Alveolar Epithelium in Host Defence: Cytokine Production

M. Liu

Infection of the respiratory system is a frequent cause of morbidity and mortality world-wide [1]. Respiratory tract infection, including croup, tracheobronchitis, bronchiolitis and pneumonia, are significant clinical problems. The increasing number of multidrug-resistant microbes has made the treatment of these infections much more difficult [1]. To further improve therapies for respiratory infection, we need to learn more about the host defence in the lung.

Host defence in the lung

The respiratory tract is accessible for potentially infective micro-organisms and noxious substances in the inhaled air. Thus, lung defence mechanisms are crucial for the effective removal of microbes and other debris from the conducting airways and alveoli [2, 3]. Host defence in the respiratory system includes three major components: *mechanical* (such as cough and mucociliary clearance), *humoral* (such as secretory immunoglobulins and complement) and *cellular* (such as alveolar macrophages, lymphocytes, and neutrophils) [4]. Recent studies have shown that the alveolar epithelium is also an important component in the host defence. It functions as a barrier to prevent the invasion of pathogens. Type II pneumocytes produce lung surfactant that can enhance the function of immune cells in the alveoli. Surfactant proteins are also important mediators of host defence. In addition, lung alveolar epithelial cells may also function as sensors for the invasion of micro-organisms and other noxious agents by producing cytokines and chemokines.

Alveolar epithelial cells as a source of cytokines and chemokines

Cytokines are extracellular signalling proteins secreted by cells, which have the ability to modify the behaviour of other adjacent cells. Cytokines are generally

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divided into pro- and anti-inflammatory mediators, and are important mediators of both innate and acquired immune defences in the lung [1]. Chemokines are chemotactic cytokines for leukocyte recruitment and activation at the sites of infection or tissue injury [2, 3]. The role of chemokines in lung host defence has been a subject of several reviews [1, 5]. They are also important mediators in acute lung inflammation [2, 3, 5, 6]. Neutrophil infiltration in the alveolar space is mainly mediated by C-X-C chemokines such as interleukin-8 (IL-8) and its rodent homologue, macrophage inflammatory protein-2 (MIP-2) [6, 7]. The C-C chemokine family, such as monocyte chemoattractant proteins (MCPs) and RANTES, activates and/or is chemotactic for macrophages, monocytes and lymphocytes.

Both *in vitro* and *in vivo* data suggest that alveolar epithelial cells can produce cytokines such as IL-6, IL-3, interferon γ, granulocyte monocyte colony-stimulating factor and tumour necrosis factor-α (TNFα) [8]. Alveolar epithelial cells can also produce a variety of chemokines such as IL-8 [9], MIP-2 [10], MCP-1 [11, 12], and RANTES [13].

During foetal lung development, the potential airway and alveolar space are filled with amniotic fluid. There are few macrophages and other immune cells in the alveoli. It is unknown how these immune cells are initially recruited and become the residents in the alveolar space after birth. Cytokines and chemokines produced by *pulmonary epithelial cells* may initiate the establishment of host defence. This is very important for newborns immediately after the birth and for children in their early childhood. Inappropriate recruitment and activation of immune cells in airway and alveoli may contribute to recurrent infections in pediatric lungs.

Cytokine and acute lung injury

The recruited inflammatory cells help remove invading organisms through phagocytic clearance. However, in addition to their defensive role, these *immune cells* are also involved in acute and chronic injury of the lung. The cytotoxic and proteolytic materials, such as neutrophil elastase, contained in these immune cells may induce lesional changes. Cytokines, especially pro-inflammatory cytokines and some chemokines, also play an important role in acute lung injury, seen in many clinical situations: severe respiratory infection, sepsis, shock, acute respiratory distress syndrome (ARDS), mechanical ventilation-induced lung injury, and ischaemia-reperfusion injury of lung transplants. Immunotherapies have been developed to inhibit pro-inflammatory cytokines [14], such as TNFα and IL-1 [15, 16], and to consequently inhibit

the acute inflammatory response. One of the problems with this approach is that it may disable host defence as well. To overcome the conflict between host defence and acute inflammatory injury in the lung, we need to understand how cytokine production from alveolar epithelial cells and other cellular sources is regulated, and to explore new strategies to control the production of cytokines and chemokines.

The interaction of leukocytes and pulmonary parenchymal cells, including alveolar epithelial cells, via cytokine signalling mediates innate and acquired immunity in lung antimicrobial host defence [1, 17]. Enhanced pro-inflammatory cytokine expression has been attempted as new therapies for lung infection, but the concern is that this strategy may exacerbate acute lung injury.

