Cytokine-mediated inflammation in acute lung injury

Richard B. Goodman a,1, Jérôme Pugin b,2, Janet S. Lee a,1, Michael A. Matthay c,∗

a Medical Research Service, VA Pulmonary Research Laboratories, Departments of Veterans Affairs and Medicine, VA Puget Sound Health Care System, VA Puget Sound Medical Center, Division of Pulmonary & Critical Care, University of Washington School of Medicine, S-111-Pulm, 1660 S. Columbian Way, Seattle, WA 98108, USA
b Department of Medicine, Division of Medical Intensive Care, University Hospital of Geneva, 24 rue Michel-du-Croix, CH-1211, Geneva 14, Switzerland
c Departments of Medicine and Anesthesiology, Cardiovascular Research Institute, University of California at San Francisco, 505 Parnassus Avenue, HSW-825, San Francisco, CA 94143-0130, USA

Abstract

Clinical acute lung injury (ALI) is a major cause of acute respiratory failure in critically ill patients. There is considerable experimental and clinical evidence that pro- and anti-inflammatory cytokines play a major role in the pathogenesis of inflammatory-induced lung injury from sepsis, pneumonia, aspiration, and shock. A recent multi-center clinical trial found that a lung-protective ventilatory strategy reduces mortality by 22% in patients with ALI. Interestingly, this protective ventilatory strategy was associated with a marked reduction in the number of neutrophils and the concentration of pro-inflammatory cytokines released into the airspaces of the injured lung. Further research is needed to establish the contribution of cytokines to both the pathogenesis and resolution of ALI.

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Keywords: Chemokine; Interleukins; Acute respiratory distress syndrome (ARDS); Pulmonary edema

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1. Introduction

Acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS), are characterized by an acute inflammatory process in the airspaces and lung parenchyma. These clinical syndromes are manifestations of the loss of barrier function of the alveolar epithelial and pulmonary capillary endothelial cells resulting in respiratory failure in critically ill patients [1]. Evidence from several clinical studies indicates that a complex network of inflammatory cytokines and chemokines play a major role in mediating, amplifying, and perpetuating the lung injury process. Simultaneous production of anti-inflammatory cytokines can potentially counteract pro-inflammatory cytokine effects and modify the intensity of the inflammatory process. The pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) have been identified in bronchoalveolar lavage fluids (BALF) from ARDS patients, but measurements of their specific antagonists IL-1RA and soluble TNF receptors (sTNFR I and II) demonstrate their simultaneous presence. Similarly, biologically significant concentrations of neutrophil chemotactic cytokines such as IL-8, growth-related oncogene (GRO)-α, ENA-78, and GCP-2 are present in ARDS BALF, but are balanced by the presence of counter-ligands such as α-2-macroglobulin, the Duffy antigen chemokine binding protein, natural neutralizing antibodies, and the anti-inflammatory cytokine IL-10 in the lung milieu. Molar concentration ratios of pro- and anti-inflammatory molecular pairs in BALF provide insight into the inflammatory balance in individual patients, and measurement of the net inflammatory activity specific to each cytokine in cellular assays is a key step in validating these calculated ratios. Finally, relating these measurements to clinical measures of lung function and clinical outcomes underscores the relevance of these investigations. Understanding the balance of pro- and anti-inflammatory cytokines in the lungs of patients with ARDS is an important step in unraveling the pathogenesis of this devastating clinical syndrome.

