10 Acute Respiratory Distress Syndrome

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INTRODUCTION

In 1967, Ashbaugh and colleagues *(1)* described a cohort of 12 patients who had acute onset of tachypnea, hypoxemia, panlobular infiltrates on chest radiograph, and decreased lung compliance. It was noted that this syndrome was similar to the infant respiratory distress syndrome, and in 1971 these same investigators coined the term adult respiratory distress syndrome (ARDS) *(2)*. Since that time, it has been noted that this same condition also occurs in children, and consequently it was renamed acute respiratory distress syndrome. In 1988, Murray and colleagues *(3)* defined ARDS via the lung injury score (LIS) based on the chest radiographic findings, the degree of hypoxemia (PaO_2/FiO_2 ratio), the level of positive end-expiratory pressure (PEEP), and the lung compliance (**Table 1**). The American-European Consensus Committee (A-ECC) was formed in 1994 to develop a universal definition of ARDS and acute lung injury (ALI). The definition, outlined in **Table 2**, included the acute nature of the disease process, oxygenation abnormalities, radiographic findings, and the exclusion of left atrial hypertension when measured, but did not include PEEP, as in the LIS *(4)*. This definition recognizes ARDS as the most severe manifestation of ALI. Although highly useful in stratifying and identifying patients for clinical studies, the definition is currently being considered for revision.

From: *Combat Medicine: Basic and Clinical Research in Military, Trauma, and Emergency Medicine*
Edited by: G.C. Tsokos and J.L. Atkins

Table 1
Murray Lung Injury Score[a]

Parameter	*Score*
Chest radiograph	
No consolidation	0
1 quadrant	1
2 quadrants	2
3 quadrants	3
4 quadrants	4
Hypoxemia (PaO_2/FiO_2)	
≥300	0
225–299	1
175–224	2
100–174	3
<100	4
PEEP (cm H_2O)	
≤5	0
6–8	1
9–11	2
12–14	3
≥15	4
Compliance (mL/cm H_2O)	
≥80	0
60–79	1
40–59	2
30–39	3
≤29	4

[a]The final value is obtained by dividing the sum of the individual component scores by 4. Scores: 0 = no injury; 0.1–2.5 = mild to moderate injury; >2.5 = severe injury (acute respiratory distress syndrome).
PEEP, positive end-expiratory pressure.

The exact incidence of ARDS has been relatively difficult to establish. A 1972 population study in New York by the National Heart and Lung Institute reported the incidence of ARDS in adults to be 150,000 cases/yr *(5)*. Other investigators have reported an incidence ranging from 1.5 to 75 patients/100,000 inhabitants/yr *(6–10)*. In children, the exact incidence has also been difficult to establish *(11)*. A prospective epidemiologic study is currently under way that makes use of the A-ECC definition of ARDS and will hopefully provide more definitive data on incidence.

Table 2
American-European Consensus Committee Definition of ARDS and ALI

	Timing	*Oxygenation*	*Chest radiograph*	*Pulmonary artery wedge pressure*
ALI	Acute onset	PaO_2/FiO_2 ratio ≤ 300mmHg (regardless of PEEP level)	Bilateral infiltrates seen on frontal chest radiograph	≤18 mmHg when measured or no clinical evidence of left atrial hypertension.
ARDS	Acute onset	PaO_2/FiO_2 ratio ≤200 mmHg (regardless of PEEP level)	Bilateral infiltrates seen on frontal chest radiograph	≤18 mmHg when measured or no clinical evidence of left atrial hypertension.

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; PEEP, positive end-expiratory pressure.

CLINICAL COURSE AND HISTOPATHOLOGY

The initial phase of ARDS (acute/exudative phase) manifestes clinically by progressively refractory hypoxemia. The chest radiograph demonstrates bilateral patchy pulmonary infiltrates (similar to those seen during cardiogenic pulmonary edema, **Fig. 1**), whereas computed tomography of the chest reveals that alveolar filling, consolidation, and atelectasis occur predominantly in the dependent lung zones. Histologic examination reveals diffuse alveolar damage, hyaline membranes, protein-rich alveolar fluid, parenchymal infiltration by neutrophils and macrophages, and disruption of the alveolar epithelium.

Although some patients will recover after this acute stage, other patients will enter a second phase known as the fibroproliferative stage. The time of onset of this stage is highly variable (3–10 days after initial onset of ARDS) but is typically characterized by the onset of lung architectural changes and persistent hypoxemia. Histologically, prominent interstitial infiltration by fibroblasts, myofibroblasts, and inflammatory cells (mostly of the mononuclear lineage) and increased collagen deposi-

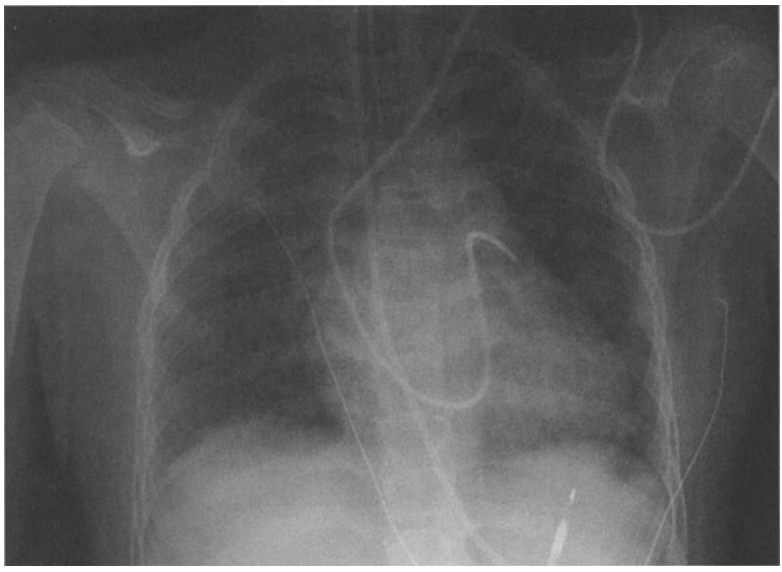

Fig. 1. Chest radiograph of a patient with ARDS illustrating bilateral diffuse alveolar infiltrates.

tion are seen. Other clinical features of the fibroproliferative stage include increased alveolar dead space and further decreases in lung compliance.

The final phase is the recovery phase, characterized by gradual resolution of the hypoxemia and improved compliance as the lung architecture is restored toward normal. The timing and duration of this stage are also highly variable. Some patients will have progressive lung fibrosis, irreversible loss of functional alveoli, and cyst formation, leading to death secondary to hypoxemia.

CAUSES AND OUTCOMES

The causative factors leading to ARDS can be broadly categorized as those that directly injure the lung and those systemic processes that cause indirect/secondary injury to the lung. Direct causes of ARDS include pneumonia, aspiration of gastric contents, lung contusion, fat emboli, near drowning, inhalation injury, and reperfusion injury. Systemic processes causing indirect/secondary injury to the lung include sepsis, multiple transfusions, pancreatitis, and polytrauma. Overall, it appears that the most common cause of ARDS is sepsis *(12,13)*.

