

Cellular mechanisms of acute lung injury: implications for future treatment in the adult respiratory distress syndrome

The adult respiratory distress syndrome is a catastrophic form of lung injury. When originally described by Ashbaugh and his coworkers in 1967 it had an associated mortality approaching 70%.¹ Over the intervening 20 years progress in developing specific treatment for it has been disappointing; treatment is in the main supportive and mortality remains at 50–70%.² The syndrome classically arises after a “latent” period of 24–72 hours following one of the varied insults that may provoke it (the most frequent being multiple trauma, septicaemia, and pancreatitis). Despite the multiplicity of initial predisposing causes, the pathological findings, by the time the patients present with lung disease, are remarkably uniform. In addition to the early histological findings of acute lung inflammation, with leakage of fluid and protein into the interstitium and airspaces, there is also evidence of type II epithelial cell and fibroblast proliferation, and even early deposition of “scar tissue” matrix proteins. This common picture suggests that a shared pathological process may operate in the early stages of most cases of adult respiratory distress syndrome regardless of the precipitating cause. Recent advances in cellular and molecular biological techniques are likely to offer new opportunities for fine dissection of the pathogenesis of adult respiratory distress syndrome in the early stages. This may lead to the identification of more accurate predictors of very high risk for the full blown clinical adult respiratory distress syndrome, and the latent period may also provide a “window of opportunity” whereby specific mechanism based treatments introduced early for patients at very high risk might be expected to attenuate the severity of lung injury or even to abort the full blown syndrome. The present state of knowledge of the early pathogenesis of the adult respiratory distress syndrome points to a complex interplay between humoral mediators released by the initiating condition and the damaging effects of injurious products released from inflammatory cells on the endothelial and epithelial cells of pulmonary microvessels.

Neutrophils and the adult respiratory distress syndrome

The neutrophil is the archetypical acute inflammatory cell. It contains various potentially histotoxic agents (table 1), many of which have been specifically implicated in inflammatory diseases.³ The neutrophil has been implicated in animal models of acute lung injury induced by circulating endotoxin,⁴ hyperoxia,⁵ and microembolisation⁶; and investigation of bronchoalveolar lavage fluid from patients with the established adult respiratory distress syndrome has shown excess numbers of neutrophils⁷ and excessive amounts of their potentially injurious agents, such as the

protease enzymes elastase⁷ and collagenase.⁸ These enzymes and hydrogen peroxide found in expired air from patients with the adult respiratory distress syndrome having assisted ventilation⁹ are thought to derive from activated inflammatory cells within injured lungs. Much evidence points to a role for the neutrophil in the development of the adult respiratory distress syndrome, though it is well recognised that the syndrome may occur in neutrophil depleted patients.¹⁰ These examples may represent a distinct subgroup within the spectrum of the adult respiratory distress syndrome, or they may reflect the part played by other inflammatory cells with a destructive potential similar to that of the neutrophil (for example, cells of the monocyte-macrophage series). Nevertheless, the neutrophil granulocyte is likely to have an important role in the adult respiratory distress syndrome associated with the most common predisposing conditions, and this article will focus on the neutrophil as exemplifying the archetypical acute inflammatory cell.

The lung normally contains a “marginated pool” of neutrophils that is in a state of dynamic equilibrium with the circulating pool. This localisation of potentially destructive cells may explain in part why the lung is at particular risk of injury triggered by systemic or distant insults in the early pathogenesis of the adult respiratory distress syndrome. Diseased lungs—for example, in chronic obstructive pulmonary disease¹¹—have an increased neutrophil burden within the pulmonary vasculature, representing enhanced potential for neutrophil mediated lung damage. It is also clear, however, that excessive neutrophil sequestration in the lung does not necessarily cause lung injury¹² and several other factors, such as the state of activation, the secretory state, and the type and degree of contact between the neutrophils and pulmonary microvascular endothelial cells, must also contribute. In the remainder of this article we will focus on the mediators that are likely to have an important action on neutrophils, the concept of a pericellular microenvironment favouring neutrophil mediated endothelial injury, and the neutrophil products that are likely to be particularly important in causing tissue injury.