Regulation of cytokine production

Investigations with macrophages, monocytes, neutrophils, and other immune and non-immune cells have yielded fruitful results regarding the regulation of cytokine production. Given space constraints, we cannot review these exciting studies; instead, several examples are given to illustrate the complexity of regulatory mechanisms of cytokine production at various levels. Recently, many cytokine and chemokine receptors have been characterized at the molecular and cellular level [18, 19]. Soluble TNF receptor [20] and naturally expressed IL-1 antagonist have been recognized as potent inhibitors to block the function of these pro-inflammatory cytokines [15, 16]. These molecules have been used in pre-clinical and clinical trials [14]. There have been exciting discoveries elucidating signal transduction pathways initiated by LPS and cytokines leading to nuclear events. The importance of protein phosphorylation, especially tyrosine phosphorylation [21], in cytokine production has been reported. Stress activated protein kinase (SAPK, also called JNK) [22], and p38MAPK [23] have been demonstrated to specifically mediate signals initiated by cytokines and other inflammatory mediators. Pharmacological agents targeting these pathways have been developed for clinical applications. The role of nuclear factor-KappaB (NFκB) as a transcriptional factor in controlling cytokine gene expression has been reported from many cell types under different experimental conditions for several cytokines [24]. In macrophages and other immune cells, cytokine synthesis can be triggered rapidly, and this apparently involves predominantly translation rather than gene transcription [25]. Detailed molecular studies have revealed A-U rich elements in the 3'untranslated region of many cytokine mRNAs [26], which play an important role in controlling cytokine protein synthesis. The intracellular transport and

secretion of cytokines is another important regulatory step for cytokine production.

Compared to the inflammatory cells, our knowledge on the regulation of cytokine production from alveolar epithelial cells is much less. In this article, we will use IL-8 and MIP-2 as examples, to discuss the induction of cytokine, the interaction of various cytokines as a network, the transcriptional regulation of cytokine gene expression, and the role of cytoskeleton in regulating cytokine secretion, to elucidate the complexity of cytokine production from alveolar epithelial cells.

Induction of cytokine from alveolar epithelial cells

Production of cytokines from alveolar epithelial cells is an important response towards a variety of stimuli from environmental factors, bacteria, viruses and other stresses. Using IL-8 as an example, many factors can directly induce this cytokine from cultured human lung epithelial cells (Table 1). IL-8 is one of the best known C-X-C chemokines to attract and activate polymorphonuclear granulocytes (PMNs) [6]. The biological activities of IL-8 include attracting neutrophils, activating surface adhesion molecules, inducing release of storage enzymes, and stimulating production of reactive oxygen metabolites [27]. IL-8 has been found to be involved in several inflammatory reaction-related diseases in the lung, for example, idiopathic pulmonary fibrosis [28], adult respiratory

Table 1. Induction of IL-8 from lung alveolar epithelial cells

Stimulus
Environmental factors and toxins
Environmental particulate, Fibrous particles, Asbestos, Silica, Dust from waster handling facilities, Coal fly ash, House dust, Smoke extract, Ragweed, Fungal allergens, Ozone
Viruses
Respiratory syncytial virus, Influenza virus, Adenovirus, Rhinovirus
Bacteria and products
Gram positive bacteria, *Mycobacterium tuberculosis*, Thermophilic bacteria, Lipopolysaccharides, *Burkholderia cepacia* products, *Pneumococcal* protein, Proteases from *Aspergillus fumigatus*, *Pneumocystis carinii* major surface glycoprotein, *Pseudomonas* nitrite reductase
Cytokines and inflammatory mediators
TNFα, IL-1α, IL-1ß, Th 2 cytokines, Neutrophil serine proteinases, Defensins, Bradykinin
Other stress
Hyperoxia, Anoxia-hyperoxia, Mechanical stretch

distress syndrome [29], and empyema [30]. A monoclonal antibody against IL-8 prevented ischaemia-reperfusion induced lung injury in a rabbit model [31].

Various environmental particulate, fibrous particles, such as asbertos and silica induced IL-8 production, as well as house dust, dust from waster handling facilities, coal fly ash and smoke extract. Fungal allergens, ragweed and other allergens in the air also stimulate alveolar epithelial cells to produce IL-8. Various viruses, such as respiratory synthial virus (RSV), adenovirus, influenza virus and rhinovirus, induced IL-8 production from alveolar epithelial cells. Replication-deficient adenoviral vectors-induced cytokine production from alveolar epithelial cells has drawn increasing attention in gene therapy-related investigations. During bacterial infection, both bacteria (such as Gram positive bacteria and *Mycobacterium tuberculosis*), and their products (lipopolysaccharides, *Burkholderia cepacia* products, pneumococcal protein, proteases from *Aspergillus fumigatus*, *Pneumocystis carinii* major surface glycoprotein, and *Pseudomonas* nitrite reductase) induced IL-8 production. Recently it has been demonstrated that primary cultured alveolar epithelial cells isolated from human lung tissues produced IL-8 [32]. Therefore, IL-8, as well as many other cytokines and chemokines, are important messengers for the host defence in the alveolar spaces, produced by alveolar epithelial cells.

