Chemokines in acute respiratory distress syndrome

Padmam Puneet,1 Shabbir Moochhala,1,2 and Madhav Bhatia1

1Department of Pharmacology, National University of Singapore; and 2Centre for Biomedical Sciences, Defence Medical and Environmental Research Institute, Singapore

Puneet, Padmam, Shabbir Moochhala, and Madhav Bhatia. Chemokines in acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 288: L3–L15, 2005; doi:10.1152/ajplung.00405.2003.—A characteristic feature of all inflammatory disorders is the excessive recruitment of leukocytes to the site of inflammation. The loss of control in trafficking these cells contributes to inflammatory diseases. Leukocyte recruitment is a well-orchestrated process that includes several protein families including the large cytokine subfamily of chemotactic cytokines, the chemokines. Chemokines and their receptors are involved in the pathogenesis of several diseases. Acute lung injury that clinically manifests as acute respiratory distress syndrome (ARDS) is caused by an uncontrolled systemic inflammatory response resulting from clinical events including major surgery, trauma, multiple transfusions, severe burns, pancreatitis, and sepsis. Systemic inflammatory response syndrome involves activation of alveolar macrophages and sequestered neutrophils in the lung. The clinical hallmarks of ARDS are severe hypoxemia, diffuse bilateral pulmonary infiltrates, and normal intracardiac filling pressures. The magnitude and duration of the inflammatory process may ultimately determine the outcome in patients with ARDS. Recent evidence shows that activated leukocytes and chemokines play a key role in the pathogenesis of ARDS. The expanding number of antagonists of chemokine receptors for inflammatory disorders may hold promise for new medicines to combat ARDS.

inflammatory mediators; polymorphonuclear leukocyte; pathogenesis; antagonists

THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) is characterized by the rapid onset of severe respiratory failure usually followed by clinical events including major surgery, trauma, multiple transfusions, severe burns, pancreatitis, and sepsis. Inflammatory mediators play a key role in the pathogenesis of ARDS (10). Clinically this condition is characterized by severe hypoxemia, diffuse bilateral pulmonary infiltrates, dyspnea, and decreased lung compliance. Originally referred to as traumatic wet lung, shock lung, or congestive atelectasis, ARDS was recognized in 1967 when the clinical, physiological, radiographic, and pathological abnormalities that were unique to a group of 12 patients were described, distinguishing them from other cases in a series of 272 patients treated for respiratory failure (4). Before 1992, the acronym ARDS represented adult respiratory distress syndrome. The American-European Consensus Committee on ARDS standardized the definition in 1994 and renamed it acute rather than adult respiratory distress syndrome because it occurs at all ages. The term acute lung injury (ALI) was also introduced at that time. The committee recommended that ALI be defined as “a syndrome of inflammation and increased permeability that is associated with a constellation of clinical, radiologic, and physiologic abnormalities that cannot be explained by, but may coexist with, left atrial or pulmonary capillary hypertension” (7). The distinction between ALI and ARDS is the degree of hypoxemia (7). ARDS is in that subset of patients at the severe end of the spectrum of ALI.

PATHOPHYSIOLOGY OF ARDS

Several investigators have reported on the clinical predispositions for ARDS. These were divided into two categories based on pathophysiological mechanisms either direct (pulmonary) or indirect (extrapulmonary) injury (29) leading to pulmonary inflammation. Direct injury includes those conditions in which a toxic substance directly injures the lung epithelium such as diffuse pulmonary infection (e.g., bacterial, viral, fungal, pneumocystis), toxic gas/smoke inhalation, pulmonary contusion, and aspiration of gastric contents (29, 105). Indirect injury is a more common predisposition and occurs by means of blood-borne systemic inflammatory processes such as sepsis, septic shock (10, 15, 29, 35, 80, 95, 100, 105, 109), acute pancreatitis (1, 4, 10, 11, 17, 59, 77, 85), and other clinical events including major surgery, trauma, multiple transfusions, dyspnea, ischemia-reperfusion injury, and decreased lung compliance (15, 84). In ARDS, the injured lung is believed to go through three phases: exudative, proliferative, and fibrotic, but the course of each phase and the overall disease progression are variable. The pathological features of the lung in ARDS derive from severe injury to the alveolocapillary unit. The morphologic picture of the lung in ARDS has been labeled diffuse alveolar damage (86) and extravasation of intravascular fluid that dominates the onset of the disease. The exudative phase occurs in the first week after the onset of the respiratory failure. The histological features are dense eosinophilic hyaline membranes and alveolar collapse. The endothelial cells swell, the intercellular junctions widen, and pinocytic vesicles increase, causing the capillary membrane to be disrupted and resulting in capillary leak and edema formation. Type I pneumocytes also

