Translational Review

Role of Chemokines in the Pathogenesis of Acute Lung Injury

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Acute lung injury (ALI) is due to an uncontrolled systemic inflammatory response resulting from direct injury to the lung or indirect injury in the setting of a systemic process. Such insults lead to the systemic inflammatory response syndrome (SIRS), which includes activation of leukocytes—alveolar macrophages and sequestered neutrophils—in the lung. Although systemic inflammatory response syndrome is a physiologic response to an insult, systemic leukocyte activation, if excessive, can lead to end organ injury, such as ALI. Excessive recruitment of leukocytes is critical to the pathogenesis of ALI, and the magnitude and duration of the inflammatory process may ultimately determine the outcome in patients with ALI. Leukocyte recruitment is a well orchestrated process that depends on the function of chemokines and their receptors. Understanding the mechanisms that contribute to leukocyte recruitment in ALI may ultimately lead to the development of effective therapeutic strategies.

Keywords: acute lung injury; inflammation; leukocytes; experimental; clinical

Acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS) is a devastating syndrome, with an incidence of approximately 200,000 in the United States and a mortality approaching 40% (1). ALI is defined by the presence of bilateral pulmonary infiltrates, severe hypoxemia, and the absence of left atrial hypertension (2). Causes of ALI may be direct (pneumonia, aspiration, inhalational injury, etc.) or indirect (sepsis, pancreatitis, blood transfusion, etc.). The pathogenesis of ALI involves inflammatory injury to the alveolo–capillary membrane, resulting in lung permeability and the accumulation of protein-rich pulmonary edema fluid in the airspaces, which, in turn, leads to the pulmonary infiltrates and hypoxemia that define clinical ALI (3). In ALI, resident lung cells are stimulated to release chemoattractants, which recruit inflammatory cells to migrate from the intravascular space across the endothelium and epithelium into the airspaces. Chemokines, which are small (8–10 kD) heparin-binding proteins, are secreted by resident lung cells in response to bacterial products and early response inflammatory mediators, and are retained by matrix heparin sulfate proteoglycans at the site of inflammation, establishing a chemokine gradient toward the inflammatory focus (4). Chemokines are classified into four subfamilies based on the positioning of specific amino acid (aa) residues in the NH_2 terminus of the protein (described subsequently here). Each subfamily binds to specific receptors on inflammatory cells, resulting in the recruitment of that cell type.

In ALI, neutrophils, followed by mononuclear cells, are recruited to the lung by chemokine gradients. There is extensive experimental evidence demonstrating the critical role of these inflammatory cells and their chemokines in the pathogenesis ALI (5–9). In this review, we discuss the major classes of chemokines and their receptors, as well as the evidence from both animal and clinical studies supporting the role that these chemokines play in the pathogenesis of ALI. Pharmacologic chemokine blockade, if it could prevent massive, dysregulated inflammatory injury to the lung without compromising host defense, may be a potential therapeutic strategy for ALI (10), a syndrome for which there are few specific treatments (3).

PATHOGENESIS OF ALI

The role of inflammation in the pathogenesis of ALI is well supported by clinical and animal studies. Bronchoalveolar lavage (BAL) (11) and lung histologic samples (12) from patients with ALI reveal the accumulation of neutrophils as well as macrophages within the airspaces, and, indeed, the degree of neutrophilic influx correlates with mortality in ALI (11). In animal models, neutrophils (13) and their products (14) cause lung injury. During ALI, resident lung cells are stimulated to release chemoattractants, which recruit neutrophils to migrate from the intravascular space across the endothelium and epithelium into the airspaces. In the presence of chemokines and other inflammatory mediators, neutrophils, which usually deform to pass through the narrow capillary lumen, become stiff, likely due to actin polymerization, and therefore become sequestered in the microvasculature (15). After sequestration, ligation of neutrophil cell surface molecules to their cognate receptors on the endothelial and epithelial cells results in tethering, rolling, firm adhesion, and crawling along the endothelium, followed by transendothelial and transepithelial migration (5, 16). During migration, large numbers of activated neutrophils release toxic mediators, including proteases, oxidants, and peptides (17, 18), in close proximity to the endothelium and epithelium. Neutrophils and their products cause endothelial and epithelial permeability, in part due to apoptotic and necrotic cell death (12, 19, 20), although, in some cases, ALI may occur in the absence of neutrophils (21), and neutrophils may migrate into the airspaces without causing permeability (22, 23). Inflammatory injury to the lung epithelium also impairs its capacity for active fluid transport out of the airspaces (24) and for surfactant