Like the incidence, the exact mortality of ARDS has been relatively difficult to determine, with reported mortality in adults broadly ranging from 45 to 92% *(6,8,10)*. Factors that predict mortality in ARDS include chronic liver disease, multiple organ dysfunction, sepsis, and advanced age. Perhaps somewhat surprisingly, initial indices of oxygenation and ventilation (including the PaO_2/FiO_2 ratio and the LIS) do not seem to predict mortality. Failure to improve lung function during the first week of ARDS, however, is a highly negative prognostic factor *(14)*. Most deaths in patients with ARDS have been associated with multiple organ dysfunction or sepsis, rather than hypoxemic respiratory failure *per se*. As will be discussed below in the therapy sections, however, it appears that lung-specific protective strategies can reduce overall mortality secondary to ARDS.

PATHOPHYSIOLOGIC MECHANISMS IN ARDS

Cytokines

One of the difficulties in elucidating the pathophysiology at play in ARDS is the multiple etiologies that have been associated with its onset. In adults, common etiologies include aspiration of gastric contents, sepsis, and major trauma *(12,13,15)*; the leading etiologies of pediatric ARDS include viral pneumonia (especially respiratory syncytial virus), bacteremia, and near-drowning *(16–18)*. Interestingly, both direct and indirect causes of ARDS are characterized by a similar cascade of pathophysiologic events. Among the most consistent findings in both human and animal studies of ARDS is the presence of increased cytokines measured either locally [from bronchoalveolar lavage (BAL) samples] or systemically (in serum samples) *(19)*. Because of their presence and multiple effects, cytokine biology has been extensively investigated in ARDS.

Cytokines are a series of soluble proteins synthesized by numerous cells including virtually every cell type in the lung: alveolar epithelium, pulmonary vascular endothelium, alveolar macrophages, lymphocytes, and interstitial cells. This heterogeneous group of peptides and glycoproteins mediates a variety of biologic functions including intercellular communication, chemotaxis, leukocyte adhesion, production of oxygen- and nitrogen-based radicals, and cell signaling. Cytokines mediate their effects by binding to receptors on the surfaces of their target cells. The receptor-ligand interaction initiates a signaling cascade that can

result in either inhibitory or stimulatory responses by the target cell *(20)*. At low concentrations cytokines act locally by both autocrine and paracrine mechanisms; however, if cytokine concentrations increase substantially, as during ARDS, they can behave as classic hormones with an endocrine effect on distant organs and tissues. Finally, at the highest plasma cytokine concentrations, the inflammatory response to these chemical signals may directly injure host tissues *(21,22)*. The most extensively studied of these molecules in ARDS are tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8).

TNF-α and IL-1 are classified as early response cytokines and are produced in response to a variety of stimuli. The innate immune system, evolutionarily designed to protect the host from a number of pathogens, mediates this early response. Microorganisms express a series of highly conserved molecular patterns that distinguish them from the host. Examples include viral double-stranded RNA, unmethylated CpG dinucleotides common to bacterial but not vertebrate DNA, mannan binding proteins of yeast, glycolipids of mycobacteria, lipoproteins of bacteria and parasites, lipoteichoic acids of Gram-positive bacteria, and lipopolysaccharide (LPS) of Gram-negative bacteria *(23–28)*. The immune active cells of the host possess specific pattern recognition receptors to detect these pathogen-associated molecules and are critical in mediating expression of the cytokines that mediate acute lung inflammation.

LPS remains an important initiator of TNF-α production. The mechanism by which this occurs has been described in a recent series of seminal investigations. The mammalian Toll-like receptors (TLRs) are key signaling receptors of innate host defense that evolved from the *Drosophila Toll* gene *(23–27,29)*. Although initially identified as a mediator of dorsoventral polarization during embryogenesis, the cytoplasmic domain of the Toll receptor was found to be structurally homologous to that of the mammalian IL-1 receptor *(30)*. This supported the concept that both Toll and mammalian TLRs may share similar signal-transduction pathways that ultimately involve cytokine production. Subsequent work has confirmed the role of TLR4 as the receptor initiating LPS signal transduction on macrophages and monocytes. LPS recognition and triggering of early cytokine expression, however, is more complex than interaction with TLR4 alone. LPS binds to the serum protein lipopolysaccharide binding protein (LBP), which

transfers LPS to CD14 that is anchored to the cell membrane by glycosylphosphoinositol. As CD14 lacks a cytoplasmic domain for signal transduction, the LPS/CD14 complex uses TLR4 as a co-receptor *(29)*. In addition, a third molecule, MD-2, is constitutively associated with TLR4 and confers enhanced LPS responsiveness to TLR4 *(31)*. Thus, LPS recognition by the host is accomplished by a complex of at least three components, CD14, TLR4, and MD-2.

TNF-α is active as a trimer and mediates its effects by binding to one of two distinct receptors (55- and 75-kDa forms) that exist on most cell types studied thus far. Administration of recombinant TNF-α in vivo results in fever, hypotension, and impaired endothelial barrier function, resulting in pulmonary edema, whereas anti-TNF-α-neutralizing antibodies prevent shock when endotoxin or Gram-negative bacteria are administered to animals *(32–36)*. A role for TNF-α in ARDS was supported by findings of increased levels of TNF-α in BAL fluids of patients with ARDS *(37,38)*. The principal biologic effects of TNF-α are highlighted just below; it appears to play a proximal role in the cytokine cascade that is observed in ARDS, characterized by a predictable order of expression of subsequent cytokines *(39)*.

Peak TNF-α levels are followed by the appearance of IL-1, which exists as two species encoded by separate genes that share little homology. IL-1β is synthesized as a proform, which is proteolytically cleaved by the IL-1β converting enzyme (ICE; or caspase-1) to its active form that appears to be responsible for the biologic effects in the circulation and lung secretions *(40, 41)*. Both TNF-α and IL-1β independently, or synergistically, are capable of regulating expression of two subsequent cytokines, IL-6 *(42)* and IL-8 *(43)*. Although the role of IL-6 in ARDS remains incompletely understood, IL-8 has been shown to recruit and activate neutrophils during ALI *(44)*.

IL-8 is a member of a large family of chemoattractant cytokines, or chemokines (reviewed in ref. *45*). A new classification for chemokines has recently been reported that separates these molecules into four classes, the CXC, CC, C, and CX_3C chemokine families, which function as potent chemotactic factors for a variety of leukocytes including neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells, natural killer (NK) cells, and T- and B-lymphocytes *(46)*. As just mentioned, CXC chemokines such as IL-8 (CXCL8), have been found to facilitate neutrophil infiltration into the lung in response to bacterial

challenge in experimental models. For example, substantial increases of CXC chemokines have been reported in animal models of *Escherichia coli* pneumonia in rabbits *(47)* and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Nocardia asteroides*, and *Aspergillus fumigatus* pneumonia in mice *(48–54)*.

It remains unclear, however, as to whether neutralization of chemokines is of benefit, as they appear to be important for bacterial clearance. In trying to establish a role for CXC chemokines in the eradication of microorganisms in the lung, Greenberger and colleagues *(48)* observed that depletion of macrophage inflammatory protein-2 (MIP-2; or CXCL2/3) during murine *K. pneumoniae* pneumonia resulted not only in reduced neutrophil recruitment to the lung, but also in reduced bacterial clearance and increased bacteremia. Since CXC chemokines employ the CXC chemokine receptor CXCR2, similar studies have targeted the CXCR2 receptor to determine the importance of CXC chemokine ligand/CXCR2 biology during pneumonia. Standiford and colleagues blocked CXCR2 and found marked reductions in lung neutrophils in response to *P. aeruginosa (52), N. asteroides (54)*, and *A. fumigatus (51)* pneumonias that were accompanied with reduced clearance of the microorganisms and increased mortality.

