Interleukin 8 and Acute Lung Injury

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Acute lung injury is a complex clinical syndrome involving acute inflammation, microvascular damage, and increased pulmonary vascular and epithelial permeability, frequently resulting in acute respiratory failure culminating in often-fatal acute respiratory distress syndrome. Interleukin 8 (IL-8), a potent neutrophil attractant and activator, plays a significant role in acute lung injury via the formation of anti–IL-8 autoantibody:IL-8 complexes and those complexes' interaction with FcγRIIa receptors, leading to the development of acute lung injury by, among other possible mechanisms, effecting neutrophil apoptosis. These complexes may also interact with lung endothelial cells in patients with acute respiratory distress syndrome. Continuing research of the role of neutrophils, IL-8, anti–IL-8 autoantibody:IL-8 complexes, and FcγRIIa receptors may ultimately provide molecular therapies that could lower acute respiratory distress syndrome mortality, as well as reduce or even prevent the development of acute lung injury altogether.


Acute lung injury (ALI) is a complex clinical syndrome for which the initial acute systemic inflammatory response causes microvascular damage, which subsequently increases pulmonary vascular and epithelial permeability, characteristically resulting in an airspace influx of protein-rich edema fluid, yielding acute respiratory failure and typically culminating in catastrophic medical or surgical illness or injury, termed acute respiratory distress syndrome (ARDS). Much of the history of ARDS is military related, and possibly the first detailed description of what we know today to be ARDS was published in 1915 by a physician who served in the Canadian Forces. Acute respiratory distress syndrome was described and named by Ashbaugh and colleagues in 1967. Today, ALI/ARDS is a relatively common condition affecting approximately 150,000 people in the United States each year, carrying a sobering overall mortality of approximately 50%. There is currently no effective treatment; patients receive supportive therapy only.

The histologic counterpart to the clinical diagnosis of ARDS is diffuse alveolar damage. It can be roughly divided into 3 phases on a continuum: (1) early or exudative phase, (2) proliferative phase, and (3) late or resolving phase. Wedge biopsy of the lung is rarely performed when the diagnosis of ARDS is clinically and radiographically straightforward; however, in difficult cases or in some cases where the patient's status deteriorates, wedge biopsy may be necessary to confirm a diagnosis. As those biopsies are performed relatively late in the clinical course of disease, they typically show proliferative-phase or resolving-phase histologic features. The exudative phase is characterized by hyaline membranes, interstitial and alveolar edema, alveolar capillary congestion, intracapillary neutrophil margination, interstitial inflammation, and microvascular thromboemboli. Proliferative-phase diffuse alveolar damage is characterized by fibroblast and type 2 pneumocyte proliferation, with associated squamous metaplasia and thromboemboli; and late or resolving phase shows remodeled lung architecture with dense fibrosis, ultimately culminating in honeycomb lung change.

HUMAN RESEARCH

Patients who develop ALI may go unrecognized for varying periods of time, only to be clinically diagnosed upon developing ARDS. Clinical research in ALI/ARDS is therefore generally focused on assisting ventilated patients with serious late-stage disease. As such, the patient cohorts in those studies are necessarily intensive care unit–based patients with dramatic mortality; indeed, many are already near death. Basic science study of ALI/ARDS is not so constrained: it examines ALI/ARDS throughout its development and progression, with the ultimate goal of identifying ALI patients early in the development of their conditions, before ARDS and its requisite mechanical ventilation. With early identification, treatment can begin earlier, possibly with molecular-based therapies, reducing the need for ventilation, potentially slowing or even stopping disease progression, and ultimately significantly reducing mortality. This line of research contemplates the possibility of future biomarker-driven ALI/ARDS diagnosis/treatment algorithms.

For more than a decade, active research for pathogenetically and prognostically important plasma and airspace molecular biomarkers has been under way. There has been significant progress in the study of the molecular basis for ALI/ARDS. For example, it has been found that extracellular histones, released in inflammation, may exacerbate endothelial dysfunction; activated protein C cleaves histones, reducing toxicity; and decoy receptor 3 plasma levels have been shown to be associated with 28-day ARDS mortality.
Also, toll-like receptor activation has been found to be important in ALI studies, both in vivo and in vitro. Further, endotoxin-mediated urokinase-type plasminogen activator receptor expression, important in neutrophil recruitment, has been found to be mediated through tyrosine phosphorylation of phosphoglycerate kinase.