(*Received in original form November 11, 2011 and in final form February 2, 2012*)

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This work was supported by in part by a University of Otago Establishment grant (M.B.), National Institutes of Health (NIH) grant K08-HL103772, a Flight Attendant Medical Research Institute (FAMRI) Young Clinical Scientist Award, a Parker B. Francis Fellowship (R.L.Z.), and by NIH grant R01-HL091958 (S.J.) and FAMRI grant YCSA_062466 (S.J.).

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Am J Respir Cell Mol Biol Vol 46, Iss. 5, pp 566–572, May 2012

Originally Published in Press as DOI: 10.1165/rcmb.2011-0392TR on February 9, 2012
Internet address: www.atsjournals.org

production, further contributing to the severity of the pulmonary edema and decreased lung compliance that characterize ALI (25). Macrophages are also recruited to the lung by chemokine gradients in ALI, and play a role in the pathogenesis and resolution of lung injury. Undoubtedly, given the critical role of inflammatory cells and their mediators in causing the endothelial and epithelial injury that characterize ALI, the chemokines that recruit these inflammatory cells to the lung are of pivotal importance in the pathogenesis of ALI (26, 27). Chemokines, including CXCL-8, CXCL-1, CXCL-5, and CCL-2, are elevated in the BAL of patients with ALI (6, 7), and levels may correlate with outcome (28). In animal models of ALI, chemokine levels are elevated in the lungs, and neutralization of chemokines (26, 27, 29, 30) or their receptors (31) can attenuate injury. Chemokine gradients are established and regulated via complex mechanisms, including proteolytic processing (32) and scavenging (33, 34).

Resolution of ALI depends on several factors. To prevent ongoing inflammatory injury to the lungs, the recruitment of inflammatory cells to the lung must be halted, and this occurs by chemokine scavenging (33, 34), followed by apoptosis (35) and clearance (36) of inflammatory cells. Resident and recruited macrophages play an important role in clearance of both injured tissue debris and apoptotic cells, and are therefore important for the resolution of inflammation. Recently, it has been demonstrated that T lymphocytes also play a critical role in the resolution of inflammatory lung injury via transforming growth factor-β–dependent mechanisms (37). As ongoing inflammation is limited, edema fluid must be cleared (25), and the injured lung must repair. Repair of the denuded lung epithelium, which correlates with prognosis (24, 38), depends on surviving alveolar type II cells, which proliferate and differentiate into alveolar type I cells. Epithelial repair in ALI depends on several pathways, including keratinocyte growth factor, hepatocyte growth factor (39, 40), and β-catenin signaling (41). Local and bone marrow–derived stem cell populations also contribute to repair of the injured lungs (42–45), and there is evidence that bone marrow–derived stems cells are recruited to the injured lung by the release of soluble factors (46). Although the mechanisms by which bone marrow–derived stem cells are recruited to the lungs have not been completely elucidated, there is significant evidence that the injured lung expresses stromal-derived factor-1 (CXCL12), which interacts with the CXCR4 receptor on the surface of bone marrow–derived stem cells. Stem cells can also express other chemokine and cytokine receptors and migrate toward other mediators, including IL-8, TNF-α, macrophage migration inhibitor factor, transforming growth factor-β, and others (47–50). The recruitment of bone marrow–derived endothelial progenitor cells to the injured lung depends on CXCL1 and CXCL2 chemokines via CXCR2 (51). Resolution of inflammation, followed by physiological repair of the lung, is required to prevent the development of fibrotic changes, which leads to increased mortality or permanent restrictive ventilatory defects (52).