MEDIATORS

Many mediators have been implicated in the pathogenesis of the adult respiratory distress syndrome yet the search for a single common final mediator for the disease process has so far been disappointing. The disease process is more likely to be provoked by a complex interplay between several important mediators, including the complement cleavage product C5a, tumour necrosis factor, platelet activating factor, leukotriene B₄, interleukin 8, and endotoxin. Not all of these mediators have identical effects on inflammatory cells. Some, including C5a, leukotriene B₄, and interleukin 8, are capable of stimulating neutrophils to secrete large quantities of potentially injurious agents (secretagogues). Others, such as bacterial lipopolysaccharide, tumour necrosis factor, and platelet activating factor, though not very effective at low concentrations in causing neutrophil secretion, may “prime” neutrophils so that they release enhanced quantities of potentially injurious agents when subsequently exposed to secretagogues or triggering agents. Acute lung injury has been produced in experimental animals after the intravenous administration of a combination of small amounts of

Table 1 Potentially histotoxic neutrophil products

Oxidants and radicals	Proteolytic enzymes
Superoxide anion	Elastase
Hydrogen peroxide	Gelatinase
Hydroxyl radical	Collagenase
Hypochlorous acid	Cathepsin
Chloramines	Lysozyme
Nitric acid	Neuraminidase
	Heparanase
<i>Others</i>	
Cationic proteins	

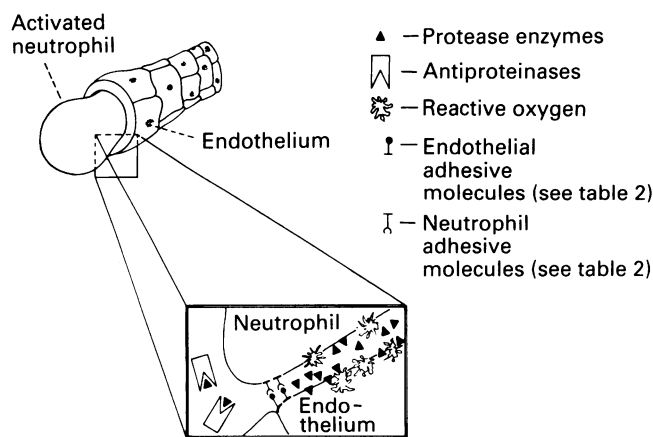


Figure 1 Concept of a restricted pericellular microenvironment favouring tissue injury at the neutrophil-endothelial interface.

bacterial lipopolysaccharide (an effective primer) and C5a (an effective secretagogue), both of which have been implicated in the adult respiratory distress syndrome, but not with either agent alone.¹³ Similar results have been obtained with neutrophil mediated endothelial injury in vitro.¹⁴ Thus we may reasonably propose that amplification mechanisms, based on the initial action of priming agents followed by the action of secretagogues, combine to enhance the potential for tissue injury in the early adult respiratory distress syndrome. Priming agents, such as bacterial lipopolysaccharide, have been shown to influence some other neutrophil functions, which could also tilt the balance of the inflammatory process towards tissue injury. These processes include increase in neutrophil adhesiveness, reduction of neutrophil chemotaxis,¹⁵ and reduction of neutrophil deformability.¹⁷ These effects are likely to help to increase the time of contact between actively secreting neutrophils and endothelial cells within the pulmonary microcirculation, thus enhancing the potential for tissue injury.

CONCEPT OF A MICROENVIRONMENT FAVOURING TISSUE INJURY

Neutrophil mediated endothelial injury does not occur in vitro without direct neutrophil adherence. The observation that neutrophil mediated degradation of the matrix still occurs in the presence of antiproteases¹⁸ has led to the concept of a restricted pericellular microenvironment serving to create a local environment between neutrophils and endothelial cells that may favour microvascular injury by several possible mechanisms (fig 1). Firstly, the most toxic reactive oxygen intermediates are such reactive molecules that they are likely to exert an effect over only a short distance in tissues; secondly, large molecular weight antiproteases, such as α_1 protease inhibitor, would be excluded, permitting unopposed action of agents such as neutrophil elastase; and, finally, some neutrophil enzymes may attain high concentrations at the cell surface instead of being dissipated into the extracellular fluid. A microenvironment favouring injury is likely to be created by the combination of changes in cell deformability and the expression of intercellular adhesive molecules, both of which have recently been shown to be greatly influenced by some of the mediators considered above. The mean diameter of the neutrophil is about 7.0 μm whereas that of the pulmonary capillary is 5.5 μm . Neutrophils must therefore deform in order to pass through a capillary. Neutrophil deformability is greatly reduced in activated states induced by C5a¹⁹ or bacterial lipopolysaccharide,¹⁷ an effect that would reduce their ability to "squeeze" through

