Chemokines in acute respiratory distress syndrome

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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 chemoattractant 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 ALI 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
become swollen with cytoplasmic vacuoles, which eventually detach from the basement membrane (25).

The proliferative phase begins as early as the third day but is most prominent in the second and third week after symptom onset. Type II cells begin to proliferate and reline the denuded basement membrane (25). Fibroblasts become pronounced in this phase. Fibroblasts and myofibroblasts migrate through breaks in the alveolar membrane into the fibrous intra-alveolar exudate, forming a cellular granulation tissue. Sparsely cellular, dense fibrous tissue forms as collagen is deposited. Epithelial cells migrate over the surface of the organizing granulation tissue and transform the intra-alveolar exudate into the interstitial tissue (106). Surfactant abnormalities are thought to occur because of damage to type II pneumocytes and because the alveolar flooding that occurs, destabilizing the surfactant monolayer in the air spaces.

The fibrotic phase can start as early as 36 h after the onset of injury; extensive remodeling of the lung by sparsely cellular collagenous tissue occurs by the third or fourth week of respiratory failure (25). Air spaces are irregularly enlarged, and there is alveolar duct fibrosis. Type III elastic collagen is replaced by type I rigid collagen over time, leading to a stiff lung. The extent of fibrosis correlates with mortality (69). Early in ARDS, pulmonary vasoconstriction, thromboembolism, and interstitial edema, all of which are potentially reversible, raise the pulmonary artery pressure. After several weeks, fibrous obliteration of the microcirculation and arterial muscularization contribute to irreversible pulmonary hypertension. Patients with ARDS also are at risk of pulmonary emboli because of immobilization and the presence of indwelling vascular catheters (103).

Polymorphonuclear leukocytes (PMNs) have been recognized as important contributors to the pathogenesis of ARDS. As a result of an exaggerated systemic inflammatory response syndrome response, leukocytes become activated within the general circulation, and some then lodge within the pulmonary microcirculation. As the condition develops, leukocytes migrate into the pulmonary interstitium, and increased endothelial permeability leads to tissue edema (75). The disruption of the epithelial and endothelial barriers in the lungs is associated with a massive increase in epithelial and endothelial permeability with accumulation of high-molecular-weight proteins that are normally excluded from the air spaces (44). This occurs together with a marked influx of PMNs, so that PMNs become the predominant leukocytes in the alveolar spaces. Normally, 90% or more of the air space cells are alveolar macrophages (AMs), <10% are lymphocytes, and only 1–2% are PMNs. In patients with ARDS, up to 90% air space cells are PMNs. When ARDS is sustained, PMNs persist in the air spaces, and the number of macrophages is reduced. When ARDS is resolved, the number of macrophages increases, but lymphocyte accumulation seldom occurs (98, 116). Surfactant abnormalities occur due to the damage of type II pneumocytes, and alveolar flooding that occurs destabilizes the surfactant monolayer in the air spaces. This dysfunction promotes alveolar collapse and worsens gas-exchange abnormalities (64).

It is now widely accepted that the formation of inflammatory mediators plays an important role in the pathophysiology of inflammation in ARDS. These mediators include tumor necrosis factor (TNF)-α; interleukin (IL)-1, -4, -6, -8, -10, and -13; substance P; platelet activating factor (PAF); complement component (C5a); adhesion molecules (e.g., vascular adhesion molecule-1, intercellular adhesion molecule-1); E- and P-selectins; L-selectin; and vasoactive mediators (e.g., nitric oxide). The transcription factor NF-κB plays a central role in the regulation of many genes responsible for the generation of mediators in inflammation. Investigations into the interactions between various cell populations have led to the concept of cytokine networking with chemokines playing a central role. One population of cells may respond directly to specific stimuli by the elaboration of a particular mediator to exert distinct effects upon another population of cells. The targets respond by producing chemokines, which may serve as feedback signals to initiate a cascade of events by activating and recruiting yet another array of target cells. The salient feature of inflammation is association of leukocyte infiltration with ALI. The maintenance of leukocyte recruitment during inflammation requires intercellular communication between infiltrating leukocytes and the endothelium, resident stromal 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 (53), which are a specific class of inflammatory mediators that play a key role in the pathogenesis of ARDS (Fig. 1).

CHEMOKINES

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 serpentine 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 carboxyl (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 3₁₀, 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 monokine induced by IFN-γ (MIG) and are structurally homologous to human growth-related oncogene (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.
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.