There is substantial clinical evidence that IL-8 is present in the lungs of patients with ARDS, and increased BAL fluid levels of IL-8 were correlated with the number of neutrophils, the severity of injury, and mortality *(55)*. Thus, cytokines are clearly present in the setting of ARDS, and further refinement of our understanding of their biologic roles in this disease state is likely to lead to more rational therapeutic targeting.

Biologic Effects of Cytokines

The cytokines described above have a variety of biologic activities that in unison can serve as crucial modifiers of the immune response.

Autocrine Activity

Cytokines, especially the early response cytokines TNF-α and IL-1β, are key amplifiers of inflammation both as proximal mediators and through synergistic activities *(39,56,57)*. Additional cytokines remain to be fully identified that probably also provide autocrine stimulation. For example, in an immune complex-mediated model of lung inflammation, blocking of the CC chemokine MIP-1α decreased the

total BAL fluid TNF-α content, suggesting that MIP-1α might function as an autocrine activator of TNF-α expression *(58)*. This finding may be relevant, as early increases in MIP-1α were associated with poor outcome in neonatal respiratory distress syndrome *(59)*. Therefore, targeting proximal cytokine mediators may dampen this autoamplification observed in the acute inflammatory response.

Regulation of Adhesion Molecule Expression

One of the more important biologic roles for cytokines in ARDS is their mediation of the endothelial cell-leukocyte adhesion cascade. A hallmark of the autopsy findings of lungs from patients succumbing to ARDS is massive neutrophil infiltration. The mechanism by which leukocytes, in particular neutrophils, are recruited from the blood to the lung has been extensively studied over the past decade (reviewed in ref. *60*). In the initial phase of leukocyte adhesion called *rolling*, selectin family members of adhesion molecules (e.g., E-selectin) are expressed on the endothelial cell surface and interact with sialylated oligosaccharides constitutively expressed on neutrophils *(61–63)*. The second phase of adhesion results from the firmer interaction between cytokine-activated β_2-integrins (e.g., CD11a,–b, and –c/CD18) that are expressed on neutrophils and their counter-receptors (e.g. intercellular adhesion molecule-1 (ICAM-1)) expressed on the endothelium *(64)*.

Once they have adhered to the pulmonary vascular endothelium, neutrophils migrate to the alveolar space via chemotactic gradients generated by chemokine release (discussed in the next section) in a manner that is partially dependent on platelet-endothelial cell adhesion *(65)*. The subsequent release by leukocytes of oxygen- and nitrogen-based radical species, proteases, and arachidonic acid metabolites all contribute to cellular dysfunction, resulting in impaired endothelial barrier function and subsequent development of pulmonary edema. With this understanding of the role of adhesion molecules in ARDS, the possibility of using antiadhesion molecule therapy has been considered. Enthusiasm for an antiadhesion molecule strategy is rightfully tempered by the appreciation that the adhesion cascade is a critical innate immune response affording host protection against invading pathogens. In light of this caveat, inhibiting leukocyte adhesion in the setting of an infectious cause of ARDS could be detrimental to pathogen eradication and consequently to patient survival.

Leukocyte Chemotaxis and Transitional Inflammation

A key characteristic of the acute inflammation associated with the development of ARDS is the recruitment of predominantly neutrophils, followed by mononuclear cells, from the blood to the air spaces of the lung *(60)*. Although these leukocytes promote the eradication of an offending pathogen, the magnitude of infiltrating cells, combined with their release of mediators, leads to further amplification of acute inflammation and tissue injury. In addition, the maintenance of leukocyte recruitment necessitates intercellular communication between leukocytes and other structural cell types including the endothelial and parenchymal cells. This intercellular communication is mediated not only by cytokines and adhesion molecules, but also by the production of chemotactic molecules, including chemokines.

Early investigations identified a series of nonspecific chemotactic molecules such as *N*-formylmethionyl peptides from bacterial cell walls, the anaphylatoxin C5a, leukotriene B_4 (LTB_4), and platelet-activating factor (PAF) that were chemotactic for leukocytes *(66,67)*. Although these molecules are important in leukocyte extravasation, they lack specificity for particular subsets of leukocytes. It became increasingly apparent that the nature of the offending stimulus variably determined the subpopulation of leukocytes elicited during an inflammatory response. Thus, it was hypothesized that a more diverse set of chemotactic factors were likely to exist that possess specific activity for subsets of leukocytes.

Chemokines are a family of low-molecular-weight proteins that share a structural homology and possess a variety of biologic activities, most notably chemotactic activity for leukocytes (reviewed in ref. *68*). As mentioned in the previous section, the chemokines have been classified into four groups on the basis of their structural cysteine motifs: C (e.g., lymphotactin); CC (e.g., MIP-1α); the CXC chemokines (e.g., interleukin-8); and the CX_3C chemokines (e.g., fractalkine). The CXC chemokines (e.g., IL-8, MIP-2/GROα, KC), designated because the first two cysteine residues are interrupted by a nonconserved amino acid, are the principal neutrophil chemoattractants. In contrast, CC chemokines [e.g., MIP-1α, monocyte chemottractant protein-1 (MCP-1)], have their first two cysteine residues adjacent and are the principal mononuclear cell chemoattractants. In studies of immune complex-mediated lung inflammation, MIP-2 appeared to mediate activation and

recruitment of neutrophils into the alveolar space, whereas MIP-1α appeared to have an autocrine effect on TNF-α expression *(69,70)*.

In the context of ARDS, the acute infiltration of neutrophils is followed by migration of mononuclear cells into the lung. Both T-cells and monocytes appear to contribute to persistent lung inflammation and subsequent fibrosis in animal models of chronic lung inflammation induced by silica inhalation, as prior depletion of T-cells ameliorates the lung injury (T. Shanley, unpublished data). Recent clinical data suggest that this late mononuclear cell recruitment, or so-called transitional inflammation, is critical to the outcome of patients with ARDS *(71)*. In this study, mononuclear cell recruitment was correlated with MCP-1 levels, suggesting that this CC chemokine is a key mediator of this process. Additionally, the number of mononuclear cells in the BAL fluid between days 3 and 7 was correlated with impairment of oxygenation in patients with ARDS. Together, these studies demonstrate a role for chemokines in mediating ARDS. Further understanding of the regulation of expression of these chemokines may identify potential therapeutic targets for interrupting the inflammatory process in ARDS.

Regulation of Inflammation by "Antiinflammatory" Cytokines

Following their discovery, cytokines were considered strictly proinflammatory molecules on the basis of their contribution to the pathophysiology of disease states such as sepsis and ARDS. More recently, an accumulating body of data supports the role of a number of cytokines as antiinflammatory molecules. Included among this group of cytokines are IL-10, IL-4, IL-13, TGF-β and in some circumstances IL-6 and a related cytokine, IL-11. Perhaps the most well studied of the antiinflammatory cytokines is IL-10, which is a potent endogenous regulator of acute lung inflammation on the basis of its ability to downregulate cytokine production by macrophages *(72)*. For example, in the setting of ALI in rats, blocking of endogenous IL-10 caused increased inflammation and pulmonary edema in association with increased levels of TNF-α and IL-1β *(73)*. This finding was supported by the finding that in the IL-10 null mutant mouse, administration of a typically sublethal endotoxin dose resulted in 100% mortality *(74)*. A correlative finding in humans was demonstrated by Donnelly and colleagues *(75)*, who showed that patients with lower levels of IL-10 in their BAL fluid had a higher mortality rate from ARDS. Similar findings have been observed using TGF-β, IL-4, and IL-13 as "monocyte deactivating

agents" that are able to decrease proinflammatory cytokine expression from effector cells such as the alveolar macrophage. These data suggest that this family of molecules serves as important counter-regulatory molecules in the setting of acute inflammatory disease states such as ARDS.