2. Pro- and anti-inflammatory cytokines implicated in ARDS

2.1. Interleukin-1β (IL-1β) and its counter-regulatory ligands and receptors

Because it can stimulate the production of a variety of chemotactic cytokines such as interleukin-8 (IL-8) [2], epithelial cell neutrophil activator (ENA-78) [3], monocyte chemotactic peptide (MCP-1) [4], and macrophage inflammatory peptide-1α (MIP-1α) [5], IL-1β has earned a position of prominence at the head of the inflammatory cytokine cascade. Several investigators have identified the presence of this pro-inflammatory cytokine in BALF from patients with ARDS [6–9]. Its naturally occurring antagonist, interleukin-1 receptor antagonist (IL-1ra), is also detectable in ARDS BALF [8–10]. It competitively inhibits binding of IL-1β to its primary cell-surface signaling receptor, IL-1RI, in 1:1 stoichiometry [11,12]. When investigators compared concentrations of this important inflammatory cytokine with its antagonist, a ratio of IL-1β:IL-1ra of 1 was found in BALF from normal volunteers, whereas, the average molar ratio was 10:1 in BALF from patients with persistent ARDS, suggesting an important role for IL-1β in maintaining a persistent inflammatory state in the lungs of patients with ARDS [8]. However, absolute values of IL-1β concentrations in BALF from ARDS patients can depend on the immunoassay selected [6–10], thus complicating the interpretation of the ratio of IL-1β:IL-1ra. For example, using different immunoassay reagents, Park et al. found an average IL-1β:IL-1ra ratio in ARDS BALF of 1:10. Additionally complicating the picture, soluble IL-1 receptor-II (sIL-1RII) is also detectable in ARDS BALF [9] and can bind and enhance the inhibitory activity of IL-1ra [13]. Also, under inflammatory conditions, IL-1RI can be shed and maintain its ability to bind IL-1β, thus hindering the IL-1β-inhibitory activity of IL-1ra [13]. Finally, successful signal transduction by the IL-1 ligand-receptor complex requires binding to a third protein, the soluble IL-1 receptor accessory protein (sIL-1RacP) [14,15]. This complexity makes mathematical predictions of the net biological activity difficult when individual cytokine values are compared, and underscores the importance of directly measuring net biological activity. When IL-1β biological activity is measured in BALF or edema fluid from patients with ARDS, there is net pro-inflammatory activity [9,16,17]. Supporting these observations several investigators have recently characterized the biological activity of IL-1β in ARDS edema fluids using microarray analysis [18] or in an in vitro epithelial cell model of wound repair [19,20]. These studies suggest a possible role of IL-1β in both the fibroproliferative and epithelial repair processes. Despite the complexities of the IL-1β system, in clinical fluids there are strong relationships between IL-1β, IL-1β:sIL-1RII, IL-1β:IL-1ra and clinical lung injury severity and outcome in patients with ARDS [9]. These clinical studies indicate that the interplay between IL-1β and its family of counter-regulatory ligands and receptors probably have an important role in the early pathogenesis of ALI and ARDS.

2.2. Tumor necrosis factor-α (TNF-α) and its counter-regulatory soluble receptors

Similar to IL-1β, TNF-α is also an important pro-inflammatory cytokine that can stimulate production of a host of other cytokines [21–23]. TNF-α has been measured in ARDS BALF and is elevated, although measured values do not predict clinical outcome [6,9,24,25]. Several studies have examined soluble TNF receptors (sTNFR) in sepsis [26–29] including a clinical trial [30]. Recently, we have reported the TNF-α, TNFR1 and TNFR2 concentrations in BALF from
patients before and after the onset of ARDS, and found that TNF-α biological activity, measured in the L-929 assay, increases over the first week of ARDS [9]. There are direct relationships between the molar ratio of TNF-α/sTNFR in BALF and severity of illness (lung compliance and severity of hypoxemia) [9]. These clinical relationships further underscore the importance of measuring TNF-α biological activity in the lungs of patients with ARDS to help determine its relevance in pathogenesis. Several studies have shown that by comparison, the biological activity of TNF-α in lung fluids from patients with ALI or ARDS is considerably less than IL-1β [16,17,19].

2.4. Macrophage migration inhibitory factor (MIF)

The biology of this pro-inflammatory cytokine has been reviewed [39]. Historically, this factor was first characterized as a nondialyzable protein which inhibited the migration of porcine peritoneal exudate macrophages in vitro [40,41], and activated the function of macrophages [42,43]. In the lung MIF is produced by alveolar macrophages and bronchial epithelial cells [44,45]. MIF and TNF-α stimulate each other’s production [39,46,47]. MIF not only potentiates the effects of endotoxin and gram positive bacterial products [47,48], but it is an essential regulator of macrophage responses to endotoxin through its regulatory effect on Toll-like receptor-4 expression [49]. By suppressing activation-induced p53-dependent apoptosis, MIF also sustains macrophage survival and function [50]. Moreover, MIF production is stimulated by corticosteroids, and MIF antagonizes the effects of corticosteroids [39]. Denny et al. have identified the presence of MIF in BALF from patients with early stage ARDS [44]. They found that MIF overrides, in a concentration-related fashion, the anti-inflammatory effects of glucocorticoids. Its role as an inflammatory mediator and its complex interplay with glucocorticoids is of particular relevance to recent and ongoing trials of glucocorticoids in sepsis and ARDS.

