NHLBI Workshop Summary

Future Research Directions in Acute Lung Injury

Summary of a National Heart, Lung, and Blood Institute Working Group

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Acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS), are syndromes of acute respiratory failure that result from acute pulmonary edema and inflammation. The development of ALI/ARDS is associated with several clinical disorders including direct pulmonary injury from pneumonia and aspiration as well as indirect pulmonary injury from trauma, sepsis, and other disorders such as acute pancreatitis and drug overdose. Although mortality from ALI/ARDS has decreased in the last decade, it remains high. Despite two major advances in treatment, low V_T ventilation for ALI/ARDS and activated protein C for severe sepsis (the leading cause of ALI/ARDS), additional research is needed to develop specific treatments and improve understanding of the pathogenesis of these syndromes. The NHLBI convened a working group to develop specific recommendations for future ALI/ARDS research. Improved understanding of disease heterogeneity through use of evolving biologic, genomic, and genetic approaches should provide major new insights into pathogenesis of ALI. Cellular and molecular methods combined with animal and clinical studies should lead to further progress in the detection and treatment of this complex disease.

Keywords: pulmonary inflammation; critical care; genomics; pulmonary edema; acute respiratory distress syndrome

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are syndromes with a spectrum of increasing severity of lung injury defined by physiologic and radiographic criteria in which widespread damage to cells and structures of the alveolar capillary membrane occurs within hours to days of a predisposing insult. In this report we will consider ALI and ARDS together, although we also specifically refer to ARDS because it has been studied as a defined entity with exclusion of patients with less severe degrees of lung injury. The time course of ALI/ARDS distinguishes these syndromes of alveolar damage from most other lung diseases, whose natural histories occur over a much longer duration, usually years. ALI/ARDS is a major cause of acute respiratory failure with high morbidity and mortality in critically ill patients (1). Recent epidemiologic data indicate that the incidence of ARDS defined by consensus physiologic criteria may account for 36,000 deaths per year in a country the size of the U.S. (2). There is reason to believe that this number will increase significantly in the future because of increasing frequency of some predisposing conditions that precipitate ALI/ARDS, such as sepsis (3). Although there is evidence that mortality in patients with ALI/ARDS may have declined over the last 10 to 15 years, it remains high (30–40%), and it is an important cause of pulmonary and nonpulmonary morbidity in patients who leave the hospital (4). Until recently, there were no specific measures that altered mortality in ALI/ARDS, and management was exclusively expectant and supportive, reflecting major deficiencies in our understanding of the cellular and molecular nature, pathogenesis, and natural history of acute alveolar injury. Recently, however, a prospective multicenter clinical trial demonstrated that a lung-protective ventilatory strategy could substantially reduce mortality (5). The results of this clinical trial suggest that there are additional opportunities to improve outcomes in ALI/ARDS if we can increase our fund of knowledge at the basic, translational, and clinical levels.

On January 21–22, 2002 the NHLBI convened a working group in Rockville, MD to consider future directions in research in ALI/ARDS. Conference participants were charged with reviewing the current state of knowledge, identifying major gaps in information and understanding, identifying promising opportunities for investigation and discovery, and devel-

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oping specific recommendations to be used by the NHLBI in planning future research in ALI/ARDS. This article summarizes presentations and discussions at the Working Group meeting. The conference also considered trends and successes in other fields that may be instructive and relevant to ALI/ARDS. As an example, management of patients with acute coronary syndromes has undergone major paradigm shifts in recent years because of insights generated from fundamental, translational, and clinical investigation; this has resulted in development of new interventional approaches and biologic and pharmacologic strategies that address thrombosis and inflammation in addition to a traditional focus on lipids and vascular remodeling and has generated ongoing investigations of these critical processes (6–8). Similarly, a new treatment for severe sepsis, recombinant activated protein C, has recently been developed based on molecular insights relevant to coagulation and inflammation that were then explored in translational and clinical studies (9–11). Sepsis is of particular interest because it is a leading predisposing condition that initiates ALI/ARDS, and it complicates ALI/ARDS induced by other causes (1).

This report of the proceedings of the Working Group meeting begins with a brief overview of the clinical features of ALI/ARDS followed by sections that consider (*1*) gaps in our understanding of basic mechanisms underlying development of ALI, (*2*) how genomics and proteomics may advance research in ALI/ARDS, (*3*) how genetic influences might be studied in patients with ALI/ARDS, (*4*) the challenges posed by the heterogeneity of the clinical phenotypes in ALI/ARDS, (*5*) how biological and clinical indices of lung injury may be useful, and (*6*) how animal models can be used to provide insights into lung injury. The final section summarizes the major recommendations for future research directions. The group did not attempt to provide a comprehensive overview of the field, as there are many fine ones available (1, 12); instead, citations outside of the field are highlighted. The report reflects common opinions of the participants. The challenges of ALI/ARDS remain substantial, and modern and evolving approaches in fundamental science (cell and molecular biology, genomics, proteomics, disease gene discovery) together with new approaches to clinical investigation provide substantial opportunities to improve prevention, treatment, and outcomes in ALI/ARDS.