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Chemokines in ARDS

Invited Review

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Chemokines are a family of mostly small (7–10 kDa), secreted proteins that function in leukocyte trafficking, recruiting, and recirculation and are characteristically basic heparin-binding proteins. They are distinguished from other cytokines by being the only members of the cytokine family that act on the superfamily of G protein-coupled serpine receptors. Although chemokines have a relatively low level of sequence identity, their three-dimensional structure shows a remarkable homology in that they all have the same monomeric fold. This fold, consisting of three β-strands, (β1, β2, and β3), a carboxy (C) terminal helix and a flexible amino (N) terminal region, is conferred to these proteins by a four-cysteine motif that forms two characteristic disulfide bridges. The α-helix folds across one face of the β-sheet. The β1-strand forms a single turn of 3_10 located near the NH2-terminal end of β1-strand, and the N-loop stretches across the β-sheet so that the NH2-terminal region is located adjacent to the β3-strand and the β1-β2-loop. The β2- and β3-strands are linked by an ordered hydrogen-bonded turn, the β2-β3-turn (5, 93) (Fig. 2).

Chemokine production can be broadly classified into the constitutively secreted and inducible. Secreted are homeostatic chemokines directing basal leukocyte trafficking and the organization of the lymphoid tissue. Stromal cell-derived factor (SDF)-1 and macrophage-derived chemokine (MDC) appear to be produced constitutively (developmentally regulated) and play a role in the basal trafficking of leukocytes. Induced are inflammatory chemokines responsible for the recruitment of leukocytes effector populations to the site of an immune reaction (12, 73). However, most chemokines are produced in response to a variety of inflammatory stimuli, including the early-response cytokines, TNF, IL-1, C5a, leukotriene B4.
Chemokines play a critical role in many pathophysiological processes such as allergic responses, infectious and autoimmune diseases, angiogenesis, inflammation, tumor growth, and hematopoietic development. Approximately 80% of these proteins have from 66 to 78 amino acids (aa) in their mature form. Although the aa sequence identity within chemokine families can be as high as 70%, there can be as little as 20% homology in the sequence between chemokines from different families (45).

**CHEMOKINE RECEPTORS**

Chemokines produce their biological effects by interacting with specific receptors on the cell surface of their target cells (46). All chemokines signal through seven transmembrane domain G protein-coupled receptors (9, 10, 12, 18). These receptors are all characterized by a heptahelical structure and belong to a superfamily of serpentine receptors coupled to guanine nucleotide-binding proteins (G proteins). Most of the chemokine receptors were identified and cloned from the immune cells that are their major target cells. There are a few receptors that bind a single ligand, and several chemokines can bind to more than one receptor (Fig. 3). Binding of chemokines to their serpentine receptors activates a complex network of intracellular signaling pathways involving a variety of second...
messenger systems, such as calcium, cAMP, and phospholipids, as well as a concerted interplay of kinase cascades downstream of small guanine triphosphatases (GTPases) such as Ras and Rac (83). It appears that the same receptor can display differential effects depending on the ligand to which it binds (67).