**NEUTROPHILS, INTERLEUKIN 8, AND ANTI–INTERLEUKIN 8:INTERLEUKIN 8 COMPLEXES**

Neutrophils have been long thought to play an important role in the development and progression of ALI/ARDS. Acute respiratory distress syndrome is characterized by increased neutrophils in airspaces and an increased proportion of neutrophils. Neutrophils mediate microvascular damage and have been shown to contribute to lung tissue damage. Alveolar neutrophil concentration correlates with both the degree of hypoxemia and increased lung permeability, and increased levels of neutrophils have been associated with poor survival.

IL-8, a potent neutrophil attractant and activator, has also been examined and found to play a significant role in ALI/ARDS. In 1992, Miller and colleagues found that IL-8 was present in significantly higher concentrations in bronchoalveolar lavage fluids from ARDS patients compared with controls, and that the ARDS patients with very high concentrations of IL-8 in bronchoalveolar lavage fluids had a higher mortality rate than patients with lower concentrations. Soon afterward, Kurdowska and colleagues found that most of the bronchoalveolar lavage fluid IL-8 from ARDS patients is associated with anti–IL-8 autoantibody, which binds with high affinity to IL-8. The anti–IL-8 autoantibody:IL-8 complexes consist of 1 immunoglobulin G (IgG) molecule (predominantly IgG3 and IgG4 subclasses) and 1 IL-8 molecule, and anti–IL-8 autoantibodies inhibit the interaction of IL-8 with its specific receptors on neutrophils, suggesting that they may regulate IL-8 activity in ARDS. Studies of ARDS patients and patients at risk who did not develop ARDS by Kurdowska and colleagues showed that there was no correlation between the concentration of IL-8 and the development or the course of ALI/ARDS. However, concentrations of anti–IL-8:IL-8 complexes on day 1 were significantly higher in ARDS patients than in at-risk patients, and there was a significant association between complex concentrations and the onset of ARDS. Further, complex concentrations were significantly higher on day 1 in ARDS patients who later died.

Examining IL-8 further, Kurdowska and colleagues found that the presence of anti–IL-8:IL-8 complexes in bronchoalveolar lavage fluids of ARDS patients was an important prognostic indicator of ARDS development and outcome, and in addition that high concentrations of complexes correlated with mortality in ALI/ARDS patients and were predictive of ARDS development in at-risk patients. The general hypothesis that began to emerge was that in overwhelming, uncontrolled infection, such as sepsis, there is a marked increase in IL-8, overwhelming removal mechanisms, resulting in markedly increased and persistent amounts of anti–IL-8:IL-8 complexes in the lung, and causing and maintaining the physiologic dysfunction that is ALI/ARDS.

**FcyRIIa**

In 2004, Krupa and colleagues, examining purified anti–IL-8:IL-8 complexes from alveolar fluid from ALI patients, found that the complexes retain the ability to trigger neutrophil chemotaxis and that they trigger superoxide and myeloperoxidase release (neutrophil respiratory burst and degranulation) from human neutrophils. The control antibody did neither. In summary, they found that anti–IL-8:IL-8 complexes purified from alveolar edema fluids from patients with ALI have the capacity to trigger an inflammatory response in the lung by attracting and activating human blood neutrophils, strongly suggesting that anti–IL-8:IL-8 complexes are involved in ALI/ARDS pathogenesis. Further, both IgG receptor FcyRIIa and IL-8 receptors were found to mediate these complexes’ chemotactic activity; however, FcyRIIa was identified as the predominant receptor. FcyRIIa was clearly found to mediate activity of anti–IL-8:IL-8 complexes in human neutrophils.

There are 2 families of stimulatory receptors that interact with immune complexes (FcyRII and FcyRIII); neutrophils express FcyRIIa and FcyRIIib. FcyRIIa, the most widespread human FcyRs and a potent inflammation activator, has a signaling motif, the immunoreceptor tyrosine-based activation motif, in the cytoplasmic tail of the ligand-binding chain; it initiates phagocytosis, antibody-dependent cellular cytotoxicity, transcription of cytokine genes, and release of inflammatory mediators. Krupa and colleagues and Fudala and colleagues have found that anti–IL-8:IL-8 complex activity is partially dependent on the activation of mitogen-activated protein kinases, that is, ERK and p38, which are important components of the FcyRIIa signaling cascade.