OVERVIEW OF ALI/ARDS

ALI/ARDS is a cause of acute respiratory failure that develops in patients of all ages from a variety of clinical disorders, including sepsis (pulmonary and nonpulmonary), pneumonia (bacterial, viral, and fungal), aspiration of gastric and oropharyngeal contents, major trauma, and several other clinical disorders including severe acute pancreatitis, drug overdose, and blood products (1). Most patients require assisted ventilation with positive pressure. The primary physiologic abnormalities are severe arterial hypoxemia as well as a marked increase in minute ventilation secondary to a sharp increase in pulmonary dead space fraction. Patients with ALI/ARDS develop protein-rich pulmonary edema resulting from exudation of fluid into the interstitial and airspace compartments of the lung secondary to increased permeability of the barrier. Additional pathologic changes indicate that the mechanisms involved in lung edema are complex and that edema is only one of the pathophysiologic events in ALI/ARDS. One physiologic consequence is a significant decrease in lung compliance that results in an increased work of breathing (13), one of the reasons why assisted ventilation is required to support most patients.

The clinical diagnosis of ALI is made by the presence of arterial hypoxemia ($Pa_{O_2}/F_{I_{O_2}}$ less than 300) and bilateral pulmonary opacities on the chest radiograph. Current definitions require exclusion of left atrial hypertension and overt heart failure by clinical assessment. It is often difficult to precisely distinguish ALI/ARDS from other diffuse inflammatory conditions of the lung and, in some cases, from high-pressure pulmonary edema. New diagnostic measures to address this issue would be useful. It is not yet clear how the physiologic alterations that define the clinical diagnosis of ALI/ARDS correlate with features that define the pathologic diagnosis, a constellation of histologic findings termed diffuse alveolar damage (14).

Until recently, management of patients with ARDS was entirely supportive with the objective of managing underlying infections medically and surgically, providing nutritional support, and employing mechanical ventilation for acute respiratory failure. In a large prospective NHLBI-supported study, mortality was reduced from 40 to 31% with the use of a ventilatory strategy that employed low Vt (6 ml/kg) and limited plateau pressure (< 30 cm H_2O) (5). The results of this trial demonstrated that use of high Vt and high airway pressures exacerbated the patient's ALI and raised interesting mechanistic questions concerning interaction of lung stretch and systemic injury that may provide additional therapeutic approaches. Also, the results of this trial raise interesting questions regarding how lung-protective ventilatory strategies work. For example, is the protection a result of decreased mechanical injury to the lung endothelium and epithelium, effects on the systemic compartment, or is there also a downregulation of proinflammatory stimuli for neutrophils and macrophages (15)? Also, are there beneficial effects from the change in the carbon dioxide tension, acid-base status, and effects on reactive oxygen and nitrogen species?

Despite this advance in ventilation of patients with ALI/ARDS, considerable work is still needed to define the basic mechanisms that (*1*) initiate lung injury before institution of positive pressure ventilation, (*2*) mediate progression of ongoing lung injury, (*3*) cause fibrosing alveolitis and pulmonary hypertension with extensive obliteration of the lung circulation in subsets of patients, and (*4*) mediate the propagation of injury from the lung to other organs in many patients with ALI/ARDS.

SYSTEMIC MANIFESTATIONS OF ALI/ARDS

A major feature of ALI/ARDS that distinguishes it from most other lung diseases such as asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis is that ALI/ARDS frequently has systemic components. Several of the major triggering conditions for ALI/ARDS, notably sepsis, nonpulmonary trauma, and shock are systemic syndromes. Furthermore, diffuse injury and infection of the lung are major causes of systemic sepsis and the systemic inflammatory response syndrome and dysregulation of cellular responses in the lung may result in circulating cytokines and other inflammatory and thrombotic mediators, as demonstrated in clinical studies and animal models (16–18). Many patients with refractory ALI/ARDS die of multiple organ dysfunction and/or sepsis, rather than isolated progressive respiratory failure (1).

A consensus of the working group was that ALI/ARDS is a systemic syndrome in virtually all cases. It is important to investigate the cellular and molecular mechanisms that mediate pulmonary injury responses to systemic insults, the factors that govern the participation of blood cells and humoral agents that mediate both pulmonary and systemic injury, and mechanisms that govern compartmentalized injury in the lung versus dissemination to systemic sites and distant organs. Systemic responses to stress and injury are incompletely characterized but involve neural, endocrine, and pro- and anti-inflammatory mechanisms that may

be either adaptive or pathologic (19). Animal studies indicate that neural stimulation modifies systemic responses to endotoxin and can differentially influence cytokine accumulation in the lung and in systemic tissues; this phenomenon involves vagal signaling of altered inflammatory cell function (20, 21). Molecular links that mediate interactions between the acute inflammatory system (innate immune effector mechanisms) and thrombotic and procoagulant responses are altered in stress and injury, as are interactions between innate and acquired immune responses (19).