CHEMOKINES

Leukocyte recruitment during inflammation requires intercellular communication between infiltrating leukocytes and the endothelium, resident stromal cells, and parenchymal cells. These events are mediated via the generation of early-response cytokines, the expression of cell surface adhesion molecules, and the production of chemotactic molecules, chemokines, which are a specific class of inflammatory mediators that play a key role in the pathogenesis of ALI (Figure 1). Chemokines are a large family of small proteins (8–10 kD) that are distinguished from other cytokines by being the only members of the cytokine family that act on the superfamily of G protein–coupled serpentine receptors. Chemokines can be classified as constitutive (developmentally regulated) or inducible (inflammatory), the latter of which contribute to ALI. Inducible chemokines were identified early in the chemokine biology field, probably for the obvious reason that they were produced at high levels in the tissues or tissue cultures in which they were identified. Chemokines have chemotactic and activating effects on leukocyte subsets, and provide a key stimulus for directing leukocytes to areas of injury (4, 53). Based on cysteine residue positioning, chemokines are classified into four subfamilies: CXC (α); CC (β); C (γ); and CX3C (δ). The CXC (α) subfamily has the first two NH_2-terminal cysteines separated by one nonconserved aa residue, the CXC cysteine motif (4). This group could be further subdivided based on the presence or absence of a glu-leu-arg (ELR) aa motif immediately preceding the first cysteine residue.

Importantly, ELR^+ CXC chemokines exert potent neutrophil chemotactic and activating properties both *in vitro* and *in vivo*. CXCL-1 (54, 55), CXCL-2 (56, 57), and CXCL-15 (58, 59) have been discovered as important neutrophil chemoattractants in mice during lung inflammation. Most chemokines that are associated with neutrophil trafficking in the lung are secreted in enormous quantities by myeloid cells, such as macrophages and neutrophils (54–57). However, CXCL15 is secreted exclusively by bronchial epithelial cells (58, 59), and CXCL5, which plays a role in neutrophil trafficking during LPS-induced lung inflammation (60), is primarily produced by alveolar type II epithelial cells (61).

The ELR^- CXC chemokines consist of three members, CXCL-9, CXCL-10, and CXCL-11. In contrast to ELR^+ CXC chemokines, ELR^- CXC chemokines exert no chemotactic effects on neutrophils, but are strongly chemotactic for activated/memory T cells and natural killer cells (18–21). The ELR^- chemokines are believed to act primarily on mononuclear leukocytes (4).

The CC chemokine subfamily (β-subfamily) has the first two NH_2-terminal cysteines adjacent to one another with no intervening aa, the CC cysteine motif (4). Target cells for CC chemokines are believed to be eosinophils, T cells, and monocytes, although recent studies have shown that these chemokines also contribute to neutrophil infiltration (62–67). The C chemokines (γ-subfamily) lack the first and third cysteines, and have a lone NH_2-terminal cysteine aa, the C cysteine motif. Two C chemokines, XCL1 and XCL2, have been described. They are predominantly expressed in the thymus, and appear to recruit immature T cells from the bone marrow (4).

The CX3C chemokines (δ-subfamily) have the first two NH_2-terminal cysteines separated by three nonconserved aa residues. CX3CL-1, the only member of this subfamily, can exist either as a membrane-anchored or a shed glycoprotein. CX3CL-1 is believed to act as a potent adhesion molecule for T cells or a chemoattractant for monocytes (4), and has recently been shown to modulate the inflammatory response in sepsis (68).