IL-8 has structural and biological similarities with MIP-2, which could represent the rodent homologue to IL-8 [6]. MIP-2 was initially purified from a mouse macrophage cell line stimulated with endotoxin [33]. Rat MIP-2 was recently cloned and expressed as a 7.9-kDa peptide [34-36] that showed dose-dependent chemotactic activity for PMNs [34]. This activity of MIP-2 has been further demonstrated in the lung from several animal models with a variety of pathogens. For examples, increased MIP-2 mRNA and/or protein was observed in the lung, in response to the intra-tracheal instillation of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, LPS and α-quartz. Intra-peritoneal administration of LPS resulted in an increase in neutrophil influx into the lung, which was at least in part due to increased levels of MIP-2 [37]. LPS induced MIP-2 production from lung explants [38]. LPS also induced MIP-2 production from primary cultured rat lung alveolar epithelial cells, which was regulated at both the transcriptional and post-transcriptional levels [10].

MIP-2 is also involved in lung injury, such as IgG immune complex-induced injury. When MIP-2 transgene was delivered into the lung with a replication-defective adenoviral vector through the intratracheal instillation, significant increase in neutrophils and alveolar macrophages was found from the lung lavage fluid [39]. Instillation of recombinant MIP-2 into the alveolar space of rats induced profound neutrophil localization both in the vascular and alveolar space [37]. In these studies, up-regulation of MIP-2 was associated with acute

lung injury. Intraperitoneal instillation of anti-MIP-2 antiserum [40] or intrapulmonary instillation of anti-MIP-2 antibodies [39, 40] decreased neutrophil influx in the lung and attenuated lung injury.

Interactions between cytokines

Micro-organisms and other pathogen-induced IL-8 and MIP-2 production can be mediated through other cytokines. TNF-α is one of the most important pro-inflammatory cytokines in the cytokine network. It is a very important mediator in host defence and in mediating acute inflammatory reactions in the lung and many other organ systems. Recent studies have demonstrated that, in response to LPS-stimulation, primary cultured rat alveolar epithelial cells produced TNF-α in a dose- and time-dependent manner [8]. TNF-α can induce IL-8 from human lung A549 cells [41-43], or from primary cultured human alveolar epithelial cell [32]. Furthermore, recombinant TNF-α induced MIP-2 production from primary cultured rat lung alveolar epithelial cells [10]. A time-delay between TNF-α and MIP-2 at both mRNA and protein levels was noted upon LPS-stimulation [8, 10]. When an antisense oligonucleotide against rat TNF-α was delivered to alveolar epithelial cells, it inhibited not only TNF-α but also MIP-2 release in a dose-dependent fashion. The inhibitory effects on these two molecules were highly correlated [10]. Neutralizing anti-TNF-α antibody also inhibited MIP-2 production [10]. These results suggested that TNF-α released from these cells might function as an alert signal to trigger the production of chemokines such as MIP-2 in rat and IL-8 in human lungs. The latter may recruit neutrophils to the alveoli where the bacteria or other pathogens have invaded. This auto-regulation of the cytokine network may be important for host defence and could be augmented during acute lung injury [10].

IL-1 is another cytokine with potent proinflammatory effects. IL-1β induced IL-8 from human lung alveolar epithelial cells [44]. IL-1α-induced neutrophil migration across A549 cell layer is partially mediated through IL-8 [45]. Incubation with neutralizing antibodies against IL-1α, IL-1β and TNF-α showed that IL-1α was the predominant soluble mediator that enhanced the mRNA expression and synthesis of IL-8 induced by RSV [46]. IL-1 receptor antagonist inhibited IL-8 expression in A549 cells infected *in vitro* with a replication-deficient recombinant adenovirus vector [47]. These results suggest that IL-1α or IL-1β could also function as autocrine regulators to stimulate IL-8 production from alveolar epithelial cells. It is worthwhile to note that it has been observed that TNF-α increased both IL-8 mRNA expression and protein production in isolated human alveolar type II epithelial cells, whereas IL-1β slightly increased IL-8 release but did not change its mRNA expression [32].