It is important to recall that the biologic effects orchestrated by the proinflammatory cytokines are a critical component of the innate immune response directed against host invasion. Accordingly, it is imperative to not assume that all critically ill patients with ARDS will benefit from inhibition of the proinflammatory response, which may lead to untoward effects from immunosuppression. Already, clinical investigators have attempted to inhibit cytokine activity, and although this strategy has proved promising in preclinical studies, their ultimate clinical efficacy in human trials has been disappointing *(76,77)*. It may be that overexpression of antiinflammatory cytokines such as IL-10 may in fact contribute to host immunosuppression, thus impairing pathogen clearance. In this context, enhancing the proinflammatory cytokine response in patients suffering from infections may improve survival. In light of this complexity, it will be necessary for the host to maintain a homeostatic cytokine balance in its attempt to fight off infection, but not at the expense of lung tissue injury. The concept of balancing proinflammatory and antiinflammatory activity in the individual patient is depicted in **Fig. 2**.

Molecular Regulation of Cytokine Gene Expression

Because of the important role cytokines play in the development, promulgation, and eventual resolution of ARDS, their molecular regulation has been a target of active investigation over the past decade. It is hypothesized that a complete understanding of the mechanism(s) of gene expression and relevant signal transduction pathways necessary for their expression will provide investigators with additional therapeutic targets in combating ARDS. Although a full description of the signaling pathways that mediate cytokine gene activation in response to a variety of stimuli is beyond the scope of this chapter, it is important to review the most notable pathways.

NF-κB Signaling

Transcriptional activation factors are proteins that bind to DNA to facilitate the transcription of DNA to messenger RNA. A key transcrip-

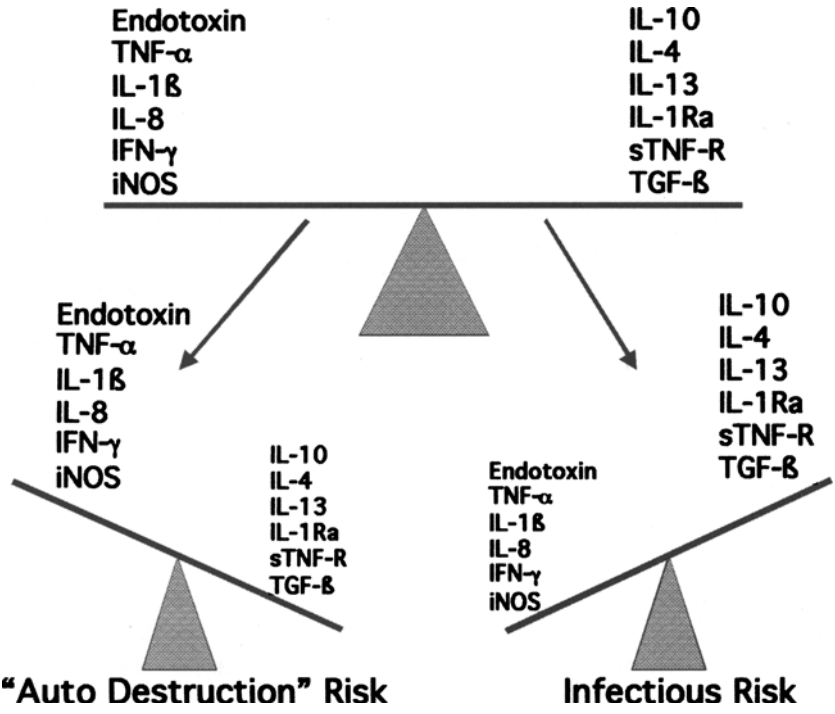

Fig. 2. Schematic depicting the balance of proinflammatory and antiinflammatory activity during ARDS. Dysregulated inflammation results in excessive production of proinflammatory mediators, causing tissue injury, whereas an overexuberant compensatory response results in immunosuppression with potential negative implications for pathogen eradication. IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthese; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; sTNF-R, tumor necrosis factor receptor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

tion factor activated by a number of stimuli associated with the onset of ARDS is nuclear factor κB (NF-κB). NF-κB is a member of the *Rel* family of transcription activation factors which is composed of two subunits (most commonly p50 and p65) that are anchored in the cytoplasm by an inhibitory subunit, IκB *(78)*. A number of stimuli can induce the nuclear translocation of NF-κB via a process that requires phosphorylation, ubiquitination, and subsequent degradation of IκB, initiated by the Iκ kinase complex *(79,80)*. Once in the nucleus, NF-κB binds to a consensus sequence of DNA in the promoter regions of a number of genes

to initiate the transcriptional process. NF-κB, either independently or in association with additional factors, regulates the transcription of multiple proinflammatory molecules including cytokines (e.g., TNFα), chemokines (e.g., IL-8), adhesion molecules (e.g., ICAM-1), and additional molecules such as inducible nitric oxide synthase (iNOS). Therefore, inhibiting this signaling pathway blocks the transcription of a number of products thought to contribute pathophysiologically to ARDS. NF-κB activation has been shown in alveolar macrophages from patients with ARDS, thereby strengthening the hypothesis that this pathway may be a valid therapeutic target *(81,82)*.

Redox State of the Cell in Relation to NF-κB

An obvious consequence of the standard therapy used to treat the hypoxemia associated with ARDS is an increase in the ambient oxygen concentration delivered to the lungs, which can contribute to oxidant stress on the lung tissue *(83)*. In addition to direct toxic effects, oxygen radicals contribute to the amplification of inflammation by inducing the transcription of proinflammatory genes such as TNFα *(84)*, IL-1, and IL-8 *(85,86)*. This oxidant-mediated pathway is at least in part dependent on activation of NF-κB *(87,88)*. As a further example of the redundancy of the regulation of the lung inflammatory response, cytokines can also mediate production of oxygen radical species. Thus, it is anticipated that targeting oxidants would ameliorate tissue injury in ARDS and blunt the inflammatory response.

MAP Kinase Pathways

Another key transcription factor that regulates the expression of a number of inflammatory molecules is activating protein-1 (AP-1). AP-1 is also a sequence-specific transcription factor composed of members of the *fos, jun,* and activating transcription factor (ATF) families (reviewed, in ref. *89*). In examining the gene products under transcriptional regulatory control by AP-1, it is clear that AP-1 and the associated upstream signaling pathways regulate a diverse set of cellular functions including inflammation, cell proliferation, apoptosis, and tissue morphogenesis. Therefore, it is necessary to understand fully the specific roles of this signaling pathway in ARDS in order to target it selectively for therapeutic intervention.

AP-1 transcriptional activity is the downstream result of a complex signal transduction cascade mediated by members of the mitogen-

activated protein kinases (MAPKs) and their upstream kinases (reviewed in refs. *90–92*). Among this set of protein kinases, three major pathways have been identified: the c-Jun NH_2-terminal kinases (JNK) pathway (also called the stress-activated MAPK or SAPK pathway); the extracellular-regulated protein kinase (ERK) pathway; and the p38 mitogen-activated kinase (p38 MAPK). All members of these MAPK families undergo activation via phosphorylation of threonine and tyrosine residues by upstream MAPK kinases (MKKs, or MEKs). These MKKs are in turn activated via phosphorylation by upstream MKK kinase (MKKKs or MEKKs). As is predicted by the complex nature of this cascade, a diverse set of stimuli can broadly influence a variety of cellular functions relevant to lung inflammation and ARDS.