Signaling pathways, such as those provided by cytokines and their receptors, may have protective or injurious effects depending on the site of challenge and whether the inflammation is local or systemic (22). The unknown aspects of these complex events are particularly relevant to ALI/ARDS that is induced as a part of systemic disorders such as sepsis, multiple trauma, and shock but also may be critical in order to understand the pathologic responses to diffuse alveolar injury caused by direct insults such as aspiration and microbial infection of the lung.

CURRENT UNDERSTANDING AND SIGNIFICANT GAPS IN KNOWLEDGE

The Working Group identified many areas in which there are significant knowledge gaps that must be addressed in order to facilitate progress in understanding ALI/ARDS and for devising new strategies for detection, prevention, management, and therapy.

The cells of the alveolar capillary membrane together with cells of the innate immune and hemostatic systems are targets of damage and effectors of injury in ALI/ARDS (1). The numbers and morphologic phenotypes of these cells are altered in development of ALI/ARDS and its progression or resolution (14). Although much has been learned about the cell biology of the alveolar capillary membrane since the clinical syndrome of ALI/ARDS was described, the functional responses, biochemical pathways, patterns of gene expression, molecular mechanisms of interaction with other cells, and responses to injury remain incompletely characterized and our understanding of these processes is embryonic at best. New approaches to ALI/ARDS are likely to require comprehensive understanding of the functions of key interacting cells. New and evolving technologies and analytic methods may provide opportunities to fill knowledge gaps.

Lung Epithelium

The important role of the alveolar epithelial barrier in the pathogenesis and resolution of ALI/ARDS has been increasingly appreciated, including the role it plays in the production of surface-active material to maintain alveolar stability as well providing salt and water transport pathways for the resolution of alveolar edema. However, our understanding of the responses of the alveolar epithelium to injury remains incomplete. Disruption of alveolar epithelial integrity is a major contributor to increased permeability and alveolar flooding with protein-rich edema fluid, a hallmark of ALI/ARDS (1). Understanding of molecular signaling pathways and mechanisms of cell–cell interaction in alveolar epithelial cells at various stages after injury is rudimentary at best. Although it is clear that alveolar epithelial Type II cells repopulate the injured, denuded alveolar barrier in ALI, very little is known about the intracellular signaling pathways that are activated or differential mechanisms of gene expression during injury, proliferation, and differentiation. For example, we know that epithelial cells produce cytokines in response to various stimuli such as LPS or lung stretch (23), but the regulatory features are not completely defined. As a second example, specific mitogens such as keratinocyte growth factor can protect the lung epithelium against experimental lung injury (24), but the mechanisms for this cytoprotective effect are not well understood.

Lung Endothelium

The responses of lung endothelial cells are also incompletely defined even though they appear to be the first cells of the lung to be altered in ALI/ARDS triggered by sepsis, trauma, and other systemic conditions. The lung endothelium, in concert with the epithelial barrier, mediates the initial change in permeability and is also critical for repair and remodeling of the alveolar capillary membrane. Endothelial heterogeneity may be a factor in the lung's responses to pathologic stimuli (25). Each short segment of a rodent lung capillary possesses a few functionally distinct endothelial cells—the pacemaker cells. These cells appear to regulate endothelial calcium within the capillary segment by generating intercellular calcium waves, and they may be the sites of inflammatory initiation in the capillary (26). More needs to be learned about the diversity of endothelial cells, particularly regarding how they regulate the onset of lung inflammation. Activation of endothelial cells, with functional changes that involve both new gene expression and constitutive pathways that do not require new gene products, occurs in both pulmonary and systemic endothelium (27). We have an incomplete picture of the molecular mechanisms that govern the responses of pulmonary endothelial cells, their interaction with alveolar epithelium, and the responses of systemic endothelium in ALI/ARDS.

Apoptosis, Necrosis, and Fibrosis

A comprehensive understanding of the mechanisms of apoptosis and necrosis in the initial injury and repair of lung epithelial and endothelial cells and other key cells involved in ALI/ARDS is lacking. These cellular processes are likely to be central to imbalances between resolution and repair versus persistence and progression in ALI/ARDS and can be influenced by biomechanical, inflammatory, and thrombotic stimuli (28). The mechanisms that govern apoptosis and necrosis in endothelial and epithelial cells in the key phases of ALI/ARDS need definition. How apoptosis and related cellular events influence fibrosing alveolitis, which is an outcome in a subset of patients with ALI/ARDS, is not known. Information is needed regarding signaling pathways, patterns of gene and protein expression, and functional responses in lung fibroblasts and mesenchymal cells that lead to dysregulated matrix remodeling and relentless fibrosis in some patients with ALI/ARDS.

It is unknown how lung endothelial and epithelial injury modify the fibrogenic response in ALI/ARDS or if apoptosis and necrosis affect the fibrotic process differently. Microarray analysis and assays of new protein expression using isolated cells in parallel with the study of animal models that include fibrosis are likely to be revealing. New approaches to the cell and molecular biology of fibrogenesis that are being applied to the syndrome of idiopathic pulmonary fibrosis may be useful in clarifying the nature of alveolar fibrosis in ALI/ARDS. A caveat is that some early events in ALI/ARDS do not involve new gene expression or changes in transcript level. Similarly, dissection of the cellular and molecular processes involved in endothelial death versus repair and other key aspects of vascular wall remodeling will be critical to understanding of the loss of pulmonary vessels and the resultant pulmonary hypertension and the increase in alveolar dead space that is also an outcome of a subset of patients with ALI/ARDS.