lung capillaries and would increase their contact with microvascular endothelial cells. There is increasing evidence¹⁹ that alterations in neutrophil deformability have direct effects on their sequestration in the lung.²⁰ Similarly, the adhesiveness of neutrophils and of endothelial cells is greatly amplified by exposure to some of the inflammatory mediators. The addition of C5a or platelet activating factor to neutrophils enhances their adhesion to endothelial cells within minutes.²¹ As endothelial cells exposed to bacterial endotoxin may take several hours to increase expression of important adhesion molecules, such as the intercellular cell adhesion molecule-1 (ICAM-1) or the endothelial leucocyte adhesion molecule (ELAM-1), these are unlikely candidates for the earliest adhesive interactions with neutrophils. Recent evidence, however, suggests that granule membrane protein-140 (GMP-140), another endothelial adhesive molecule, may be expressed very early after endothelial stimulation. The recent explosion of knowledge of neutrophil-endothelial adhesion²² may produce special therapeutic opportunities in patients at risk of the adult respiratory distress syndrome (see below), though we have not yet established which mechanisms are the most important or the relative importance of deformability and adhesive mechanisms in the early stages of the syndrome. In addition to the mechanism of neutrophil endothelial contact the dynamics of the interaction have to be considered, and also the amplification effects of further neutrophils released from the bone marrow, which are similarly exposed to modulating agents both systemically and within the pulmonary microvasculature. For example, an actively secreting neutrophil that remains in contact with an endothelial cell for several seconds is likely to have a more destructive potential than one in contact for only a few milliseconds. The combination of trace concentrations of bacterial lipopolysaccharide (a priming agent) and formyl-methionyl-leucyl-phenylalanine (FMLP, a secretagogue) not only promotes neutrophil secretion but also exerts supra-additive effects on neutrophil adhesiveness in vitro²³ and on neutrophil sequestration in the pulmonary vasculature,¹³ an effect that persists in vivo for several hours after the intravenous injection of the combination of these agents.

POTENTIALLY INJURIOUS PRODUCTS

Neutrophils contain more than 30 agents with proved capacity in the activated state to injure tissues; some of the more important ones are shown in table 1. Over the last few years the spotlight has focused on reactive oxygen intermediates as the most important primary agents in neutrophil mediated tissue injury. More recently, however, attention has moved to indirect actions of reactive oxygen intermediates, in particular secondary effects that might be exerted by their inactivation of antiproteases.²⁴ In a recent study of neutrophil mediated endothelial injury inhibitors and scavengers of reactive oxygen intermediates did not prevent neutrophil mediated endothelial injury, whereas the addition of a specific neutrophil elastase inhibitor alone attenuated injury.²⁵ Neutrophil elastase has been found in increased quantities in the lavage fluid from patients with the adult respiratory distress syndrome.⁷ The cellular toxic mechanisms of this enzyme are uncertain; although its ability to digest elastin and other proteins as well as being highly cationic may be contributory mechanisms, other relevant effects may yet be elucidated. This enzyme is emerging as one of the primary candidates responsible for tissue injury in neutrophil mediated inflammatory diseases and the adult respiratory distress syndrome in particular.

Within this framework we can now construct a likely sequence of events following the initial provoking condi-

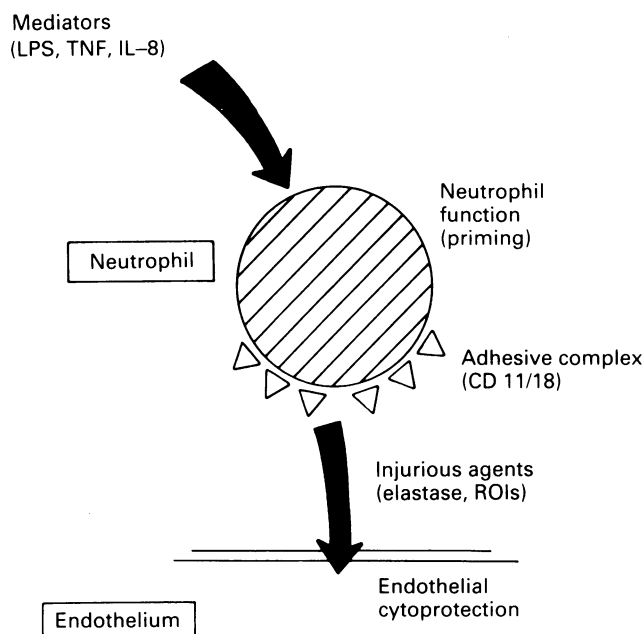


Figure 2 Potential "attack" points for therapeutic intervention. ROIs—reactive oxygen intermediates.

tion. In response to an often distant insult mediators are released that prime and trigger neutrophils and other inflammatory cells to secrete large amounts of histotoxic agents. These activated neutrophils are less deformable and express greatly increased numbers of adhesion receptors, thus increasing and prolonging contact with the endothelium and creating a restricted neutrophil-endothelial interface which provides a microenvironment favouring cell injury. Amplification of injury occurs by the accumulation of newly released bone marrow neutrophils, which also become primed, triggered, and sequestered in the lung. The subsequent endothelial and epithelial injury allows leak of protein rich exudate into the interstitium and alveolar spaces, leading to cytokine cascades and repeated epithelial injury, which may promote replication of fibroblasts and deposition of matrix.