Neutrophil apoptosis has been shown to be delayed in ALI/ARDS patients. Fudala and colleagues, examining whether anti–IL-8:IL-8 complexes play a role in modulating spontaneous apoptosis of normal human neutrophils, measured enzymatic activity of caspase 3, evaluated morphologic changes, and determined the extent of DNA regulation. Samples containing the complexes, but not samples from which complexes were removed, inhibited neutrophil apoptosis. The anti–IL-8:IL-8 complexes purified from alveolar edema fluids from patients with ALI have the capacity to inhibit apoptosis of human neutrophils by engaging FcyRIIa. Samples containing anti–IL-8:IL-8 complexes have been shown to induce an increase in antiapoptotic protein Bcl-XL and to suppress caspase 3 and caspase 9. Fudala and colleagues demonstrated for the first time that anti–IL-8:IL-8 complexes are able to prolong neutrophil life. Fudala and colleagues also established that FcyRIIa mediates antiapoptotic activity of the complexes, and found that the key components of the FcyRIIa signaling pathway—Src, Syk, PI3 kinase, and ERK—may be involved in the complexes’ neutrophil apoptosis regulation. Fudala and colleagues showed that interaction between FcyRIIa and Src tyrosine kinase family triggers activation of Syk-tyrosine kinase, PI3 kinase, Akt, or ERK, leading to an increased level of antiapoptotic protein Bcl-xL, inhibition of proapoptotic proteins Bax and Bak, and activation of caspase 3 and caspase 9, causing neutrophil apoptosis suppression.

**CONFOCAL MICROSCOPY**

Allen and colleagues visualized anti–IL-8:IL-8 complexes by laser confocal microscopy in injured human lung; this was the first report showing the presence of anti–IL-8:IL-8 complexes in the lungs of ARDS patients and the colocalization of the complexes with FcyRIIa. Colocalization...
was blocked with anti-FcγRIIa. These findings do not prove that the complexes play a causative role in ARDS; however, they support the hypothesis that the complexes contribute to the pathogenesis of ARDS. The formation of anti–IL-8:IL-8 complexes in the lung in ALI/ARDS is probably due to increased production of both IL-8 and anti–IL-8 autoantibodies in response to ongoing inflammation. The clinical significance remains to be established.

**ENDOTHELIUM**

Anti–IL-8:IL-8 complexes have also been studied in relation to endothelial cells. Krupa and colleagues studied human and mouse lungs and human umbilical vein endothelial cells, and found that anti–IL-8:IL-8 complexes promote an inflammatory phenotype of human umbilical vein endothelial cells via interaction with FcγRIIa. Human umbilical vein endothelial cells became activated, with increased phosphorylation of ERK, JNK, and Akt and augmented nuclear translocation of nuclear factor–κB. The complexes also upregulated intracellular adhesion molecule 1 (ICAM-1) expression on human umbilical vein endothelial cell surfaces. Increased ICAM-1 levels were detected on mouse lung endothelial cells where lung injury had been induced by generating immune complexes in alveolar spaces. Expression of ICAM-1 was unchanged in the lungs of γ-chain–deficient mice, lacking receptors that interact with immune complexes.

Our prior finding of anti–IL-8:IL-8 complexes associated with FcγRIIa in ARDS lung tissue did not specify with which cells the complexes interacted. We evaluated human control and ARDS tissue for the presence of complexes in endothelial cells. Complexes can be detected by evaluating colocalization between IL-8 and FcγRIIa, with CD34 as an endothelial cell marker. We found that IL-8 colocalized with FcγRIIa in ARDS tissue, indicating interaction of anti–IL-8:IL-8 complexes with endothelial cells in ARDS lung tissue. FcγRIIa was also identified in normal lung tissue; however, IL-8 and colocalization between IL-8 and FcγRIIa was minimal. We also observed elevated expression of ICAM-1 on endothelial cells that showed positive staining for complexes in ARDS lung tissue. The ICAM-1 on the cell surface was associated with positive staining for complexes. Presence of complexes bound to FcγRIIa on the surface of activated endothelial cells indicates that these complexes may interact with lung endothelial cells in ARDS patients. The complexes may possibly exhibit their proinflammatory activity, adding to the severity of the inflammatory response in these patients.

In summary, our data show that complexes purified from edema fluid from ALI patients have the ability to induce endothelial cell activation by the engagement of FcγRIIa, and that analogous murine complexes behave similarly, making this scenario even more likely. Overall, our studies implicate antichemokine autoantibody:chemokine immune complexes as triggers of endothelial cell alterations that may lead to their developing a proinflammatory phenotype.