The results from cell lines such as A549 cells need to be interpreted with caution.

Transcriptional regulation of cytokine gene expression in alveolar epithelial cells

The gene expression of cytokine is regulated at the transcriptional level that is mediated via intracellular signal transduction pathways. Activation of nuclear factor NF-κB is one of the most important regulatory mechanism for IL-8 gene expression induced by RSV, rhinovirus, and human immunodeficiency virus type 1 protein R in human lung epithelial cells. NF-κB also plays an essential role in regulation of IL-8 gene expression induced by nitrite reductase from *Pseudomonas aeruginosa* in respiratory epithelial cells [48]. Asbestos fibers also stimulated DNA binding activity to the regulatory elements in the IL-8 promoter, binding sites of NF-κB- and NF-IL-6-like transcription factors [49]. Another important transciptional activation is through Activator protein-1 (AP-1), which consisted of Jun and Fos proteins. Although both H_2O_2 and TNFα can induced IL-8 production in lung epithelial cells, they induce differential binding of the redox-responsive transcription factors to the IL-8 promoter. H_2O_2 activates AP-1 but not NF-κB in A549 cells, whereas TNFα activated both AP-1 and NF-κB [41]. TNFα-induced NF-κB activation and IL-8 release in A549 cells can be inhibited with the proteasome inhibitor MG-132, which blocks the degradation of NF-κB complex [43]. AP-1 is also the preferred transcription factor for cooperative interaction with NF-κB in RSV-induced IL-8 gene expression in airway epithelium [50].

The transcriptional activation of IL-8 is mediated through intracellular signal transduction pathways. Asbestos-inducible IL-8 secretion was suppressed by staurosporine, an inhibitor of PKC, and also by inhibitors of tyrosine kinase such as herbimycin A and genistein. The suppression effect paralleled the effect of these inhibitors on asbestos-induced DNA binding to the NF-κB - and NF-IL-6-like binding sites of the IL-8 promoter [49]. Mechanical stretch-induced activation of mitogen-activated protein kinases (MAPK), including c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK) [51]. These MAPK isoforms could be involved in the regulation of transcriptional factors, especially AP-1. IL-8 production in type II alveolar cells is associated with the activation of JNK [52]. The IL-1ß induced JNK activation is through RhoA, a small G protein, whereas H_2O_2-induced JNK activation is through phosphoinosital-3 kinase and phospholipase A2 pathway [53]. RSV infection results in activation of multiple protein kinase C (PKC) isoforms leading to activation of MAPK [54]. Activation of ERK2 by RSV in A549 cells

is linked to the production of IL-8 [55]. Further investigation of these pathways may lead to specific regulation of cytokine production from lung alveolar epithelial cells.

Role of cytoskeleton in LPS-induced cytokine secretion from alveolar epithelial cells

The cytoskeletal system of cells is composed of microfilaments, microtubules and intermediate filaments. Recent studies have shown that both microfilaments and microtubules are involved in regulating cytokine production from alveolar epithelial cells. Importantly, these effects appear to be opposite to that in inflammatory cells.

LPS suppressed macrophage phagocytosis by affecting microfilament and microtubule structures [56]. LPS induced a rapid reorganization of F-actin assembly in macrophages [57], increased stiffness and F-actin assembly in monocytes [58] and enhanced a chemotactic factor induced actin polymerization in neutrophils [59]. In contrast, LPS reduced polymerization of microfilaments in primary cultured rat alveolar epithelial cells [60, 61]. Cytochalasin D (CytoD), a microfilament-disrupting agent, blocked LPS-induced TNFα gene expression and/or protein synthesis in macrophages [62]. In contrast, CytoD enhanced LPS-induced TNFα production from rat pneumocytes. A membrane-permeable cyclodepsipeptide, jasplakinolide, can induce actin polymerization and stabilize pre-existing actin filaments [63]. When cells were treated with jasplakinolide, it inhibited LPS-induced TNFα production from rat pneumocytes, but enhanced it from macrophages [60]. The LPS-induced depolymerization of microfilaments has similar effect on LPS-induced MIP-2 production from these cells [61]. Mechanical stretch-induced cytoskeletal deformation enhanced MIP-2 secretion from primary cultured foetal rat lung cells [64].

Disassembly of actin filaments has been found from many non-immune cells that play a significant role in secretion. The cytoskeletal structure endows the cell with a very crowded cytoplasm, and the integrated organization of the cytoskeleton and membrane systems may provide an important barrier to the free diffusion of secretory vesicles [65]. During resting conditions the actin cytoskeleton, localized under the plasma membrane, may prevent secretory granules from reaching their exocytic destination. Upon stimulation, microfilaments may be disassembled or rearranged to allow secretory granules to reach the site of exocytosis [66].