Posttranscriptional Modification of mRNA

Altering the signal transduction pathways reviewed just above will have a predominant effect on the transcription of proinflammatory genes (i.e., the mRNA species). These mRNA species, however, must still undergo translation to the final protein product in order to exert their biologic activity. This process allows for posttranscriptional modification of the mRNA, thus influencing the amount of translation that can occur. An important mechanism that regulates the amount of translated product is mRNA destabilization. Many cytokines, including TNFα, IL-1β, and IL-8, possess copies of an AU-rich element (ARE) in the nucleotide base sequence of the 3'-untranslated region (3'-UTR) of their mRNA *(93,94)*. This ARE sequence provides a target for both stabilizing and destabilizing proteins (e.g., ribonucleases), thus serving to alter the length of time a transcriptional product can be translated to protein *(95)*. Because these sequences are most common in genes characterized as constitutively silent but acutely transcribed, such as cytokines, targeting this mechanism may prove selective for degradation of those molecules contributing to the pathophysiology in ARDS.

As a relevant example to the pathophysiology of ARDS, the posttranscriptional regulation of TNFα synthesis has been increasingly understood *(96)*. Recent investigations have identified a series of proteins that bind to an ARE sequence in the 3'-UTR of the TNFα transcript. These include tristetraproin (TTP) and AUF1, which destabilizes the mRNA, and HuR, which stabilizes TNFα mRNA. Members of the RNA recognition motif family of RNA binding proteins such as TIAR

and a related homolog, TIA-1, also appear capable of binding to the TNFα ARE and repressing the initiation of translation. Inhibition of the p38 and ERK MAP kinase pathways appears to regulate the interaction of these RNA binding proteins to the 3'-UTR, affecting the rate of transcription and possibly translation of the mRNA species. This tightly regulated system of posttranscriptional modification provides an additional therapeutic target in combating the cytokine production associated with pathologic disease states such as ARDS.

CONVENTIONAL THERAPEUTICS

Therapy for ARDS begins by addressing any treatable, underlying cause of ARDS, such as sepsis, pneumonia, or pancreatitis (reviewed in ref. *97*). Beyond this, with a few exceptions, most therapies specifically directed at the pathophysiologic mechanisms described just above remain experimental or have not shown any benefit in clinical trials (reviewed in ref. *8*). Thus, at present, most therapies for ARDS are primarily supportive. Furthermore, consideration of any therapy for ARDS must take into account the fact that most patients with ARDS do not die from respiratory failure, rather, as mentioned in the beginning, most patients with ARDS die as a result of sepsis or multiple organ failure. Nevertheless, it is expected that therapy specifically directed toward ARDS would have the potential to reduce the incidence of all causes of death associated with ARDS.

Conventional Mechanical Ventilation

As is the case for all patients with critical illnesses, maintaining adequate oxygen delivery is an important therapeutic goal in the management of ARDS. In the patient with ARDS this goal is achieved with the usual strategies of fluid management, achievement of adequate hematocrit, achievement of adequate oxygen saturation, and the use of appropriate inotropes and vasopressors to maintain adequate cardiac output. Unique to the patient with ARDS is the need for respiratory support, most typically in the form of mechanical ventilation.

A select group of patients with respiratory failure has the potential to be managed with noninvasive positive-pressure ventilation (NPPV). The most common indication for NPPV appears to be acute hypercapnic respiratory failure, particularly in the setting of chronic obstructive pulmonary disease *(98,99)*. Additionally, it has been suggested that

patients with hypoxemic respiratory failure do not receive the same benefit from NPPV as do patients with hypercapnic respiratory failure *(100)*. Nevertheless, there are reports of successful NPPV in the setting of ARDS *(101,102)*. Thus, NPPV may be considered in some selected patients with ARDS (i.e., patients with chronic obstructive pulmonary disease and patients with milder forms of ARDS), but it should be kept in mind that the vast majority of patients with ARDS require endotracheal intubation and mechanical ventilation.

Increasing mean alveolar pressure (mP_{alv}) is currently considered the key component of mechanical ventilation support for ARDS. Increased mP_{alv} allows for recruitment of alveoli and for reduction of FiO_2 to "nontoxic" levels (<60%). There are several ways to increase mP_{alv}, but PEEP appears to be the most effective with respect to lung mechanics and avoidance of ventilator-induced lung injury (VILI). Typically, PEEP levels are increased incrementally until the FiO_2 can be reduced below 60% while maintaining a systemic oxygen saturation > 90%. Recent literature has advocated the use of pressure-volume curves for the optimal setting of PEEP *(103)*. In this strategy, PEEP is set at or above the lower inflection point of the pressure-volume curve, with the goal of maintaining alveolar patency and eliminating repeated closure and opening of alveoli (the open lung approach). Thus, most patients with ARDS will require PEEP levels in the range of 10–15 cm H_2O. The efficacy of this strategy is being rigorously tested in a current trial conducted by the ARDS Clinical Network, which is comparing higher PEEP/lower FiO_2 with lower PEEP/higher FiO_2 in patients with ARDS (*see* www.ardsnet.org).

The main negative consequences of PEEP include barotrauma, alveolar distension with CO_2 retention, and decreased cardiac output secondary to increased intrathoracic pressure. Fear of barotrauma should not preclude the aggressive use of PEEP *a priori*, given the potential benefits of PEEP. Increases in $PaCO_2$ secondary to alveolar distension can be well tolerated physiologically (permissive hypercapnia); when they are excessive, they can be corrected by lowering PEEP as long as it does not compromise oxygenation. Finally, reductions in cardiac output can be overcome by appropriate augmentation of preload and appropriate use of intravenous inotropes. In this setting, data derived from a pulmonary artery catheter may provide valuable guidance.

Inverse Ratio Ventilation and High-Frequency Ventilation

Apart from PEEP, there are other available modalities for increasing mP_{alv} in the setting of ARDS. Inverse ratio ventilation (IRV) makes use of supraphysiologic inspiratory cycles such that the inspiratory-to-expiratory time ratio is greater than 1:1 *(104)*. This strategy substantially increases mP_{alv}, thereby increasing alveolar recruitment and improving oxygenation. Whether improvements in oxygenation are caused by increased inspiratory time *per se*, or increased intrinsic PEEP, is a matter of debate. Studies using historical controls suggest that IRV can improve the outcome of ARDS *(105–110)*. However, when considering the use of IRV, it should be kept in mind that there are no large, prospective randomized trials comparing IRV with conventional ventilation in ARDS.

High-frequency ventilation (HFV), in the form of either high-frequency jet ventilation (HFJV) or high-frequency oscillatory ventilation (HFOV), is another alternative means of increasing mPalv in the treatment of ARDS *(111)*. HFV has theoretical appeal in ARDS because it makes use of small tidal volumes, while maintaining alveolar recruitment, thus potentially reducing VILI. A large experience in adults with HFJV ventilation suggests that there is no benefit with respect to mortality *(111)*. Experience in pediatric patients, however, suggests that HFOV may provide some benefit *(112,113)*. Overall, there is continued enthusiasm for the use of HFV in the setting of ARDS, but its true benefit remains to be established.