**MICE STUDIES**

Further, we used a mouse model to evaluate the contribution of anti–KC autoantibody:KC complexes to lung inflammation and injury. (KC is a mouse equivalent of human IL-8.) Both WT and γ-chain–deficient mice lacking stimulatory receptors for IgG immune complexes (FcγRs) were studied. Autoantibodies to KC (in plasma and alveolar spaces) were induced by immunization with KC, which was administered intratracheally to generate anti–KC:KC complexes in the lung. Presence of anti–KC:KC complexes was associated with development of severe pulmonary inflammation that was dramatically suppressed in γ-chain–deficient mice. Moreover, the best performing biomarkers in patients with ALI/ARDS were the neutrophil chemotactic factor, IL-8, and surfactant protein D, a product of alveolar type 2 cells, supporting the concept that acute inflammation and alveolar epithelial injury are important pathogenic pathways in human ALI/ARDS.

**CONTINUING RESEARCH**

Investigations of signaling pathway proteins associated with anti–IL-8:IL-8 complex/FcγRIIa involvement with ALI/ARDS are ongoing. Several avenues are currently being explored. For example, Fudala and colleagues found that nuclear factor–κB and Fos-related antigen 1 (a member of the activator protein 1 family) are elevated in ARDS lung tissue, suggesting that continued examination of how proinflammatory signaling involving nuclear factor–κB initiates epithelial cell dysfunction and apoptosis, including inflammatory cascade molecules—cytokines and adhesion molecules—could be beneficial. Neutrophil α-defensins have been shown to mediate ALI via lipoprotein-related receptor–mediated loss of capillary-epithelial barrier function in mice. Continued examination in this area could lead to a potential new approach to intervention. Urokinase plasminogen activator is elevated in ALI where pulmonary arterial contractility and permeability are disrupted; and in nonpathologic concentrations, urokinase plasminogen activator reduces pulmonary arterial contractility and increases permeability via activation of N-methyl-d-aspartate receptor 1. Continued manipulation of this process may lead to methods of affecting the fibrinolytic activity in ALI/ARDS therapeutically. Porcine lungs, exposed to wood bark smoke, develop ALI. Midde and colleagues have shown that smoke induces plasminogen activator inhibitor 1, in part by stabilization of plasminogen activator inhibitor 1 messenger RNA. This newly recognized pathway may promote extravascular fibrin deposition and lung dysfunction in ALI. Further developments in this area might produce successful therapies for ALI/ARDS.

Other potentially fruitful avenues of ALI/ARDS study include expanding on studies of non–ALI/ARDS patients, examining, for example, the roles of tissue plasminogen activator, granulocyte/macrophage colony-stimulating factor, and N-methyl-d-aspartate receptor in patients with ALI/ARDS.

**CONCLUSION**

Lung cancer diagnosis and therapy has recently undergone a molecular revolution, and our understanding of interstitial lung diseases has shown remarkable advances. However, ALI prognosis has shown only modest improvements, almost exclusively based on better supportive care. Moreover, the bulk of ALI research focuses on providing better supportive therapy. Even so, the molecular study of ALI is well under way.

In 2010, Ware and colleagues, examining a combination of clinical predictors of ALI and 8 biomarkers studied in ALI, found that IL-8 and surfactant protein D performed best, supporting the concept that acute inflammation and alveolar epithelial injury are important pathogenic pathways in
lungs injury.28 Future molecular research of ALL, including the role of neutrophils and IL-8, may provide an understanding for which molecular therapies may not only lower ARDS mortality, but also provide treatments that reduce or even prevent the development of ALL altogether.

References

Submissions Now Accepted for CAP ’14 Abstract Program

Abstract and case study submissions are now being accepted for the College of American Pathologists (CAP) 2014 meeting, which will be held September 7th through the 10th in Chicago, Ill. Submissions for the CAP ’14 Abstract Program will be accepted from:

Monday, January 13, 2014 through Friday, March 14, 2014

Accepted submissions will be published as a Web-only supplement to the September 2014 issue of the Archives of Pathology & Laboratory Medicine and will be posted on the Archives Web site. Visit the CAP ’14 Web site at www.cap.org/cap14 to access the abstract submission site and additional abstract program information as it becomes available.