Cytoskeletal elements, particularly microtubules and their associated motor proteins, are fundamental in facilitating delivery of transport intermediates between spatially segregated organelles and determine the steady-state locali-

zation of the organelles [67]. Eukaryotic cells have highly regulated membrane transport systems that mediates exchange of protein and lipid between distinct membrane-bound compartments of organelles, including the endoplasmic reticulum (ER), Golgi, transport intermediates and others. In higher eukaryotic cells, the ER and Golgi complex are spatially segregated. The ER network with branching membrane tubules extends outward along microtubules throughout the cell [68], while Golgi cisternae are clustered around microtubules near the perinuclear microtubule organizing centre. Transport intermediates arising from peripheral ER sites, thus, often travel considerable distances to reach the Golgi complex [69]. Therefore, microtubules may play an important role in the intracellular transport of cytokine molecules.

MIP-2, as well as most cytokines and chemokines, are synthesized as precursor polypeptides, containing cleavable N-terminal signal or targeting sequences for transport through the ER-Golgi pathway [70]. When cells were incubated with brefeldin A (BFA), which blocks the ER-to-Golgi transportation, LPS-induced MIP-2 production was inhibited in a dose-dependent manner [71]. Microtubules have been recognized as secretory "highways" in the cell [65]. Membranes move along microtubules in both directions between the ER and Golgi, and at the steady state, forward (ER-to-Golgi) and reversed transportation is in balance [65]. Microtubules are also involved in transportation of secretory vesicles from the Golgi to the plasma membrane [67, 69]. Using fluorescent and immunofluorescent staining and confocal microscopy, it was found that LPS reduced polymerization of microtubules [60, 71], whereas LPS increased the number, length, and stability of microtubules in mononuclear phagocytes [72]. When alveolar epithelial cells were pre-incubated with various concentrations of microtubule-disrupting agents, colchicine or nocodazole, LPS-induced-MIP-2 production was further enhanced in a dose-dependent fashion. Alternatively, when cells were stimulated with various concentrations of LPS in the presence of colchicine or nocodazole, both agents increased LPS-induced MIP-2 production over a wide range of LPS concentrations. Taxol, a microtubule-*stabilizing* agent, partially inhibited LPS-induced MIP-2 production [71].

Although both the anterograde and retrograde traffic depend upon microtubules, LPS may selectively block the retrograde transportation from the Golgi back to the ER. Microtubules are very important in maintaining the intracellular localization of the ER and Golgi complex, which are essential in determining the pathways for secretory proteins. When cells were treated with nocodazole, depolymerization of microtubules leads to redistribution of the ER and Golgi [73, 74]. LPS-induced depolymerization of microtubules, especially in the presence of nocodazole or colchicine, may change the distribution of the ER

and Golgi in alveolar epithelial cells, which may lead to a microtubule-independent secretion from the ER to plasma membrane. These two mechanisms may be both involved in LPS-induced secretion of MIP-2 as well as other cytokines from alveolar epithelial cells.

Based on the roles of the cytoskeleton in secretion, both microfilaments and microtubules may be involved in regulating cytokine transportation in peumocytes through different mechanisms. The effects of LPS on the cytoskeleton and the roles of the cytoskeleton in mediating LPS-induced TNFα production in alveolar epithelial cells are opposite to that in immune cells. Selective inhibition of cytokine production from different cell types could be beneficial. For example, ventilation-induced TNFα could be mainly from alveolar epithelial cells. If we block TNFα produced from alveolar epithelial cells, while maintaining the ability of alveolar macrophages to produce the cytokine, lung injury might be ameliorated without compromising host defence.

Conclusions

In this chapter, we described the role of alveolar epithelial cells in the host defence in the lung, as a source of cytokines. Using IL-8 and MIP-2 as examples, it can be see that cytokines can be induced by a variety of environmental factors, bacteria, viruses, and other pathogens. Cytokine production is regulated as a network via autocrine and paracrine mechanisms. The intracellular signal transduction and transcriptional regulation of cytokine production from alveolar epithelial cells are complex. The cytoskeletal system plays an important role in controlling cytokine secretion from alveolar epithelial cells. This effect seems to be opposite between alveolar epithelial cells and other immune cells, which provide an opportunity to selectively control the cytokine production from a different cellular source. This concept may have significant clinical impact to reduce acute inflammatory response, but keep the host defence response intact.

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