Lung Protective Strategies

The use of mechanical ventilation presents a clinical paradox. On the one hand, it provides life-sustaining support to allow sufficient time for recovery. On the other hand, the use of high concentrations of oxygen and the stretching forces of positive pressure ventilation can be directly injurious to the lung.

Lung toxicity related to high concentrations of oxygen (hyperoxia) has been recognized for many years. Hyperoxia is directly toxic to lung parenchymal cells by the generation of oxygen-related radicals and by impacting the signal transduction pathways of lung parenchymal cells *(114)*. Indeed, in the words of Fridovich *(115)*, "The aerobic lifestyle offers many advantages but is fraught with danger." Although the "safe" level of oxygen during ARDS is not known, a reasonable goal

appears to be achievment of an $FiO_2 < 60\%$. Thus the general recommendation exists of titrating PEEP to a level that allows reduction of FiO_2 below 60%.

The concept of VILI secondary to mechanical forces has generated a great deal of clinical and investigative interest in the last decade. VILI is a manifestation of direct physical damage to lung parenchyma, as well as stretch-induced changes in lung parenchymal signal transduction pathways. This latter concept is embodied in the term *mechanotransduction*, which describes how physical forces change gene expression patterns in the lung, thus leading to potentially important negative consequences such as increased inflammation and alterations of ion channels. Multiple experimental models and clinical studies have documented the physiologic relevance of VILI *(116–125)*.

Recognition of the influence of VILI on the course of ARDS has led to the clinical use of lung protective strategies. These strategies seek to use mechanical ventilation forces in a manner that limits the degree of VILI. As described above, the appropriate use of PEEP to prevent cyclic opening and collapse of alveoli and the use of HFV are two examples of lung protective strategies. A more recent, and seemingly more successful, lung protective strategy has been to reduce tidal volume (6 mL/kg) below the more traditional volumes (12 mL/kg) used in clinical practice. Numerous studies have suggested clinical benefits of this low tidal volume strategy *(126–129)*. A recent trial conducted by investigators in the National Institutes of Health ARDS Clinical Network provides the most definitive evidence that a low tidal volume strategy is beneficial for patients with ARDS *(130)*. This trial enrolled over 800 patients who were randomized to a conventional ventilation group (using 12 mL/kg tidal volume) or an experimental group (using 6 mL/kg tidal volume). The trial was terminated early after a planned interim analysis because patients ventilated with the low tidal volume strategy had a mortality rate of 31.3% compared with 39.8% in the patients treated with conventional tidal volumes ($p = 0.01$). Details regarding the ARDS Network and this specific clinical trial can be found at the website: www.ardsnet.org. Based on these data, the use of low tidal volumes can now be considered as standard in the management of ARDS.

Permissive Hypercapnia

One physiologic consequence of a low-volume ventilation strategy is increased $PaCO_2$ *(128,129)*. Allowing $PaCO_2$ to rise in an attempt to limit VILI is known as *permissive hypercapnia*. An alternative term, coined by Robert S.B. Clark, is *submissive hypercapnia*, which describes extreme elevations of $PaCO_2$ in the most severe forms of ARDS. Although hypercapnia can cause pulmonary hypertension, increased intracranial pressure, and cardiovascular dysfunction, several clinical studies suggest that hypercapnia is well tolerated in patients with ARDS *(128,129)*. One study reported a mean $PaCO_2$ of 66.5 torr (range 38–158) and a mean pH of 7.23 (range 6.79–7.45), with a mortality rate of 26.4% *(129)*. Although most clinicians tolerate a pH of approximately 7.25, below this level there is considerably less consensus. When pH drops below this level, some clinicians will tolerate the lower pH, some will increase ventilation, and others will administer intravenous base agents. Which of the three approaches is most appropriate remains to be determined and for now should probably be dictated by the needs of the individual patient.

Prone Positioning

Chest computed tomography illustrates how heterogenously the lung parenchyma is affected in patients with ARDS. When in a supine position, the dependent areas (dorsal) tend to be fluid-filled and collapsed, whereas the nondependent areas (ventral) tend to be well ventilated, thus causing significant mismatch of ventilation and perfusion. Positioning patients in a prone position allows for improved ventilation/perfusion matching and has been shown in several clinical studies to improve oxygenation in patients with ARDS *(131–141)*. Complications of prone positioning include accidental extubation, pressure sores, catheter dislodgement, and, in some patients, worsening oxygenation. Overall, however, prone positioning is well tolerated and clinically feasible. The feasibility of positioning patients in the prone position is illustrated in **Fig. 3**. which depicts a 180-kg patient in the prone position. Our clinical experience indicates that the response to prone positioning is variable from patient to patient, with some patients achieving greater improvements in oxygenation than others, and other patients requiring relatively frequent changes from the supine position to the prone position. In the absence of a large prospective trial examining prone positioning in patients with ARDS, our most reasonable recom-

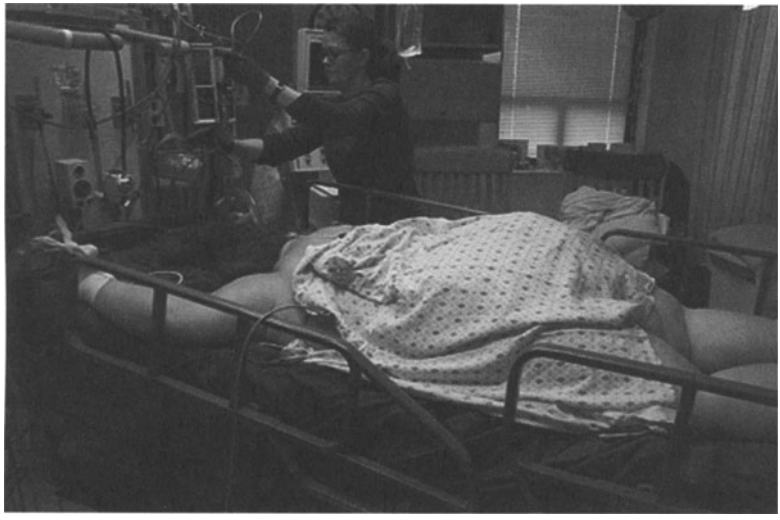

Fig. 3. A 180-kg patient in the prone position, illustrating that this position is feasible in virtually any patient with ARDS.

mendation is that the prone position should be considered in most patients with ARDS.

Fluid Management

Titrating preload and concomitant hemodynamic variables to supraphysiologic values in patients with ARDS cannot be recommended based on the current literature. Certainly, titrating these variables to normal seems to be a prudent recommendation. Furthermore, because pulmonary/alveolar edema is an important component of ARDS, it has been suggested that significantly reducing extravascular lung water, with fluid restriction and administration of diuretics, is beneficial for patients with ARDS. The literature supporting or refuting this approach is somewhat controversial and relies heavily on the use of pulmonary artery catheters, which has also come under strong criticism recently. Recent clinical data, however, suggest that pulmonary edema during ARDS results not only from an imbalance of alveolar-epithelial permeability but also from impaired alveolar fluid clearance by the alveolar epithelium *(142–144)*. In addition, patients with ARDS who have relatively normal alveolar fluid clearance have a better survival rate than patients who have a lower than normal alveolar fluid clearance rate

(143). Based on the available data, the "best" approach seems to be avoidance of hypervolemia and attempts to reduce of extravascular lung water to a level that does not compromise cardiac output (*euvolemic dehydration*, a term coined by Alan B. Fields). Admittedly, judging the latter can be problematic and relatively subjective. Future therapies aimed at restoring normal alveolar fluid clearance hold the promise of more specifically managing the pulmonary edema associated with ARDS.