An approach for the future treatment of ALI/ARDS could involve stem cells to replace dying lung cells. A similar strategy has been proposed for brain, heart, and liver diseases. The inflammatory response includes release of cells from the bone marrow such as mature and immature neutrophils and stem cells.

Elegant bone marrow reconstitution studies have demonstrated that these stem cells have the potential to differentiate into endothelial, mesenchymal, and epithelial cells (29–31). The proliferative potential of fully differentiated capillary endothelial cells and epithelial Type I cells is very poor in the lungs. The potential for stem cells to help in the repair of injured lung tissue has not been explored and requires a better understanding of stem cell release, targeting, proliferation, and differentiation in lung tissue. Supplementing this release by intravenous infusion of donor bone marrow–derived stem cells might be useful in ALI/ARDS, as the pulmonary microvasculature would be the first capillary bed that these cells would encounter, enhancing their entrapment and accumulation. Newly implanted endothelial cells may help restore the lung capillary network after ALI, although considerable work will be needed to develop approaches for delivery of viable cells to injured areas of the lung.

Mechanical Forces and Lung Injury

There is incomplete understanding of how normal lungs deform during breathing and to what stresses key lung cells are exposed (32). Lung injury may greatly alter these complex forces. Furthermore, there is uncertainty about the molecular mechanisms involved in mechanosensing and mechanotransduction (33). Recent observations suggest that lung stretch induces local and/or systemic cytokine release (23), yet it remains unclear if the proinflammatory deformation response is a consequence of cellular stress failure (34) or of stretch-mediated signal transduction. Integrated studies examining molecular pathways, individual cellular responses, and responses of the intact lung will be required to determine how mechanical ventilation modifies the dynamic interactions of lung cells in ALI/ARDS.

The effect of the mechanical stresses applied to the lung may extend far beyond the thoracic structures to systemic organs. Recent studies have demonstrated that mechanical ventilation can release mediators (35) that may translocate into the systemic circulation (23). This may contribute to the high prevalence of multiple organ failure in patients with ALI/ARDS. This hypothesis was strengthened by the recent low V_T study that demonstrated a systemic component in ALI/ARDS that was altered by the ventilatory strategy (5). Injurious ventilatory strategies can lead to loss of compartmentalization in the lung and may cause translocation of mediators, endotoxin, and bacteria (36, 37) from the lung to the systemic circulation. Examination of the physiological, biological, and genetic basis of stress-induced injury to the lung in health and disease is thus a fertile area of future research.

Innate Immunity

There is a lack of understanding of how cells of the innate immune system (neutrophils, monocytes, macrophages, natural killer cells, dendritic cells, and others) and cells involved in hemostasis and thrombosis (platelets and endothelial cells, together with interacting leukocytes) are dysregulated in ALI/ARDS. Although it was originally believed that increased permeability pulmonary edema and other features of ALI/ARDS could be explained by endothelial and epithelial alterations alone, it is now clear that acute inflammation, thrombosis, and activation of the coagulation system are involved. There is evidence that dysregulation of innate immune and thrombotic cascades contribute to both the initiation and progression of ALI/ARDS (1, 38). Recent studies of human leukocytes and platelets have identified previously unrecognized surface receptor interactions, intracellular signaling pathways, mechanisms of gene product expression, and functional responses that are directly relevant to ALI/ARDS and to triggering conditions such as sepsis (39–43).

The toll-like receptors of innate immune cells and endothelial cells, which recognize pathogen-associated molecular patterns in LPS and other microbial factors, are another group of factors that are critical to inflammation and injury but were only recently discovered. Toll-like receptors are linked by intracellular signal transduction cascades to activation of the nuclear factor-κB family of transcription factors and induction of tumor necrosis factor α and other nuclear factor-κB–dependent gene products. Their engagement is central to host defense and sepsis, septic shock, and their complications including ALI/ARDS (44). The recent explosion of information regarding toll-like receptors illustrates the point that much remains unknown regarding cellular responses and molecular signaling in the innate immune system. Precise understanding of compartmentalized versus dysregulated systemic inflammation will depend on additional insights into the biology of these cells and the molecular systems that regulate them, including biologically active lipids, chemokines, and cytokines.

Thrombosis and Inflammation

Additional knowledge regarding the mechanisms that link acute inflammatory responses to thrombosis and to activation of the coagulation cascade is also important because there is substantial evidence that microvascular thrombosis and dysregulated intracellular and extracellular fibrin deposition are early events in ALI/ARDS and in experimental ALI (1, 45). Recombinant activated protein C, recently approved for treatment of severe sepsis, interrupts both inflammatory and thrombotic responses (10, 46, 47). Recombinant platelet activating factor acetylhydrolase also may reduce mortality and organ failure in sepsis and trauma and diminish inflammatory and thrombotic signaling (47). The development of both of these therapies can be traced to fundamental cell and molecular biology experiments in parallel with preclinical animal studies.