CHEMOKINE RECEPTORS

Chemokines produce their biological effects by interacting with specific receptors on the cell surface of their target cells. Chemokine receptors have a seven-transmembrane structure and couple to G protein for signal transduction, making them members of a large protein family of G protein–coupled receptors. Chemokine receptors are divided into different families, CXC chemokine receptors, CC chemokine receptors, CX3C chemokine receptors, and XC chemokine receptors, which correspond to the four subfamilies of chemokines described previously here. There are a few receptors that bind a single ligand, and several chemokines can bind to more than one receptor, giving rise to

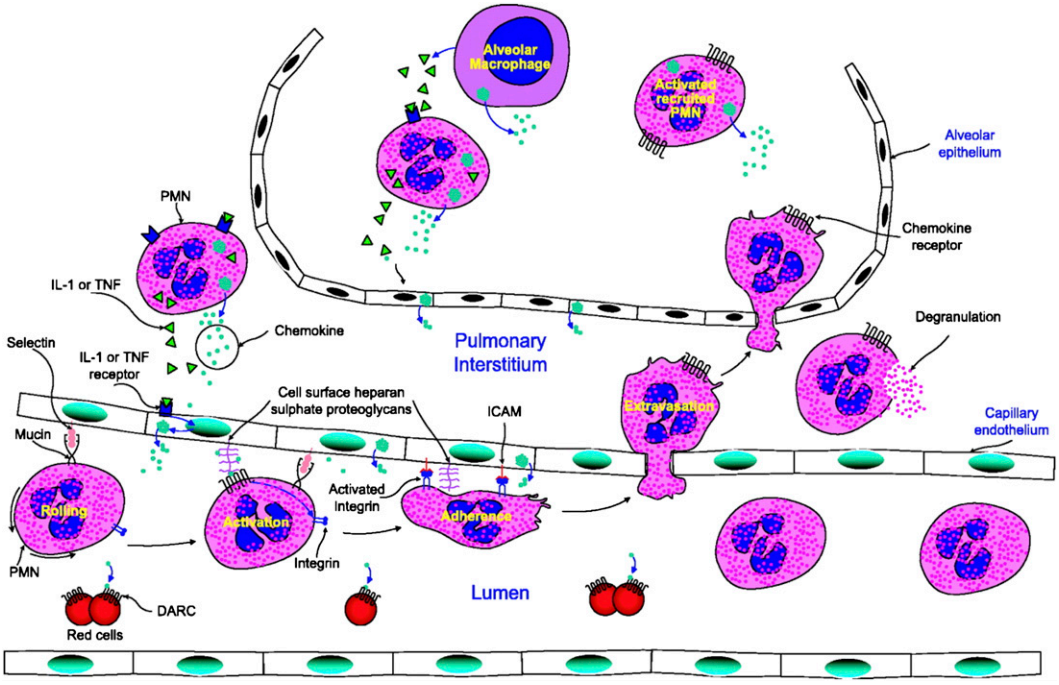

Figure 1. Polymorphonuclear cell (PMN) chemotaxis into the lung in acute lung injury (ALI). Chemokines are secreted at the site of inflammation by resident tissue cells, leukocytes, and cytokine-activated endothelial and epithelial cells. Chemokines are locally retained by matrix heparan sulfate proteoglycans establishing a chemokine gradient surrounding the inflammatory stimulus. PMNs roll over the endothelium in a selectin-mediated process. Chemokine signaling activates leukocyte integrins, leading to firm adherence and extravasation. The PMNs then pass out of the blood vessel and move up the concentration gradient of the chemotactic peptides toward the site of inflammation. The Duffy antigen receptors for chemokines (DARC) functions as a sink, removing the chemokines from circulation and thus helping to maintain the tissue blood stream gradient. In ALI, the capillary endothelium and alveolar epithelium have separate injuries. Macrophages are recruited to the lung by chemokine gradients in ALI and play a role in the pathogenesis and resolution of lung injury. Activated alveolar macrophages release TNF-α and IL-1β and, in response to this, other cells of the alveolar environment produce CC and CXC chemokines, which, in turn, activate the inflammatory cascade, resulting in PMN migration into the lungs (reproduced by permission from Ref. 2).

an apparent "redundancy." It appears that the same receptor can display differential effects depending on the ligand to which it binds (69, 70). Table 1 summarizes our current understanding of chemokines and their receptors.