2.5. HMG-1

High-mobility group (HMG)-1 protein is known as a DNA-binding protein that facilitates gene transcription, and regulates the activity of steroid hormone receptors. Recently, Tracey and coworkers reported that HMG-1 was also a late mediator of delayed endotoxin lethality by activating downstream cytokine release [51]. HMG-1 given intratracheally to animals produces an acute inflammatory injury to the lungs [52]. HMG-1 induced an neutrophilic inflammatory lung edema and local production of IL-1β, TNF-α, and the chemokine macrophage-inflammatory protein (MIP)-2. Endotoxin-induced lung inflammation was blunted by the administration of anti-HMG-1 antibodies, suggesting a role for the new mediator in lung injury to bacterial products [52]. Interestingly, Sappington et al. have reported that HMG-1 induces an increase in the permeability of the gut barrier, a phenomenon that is dependent on the production by intestinal cells of nitric oxide and peroxynitrite [53]. Whether HMG-1 participates in the increased permeability of the alveolar capillary barrier, a hallmark of ARDS, remains to be determined.

3. Chemotactic cytokines implicated in ARDS and the inflammatory cells they recruit

3.1. Neutrophils (PMN)

Several bronchoalveolar lavage studies in patients with ARDS identify the acute inflammatory cell populations, their evolution over time, and their importance with respect to clinical outcomes [54–59]. Neutrophils (PMN) are abundant in BALF from patients with ARDS, and PMN products in BALF correlate with the physiological abnormalities that occur [56,60]. Moreover, persistence of high numbers of PMN in BALF after the first week of ARDS is associated with mortality, particularly in patients with sepsis [59]. Although it is clear that lung cellular injury and clinical ARDS can occur in neutropenic patients from ionizing radiation or anti-metabolites [61,62], it is also true that after the initial lung injury, lung dysfunction can acutely worsen during the resolution of neutropenia as PMN are recruited to the injured lungs [63,64]. Several studies have found that circulating PMN from patients with ARDS have enhanced responses to activating stimuli [65,66], although there is controversy on this issue [57]. PMN products are abundant in the BALF of patients with ARDS [56,67–69], but by contrast, when PMN are recruited into the normal human lung simply by instilling the chemoattractant LTβ4, PMN can migrate without causing injury to the endothelial or epithelial barriers of the lung [70]. However, in several experimental
Table 1 Animal models determining the contribution of PMN to lung injury