These later stages of the adult respiratory distress syndrome are likely to have been reached by the time the patient presents with full blown lung disease, when it is difficult to conceive of any simple therapeutic approach. But the existence of a latent period before the onset of lung disease in most cases and new knowledge of the likely early cellular and molecular events are likely to provide special opportunities for incisive treatment in patients at high risk of this catastrophic condition.

Newer therapeutic strategies

As yet there is no effective pharmacological therapy for the adult respiratory distress syndrome and treatment is supportive. Treatment with corticosteroids is controversial. High dose corticosteroids did not improve outcome in controlled clinical trials²⁶ and may even be detrimental in cases of sepsis. Many agents have been investigated for a possible beneficial effect in the disease process. These include cyclo-oxygenase inhibitors (non-steroidal anti-inflammatory agents such as lonazolac),²⁷ oxygen scavenging agents, such as *N*-acetylcysteine,²⁸ and vasodilators, such as prostacyclin.²⁹ Pentoxifylline, a methylated xanthine, has received particular attention recently because it has exerted a beneficial effect in several models of acute lung injury.^{30,31} The reduction of pulmonary oedema formation induced by pentoxifylline might be due to its anti-inflammatory effects on neutrophils,³² which may operate

in several ways, including reduction of superoxide production³³ and increase in neutrophil filterability.³⁴

Rational and safe therapeutic strategies, however, are likely to derive only from a detailed understanding of the cellular and molecular mechanisms of the early adult respiratory distress syndrome. The interactions between inflammatory cells (neutrophils) and microvascular endothelial cells provide several potential "attack" points (fig 2).

MEDIATORS INFLUENCING INFLAMMATORY CELLS

Although the mediators that influence inflammatory cells are probably numerous, bacterial lipopolysaccharide, tumour necrosis factor, and interleukin-8 are likely to be particularly important. Although such mediators are possibly generated too early after the provoking insult to be a useful target, they may also play a part later in important amplification events; there are suggestions that antiendotoxin antibodies may have been protective in some (but not all) studies of sepsis.³⁵

ABNORMAL NEUTROPHIL SEQUESTRATION

The molecular identification of specific intercellular adhesive processes is likely to provide special opportunities for disrupting intercellular microenvironments that favour injury. Monoclonal antibodies against molecules of the leucocyte integrins, selectins, and the immunoglobulin supergene family are available (table 2). These, however, are also likely to prevent neutrophil migration in support of host defence in other organs, which in patients with trauma and sepsis are particularly susceptible to secondary infection.

INJURIOUS INFLAMMATORY CELL PRODUCTS

Development of treatments directed against injurious inflammatory cell products have been hampered by our awareness of the multiplicity of neutrophil products with the potential to injure tissues, plus our uncertainty about which of the many agents are likely to be particularly important clinically. Many of the agents produced so far have been rather non-specific in their action or too toxic for clinical use. Nevertheless, neutrophil elastase is assuming prominence as a neutrophil product of great importance in several inflammatory diseases and considerable effort is likely to be made by the pharmaceutical industry in identifying safe and specific inhibitors of this enzyme.

CELLULAR DEFENCES

Endothelial cells appear to tolerate a certain degree of proteinase or oxidative load without irreversible damage,

Table 2 Adhesive molecules and ligands relevant to neutrophil-endothelial interactions

Cell type and workshop cluster designation	Common nomenclature	Ligands
Neutrophil		
CD11a/CD18	LFA-1	ICAM-1 ICAM-2
CD11b/CD18	CR3	C3bi ICAM-1
CD11c/CD18	P150, 95	Fibrinogen C3bi?
W/D	LAM-1 (MEL-14 Mouse)	Endothelial ligand Fibrinogen
Endothelium		
CD62	GMP-140 Padgem ELAM-1	Sialylated Lewis x antigen (CD15 on neutrophil)

yet very little is known about the protective mechanisms. Other cytoprotective systems (for example, in paracetamol induced hepatocyte toxicity) are better understood, and elucidation of endothelial cytoprotective mechanisms—against neutrophil elastase, for example—should provide opportunities for boosting such mechanisms and thereby attenuating microvascular injury.

There are many strands in the web of the inflammatory response, and removal of a single strand is unlikely to inhibit progression of the disease process. Inhibition of two or three key events early in the inflammatory process would be more likely to attenuate the disease. Although the above strategies represent exciting future therapeutic options, overzealousness should be tempered by our knowledge that the destructive elements of the inflammatory response may also, paradoxically, be important in host defence. Major progress is likely to be made by fine dissection of injurious mechanisms to distinguish these from beneficial processes, and by more clearly defining the temporal stages of inflammatory disease in the hope of identifying a stage or stages when a particular cell or process is more critical to the disease process than it is to host defence.

SC DONNELLY
C HASLETT
Respiratory Medicine Unit,
City Hospital,
Edinburgh EH10 5SB

Reprint requests to: Professor C Haslett

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