In addition to these chemokine receptor subtypes, several virus-encoded proteins have been identified that have sequence homology and share the serpentine structure of the cloned chemokine receptors, and have therefore been termed "virocepters." For example, Duffy antigen receptor for chemokines (DARC), a protein first identified in human erythrocytes as a CXCL-8–binding protein, has been shown to bind both CC and CXC chemokines with high affinity. DARC is identical to the Duffy blood group antigen, a receptor for the malarial parasite *Plasmodium vivax*. This receptor has no signal-transducing activity, and may act as a sink, mopping up excess free chemokines and thereby preventing inappropriate activation of circulating leukocytes. DARC selectively binds certain proinflammatory chemokines and down-regulates their activities upon binding (71). Other receptors with a similar action are D6 (72) and CCX/CKR (73). Finally, there is also evidence that CXCR7 can function as a sink for CXCL12 (74).

CHEMOKINES IN ALI—EXPERIMENTAL

To investigate the role of chemokines in animal models of ALI, experimental tools include receptor-neutralizing antibodies, modified chemokines that act as receptor antagonists, and small molecule receptor antagonists. In addition, approaches that inhibit chemokine synthesis and mice deficient for selected chemokines or their receptors can also be used.

Studies employing these approaches have contributed substantially to our understanding of chemokine function in ALI. Plasma levels of both CCL-2 and CXCL-1 are increased in ALI associated with acute pancreatitis (75). Treatment with an anti–CXCL-1–neutralizing antibody reduces the inflammatory response in this model of ALI (29). In addition, treatment with antileukinate, an antagonist of the CXCR2 receptor (that binds CXCL-1), protects mice against ALI associated with acute pancreatitis (76). In septic peritonitis, the absence of CXCR2 has been reported to protect mice from septic injury, potentially by delaying inflammatory cell recruitment and enhancing CXCL10 expression in the peritoneum (77). Regarding chemokine ligands, CXCL1, CXCL2, and CXCL5/LIX are important CXC chemokines in ALI (34, 60, 61, 78). CXCL-2 has been shown to play an important role in murine models of *Klebsiella* pneumonia and septic peritonitis (79, 80). The absence of CXCL-2 was deleterious to the clearance of infection due to decreased neutrophil responses. In addition, CCL-3 mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia (81). Lung inflammation in hyperoxia has been shown to be prevented by anti-chemokine treatment in newborn rats (82). Moreover, ELR$^-$ CXC chemokines have been shown to contribute to inflammation in hemorrhage-associated ALI (83–85). Deletion of CCR1 is associated with protection from pulmonary inflammation secondary to acute pancreatitis in the mouse (62). Treatment with Met-RANTES (methionine–regulated upon activation, normal T cell expressed and secreted), a CCR1 antagonist, protects mice against acute pancreatitis–associated lung injury (64). Furthermore, treatment with BX471, a small-molecule CCR1 antagonist, protects mice against lung injury associated with acute pancreatitis and sepsis (65, 66). Studies with transgenic mice that overexpress CCL-2 in type II alveolar epithelial cells have shown that CCL-2 is sufficient for the chemotaxis of monocytes and lymphocytes in transgenic mice, but requires an additional stimulus for inflammatory activation (86). CCL-2 acts as an efficient neutrophil chemoattractant in mice in the context of acute and chronic inflammation (87, 88). Intratracheal instillation of CCL-2 in mice has been shown to cause increased alveolar monocyte accumulation in the absence of lung inflammation, whereas combined CCL-2/LPS challenge provoked acute lung inflammation with