FUNCTIONAL GENOMICS, PROTEOMICS, AND OTHER NEW METHODS

Modern approaches to cell and molecular biology provide significant opportunities to generate new information relevant to ALI/ARDS. In many cases, these methods can be applied or adapted to experiments with lung or other complex tissues as well as with isolated primary or surrogate cells and can be used with appropriate modification in analysis of clinical samples. Thus, there is the potential for rapid integration of basic and clinical data in a translational fashion. Genomic approaches using microarrays and other methods to display multiple DNA sequences have rapidly become major tools in biologic investigation and are increasingly being applied in critical care medicine. Microarray analysis can be applied to isolated cells and normal and diseased tissues (48–50). Laser capture microdissection, real time polymerase chain reaction, and additional analytic techniques enhance the power of this technology (51). "Functional genomic" approaches are increasingly being applied to questions in pulmonary and critical care medicine (52) and have great potential to address many unanswered questions related to changes in phenotypes of key cells in ALI/ARDS. For example, injury mechanisms mediated by release of granular enzymes or oxygen radicals by neutrophils, aggregation of neutrophils, monocytes and platelets, and activation of the clotting cascade use constitutive biochemical pathways and do not require induction of new transcripts or expression of new proteins. Furthermore, transcript abundance does not unequivocally reflect abundance of corresponding proteins or the physiological results. Critical messenger RNAs are under stringent posttranscriptional control in many cases. Constitutively expressed but repressed messenger RNAs can be translated to proteins in response to cellular signals that are relevant to ALI/ARDS and its systemic manifestations (42, 43, 53). Thus, studies of acute constitutive cellular and functional responses should not be abandoned in wholesale fashion but should be used in a complementary fashion with gene analysis strategies.

Evolving proteomic technologies have the potential to identify each protein expressed in isolated cells or in complex tissues such as injured lung and also to determine posttranslational modifications, protein–

protein interactions, and other critical features that define phenotype and regulate cellular function (54). Proteomic approaches can provide physiologic relevance that is not inherent in measuring transcript levels and profiles (53). New proteomic strategies based on isotope-coded affinity tags can accurately quantify individual proteins in complex mixtures, a capability that has been lacking in the past, and can also provide sequence information linking descriptive and quantitative proteomics (54). Proteomic analysis of clinical samples may be useful in establishing molecular patterns that are characteristic of individual diseases or disease stages (55).

The most effective use of proteomic strategies, as with genomic technology, will be in combination with traditional approaches to determine the functions of key proteins and their effects on cell and tissue behavior. In many cases these experimental strategies will require new reagents, databases, and bioinformatic tools to handle large amounts of complex data (50, 51, 54)

GENETICS OF ALI/ARDS

A major gap in our knowledge is the extent to which genetic features interact with environmental variables to influence susceptibility to ALI/ARDS. In sepsis, trauma, and other triggering conditions, only a subset of patients develops ALI/ARDS even among those in whom the pathologic stimuli are apparently equivalent (1, 3, 5, 11), suggesting that there are genetic features that may influence its onset. Similarly, we do not know what genetic features influence the natural history of ALI/ARDS once it is established or how genetic variables interact with environmental factors to determine resolution and repair versus persistence and progression of acute lung inflammation and injury. Furthermore, we have little understanding of genetic features that regulate compartmentalization of inflammation and injury in one case and engender a systemic response in another (19).

Human genetic syndromes are commonly classified as simple Mendelian disorders or as multifactorial complex diseases. In Mendelian diseases, a mutation in one or both alleles of a single disease gene leads to a pathologic phenotype, whereas complex genetic syndromes are generally caused by mutations in more than one gene with significant contributions from the environment usually influencing the manifestations of the disease. Additional features of complex genetic diseases include large phenotypic variation, incomplete penetrance, late age of onset, the potential for locus heterogeneity, and lack of easily identified large families because of a complicated pattern of heritability. The frequency of alterations in disease genes is variable in multifactorial disorders, potentially making them difficult to identify. Recent identification of alleles of the *NOD 2* gene as variants that predispose to Crohn's disease (56, 57) illustrates many of these points. Complex human disorders may also result from multiple rare variant mutations in a single gene leading to similar or identical phenotypes (58). Finally, many traits of clinical significance in complex diseases have continuous variation rather than discrete phenotypes; interacting genes that influence variation in these traits are located in chromosomal regions termed quantitative trait loci. Mapping quantitative trait loci to identify genes involved in polygenic traits has been used for analysis of complex human diseases, including asthma, arteriosclerosis, and diabetes (59).