<table>
<thead>
<tr>
<th>Authors</th>
<th>Model</th>
<th>Animal</th>
<th>Method to determine PMN role</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ismail et al.</td>
<td>PMA-stimulated PMN infusion</td>
<td>Isolated, perfused rat lungs</td>
<td>Anti-Mo1 blocking mAb</td>
<td>1987</td>
<td>[160]</td>
</tr>
<tr>
<td>Velder et al.</td>
<td>Hemorrhagic shock and reperfusion</td>
<td>Rabbits</td>
<td>Anti-CD18 blocking mAb</td>
<td>1988</td>
<td>[161]</td>
</tr>
<tr>
<td>Guice et al.</td>
<td>Lung injury associated with cerulein-induced acute pancreatitis</td>
<td>Rats</td>
<td>Anti-PMN antibody</td>
<td>1989</td>
<td>[162]</td>
</tr>
<tr>
<td>Sekiya et al.</td>
<td>Zymosan or BCG-induced inflammatory edema</td>
<td>Rats</td>
<td>Anti-PMN antibody</td>
<td>1990</td>
<td>[163]</td>
</tr>
<tr>
<td>Lo et al.</td>
<td>TNF, PMA infusion</td>
<td>Isolated, perfused guinea pig lungs</td>
<td>Anti-CD18 or Anti-ICAM-1 blocking mAb</td>
<td>1992</td>
<td>[164]</td>
</tr>
<tr>
<td>Windsor et al.</td>
<td>IV pseudomonas infusion</td>
<td>Pigs</td>
<td>Anti-TNF to prevent CD-18 upregulation by PMN</td>
<td>1993</td>
<td>[165]</td>
</tr>
<tr>
<td>Sekido et al.</td>
<td>Lung reperfusion injury</td>
<td>Rabbits</td>
<td>Anti-IL-8 antibody</td>
<td>1993</td>
<td>[166]</td>
</tr>
<tr>
<td>Inoue et al.</td>
<td>Lung injury associated with surgically-induced necrotizing pancreatitis</td>
<td>Rats</td>
<td>Anti-PMN antibody</td>
<td>1993</td>
<td>[167]</td>
</tr>
<tr>
<td>Folkesson et al.</td>
<td>Acid aspiration lung injury</td>
<td>Rats</td>
<td>Anti-IL-8 antibody</td>
<td>1995</td>
<td>[90]</td>
</tr>
<tr>
<td>Yokos et al.</td>
<td>Endotoxemia following priming with endotoxical heat-killed Step</td>
<td>Rats</td>
<td>Anti-IL-8 antibody</td>
<td>1997</td>
<td>[87]</td>
</tr>
<tr>
<td>Mukada et al.</td>
<td>Endotoxemia following priming with endotoxical heat-killed Step</td>
<td>Rats</td>
<td>Anti-IL-8 antibody</td>
<td>1998</td>
<td>[168]</td>
</tr>
<tr>
<td>Folsz et al.</td>
<td>Hyperoxic lung injury</td>
<td>Mice</td>
<td>Anti-PMN antibody</td>
<td>1999</td>
<td>[169]</td>
</tr>
<tr>
<td>Beloferi et al.</td>
<td>Ventilator-induced lung injury</td>
<td>Mice</td>
<td>Anti-CXCR2 antibody</td>
<td>2002</td>
<td>[153]</td>
</tr>
</tbody>
</table>

PMN: polymorphonuclear neutrophil; PMA: phorbol 12-myristate 13-acetate; BCG: Bacillus Calmette-Guerin; ICAM: intercellular adhesion molecule.

models, depletion of neutrophils or blockade of their recruitment attenuates lung injury from clinically relevant insults (Table 1). Thus, although PMN are neither necessary nor sufficient to cause lung injury in humans, PMN probably contribute to the pathogenesis. It seems likely that interactions between migrating PMN, pro-inflammatory cytokines in the microvascular and tissue environments, and activated or damaged endothelial and/or epithelial cells act in concert to produce the lung dysfunction that is seen in ARDS.

3.2. Neutrophil chemoattractants

PMN must be recruited from the blood stream in order to gain access to the alveolar space and airways. A variety of chemoattractants exist, and several of the most potent PMN chemoattractants have been studied in BALF from patients with ARDS. Early studies to identify the specific mediators responsible for PMN recruitment in the lungs of ARDS patients have excluded some potential candidate molecules. Parsons and coworkers demonstrated the presence of PMN chemoattractive activity in ARDS BALF, and showed that it was not attributable to the components of the complement cascade [60,71]. Similarly, the chemotactically active cleavage product of platelet basic protein, neutrophil activating peptide-2 (NAP-2) is not present in ARDS BALF [72].

3.2.1. The CXC chemokines

Over the last 15 years, a family of cytokines which are chemoattractive for PMN, known as the CXC chemokines, has been described and characterized. An important sub-class known as the ELR+ CXC chemokines contain a glutamyl-leucyl-arginine (ELR) motif that is critical to their neutrophil binding and chemoattractive functions [73-74]. The ELR+ CXC chemokines, IL-8, ENA-78, and GRO-α, GRO-β, and GRO-γ and granulocyte chemoattractant peptide (GCP)-2, are all produced by human alveolar macrophages [75,76], and IL-8, ENA-78, and GRO-α are present in biologically significant concentrations in BALF from patients with ARDS (Fig. 1) [8,77]. Their concentrations correlate with PMN studies (Table 2) [8,77-79]. As long as patients are sick enough to require continued mechanical ventilation, the concentrations of these CXC chemokines are logarithmically elevated above normal [8]. Although GRO-α and ENA-78 concentrations are higher than IL-8 concentrations, IL-8 is the predominant chemoattractant in ARDS BALF [8]. Specific antibodies to IL-8 substantially
block the chemotactic activity of BALF from patients with ARDS [8,78].