CHEMOKINE SUBFAMILIES

On the basis of cysteine residue positioning, chemokines are classified into four subfamilies. The CXC subfamily (α-subfamily) has the first two NH2-terminal cysteines separated by one nonconserved aa residue, the CXC cysteine motif (53). CXC chemokines are clustered on human chromosome 4 and exhibit between 20 and 50% homology at the aa level, with the exception of SDF-1, which is found on human chromosome 10 (18, 100, 101). 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. ELR + chemokines bind to CXCR2 and have generally been believed to act as neutrophil chemoattractants and activators. The ELR−chemokines generally bind to CXCR3-XCR5 and act primarily on mononuclear leukocytes (9, 10, 12, 18). CXC chemokines have been found to be produced by an array of monocytes, AMs, neutrophils, platelets, eosinophils, mast cells, T lymphocytes, natural killer cells, keratinocytes, mesangial cells, hepatocytes, fibroblasts, smooth muscle cells, mesothelial cells, and endothelial cells (100). The murine homologs to the CXC chemokine family include KC, macrophage inflammatory protein-2 (MIP-2), and monocyte induced by IFN-γ (MIG) and are structurally homologous to human growth-related oncoprotein (GRO-α, -β, -γ), 10-kDa IFN-γ-inducible protein (IP)-10, and MIG, respectively. No murine or rat structural homolog exists for human IL-8.

The CC chemokine subfamily (β-subfamily) has the first two NH2-terminal cysteines adjacent to one another with no intervening aa, the CC cysteine motif (53). CC chemokines are clustered on human chromosome 17, with the exception of MDC on chromosome 16, MIP-3α on chromosome 2, and MIP-3β on chromosome 9. They exhibit between 28 and 45% homology at the aa level. The receptors for this group are designated CCR1–CCR11. Target cells for different CC family members include most types of leukocytes (9, 10, 12, 18). Eotaxin, for example, is a potent eosinophil chemoattractant and induces their degranulation, whereas monocyte chemoattractant protein (MCP)-1 and regulated on activation normal T cell expressed and secreted (RANTES) are chemoattractants for T cells and monocytes, respectively (90). However, recent studies have shown that these chemokines display a significant increase in neutrophil infiltration (80). The C chemokines (γ-subfamily) have one lone NH2-terminal cysteine aa (they lack the first and third cysteines), the C cysteine motif. Lymphotactin is the lone member of this family and located on human chromosome 1, both human and mouse. Lymphotactin receptor is designated XCR1. Lymphotactin is predominantly expressed in the thymus and appears to recruit immature T cells from the bone marrow (54).

The CX3C chemokines (δ-subfamily) have the first two NH2-terminal cysteines separated by three nonconserved aa residues.

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Fig. 2. Ribbon diagram illustrating the monomer 3-dimensional structure of IL-8. The NH2 and COOH termini and the secondary structure elements are labeled in the IL-8 structure (5). [Image prepared using Protein Explorer 1.982 by Eric Martz (http://proteinexplorer.org).]
Fractalkine (Neurotactin) is the only member of this subfamily and is located on human chromosome 16 and mouse chromosome 8 (5, 53, 101). The fractalkine receptor is known as CX3CR1 (9, 10, 12, 18). Fractalkine can exist either as a membrane-anchored or as a shed glycoprotein, which acts as a potent adhesion molecule or chemoattractant, respectively, for T cells and monocytes (118). Cells expressing the chemokine receptor CX3CR1 bind fractalkine with high affinity (44). In contrast to other chemokines, fractalkine is not synthesized by leukocytes but is expressed on activated endothelial cells (42, 78). This suggests a novel role for this chemokine as an adhesion molecule. Other chemokines induce cell adhesion but do so indirectly by upregulation of integrins, a process that is dependent on G protein activation. Haskell and colleagues (44) were able to demonstrate that fractalkine mediates rapid firm adhesion that is independent of G protein activation. Furthermore, the adhesion of CX3CR1-
expressing cells to fractalkine did not enhance integrin-dependent adhesion, suggesting that fractalkine may provide an alternative to integrin-mediated adhesion. Efsen and colleagues (27) showed that the fractalkine system is upregulated during liver damage and suggested that fractalkine may play a role in the recruitment and adhesion of inflammatory cells and in the biology of liver epithelial cells.