Corticosteroids

There has been interest in the therapeutic use of high-dose corticosteroids in ARDS and septic shock since the early 1960s. This strategy is based on the sound pathophysiologic concept that a great deal of the organ injury seen in these clinical syndromes is a manifestation of dysregulated inflammation. Because corticosteroids are such potent antiinflammatory agents, it has been postulated that they can substantially attenuate organ injury associated with ARDS. Despite this background, however, a beneficial effect of corticosteroids has not been established in patients with ARDS *(144–146)*. Recently, there has been renewed interest in the use of corticosteroids in patients with "late" or "unresolving" ARDS *(147–149)*. The "new" strategy uses a longer course of therapy, at lower doses, compared with the original trials of corticosteroids in early ARDS. Meduri and colleagues *(149)* performed a randomized trial involving 24 patients with "unresolving" ARDS, defined as patients showing no improvement on day 7 of their disease process. Administration of corticosteroids to these patients had several benefits, including improvement in lung injury score, improvement in PaO_2/FiO_2 ratio, decreased multiple organ dysfunction, and decreased mortality. Although we await the results of the Late Steroid Rescue Trial being conducted by the ARDS Network (*see* www.ardsnet.org), the use of corticosteroids in unresolving ARDS may be considered in clinical practice.

EXPERIMENTAL THERAPIES

Targeting Cytokine Production

Although a number of cells produce cytokines, those of the mononuclear-leukocyte lineage, such as the peripheral blood monocyte (in indirect lung injury, e.g., sepsis) and the alveolar macrophage (in the

direct lung injury setting, e.g., pneumonia), appear to be the principle sources. As noted in the cytokine discussion above, a number of agents have shown promise in "deactivating" these cells as a means of inhibiting cytokine production. Antiinflammatory cytokines such as IL-10 *(72,150)* and transforming growth factor-β (TGF-β) *(151)* display potent monocyte deactivating properties and have been touted as therapeutic candidates in ARDS. IL-10 has demonstrated particular promise as it inhibits a variety of biologic functions that are fundamental to the development and promulgation of ARDS. First, it inhibits the synthesis of a number of cytokines, which would have an additional benefit of impairing the autocrine effect of these molecules *(72)*. Second, it inhibits the endothelial cell-leukocyte adhesion cascade by regulating adhesion molecule expression *(152)*. Third, it inhibits NF-κB nuclear activation via its ability to inhibit the Iκ kinase complex *(153,154)*. Fourth, IL-10 increases the expression of naturally occurring cytokine antagonists such as the IL-1 receptor antagonist (IL-1Ra) protein *(155)*. Finally, IL-10 may destabilize the mRNAs of cytokines, resulting in decreased translation *(156)*. In light of the multiple mechanisms by which IL-10 and other regulatory cytokines can regulate inflammation, exogenous administration of these molecules may be a potentially promising strategy.

Alternatively, increasing the endogenous production of these cytokines via pharmacologic means may accomplish similar functions. Several agents can increase the production of IL-10, including interferon-α, glucocorticoids, prostaglandin E_2, chlorpromazine, and cyclosporin (reviewed in ref *157*); however, the ability of these agents to control the inflammatory response in human ARDS remains to be determined.

Cytokine Neutralization

Because of the proximal role that cytokines play in the inflammatory cascade and their autocrine amplification effects, investigators have traditionally attempted to block their activity directly either by antibody neutralization (e.g., anti-TNF-α antibody) or receptor blockade (e.g., IL-1Ra). Although these strategies proved promising in preclinical trials, their ultimate clinical efficacy in human trials has been disappointing (reviewed in refs. *158* and *159*). The reasons for this are multiple, including inaccurate modeling of the human disease state, poor identification of underlying risk factors, and limitations on statistical power analysis. Other factors weighing against the success of

this strategy include the fact that cytokines are likely to be increased prior to the clinical presentation of a critically ill patient. Also, the cytokine cascade is redundant, making it unlikely that inhibition of a single cytokine will prove beneficial in the context of the limited clinical trials. Most importantly, as stated above, it is unlikely that all patients with ARDS are battling with uncontrolled proinflammation. It is probable that a subset of individuals are existing in a relatively immunocompromised state caused by overexpression of antiinflammatory molecules and cytokines, leaving the host at substantial risk for overwhelming infection as the cause of respiratory failure *(158,160)*.

Inhibition of Signal Transduction Pathways

Cytokine gene expression, and thus cytokine-induced biologic responses, are under the control of specific signaling pathways some of which were reviewed in the Pathophysiologic Mechanisms section above. As mentioned, the NF-κB pathway appears to be involved in the pathophysiology of ARDS. Consequently, investigators have employed a variety of agents to inhibit this pathway at different sites in order to modulate gene expression. Therapeutic targets have included the phosphorylation, ubiquitination, and proteasomal degradation of IκB. That the initial phosphorylation step can be targeted is supported by the observation that expression of a dominant-negative serine 32 (the amino acid targeted for phosphorylation) mutant IκBα blocked activation of NF-κB by TNFα *(161)*. Several novel compounds, including antioxidants, have been designed that also block the phosphorylation of IκBα independent of MAP kinase pathways *(162,163)*. The final step of proteasomal degradation has been successfully targeted for NF-κB inhibition, resulting in diminished cytokine production and cytokine-mediated adhesion molecule expression *(164,165)*.

An alternative strategy for NF-κB inhibition is overexpression of its inhibitory protein, IκBα. Adenovirus-directed expression of IκBα reduced expression of endothelial cell adhesion molecules, as well as the cytokines IL-1, IL-6, and IL-8 *(166)*. More recently, this pathway was shown to be dependent on tyrosine kinase- and phosphatidylcholine-specific phospholipase C in primary human alveolar macrophages, thus providing additional targets for inhibition with agents such as genistein and tyrophostin *(167)*. Also, as this pathway has been demonstrated to be exquisitely sensitive to oxidative stress (reviewed in ref.

168), several antioxidants including pyrrolidinedithiocarbamate (PDTC) *(163)*, *N*-acetylcysteine *(163)*, vitamin D *(169)*, and sesquiterpene lactones *(170)* have demonstrated the ability to block NF-κB inhibition and subsequent gene expression. Corticosteroids (described above in the therapy of ARDS) are also potent inhibitors of the NF-κB pathway.

Finally, an interesting characteristic of this signaling pathway has been its regulation by the heat shock or stress response. This is a conserved, cytoprotective response characterized by rapid expression of stress proteins, including heat shock proteins (reviewed in ref. *171*). Induction of the heat shock response has been shown to impact on the NF-κB signaling pathway. For example, in cultured human respiratory epithelial cells, heat shock decreased NF-κB translocation in a manner associated with increased IκB expression *(172)*. This strategy has been employed to inhibit induction of iNOS *(173)*. Although it is impractical to subject patients to thermal stress, nonpharmacologic inducers of this stress response such as prostaglandin A_1 *(174)* and zinc *(175)* may prove beneficial.

In light of the ubiquitous role of NF-κB in regulating a number of cellular functions, the practical nature of a strategy of NF-κB inhibition needs to be addressed in vivo; however, the current experimental data substantiate the potential role of the NF-κB pathway as a therapeutic target.