In most cases, susceptibility to ALI/ARDS, and the natural history of these syndromes once they are established, is likely governed by genetic features together with environmental variables in the complex fashion outlined previously. Studies of large families, evaluation of large archives of small families, and analysis of affected sibling pairs are major gene discovery approaches that have been used in asthma and other complex diseases; however, these investigational tools are not available for ALI/ARDS. Furthermore, there is substantial heterogeneity in ALI/ARDS (*see* next section), and there are no unique, easily measured markers or combinations of markers that can yet be used to define the syndromes in general or to more precisely characterize subsets or phenotypes (60). Heterogeneity is a particularly confounding problem in establishing genetic and environmental contributions to complex diseases.

There is suggestive evidence for genetic variations in ALI/ARDS and in predisposing conditions such as sepsis. Polymorphisms in genes related to inflammatory markers (tumor necrosis factor α, surfactant proteins, and interleukin-6), angiotensin converting enzyme, and pathogen receptors (CD14, toll-like receptors) appear to correlate with incidence and/or outcome of sepsis (61, 62) and/or ALI/ARDS (63–65). These observations used case–control association studies. Although association studies pose significant logistic challenges in critically ill patient populations and have limitations, they are the most immediately applicable approach to ALI/ARDS. Family-based association studies and case-controlled association studies may be beneficial to explore in well-defined subgroups of patients with ALI/ARDS.

Positional cloning has been used with success in the study of complex diseases. However, availability of the sequence of the human genome and new technologies that link gene discovery to biologic function will likely provide accelerated insight into multifactorial syndromes such as ALI/ARDS and will use new approaches (66). These include identification and establishment of the significance of single-nucleotide polymorphisms and other variations in the human genome; methods to further characterize quantitative trait loci; identification of new pathways of cell function and regulation and refined analysis of known metabolic pathways based on coregulated genes; new analysis of mechanisms of transcriptional and posttranscriptional regulation, protein–protein interactions, and cross talk between signaling cascades; genotype–phenotype correlations that identify gene–gene interactions and other modifying features that lead to variations in the clinical phenotypes; and new approaches to dissecting interactions between genes and environment involving epidemiologic studies of carefully characterized populations. Robust databases that link disease records, family records, and other data sets that can be integrated for biomedical research will be important tools. Application of the new genetic approaches will also require parallel refinement of the clinical phenotypes of diseased patients and control populations, a particular challenge in the case of ALI/ARDS.

Postgenomic approaches will continue to depend on genetically modified mice and other model systems. Mapping of traits, generation of knockouts and transgenics, and mutational strategies with genome-wide analysis are highly tractable in mouse models and can circumvent some of the problems of human patient populations, including heterogeneity. Genetic and genomic approaches using mice and other model species have promise for dissection of the complexity of cardiovascular diseases (67) and other disorders. Some of these approaches have recently been applied in mouse models of lung injury (68).

HETEROGENEITY OF PHENOTYPE IN ALI/ARDS

The heterogeneity of inciting factors and outcomes in ALI/ARDS creates complexity and uncertainty in the study of this syndrome. In order to better understand specific mechanisms of ALI under different clinical conditions as well as to probe genetic and environmental influences, it is important that more information be obtained regarding characterization of subgroups of patients with ALI/ARDS. For example, the incidence, natural history, and outcome of ALI/ARDS induced by sepsis and trauma are quite different in some senses, but the factors contributing to this heterogeneity are not clear (1).

Patients develop pneumonia from several different etiologies, including gram-positive or gram-negative bacteria, viruses, or even

fungi or parasites, particularly in the presence of immunosuppression, and the signaling pathways that regulate the inflammatory response to any particular organism depend on the initial molecular interactions between the host cells and the organism (39, 44). A better understanding of bacterial genetics and the specific virulence factors of infecting bacteria should provide new approaches to understanding susceptibility to pneumonia and ALI/ARDS both as a cause of injury and as a cause of nosocomial pneumonia in patients with ALI/ARDS (17). A better understanding of why some patients with sepsis develop ALI is needed. Is there a host susceptibility to sepsis so that patients who are more likely to upregulate gene expression for proinflammatory cytokines are therefore more likely to develop ALI? This hypothesis is plausible but other factors may also be involved, such as the ability of the host to confine an infection to a localized area. For example, the ability to mount a local proinflammatory response may be important in containing bacterial infection to one portion of the lung, thus preventing dissemination of cytokines and bacterial products to the circulation that could result in multiple organ failure and diffuse lung injury if not locally contained (19). A more detailed understanding of the pathogenesis of clinical lung injury, including biochemical markers, studies of lung pathology, and integrated studies using genomics and proteomics may provide more insight into individual patient susceptibility and potentially identify ALI/ARDS subgroups. It is possible that one or more specific markers may apply to one phenotypic ALI/ARDS subgroup but not to another. Similar approaches are being used in other human diseases (55).

CLINICAL AND BIOCHEMICAL PREDICTORS OF ALI

The search for biochemical and clinical predictors or markers of ALI has been hampered by a number of factors, including (*1*) the lack of correlation between the clinical diagnosis and the pathogenesis, (*2*) the recognition that patients at risk for and with ALI are heterogeneous, (*3*) the continuous discovery of new mediators and modulators of inflammation, (*4*) the recognition that the development of ALI is likely the result of a balance of mediators and modulators of inflammation, and (*5*) the increasing awareness of the importance of correlating biochemical markers with physiologic variables. Although the recognition of these factors has complicated the quest for markers in ALI/ARDS, each of these factors provides unique new opportunities for their study.