TABLE 1. CHEMOKINES AND CHEMOKINES RECEPTORS

Systemic Name	Other Name(s)	Receptor(s)
CCL1	I-309	CCR8
CCL2	MCP-1	CCR2
CCL3	MIP-1α	CCR1,CCR5
CCL4	MIP-1β	CCR5
CCL5	RANTES	CCR1,CCR3, CCR5
CCL6	—	—
CCL7	MCP-3	CCR-1, CCR-2, CCR-3
CCL8	MCP-2	CCR-3
CCL9	—	—
CCL10	—	—
CCL11	Eotaxin	CCR-3
CCL12	—	CCR-2
CCL13	MCP-4	CCR-2, CCR-3
CCL14	HCC-1	CCR-1
CCL15	HCC-2	CCR-1, CCR-3
CCL16	HCC-4	CCR-1
CCL17	TARC	CCR-4
CCL18	DC-CK1	—
CCL19	MIP-3β	CCR-7
CCL20	MIP-3α	CCR-6
CCL21	SLC	CCR-7
CCL22	MDC	CCR-4
CCL23	MPIF-1	CCR-1, CCR-12
CCL24	MPIF-2	CCR-3
CCL25	TECK	CCR-9
CCL26	Eotaxin-3	CCR-3
CCL27	CTACK	CCR-10
CCL28	MEC	CCR-10
XCL1	Lymphotactin	XCR1
XCL2	SCM1-α	XCR1
CXCL1	GROα, CINC, KC	CXCR2
CXCL2	GROβ, MIP-2	CXCR2
CXCL3	GROγ	CXCR2
CXCL4	PF4	—
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1, CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL9	MIG	CXCR3
CXCL10	IP-10	CXCR3
CXCL11	I-TAC	CXCR3, CXCR7
CXCL12	SDF-1α, SDF-1β	CXCR4, CXCR7
CXCL13	BCA-1	CXCR5
CXCL14	BRAK	—
CXCL15	Lungkine	—
CXCL16	—	CXCR6
CXCL17	VCC-1	—
CX3CL1	Fractalkine	CX3CR1

Definition of abbreviations: BCA-1, B cell–attracting chemokine-1; BRAK, breast and kidney-expressed chemokine; CINC, cytokine-induced neutrophil chemoattractant; CTACK, cutaneous T cell–attracting chemokine; DC-CK1, dendritic cell chemokine 1; ENA, epithelial-derived neutrophil-activating peptide; GCP, granulocyte chemotactic protein; GRO, growth-related oncogene; HCC, human CC chemokine; IP, IFN-γ–induced protein; I-TAC, interferon-inducible T cell alpha chemoattractant; KC, keratinocyte chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MEC, mucosa-associated epithelial chemokine; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factors; NAP, neutrophil-activating peptide; PF4, platelet factor-4; RANTES, regulated upon activation, normal T cell expressed and secreted; SCM, single C motif; SDF, stromal-derived factor; SLC, secondary lymphoid-tissue chemokine; TARC, T cell–directed CC chemokine; TECK, thymus expressed chemokine; VCC, vascular endothelial growth factor–coregulated chemokine.

