in response to exposure to tobacco smoke to produce a non-complement-derived substance which is chemotactic for neutrophils, the net PMN content (and protease content) of the lungs of smokers is therefore higher than in non-smokers. To a small but perhaps clinically important extent, this effect is amplified by the tendency of smokers to have higher white blood cell counts, and to have granulocytes which are metabolically more active in both the resting and stimulated state. While compensatory increases in the levels of anti-proteases might occur, they are not adequate to offset the increase in protease exposure, especially since the anti-proteases in smokers' lungs appear to be oxidized and thereby rendered less potent, slowly progressive, destructive lung disease results.

We have also observed in pilot studies that the exposure of some persons' plasmas to tobacco smoke leads to the generation of a complement-independent factor which aggregates PMNs and provokes them to produce \( \cdot O_2^- \) in increased amounts. However, this method of stimulating granulocytes is of clinical import in chronic lung disease remains to be determined. However, Petrone et al have described a PMN chemotactic substance generated when \( \cdot O_2^- \) is generated; it appears to be an oxidized lipid bound to albumin. Therefore, the generation of \( \cdot O_2^- \) by stimulated PMNs or by tobacco smoke components directly would be expected to recruit still more granulocytes to the scene, resulting in a still higher protease load.

Fairly obviously, this concept has important therapeutic implications. Methods of inducing increased production of anti-proteases might be developed—analagous to the use of attenuated androgens to promote production of the C' inhibitor in hereditary angioedema—to limit the amount of damage done by a given amount of protease; supplemental (pharmacologic) antiproteases might be devised. One might also be able to reduce the protease load itself, by blocking the generation of chemotaxins (? antioxidants; ? identifying and removing the inciting substances from tobacco smoke), or by blunting PMN response to them. Of course, the simplest way to decrease the protease load is already known to be highly clinically effective; unfortunately, too many people have become too severely habituated to find it easy to stop smoking.

**Hyperoxic Lung Injury**

It has been recognized for at least 15 years that the use of very high inspired oxygen tensions for prolonged periods of time may lead to pulmonary injury. Because a common context for such injury is mechanical ventilation (whereby high oxygen fractions may be delivered at greater than atmospheric pressure), this has sometimes been informally referred to as "respirator lung." When I was a house officer, I was taught that this was simply a direct toxic effect of the oxygen upon lung cells; more recent evidence suggests that there may be more to the story than that.

In an interesting series of experiments, Drs. Richard Fox and John Repine (formerly of the University of Minnesota; now with the Webb-Waring Lung Institute) and their co-workers argued for a role of phagocytes in this syndrome, as well. When isolated lung slices were exposed to hyperoxia, lung cells—apparently macrophages—were found to release a factor which was chemotactic for granulocytes. The addition of granulocytes to this system led to destruction of lung parenchyma (assessed by radiochromium release), which was inhibitable by antioxidants or free-radical scavengers. The hypothesis then becomes attractive that some of the damage might be worked by toxic oxygen species released from recruited granulocytes in hyperoxic patients' lungs, rather than just by the inspired oxygen per se. It is possible, however, to work oxidant lung injury even in the absence of PMNs, the relative import of different sources of toxic oxygen in clinical lung injury remains to be determined.

**Therapeutic Implications**

The recognition that granulocytes may play a key role in the development of a number of types of pulmonary injury allows one to consider new modes of therapy, as well as propose mechanisms for some old therapies. For example, granulocyte emobilization to the lungs, or granulocyte response to chemotactic stimuli elaborated from the lungs, might be inhibited by high-dose corticosteroids or by non-steroidal anti-inflammatory agents. The elaboration of the chemotactic stimuli themselves may also in some contexts prove liable to pharmacologic manipulation. Further, as mentioned earlier, the body's defenses against stimulated granulocytes may be enhanced, either by providing protease inhibitors and oxidant detoxifiers, or by stimulating the host's own production of such defenders.

Of course, the interaction of granulocytes with other cell types and biologic systems is just beginning to be understood, and will almost certainly provide other sites at which granulocyte-dependent pathophysiology may be interrupted. Further, the interaction among potential drugs, including synergy in inhibition of granulocyte responsiveness, may well be exploited.

**Conclusion**

The stimulated granulocyte is an important effector cell in a variety of pulmonary injuries. Recognition of this fact has prompted a large number of studies, attempting to clarify the role of PMN in greater detail, including its interactions with other cells and systems. Such clarification, in turn, promises both to expand our understanding of pulmonary disease and to provide us with valuable new therapies.

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Neutrophil Depletion Does Not Prevent Oxygen-Induced Lung Injury in Rabbits*

J. Usha Raj, M.D.; and Richard D. Bland, M.D.

Recent reports suggest that polymorphonuclear leukocytes play an important role in many types of lung injury,1,2 including endothelial damage and edema from prolonged oxygen breathing.3,4 Other studies indicate that pulmonary endothelial injury from oxygen can occur even in the absence of neutrophils.5,6 We therefore designed experiments to see if neutrophil depletion would prevent or reduce the severity of lung injury caused by prolonged oxygen breathing. We found that neutropenia, induced by nitrogen mustard, had no effect on survival time or lung water content of adult rabbits that continuously breathed pure oxygen at 1 atmosphere of pressure.

We used 38 New Zealand white rabbits that we divided into 4 groups. We pre-treated 11 rabbits (group 1) with nitrogen mustard intra-arterially, 1.5 mg/kg body weight every third day, until the circulating neutrophil count decreased to <50 cu mm of blood, after which we placed the rabbits in oxygen. Nine other rabbits (group 2) received no nitrogen mustard and had normal leukocyte counts in their peripheral blood during oxygen breathing. We killed 18 additional control rabbits that had no supplemental oxygen: 9 were neutropenic from nitrogen mustard (group 3) and 9 were untreated (group 4).

We placed a catheter in the aorta of all the rabbits so that we could give drugs and measure systemic blood pressure and partial pressures of oxygen and carbon dioxide in arterial blood. We also placed a Swan-Ganz catheter in the pulmonary artery of 3 rabbits in each group so that we could measure their pulmonary arterial pressure. All rabbits received antibiotics daily. We studied them one at a time in a Lucite chamber, through which flowed either oxygen (partial pressure >710 mm Hg) or air. While the rabbits breathed oxygen, we made daily measurements of vascular pressures, circulating neutrophils, arterial pH and partial pressures of oxygen and carbon dioxide in arterial blood. When the rabbits died, we immediately removed their lungs for measurement of extravascular water,5,6 and we froze a block of tissue in liquid nitrogen for microscopy. In addition, we fixed in formalin a piece of lung from four rabbits in each group, and we made thin sections for determining the number and distribution of neutrophils in the lung.

There was no significant difference between neutropenic and non-neutropenic oxygen-treated rabbits with respect to vascular pressures, arterial pH and partial pressures of oxygen and carbon dioxide in arterial blood. Table 1 is a summary of results. All oxygen-treated rabbits died of respiratory failure from pulmonary edema. Neutrophil depletion decreased the number of neutrophils in the lungs of rabbits that breathed oxygen, but it had no significant effect on survival time or lung water content. Air-breathing rabbits, treated with nitrogen mustard and killed after

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Lung Defense, Injury and Repair