Patients with a higher severity of illness score are more likely to develop ALI/ARDS and die of lung injury. However, the search for a pulmonary-specific variable has been challenging. Indices of hypoxemia have not been predictive of clinical outcome (5). One recent study established that an elevated dead space fraction has an independent predictive power for identifying patients more likely to die (13). This finding needs to be explored in conjunction with detailed biochemical studies and other methods to determine the pathologic and physiologic basis for a high dead space fraction. Procoagulant mechanisms are activated both in the circulation and the distal airspaces of the lung in patients with early ALI and may ultimately provide markers of alveolar capillary damage or vascular occlusion (45). More work is needed to evaluate products of endothelial injury as well as mechanisms that reflect endothelial, neutrophil, and thrombotic interactions. Markers of endothelial injury such as von Willebrand factor that reflect pulmonary and systemic endothelial injury may prove useful.

Although measurement of soluble biochemical markers in plasma, edema fluid, and bronchoalveolar lavage fluid has provided insights into mechanisms of lung injury (69), they may not necessarily reflect critical molecular events at the cell surface. In juxtacrine signaling between cells, the signaling molecule remains associated with cellular plasma membranes and is not released into solution (27). Thus, it may not be detected in bronchoalveolar lavage or other fluid samples and/or its concentration in fluid samples may bear little relationship to its signaling actions. Also, antigenic measurement of cytokines in edema fluid or bronchoalveolar lavage may not correlate well with biological activity. Both interleukin-β and tumor necrosis factor α can be detected by immunoassays in the distal airspaces of the lung in patients with early ALI, but interleukin-1β has more biological activity than tumor necrosis factor α (70).

ANIMAL MODELS OF LUNG INJURY

Animal studies that have attempted to mimic human ALI/ARDS have been useful and will likely continue to provide valuable observations regarding both the mechanisms underlying the pathogenesis, progression, and resolution of this syndrome and ways in which its course can be modulated therapeutically (39, 68, 71–76). The lack of an animal model that unequivocally mimics key aspects of human ALI/ARDS has been limiting in mechanistic studies and in providing meaningful and rapid extrapolations to the clinical syndrome. Furthermore, there is uncertainty as to which of the many available animal models best reflects the human clinical syndrome. Animal models are usually monitored over a shorter term than the human syndrome, which requires hours or days to develop. Most animal models do not include ventilation and fluid management interventions, features that may be crucial factors in determining outcome in humans. A major question is whether the pathogenesis of human ALI/ARDS varies with risk factors, such as trauma and sepsis. This has led to studies comparing and contrasting the mechanisms of injury in animal models of endotoxin and hemorrhagic shock–associated ALI (71). Administration of LPS alone does not completely mimic the systemic or pulmonary effects of bacteremia or endotoxemia, contributing to the use of more complex models such as cecal ligation and puncture (72). Furthermore, many workshop participants suggested that two-hit models (73–76) might be more appropriate to reflect common comorbidities and risk factors commonly present in these patients. Continued study of the heterogeneity of precipitating causes of ALI/ARDS is needed.

The traditional knockout and transgenic approaches to delete or add a gene will remain useful, but more sophisticated approaches to modify genes and to conditionally express or knock out genes are needed to better understand the role of particular molecules and pathways (67). In combination, these studies will elucidate interactions and redundancies in pathways and molecular functions. Furthermore, cell- and organ-specific approaches, either through bone marrow reconstitution or selection and manipulation of promoter function, will also prove valuable for understanding the complexities of cell function and interaction in the lungs and to provide potential therapeutic targets. Furthermore, animal studies can also facilitate understanding of the heterogeneity of phenotype using chemical mutagenesis or gene mapping and linkage studies in mice of specific genetic backgrounds that display variable responses to experimental lung injury. Finally, the availability of the rat genome will be extremely useful (77), as opportunities for comprehensive physiologic studies of lung injury are better in rats than in mice.

Animal models that facilitate "fast track" analysis of therapeutic leads and combinations of therapeutic agents would be expected to accelerate clinical application of basic discoveries. One approach that may prove useful for identifying clinical targets would involve gene profiling and proteomic evaluation of tissue from one or more animal models of lung injury that reflect phenotypic subgroups of ALI/ARDS. The effect of agents having the potential to diminish the severity of systemic and pulmonary

injury on genomic and proteomic variables could then provide mechanistic insights to determine if similar pathways were being altered in human ALI/ARDS and help to narrow the field of candidate genes for human ALI/ARDS. Animal models could and should focus on both systemic and pulmonary injury and may also provide insights about the interactions between them. For example, recent studies of an intraperitoneal sepsis model in rats using a limited gene microarray revealed interesting results regarding the host response in lung, kidney, brain, and lymphoid tissues (72). Studies in animal models with clinical relevance to ALI/ARDS would facilitate the design of clinical trials focused on diminishing clinical lung injury and the associated nonpulmonary organ dysfunction, including renal failure and hepatic and hematologic dysfunction, as well as the consequences of shock states produced by sepsis and trauma.