In addition, several virus-encoded proteins that have sequence homology and share the serpentine structure of the cloned chemokine receptors have been identified and termed as viroceptors. A protein first identified in human erythrocytes as an IL-8-binding protein has been shown to be a novel chemokine-binding protein that binds both CC and CXC chemokines with high affinity (46). This protein, known as Duffy antigen receptor for chemokines (DARC), is identical to the Duffy blood group antigen, a receptor for the malarial parasite Plasmodium vivax (45). This receptor has no signal transducing activity and may act as a sink, mopping up excess free chemokines and preventing inappropriate activation of circulating leukocytes (55, 118). DARC is selectively expressed at the mRNA and protein levels in the high endothelial venules of unstimulated lymph nodes. DARC selectively binds members of the proinflammatory chemokines but not lymphoid chemokines that are normally expressed in high endothelial venules. DARC downregulates activities of proinflammatory chemokines upon binding (51).

**ROLE OF CHEMOKINES IN ARDS**

The hallmark of pulmonary infiltration associated with ALI is the presence of infiltrating leukocytes. Leukocyte migration
is directed largely by chemokines. The interrelationship of early-response cytokines, adhesion molecules, and chemokines orchestrates the recruitment of neutrophils into the lung (Fig. 1).

AMs are a major source of chemokines in the air spaces and produce IL-8, GRO-related peptides, and epithelial neutrophil-activating protein (ENA)-78. AMs respond directly to bacterial products such as bacterial lipopolysaccharide (LPS) and gram-positive cell wall products such as leipoteichoic acids. On a quantitative basis, IL-8 is the most abundant product following LPS stimulation (35). Other cells of the alveolar environment also produce α- and β-chemokines but do so in response to the proinflammatory cytokines TNF-α and IL-1β and not directly in response to bacterial products like LPS (87, 88, 97, 120). Mounting evidence suggests that MCP-1 and its hematopoietic cell receptor CC chemokine receptor 2 (CCR2) are involved in inflammatory disorders of the lung (89).

**EXPERIMENTAL EVIDENCE**

The direct homolog of MCP-1 in rat often referred to as JE has been shown to be as a potent monocyte chemoattractant and activator in rat models. Plasma levels of both MCP-1/JE and the CXC chemokine cytokine-induced neutrophil chemoattractant (CINC), the homolog of the human chemokine GRO-α, were increased in inflammatory conditions in rat models (17, 121). Strategies that block CINC activity reduce the effect of the inflammatory response in some of these models of injury (11, 21, 23, 119). In a recent study, we have shown that treatment with neutralizing antibody against CINC protects rats against acute pancreatitis-associated lung injury (11). An increase in pancreatic MCP-1 mRNA on induction of pancreatitis was observed in a rat model (38). The proinflammatory CXC chemokines GRO, CINC-2α, and MIP-2 are a closely related family of neutrophil chemokinators. Vandeslitt and colleagues (112) have reported that freshly isolated alveolar type II cells express these chemokine mRNAs at much higher levels than do freshly isolated type I cells or AMs. Type II cells also express CXCR2, the receptor for these chemokines. Lung injury caused by acid or *Pseudomonas aeruginosa* caused an increase in alveolar type II cell expression of chemokine mRNAs and GRO protein (112).