Adding another level of complexity, there are two CXC chemokine receptors on human PMN, CXCR1 and CXCR2 [80,81]. IL-8 and GCP-2 can bind to either receptor with high affinity, whereas ENA-78, GRO-α, GRO-β, and GRO-γ bind with high affinity only to CXCR2 and only bind with low affinity to CXCR1 (Fig. 2) [82–84]. On ligand stimulation both receptors are rapidly internalized. However, CXCR1 is rapidly re-expressed (within minutes), whereas the re-expression of CXCR2 is considerably slower [85]. In the presence of a systemic inflammatory process such as severe sepsis, CXCR2 is tonically down-regulated and the function of only one receptor predominates, CXCR1 [86]. Thus, of the multiple neutrophil chemotactic factors produced in humans, there appears to be a small group that is particularly relevant to patients with ARDS, with IL-8 and its cognate receptor, CXCR1, being the dominant receptor–ligand pair.

Several studies in animal models of lung inflammation and injury provide proof of concept for IL-8 as the dominant PMN chemoattractant. Monoclonal antibodies to IL-8 significantly reduce lung injury and PMN migration in endotoxemia models [87–89], and in an acid aspiration model [90]. These data argue strongly for the role of IL-8 in the acute inflammatory response to cellular activation or injury.

3.2.2. The Duffy antigen chemokine binding protein

There are also endogenous molecules that sequester or modulate the activity of chemokines. The Duffy antigen is a minor blood group antigen and the receptor for Plasmodium vivax required for erythrocytes invasion [91]. The Duffy antigen binds ELR+ CXC chemokines and several CC chemokines with high affinity [92–95]. The Duffy antigen on erythrocytes is postulated to sequester chemokines in the circulation because IL-8 bound to erythrocyte Duffy antigen does not stimulate neighboring neutrophils [92]. Teleologically, this process would help contain these chemotactic cytokines at organ sites of inflammation, prevent systemic activation of leukocytes, and help maintain a concentration gradient between the blood stream and an inflamed organ.

### Table 2

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>0.41</td>
<td>0.011</td>
<td>0.54</td>
<td>0.001</td>
</tr>
<tr>
<td>GCP-2</td>
<td>0.20</td>
<td>0.1</td>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>ENA-78</td>
<td>0.29</td>
<td>0.00</td>
<td>0.35</td>
<td>0.009</td>
</tr>
<tr>
<td>GRO-α</td>
<td>0.08</td>
<td>0.6</td>
<td>0.06</td>
<td>0.7</td>
</tr>
<tr>
<td>Dependent variable</td>
<td>percentage of PMN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spearman correlation coefficients.</td>
<td>*P ≤ 0.05, **P ≤ 0.01.</td>
<td></td>
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<td></td>
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</tbody>
</table>
However, Duffy antigen’s role in inflammation is more complex. It is also expressed on high endothelial venules of lymphoid tissue [96], post-capillary venular endothelial cells [97], and capillaries of various organs [98,99], precisely where leukocytes emigrate. Endothelial expression of Duffy antigen is preserved even in individuals who do not express Duffy antigen on their erythrocytes [100]. Furthermore, Duffy antigen expression is up-regulated on endothelial cells at sites of inflammation [99], with its up-regulation corresponding to areas of leukocyte accumulation [101–103]. The site specific and inductive expression of endothelial Duffy antigen implicates its relevance in inflammation.