Posttranscriptional Strategies

In addition to conferring mRNA instability, the characteristic AU-rich sequence in the 3'-UTR of many cytokine mRNAs results in diminished translational efficiency, as has been shown for TNF-α *(176)*. These findings have prompted investigators to develop strategies for selectively degrading mRNA by decreasing mRNA half-life. As mentioned in the Targeting Cytokine Production section above, IL-10 has been shown to suppress cytokine production posttranscriptionally by promoting mRNA degradation *(151)*. Interestingly, thalidomide has been shown to decrease the half-life of TNF-α mRNA in a selective manner *(177)*. Investigators are actively developing systems that test the ability of pharmacologic agents to target this mechanism selectively *(178,179)*.

Some studies have addressed the role of genetic variation, or polymorphisms, in 3'-UTR of cytokine genes. It was felt that genetic alter-

ations in these sequences might contribute to the variable responses to inflammatory stimuli that are observed among individual patients. Although mutations in the 3'-UTR of the TNF-α gene in mice were shown to contribute to TNF-α-mediated diseases *(180)*, a subsequent study in a population of pediatric patients with autoimmune diseases was unable to confirm a significant frequency of mutations in this area *(181)*. Nevertheless, an increasing number of studies is aimed at determining the effect of polymorphisms in DNA sequences, which regulate cytokine gene expression *(182)*. For example, polymorphisms in the IL-10 promoter region as well as in the 5'-upstream sequence of TNF-α may regulate an individual's immune response to meningococcal disease *(183)* and heart transplantation *(184)*. It is anticipated that in the future genetic markers will assist the clinician in predicting the degree of inflammatory response each individual patient with ARDS is likely to display.

A novel approach for interfering with posttranscriptional processing of the mRNA for a gene product involves the introduction of an antisense oligonucleotide sequence. In using this strategy, DNA fragments that are complementary to the transcribed mRNA are introduced into the cell by a variety of transfection protocols. The complementary strand binds to the native mRNA, making it inaccessible to the translational machinery of the cell and thereby inhibiting protein expression. For example, in human umbilical vein endothelial cells and carcinoma cells, an antisense oligonucleotide for ICAM-1 mRNA inhibited its expression *(185)*. A similar antisense strategy has been employed by these same investigators to decrease functional protein expression of ICAM-1 in two in vivo models *(186,187)*. The antisense oligonucleotide delayed cardiac allograft rejection *(188)* and also inhibited LPS-induced neutrophil accumulation in the alveolar space *(189)*. Antisense strategies have been employed to block expression of other cytokine gene products as well *(190)*. The difficulties inherent in this strategy are analogous to those encountered with gene therapy: synthesizing an effective oligonucleotide, delivery of the sequence into the cell with specificity, and achieving sufficient inhibition of protein expression.

Blocking Adhesion Molecules

As our understanding of the role of adhesion molecule expression has unfolded, the goal of antiadhesion molecule therapy has become an

intriguing pursuit. Numerous preclinical animal trials have demonstrated that antiadhesion molecule antibodies such as anti-ICAM-1 *(187,191)*, anti-E-selectin *(192)*, anti-L-selectin *(193)*, and anti-P-selectin *(194)* are able to inhibit neutrophil accumulation in the lung and subsequent tissue injury. Despite these encouraging results, to date, no human trials have successfully used antiadhesion molecule strategies. The leukocyte-adhesion molecule cascade is a necessary host response, as evidenced by individuals who suffer recurrent bouts of infection as a result of leukocyte adhesion deficiency (LAD) syndromes 1 and 2. The molecular bases of these defects are absent expression of the β-integrins (counter-receptor for ICAM-1) in LAD-1 and absence of sialyl-Lewis X (carbohydrate ligand for selectins) in LAD-2 *(195)*. In light of this, disrupting this cascade in the setting of an invading organism may be detrimental to the host. Thus, antiadhesion molecule strategies are likely to face stringent safety trials.

Blocking of Chemokines or Chemokine Receptors

As mentioned in the Pathophysiologic Mechanisms section above, chemokines appear to play a central role in the activation and recruitment of neutrophils to the lung in ARDS. As such, chemokines have become important therapeutic targets in many inflammatory states including ARDS, rheumatoid arthritis, and AIDS. Currently two mechanisms are being employed. Monoclonal antibodies directed against IL-8 have been shown to decrease neutrophil influx and tissue injury in a number of animal models of lung injury *(196–198)*. Because of these encouraging preclinical results, anti-IL-8 antibody is likely to be tested in human ARDS in the very near future. In addition to antibody neutralization, targeting the chemokine receptors has become a hotly pursued field over the past 2 yrs (reviewed in ref. *199*). It is probable that chemokines will be successfully inhibited in both a selective and effective manner in the near future.

APPLICATION OF GENOMICS TO ARDS

As has been emphasized, ARDS is a highly heterogenous disease process with respect to both etiology and outcome. Variable outcomes are particularly frustrating when one considers that one patient with ARDS may survive, and another patient of similar age, having an identical cause of ARDS and similar comorbidities, may die. These highly

divergent outcomes can, at times, be explained by management strategies. Recent progress in genomics, however, suggests that the basis of these variable outcomes may lie in the genetic background of the individual patient. That is, some individuals with ARDS may have a genetic background predisposing him or her to a more severe manifestation of ARDS and are consequently more likely to die as a result of ARDS. The evolving field of genomics holds the promise of elucidating a genetic predisposition to ARDS *(200–202)*. Although no clear ARDS gene or marker has been established to date, there is good evidence that mutations, or polymorphisms, in surfactant protein genes can impart a phenotype characterized by the propensity to develop interstitial lung disease and/or ARDS *(203–205)*. In addition, polymorphisms of cytokine genes have been associated with increased mortality in sepsis *(206–208)*. Because the development of sepsis is so closely linked, clinically and pathophysiologically, to the development of ARDS, it is expected that similar associations will be found between cytokine gene polymorphisms and the course of ARDS.

Important tools for the application of genomics to the study of ARDS include the recent sequencing of the human genome, microarray technology, and bioinformatics *(200–202,209)*. The human genome sequence provides the blueprint for potentially understanding how an individual's genome impacts the development and outcome of virtually any disease process. Microarray technology allows for measuring the simultaneous expression of thousands of gene products and polymorphisms, which can subsequently be analyzed by the evolving field of bioinformatics. With these tools it may become possible to characterize the host response further during ARDS at the genomic level. These types of studies are eagerly awaited as they hold the promise of significantly increasing our understanding of ARDS. Specifically, it is hoped that individual patients can be more thoroughly characterized (i.e., immunophenotyped); as a consequence, immune- or inflammatory-modulating therapies can be more specifically tailored to the needs of the individual patient.

SUMMARY

ALI in its most severe form of ARDS continues to be a major cause of mortality in critical care medicine. It is clear that cytokines contribute to this pathophysiologic state via receptor-mediated signaling

pathways that effect target cell responses. The application of molecular biology techniques to the field of critical care medicine has both improved our understanding of this biologic response and identified a number of potential therapeutic targets. Although in vitro and animal model data have demonstrated the amelioration of the inflammatory response and lung injury by these strategies, the modalities that have been tested in humans thus far have proved ineffective. It is hoped that further understanding of the fundamental biology, improved identification of the individual patient's pro- versus antiinflammatory cytokine state, and application of therapies directed at multiple sites of action may ultimately prove beneficial for patients suffering from ARDS.

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