The deletion of CCR1 receptor for MIP-1α and RANTES is associated with protection from pulmonary inflammation secondary to acute pancreatitis in the mouse (33). MCP-1 overexpression in the lung did not cause any lung inflammation but resulted in increased monocyte and lymphocyte infiltration into the airways (40). MCP-1 was shown to behave as an efficient neutrophil chemoattractant in mice in the context of chronic inflammation (50). More recently, a broader role for MCP-1 in the systemic inflammatory response has been suggested. After endotoxin challenge in baboons, there is an increase in TNF levels at 2 h postchallenge, which is followed at 4 h with a peak in MCP-1 levels (49). Administration of exogenous MCP-1 protects mice from a lethal challenge of bacteria or endotoxin; MCP-1 seems to shift the balance in favor of anti-inflammatory cytokines, with an increase in IL-10 and a decrease in IL-12 (122). MCP-1 not only prevented death in normal mice challenged with *Pseudomonas* but also effectively protected cyclophosphamide-induced leukopenic mice against *Pseudomonas* infection (76). Pretreatment with a FasL antagonist, a decoy receptor 3 analog (Dr3 analog), reduced neutrophil infiltration into the air space and resulted in a highly significant reduction in the levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), MIP-2, and KC in bronchoalveolar lavage (BAL) fluid in a murine model of lung injury (117). Inosine, an endogenous purine, downregulated the LPS-induced expression of TNF-α, IL-1β, IL-6, and MIP-2 and tended to reduce MIP-1α, whereas it enhanced the production of IL-4 in ARDS in an inbred BALB/c mice challenged with intratracheal LPS (62).

Neutrophil-derived α-defensins increased IL-8 expression, ENA-78, MCP-1, and GM-CSF release from A549 cells, whereas in primary bronchial epithelial cells only IL-8 and IL-6 were increased. Pretreatment with dexamethasone significantly reduced defensin-induced IL-6, IL-8, and ENA-78 synthesis in airway epithelial cells (111). IL-8-mediated neutrophil migratory activity in the early postinjury phase, before the development of ARDS, may be a crucial factor in the etiology of ARDS (79).

Intratracheal instillation of MCP-1 in mice was recently shown to cause increased alveolar monocyte accumulation in the absence of lung inflammation, whereas combined JE/MCP-1/LPS challenge provoked acute lung inflammation with early alveolar neutrophil and delayed alveolar monocyte influx. LPS induces protein tyrosine phosphorylation and NF-κB binding activity. These events were significantly inhibited by β-lapachone. Furthermore, β-lapachone in vivo protected against the induction of lung edema, lung inducible nitric oxide synthase (iNOS) protein expression and NF-κB activation, lethality, and increased plasma nitrite and serum TNF-α levels induced by LPS (108) in a mouse model. The role of resident AMs in these leukocyte recruitment events and related phenomena of lung inflammation was evaluated. Depletion of resident AMs by pretreatment of mice with liposomal clodronate did not affect the JE/MCP-1-driven alveolar monocyte accumulation, despite the observation that resident AMs constitutively expressed the JE/MCP-1 receptor CCR2. In contrast, depletion of resident AMs largely suppressed alveolar cytokine release as well as neutrophil and monocyte recruitment profiles upon combined JE/MCP-1/LPS treatment. Increased lung permeability was still observed in resident AM-depleted mice undergoing JE/MCP-1/LPS challenge. Collectively resident AMs were not involved in JE/MCP-1-driven alveolar monocyte recruitment in noninflamed lungs but largely contribute to the alveolar cytokine response and enhanced early neutrophil and delayed monocyte influx under inflammatory conditions (JE/MCP-1/LPS deposition) (67). β-Lapachone could also inhibit the production of TNF-α induced by LPS. LPS induces protein tyrosine phosphorylation and NF-κB binding activity.

Receptors in disease models can be neutralized using neutralizing antibodies, modified chemokines that act as receptor antagonists, and small molecule receptor antagonists. Passive immunization of animals with neutralizing antibodies to IL-8, before reperfusion of the ischemic lung, prevented neutrophil extravasation and tissue injury, suggesting a casual role for IL-8 in a rabbit model of lung ischemia-reperfusion injury (100). The IL-8 levels are higher in animals with lethal bacteraemia than in those with sublethal endotoxemia. However, administration of an anti-IL-8 antibody only marginally im-
proven survival in endotoxin-induced acute lethality in *Propionibacterium acnes*-primed rabbits (48). Receptor activation is followed by receptor phosphorylation and subsequent desensitization to further stimulation. The major mechanism of desensitization is through sequestration of the receptor via clathrin-coated pits into early endosomes. Blocking the endocytosis of CXCR2 severely attenuates ligand-mediated chemotaxis but has no effect on MAPK activation. This implies that a desensitization-resensitization process is required for chemotaxis to a continuous signal generated over a concentration gradient, but not for downstream signaling (120). In contrast to the ligand-induced desensitization that is seen with CXCR2, the downregulation of CXCR1 and CXCR2 that is seen in response to TNF-α and LPS appears to be due to proteolytic cleavage of the receptors, as metalloprotease inhibitors block the inhibitory effect of LPS and TNF-α on IL-8-mediated calcium mobilization and neutrophil chemotaxis (100).