CONCLUSIONS AND RECOMMENDATIONS

There are major unanswered questions about ALI/ARDS that will require continued research efforts at the basic, translational, and clinical levels. Recent successes in the treatment of critically ill patients provide an excellent opportunity for rapid progress, as do new and evolving biologic, genomic and genetic approaches. Investigations and collaborative efforts that include cellular and mechanistic studies combined with animal and clinical studies will be the key to improving detection and treatment of this complex syndrome.

1. Continued support for high quality collaborative multicenter clinical trials, as well as individual investigator-initiated studies, is crucial for continued progress in prevention and treatment of ALI/ARDS. Studies of multiple drug treatments for this complex syndrome may be needed. Future studies could be broadened to include common comorbid conditions such as renal failure and sepsis. Support for mechanistic/pathogenic investigations in conjunction with clinical trials is essential. The smaller clinical investigations conducted at single centers continue to have value because of complex patient populations and to test new treatments for evidence to support further evaluation in larger multicenter trials.
2. Strategies for robust and facile analysis of genomic and proteomic data from studies in animal models, including genome sequencing databases, tissue- and species-specific microarrays, online archiving of serial microarray and other data, and other resources should facilitate translation of animal experiments to clinical relevance.
3. Studies that correlate biochemical and biological markers with clinical variables should continue in order to address the issue of heterogeneity, to more precisely define the phenotypes of patients with ALI/ARDS, to permit genetic analysis, and to facilitate clinical trials of new candidate therapies. Panels of biological and biochemical markers, in conjunction with clinical indices, may be needed to generate a prognostic index in ALI/ARDS. Markers that reflect systemic events (coagulation and inflammatory markers) as well as those that reflect alterations in lung endothelial and epithelial cells and fibrotic processes should be included. Pulmonary edema fluid, circulating cells, DNA samples, and plasma should be obtained in clinical studies.
4. Accrual and collections of well-characterized lung tissue samples from patients with ALI/ARDS will facilitate application of modern analytic techniques (laser capture microdissection, microarray interrogation, proteomic analysis) to define the molecular phenotype of key lung cells and structures.
5. Future research should build in part on the recently demonstrated new treatments for ALI/ARDS and sepsis. More basic research is needed to detail the cellular, molecular, and physiologic mechanisms that protect the lung from biomechanical stress and injury and potentially modulate systemic inflammation. More studies are needed to explore the role of the inflammatory and coagulation pathways in inducing organ injury in sepsis and other injury states and in modulating ALI. Both basic and clinical studies are needed. Although ARDS is a uniquely human disease, animal models are relevant to several different features of clinical ALI. Better understanding is needed to determine which animal models are most relevant to the human disease. "Two hit" models may better reflect comorbid conditions and other pre-existing variables and may be more informative in dissecting the roles of the innate immune system and specific mechanisms of lung injury. Animal models that focus on systemic as well as pulmonary injury may prove to be particularly relevant to human ALI. Genetically altered mouse models will also be useful. Lung- and tissue-specific conditional knockouts, overexpression of candidate genes, and other genetic models should be of value in dissecting the host response to pathological conditions that model human lung injury.
6. Because infection is the most frequent cause of ALI/ARDS, the influence of bacterial and viral genetics on host response that influence lung injury needs to be better understood.
7. Research to study and improve the resolution of ALI, including mechanisms to modulate clearance of edema fluid, soluble and insoluble proteins, inflammatory cells, and remodeling of the lung is needed. The potential for enhancement of lung repair with stem cell therapy should be explored.
8. More emphasis is needed on understanding cell-to-cell communication in the lung, including the mechanisms that transmit signals between endothelial and epithelial cells and mechanisms of cell-to-cell interaction in inflammatory and thrombotic responses. The signals and mechanisms that determine normal cell turnover and influence apoptosis and necrosis in lung injury need to be elucidated. Advanced imaging modalities, such as microcomputerized tomography and confocal microscopy, should be refined to generate data on cell and parenchymal microstrains in intact lungs, so that molecular research on mechanosensing and mechanotransduction can be interpreted in an appropriate context. Similarly, refined imaging techniques that are applicable to inflammatory cell trafficking and cell–cell interactions will likely be informative.
9. Studies that use cell biological approaches including single and multiple cell type cultures should be integrated with animal and human studies.
10. More knowledge is needed to study the extent to which genetic factors influence the development, severity and time course of development and resolution of ALI/ARDS as well as the development of multiple organ failure and other systemic sequelae. Development of genetic tools and resources specifically relevant to ALI/ARDS, triggering conditions, and systemic manifestations will likely facilitate characterization of genetic and environmental variables that influence ALI. Case–control association studies, although of lesser power, may be useful.

11. Interaction of the new NHLBI Specialized Centers of Clinically Oriented Research programs in ALI with the ARDS clinical trials network should be encouraged. Progress will be greatly enhanced with vertically and horizontally integrated collaborative studies.

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