There is evidence to support a conceptually distinct role for Duffy antigen on endothelial cells from Duffy antigen on erythrocytes. While Duffy antigen serves as a sink for chemokines on erythrocytes, Duffy antigen may participate in the transport of chemokines across endothelial cells and, therefore, contribute in regulating inflammatory cell recruitment to tissue sites of inflammation [100,104–106]. Duffy antigen facilitates chemokine movement across endothelial cells in vitro [104]. Venular endothelial cells in rabbit skin bind, internalize, and transcytose $^{125}$I-IL-8 in vesicles from the albuminal to luminal surface where it is subsequently presented to circulating leukocytes [105]. Although, in these early studies the molecule(s) participating in binding, internalization, and transport of chemokine remained unidentified, the characteristics of binding IL-8 and RANTES but not MIP-1α were identical to those of the Duffy antigen [105]. Others have shown that Duffy antigen is expressed on both the apical and basolateral membrane of endothelial cells, localizes to calveolae [98], and that internalization of chemokine by Duffy antigen does not result in degradation of chemokine [100]. Finally, Duffy expression enhances neutrophil migration across endothelial monolayers in vitro and in vivo [104]. Taken together this body of literature indicates that Duffy antigen actively participates in chemokine-mediated inflammation, acting as a shuttling molecule for chemokines across the endothelium. Duffy antigen is a functionally relevant molecule in the lungs, although its contribution to the neutrophilic component of ALI and ARDS remains to be elucidated.

3.2.3. $\alpha$-2-Macroglobulin

$\alpha$-2-Macroglobulin ($\alpha$-2-M) can also play an important role in modulation of the biological activity of chemokines in the lungs of patients with ARDS. $\alpha$-2-M is a carrier protein in plasma for a variety of cytokines including IL-8 [107–109], TNF-α [110,111], IL-1β [112], IL-6 [113], transforming growth factor-β [114–121], and platelet-derived growth factor [122,123]. The molecular interactions between these cytokines and $\alpha$-2-M may differ with each cytokine and may be covalent or ionic [110–113,124]. Kurdowska et al. have extensively studied the interactions of $\alpha$-2-M with the CXC chemokine, IL-8, and measured its concentrations in lung fluids from patients with ARDS [107–109,125,126]. IL-8 binds with a $K_d$ of 30 nM, similar to IL-8’s affinity for its receptors on neutrophils, CXCR1 and CXCR2, and other chemokines such as GRO-α compete with IL-8 for binding to $\alpha$-2-M [109]. The majority of $\alpha$-2-M in BALF from patients with ARDS is complexed to neutrophil elastase [127], and although <1% of $\alpha$-2-M is associated with IL-8, 2–60% of IL-8 in lung fluids from patients with ARDS is bound to $\alpha$-2-M [109]. Further, binding by $\alpha$-2-M impairs recognition of IL-8 in standard ELISA assays [126]. Although binding of IL-8 to $\alpha$-2-M protects it from proteolytic degradation, preserving its chemotactic activity, this binding could also prevent IL-8 from binding to matrix proteins, thus facilitating its clearance by macrophages via the $\alpha$-2-M receptor [125,126] similar to other cytokines [128,129]. In a rabbit model of lung inflammation, endotracheal instillation of IL-8 was compared with instillation of IL-8 complexed to rabbit $\alpha$-macroglobulin. Although IL-8-$\alpha$M complexes retained chemotactic activity in vitro, their in vivo activity was markedly diminished, presumably as a result of enhanced clearance [108]. Thus, depending on the surrounding milieu...
5. Mechanical ventilator-induced lung injury (VILI)