Another model of ischemia-reperfusion injury demonstrating the importance of cytokine cascades between the liver and lung demonstrated that hepatic ischemia-reperfusion injury and the generation of TNF could result in pulmonary-derived ENA-78. The production of ENA-78 in the lung was correlated with the presence of neutrophil-dependent lung injury, and passive immunization with neutralizing ENA-78 antibodies resulted in significant attenuation of lung injury (21–24). When injured, epithelial cells secrete the chemokine KC (94). In matriksin null mice, neutrophils remained confined in the interstitium of injured lungs and did not advance into the alveolar space. Impaired transepithelial migration was accompanied by a lack of shed syndecan-1 and KC in the alveolar fluid (61). Rats were immunized with neutralizing KC (homologous to human GRO-α) antibodies before intratracheal LPS challenge, and it was found that there was a 71% reduction in neutrophil accumulation within the lung. MIP-2 plays a key role in the pathophysiology of acute pancreatitis, and MIP-2 blockade may improve the outcome of the disease (82). Neutralization of MIP-2 (murine homolog of human GRO-β) results in both reduction in the recruitment of neutrophils in the lung and pulmonary clearance of bacteria. The depletion of MIP-2 in this model during bacterial pneumonia was associated with a higher mortality (39). Furthermore, these same investigators have found that lung-specific transgenic expression of KC enhances resistance to *Klebsiella* pneumonia and improves survival in mice (80, 107). MCP-2 is chemotactic for monocytes without increasing intracellular calcium (96). In contrast, MCP-2 is much more prominent in transgenic mice on a BALB/c background than in those on a C57BL/6 background (80). It would be interesting to determine whether the difference in lung neutrophilia seen in the BALB/c and C57BL/6 mice in allergic inflammation is at least partly because of differential RANTES production in the mice. When RANTES expression was induced in the transgenic mice, a significant increase in the number of neutrophils was observed in BAL fluid (10–30% of total cells) (80). Previously, RANTES was implicated in eosinophil chemotaxis based on blocking studies with antibodies (58, 113) as well as on studies that used the RANTES receptor antagonist Met-RANTES (28, 34).

The studies with Met-RANTES in rodent models allow certain conclusions to be drawn. Treatment with Met-RANTES protected mice against acute pancreatitis-associated lung injury, with little or no protection against local pancreatic damage (13). Many of the models studied with Met-RANTES have been Th1-mediated inflammatory disease models. However, Met-RANTES also had an anti-inflammatory effect in a model of Th2 inflammation. It was efficient in preventing eosinophil recruitment in an airway inflammation model (34). In a model in which bacteria induced the recruitment of dendritic cells to the trachea, significant inhibition was observed when Met-RANTES was administered 1 and 3 days before the antigen challenge (102). Several drug companies have identified potent small molecule antagonists of the CXCR2 and CCR1 chemokine receptors. These should find broad utility in a variety of acute and chronic inflammatory diseases. For example, the CCR1 receptor antagonists have nanomolar affinity for the receptor and potently inhibit the ability of the ligand MIP-1α and RANTES to induce cellular migration of T cells and monocytes (45). The deletion of CCR1 receptor is associated with protection from pulmonary inflammation secondary to acute pancreatitis in the mouse (76). PMN counts were significantly higher in BAL fluids of LPS-induced lung injury in a rat model (110). Intraperitoneal injections of LPS into rabbits induce a massive infiltration of neutrophils into the pleural cavity (34). The administration of an anti-IL-8 antibody reduces neutrophil infiltration in this model, implicating IL-8 as a key mediator in endotoxin-induced pleurisy (63). Intraperitoneal administration of anti-CD44 MAb in mice prevented both lymphocyte and eosinophil accumulation in the lung and also blocked chemokines in BAL (52).