### Table 1. CC, C, CXC, and CX3C chemokine/receptor families

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<th>Systemic Name</th>
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<th>Alternative Name</th>
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Inflamm, inflammatory chemokines; homeo, homeostatic chemokines; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T cell expressed and secreted; TARC, thymus- and activation-regulated chemokine; SLC, secondary lymphoid-tissue chemokine; MDC, macrophage-derived chemokine; MPIF, myeloid progenitor inhibitory factor; TECK, thymus-expressed chemokine; CTACK, cutaneous T cell-attracting chemokine; GRO, growth-related oncogene; ENA, epithelial neutrophil-activating protein; GCP, granulocyte chemotactic protein; NAP, neutrophil attractant/activation protein; MIG, monokine induced by IFN; IP-10, 10-kDa IFN-γ-inducible protein; I-TAC, IFN-γ-inducible T cell α-chemoattractant; SDF, stromal cell-derived factor.
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 virocepters. 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 leupeptinoidoic 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 CX3 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 CX3 chemokines GRO, CINC-2α, and MIP-2 are a closely related family of neutrophil chemotactants. Vanderbilt 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 FaslL antagonist, a decoy receptor 3 analog (DcR3 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 causal role for IL-8 in a rabbit model of lung ischemia-reperfusion injury (100). The IL-8 levels are higher in animals with lethal bacte- remia than in those with sublethal endotoxemia. However, administration of an anti-IL-8 antibody only marginally im-
proved 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 metalloproteinase 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 matriylsin null mice, neutrophils remained confined in the interstitium of injured lungs and did not advance into the alveolar space. Impaired transmepithelial migration was accompanied by a lack of both 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 pneumoniae* and improves survival in mice (80, 107). MCP-2 is chemotactic for monocytes without increasing intracellular calcium (96). In contrast, MIP-1α mobilizes calcium in neutrophils without causing chemotaxis (68). CD40, a member of the TNF receptor family, is expressed on a variety of hematopoietic cells and is crucial in orchestrating both humoral and cellular immune responses. CD40 ligand-deficient mice were protected against acute pancreatitis-induced lung injury (33). CCR8 knockout mice showed significant decrease in Th2 responses, resulting in reduced eosinophilia in two models of airway inflammation as would be predicted if this receptor is a selective Th2 chemokine receptor (19).

The CC chemokine RANTES was initially shown to be chemotactic for T cells and monocytes (40) but has subsequently been shown to be a potent eosinophil chemottractant. RANTES can be expressed by a variety of cell types including lung epithelial cells (57). To determine the effect of RANTES expression alone in vivo, Pan and colleagues (80) generated transgenic mice that overexpress human RANTES especially in lung in an inducible fashion. The airways of the transgenic mice overexpressing RANTES displayed a significant increase in neutrophil infiltration compared with that in control mice. RANTES expression also induced expression of the chemokine genes MIP-2, IP-10, and MCP-1 in the lungs of the transgenic mice. Although neutrophils were increased in the airways of the mice, the lung parenchyma did not show increased neutrophil numbers or any inflammation. The increased neutrophil chemotaxis induced by RANTES was 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 effective in preventing eosinophil recruitment in an airway inflammation model (34). In a model in which bacteria induced the recruitment of dendrite 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). Intratracheal 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,
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 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.

ACKNOWLEDGMENTS

The authors thank Philip Moore for a critical reading of the manuscript.

GRANTS

The authors acknowledge grant support from National Medical Research Council, Defence Science and Technology Agency-National University of Singapore Joint Applied R&D Co-operation Programme, Biomedical Research Council, and Academic Research Fund.

REFERENCES

Invited Review

CHEMOKINES IN ARDS

Chemoattractants (chemokines) are key players in cellular cross-talk during disease development and as therapeutic targets in the treatment of acute respiratory distress syndrome (ARDS). They elicit bidirectional recruitment of innate and adaptive immune cells and control the relative composition of the immune cell infiltrate. The chemokines are subdivided into subfamilies with shared structural and biological properties, and each subfamily is further subdivided into distinct ligand-receptor pairs with different tissue distribution and cellular responses. They are released by diverse cell types, including immune cells, epithelial cells, and keratinocytes, and are involved in multiple steps of the disease process. This review covers the current understanding of the role of chemokines in ARDS, highlighting their import in the future development of therapeutics.