Dunn and Pugin developed an innovative plastic lung model to study the effects of mechanical stress on lung cells in vitro [140]. Primary human lung cells or cell lines were cultured on collagen I-coated silastic membranes that formed the bottom of wells of a 12-well plate; a Plexiglas lid was bolted down in an airtight manner, and connected to a mechanical ventilator. They examined primary alveolar macrophages, monocyte-derived macrophages, THP-1 cells, type II-like A549 and bronchial ECV and EA-hy926 cell lines, and primary lung fibroblasts. They found that IL-8 and matrix metalloproteinase-9 production and NF-κB translocation was most pronounced in alveolar macrophages simply as a result of ventilation, although under certain conditions A549 cells will upregulate IL-8 production with stretch alone [141]. However, when cells were simultaneously stimulated with endotoxin, the combined effect resulted in the production of significantly greater amounts of TNF-α and IL-6 than endotoxin-stimulated cells without ventilation (Fig. 3) [140]. Further, in animal models of VILI, anti-TNF-α antibody improved oxygenation and respiratory compliance, decreased leukocyte accumulation and histologic inflammation, and IL-1ra reduced lung albumin, elastase and PMN concentrations [142,143]. Interestingly the intracellular signaling molecules that are ‘turned on’ by cyclic stretch are the same as those utilized by inflammatory cytokines and bacterial products. These include enzymes of the NF-κB pathway, and stress kinases of the mitogen-activated protein kinase (MAPK) family, in particular p38 [144]. The synergy between inflammatory stimuli and ventilator-induced lung cell stretching observed in animals and in vitro models could be explained by both an increased transcription of target inflammatory genes due to a cooperation between transcription factors, and by a stabilization of messenger RNAs from inflammatory gene products [145]. A better understanding of pathways and mediators implicated in the lung cell activation in response to cell overstretching may lead to specific therapies aimed at reducing ventilator-induced lung injury.

PMN are important effector cells mediating tissue injury in VILI [146]. Several studies indicate that overventilation with high tidal volumes or positive end-expiratory pressure results in the accumulation of PMN, particularly in the vasculature [147–149]. Neutrophil depletion with nitrogen mustard was protective in a ventilated, surfactant-depletion model of lung injury in rabbits [150]. Injurious ventilatory
strategies can result in neutrophil recruitment to the lung [151].

Ranieri and coworkers performed a randomized controlled trial in 44 patients with ARDS to test the hypothesis that mechanical ventilation induces a pulmonary and systemic cytokine response that can be minimized with a protective strategy of mechanical ventilation [152]. They found that patients who received the lung-protective strategy had reductions in BALF concentrations of PMN, TNF-α, IL-1β, sTNFRI, and IL-8, and in plasma and BALF concentrations of IL-6, sTNFRII, and IL-1ra. This clinical study demonstrated that mechanical ventilation can induce an inflammatory cytokine response that may be attenuated by a strategy to minimize overdistention and recruitment/derecruitment of the lung.

Recently, Belperio et al. used a mouse model of ventilator-induced lung injury, mechanical ventilation to define the role of ELR+ CXC chemokine production and neutrophil recruitment in the lungs, as well as lung injury [153]. These investigators compared high- and low-stretch ventilation in the presence and absence of blocking antibody to CXCR2 and in CXCR2 knock-out mice. Because mice lack a homologue of IL-8 and its receptor, CXCR1, there are limitations on the specific relevance of these studies, but because no CXCR1 blocking reagents exist, such studies cannot be done in higher mammals. These investigators demonstrated that this model of VILI is characterized by the presence of neutrophils, ELR+ CXC chemokines, and histologically defined lung injury. Blockade or genetic deletion of CXCR2 resulted in substantially reduced PMN accumulation and lung injury. These studies demonstrate an important role for ELR+ CXC chemokines in the pathogenesis of VILI. They suggest that VILI and ALI/ARDS share a common underlying process of chemokine-mediated PMN recruitment, and that ventilator-induced lung injury may result in the persistence and severity of ALI/ARDS in some individuals by potentiating this shared pathologic process [153].

After a clinical controversy in the literature from a series of smaller studies [152,154–156], a phase-III trial by the NHLBI-sponsored ARDS Network demonstrated that reduction of ventilator tidal volumes resulted in a 22% reduction in mortality in patients with ARDS [157]. This study highlights the concept that VILI contributes to and perpetuates ALI and ARDS, and translates into clinical practice more than a decade of research focused on animal models and basic mechanisms of ventilator-induced lung injury [158].

6. Conclusions

Although a variety of injurious events and mediators can lead to ALI and ARDS depending on the clinical risk group, cytokine-mediated inflammation is shared in all of these patients. This inflammatory process contributes to
the severe dysfunction of the lungs and to the injury of resident epithelial and endothelial cells. Understanding the basic cellular and mechanisms and the balance of pro- and anti-inflammatory cytokines and chemokines in the lungs of patients with ARDS is an important step toward understanding the pathogenesis of this devastating clinical syndrome, and in designing effective strategies to minimize the clinical severity and mortality of ALI and ARDS.

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