early alveolar neutrophil and delayed alveolar monocyte influx (89). In one article, however, CCL-2 was reported to protect mice in lethal endotoxemia (90). Recently, CCL-2 has been shown to regulate pulmonary host defense via neutrophil recruitment during *Escherichia coli* infection (88). Treatment with bindarit, an inhibitor of CCL-2 synthesis, both prophylactically and therapeutically significantly reduced CCL-2 levels in the liver and lungs in cecal ligation, puncture-induced sepsis, and LPS-induced endotoxemia. In addition, prophylactic and therapeutic treatment with bindarit significantly protected mice against sepsis and endotoxemia, as evidenced by the attenuation in lung and liver myeloperoxidase activity, an indicator of neutrophil recruitment. The protective effect of bindarit was further confirmed by histological examination of lung and liver sections (91). In addition, we have recently shown that administration of CX3CL-1 modulates inflammatory ALI in a murine model of sepsis (68). In recent years, hydrogen sulfide and substance P have been identified as mediators of inflammation in ALI associated with acute pancreatitis, sepsis, and severe burns. Both hydrogen sulfide and substance P contribute to inflammation in these conditions via activation of chemokines (92–103).

In summation, over 30 different chemokines and over 20 different receptors, with overlapping functions, have been identified, and it is likely that additional chemokines and chemokine receptors exist that are as yet undiscovered. Despite the complexity and apparent redundancy of this system, we believe that specific chemokine receptor antagonists that interfere with leukocyte migration and activation could be therapeutically useful in ALI.

CHEMOKINES IN ALI—CLINICAL

Evidence from clinical studies confirms the key role for chemokines in the pathogenesis of ALI. An early study showed significantly higher CXCL-8 levels in patients with ARDS plus pneumonia than in those with ARDS or pneumonia alone (104). In subsequent studies, plasma and BAL levels of chemokines, such as CXCL-8, CXCL-1, CXCL-5, and CCL-2, have all been found to be elevated in patients at risk for, and with established, ARDS (6, 105). Early enhanced neutrophil migratory activity due to elevated pulmonary concentrations of CXCL-8 may be critical to the neutrophil infiltration that is characteristic of ARDS in patients who later develop ARDS (106). Circulating levels of CXCL-8, CXCL-1, and CXCL-5 are elevated in severe acute pancreatitis, and are predictors of disease severity (107). In a recent study, a panel of seven plasma biomarkers (CXCL-8, receptor for advanced glycation end products, procollagen peptide III, brain natriuretic peptide, angiopoietin-2, IL-10, and TNF-α) could accurately differentiate patients with trauma-induced ALI from patients who have undergone trauma without ALI (108). In another recent multicenter study, it has been shown that, when combined with clinical data, plasma biomarkers (CXCL-8, intercellular adhesion molecule-1, von Willebrand factor, soluble TNF receptor-1, and surfactant protein-D) measured at the onset of ALI can improve the accuracy of risk prediction. A reduced set of three biomarkers (CXCL-8, soluble TNF receptor-1, and surfactant protein-D) had nearly equivalent prognostic value. In summation, biomarkers including chemokine levels may be useful for identifying a population at high risk of ALI (109). Moreover, the results of these studies and preclinical research with experimental animal models of ALI suggest that chemokines can act as therapeutic targets for ALI of different etiologies.

CONCLUSIONS

ALI is a devastating clinical syndrome. Although there are no specific therapies for ALI, there has been progress in the management of patients with ALI using lung-protective ventilation methods and careful fluid management. Chemokines are critical for neutrophil and macrophage recruitment to the lungs in ALI. An ideal therapeutic strategy for ALI would attenuate the extensive tissue-destructive potential of recruited leukocytes without impairing host defense mechanisms. Based on the findings from

experimental and clinical studies of ALI, our understanding of the molecular and cellular mechanisms that regulate the pathogenesis of ALI has improved substantially over recent years. Development of unique tools, such as gene-deficient mice and blocking antibodies for chemokines and/or their receptors, along with biomarker discoveries in ALI, have contributed to these new insights. The future challenge will be to apply our current understanding of chemokine function to develop therapeutic methods to modulate the destructive potential of inflammatory lung injury. Specific pharmacological inhibitors of chemokines and/or their receptors may be useful therapies for ALI, although the timing and specificity of such therapies must be exquisite so as not to limit the critical host defense functions of inflammatory cells (110).

Author disclosures are available with the text of this article at www.atsjournals.org.

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