**CLINICAL EVIDENCE**

The α- and β-chemokines are present in the lungs of patients with ARDS (64). The α- and β-chemokines are produced in vitro by cells or cell lines derived from the human lung (35, 71,
99, 103). One study showed significantly higher IL-8 levels in patients with ARDS plus pneumonia than in those with ARDS or pneumonia alone (20). Consistently elevated levels of IL-8 and IL-6 in ARDS, severe pneumonia, or both distinguished these entities from cardiogenic pulmonary edema in a study performed by Schutte et al. (92). In patients with established ARDS, IL-8 most consistently correlates with neutrophil concentration in BAL but not with the severity of lung injury or subsequent clinical course (36, 72). IL-8 levels are higher in patients with sepsis- vs. nonsepsis-induced ARDS (72), but this finding has been contradicted in some studies (91). Patients who progressed to ARDS had significantly greater BAL levels of IL-8 than those patients who failed to develop ARDS. Interestingly, plasma levels of IL-8 from these patients were not found to be significantly different from patients who developed ARDS compared with those who did not develop ARDS (100). IL-8, GRO, ENA-78, and MCP-1 have all been found in BAL fluid of patients at risk for, and with established, ARDS (26, 36, 72, 114). Although other potent leukocyte chemoattractants also exist, including the complement component C5a, and the low-molecular-weight lipids LTB4 and PAF, the neutrophil chemotactic activity in BAL is due predominantly to IL-8 and not to C5a (36, 81). The relationship between IL-8 and PMN grows stronger with time in patients with persistent ARDS (65). Early enhanced neutrophil migratory activity coupled with elevated pulmonary concentrations of IL-8 may be central to the establishment of the neutrophil infiltration that is characteristic of ARDS in patients who later develop ARDS (79). Other chemokines in ARDS BAL also are likely to contribute to PMN recruitment. The mRNA expressions of IL-1β, TNF-α, IL-6, and IL-8 and iNOS were upregulated in late ARDS patients. Expressions of these genes in the acute phase of septic ARDS were most distinct (43). BAL from patients at risk of developing ARDS, with early-phase ARDS, and with late-phase ARDS contained increased levels of TNF-α, but not of transforming growth factor-β1, compared with BAL from control patients. Neutralization of TNF-α inhibited the cytotoxic activity on endothelial cells of part of the early-phase ARDS BAL (41). The clinical benefit of corticosteroids is still controversial, and in fact, a multicenter trial showed no preventive effect of steroid therapy for ARDS (14). Patients in the late ARDS group who received large-dose methylprednisolone showed less expression of inflammatory cytokines mRNA (30, 47). Because anti-inflammatory genes may also be suppressed by corticosteroids, selective suppression of specific molecules as in anti-IL-8 therapy (30) might be more important for successful treatment of ARDS. Several investigators have suggested that the use of corticosteroids in the late phase of ARDS improves lung function and survival (70), whereas trials of short-term, high-dose steroid therapy failed to show an improvement in mortality in patients at risk of or early ARDS (8).

Patients with ALI/ARDS had significantly higher concentrations of α2-macroglobulin compared with control patients. IL-8 bound to α2-macroglobulin retained its biological activity, and this fraction of IL-8 was protected from proteolytic degradation. Thus complex formation may modulate the acute inflammatory process in the lung (56). The concentration of GRO was higher than that of IL-8 in patients with ARDS (114). Concentrations of GRO and ENA-78 exceed the concentration of IL-8 in BAL throughout most of the course of ARDS, despite the fact that depletion studies with antibodies to IL-8 suggest that IL-8 was the dominant PMN chemoattractant in the fluids studied (36). Among 11 patients with acute pancreatitis, ALI patients had significantly higher IL-8, IL-6, and phagocyte CD11b expression levels than did non-ALI patients, whereas among 14 patients with massive transfusion, respective findings in ALI and non-ALI patients were comparable. Results give credence to the view that systemic inflammation plays a role in development of ALI triggered by pancreatitis (104). Various members of the GRO subfamily of proteins have also been identified as products of human AMs, but there has been controversy regarding which of the subfamily members are produced under conditions of LPS stimulation (6, 55). Because of their remarkable similarity, part of this controversy likely results from the difficulty in designing strategies that reliably distinguish the three cDNAs. LPS-stimulated human AMs transcribe all three GRO subfamily genes (74). Intravenous injection of LPS into a normal human volunteer causes a rapid increase in plasma CXCL8 levels, peaking at 2 h and returning to baseline levels within 5 h after LPS injection (63). Moreover, an elevation in plasma CXCL8 levels precedes neutrophil accumulation and activation, as shown by an elevated neutrophil-derived elastase level (84). IL-8 binds with high affinity to both of the PMN cell surface CXC receptors (CXCR1 and CXCR2), whereas the GRO subfamily members ENA-78 and neutrophil attractant/activation protein-2 bind with high affinity only to CXCR2 (2, 60, 115).

Pancreatitis patients who developed ALI had significantly higher serum concentrations of IL-8, IL-6, and CD11b expression (indicative of neutrophil activation) compared with pancreatitis patients who did not develop ALI (77). The mRNA expressions of IL-1β, TNF-α, IL-6, and IL-8 in the acute phase of septic ARDS were most distinct. Plasma levels of GRO-α and ENA-78 were raised in addition to those IL-8 in patients with severe acute pancreatitis. This suggests that all three chemokines are involved in the inflammatory response in this condition (95). MCP-1, which regulates monocyte recruitment, is detectable in ARDS BAL at the onset of ARDS and persists in the lungs of patients with sustained ARDS (36). MCP-1 has been shown to be increased in patients with sepsis and shock and correlates with increased survival (16, 36, 37). There was no correlation between MCP-1 levels and leukocyte numbers, suggesting an alternative biological function for MCP-1 other than leukocyte recruitment (16). Recently, polymorphism for MCP-1 has been described with increased cytokine-induced release of MCP-1 by monocytes, and these studies identify potentially important roles for MCP-1 in these lung inflammatory disorders. MCP-1 inhibition in patients with ARDS may be hazardous by interfering with defense against bacteremia (89). 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 patients (66).

There are >30 different chemokines and >20 different receptors, with overlapping functions (Table 1, Fig. 1). Despite the complexity and apparent redundancy of the chemokines and receptors with overlapping functions, specific receptor
antagonists that interfere with leukocyte migration and activation could be useful in ARDS.

CONCLUSION

Despite improved understanding of the pathogenesis of ARDS, pharmacological modalities have been unsuccessful in decreasing mortality. Various medications directed at key stages of the pathophysiology have not been as clinically efficacious as the preceding experimental trials indicated (109). None of the randomized clinical trials using new therapeutic agents have shown an improvement of patient outcome. In summary, the severity of ARDS depends on interplay of pro- and anti-inflammatory mediators that start soon after the initiation of lung injury. The stage of ARDS at the time of intervention, to enable treatment to inhibit the progression of the disease; type of injury, direct or indirect; early recognition; and combined treatment and prevention all must be considered for future trials. Although the current focus has been to develop antagonists for specific receptors, the pleiotropy of the chemokines and their receptors may necessitate the use of multiple antagonists targeting multiple receptors to achieve complete inhibition of function. In addition, understanding the biological and biochemical markers involved in the complex inflammatory response of ARDS offers the possibility of future investigations to target treatment on these mediators to predict more accurately either the onset or outcome of the injury that occurs in the lungs. Cellular and molecular methods combined with animal models and clinical studies may lead to further progress in detection and treatment of ARDS.

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