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Neutrophils in acute lung injury

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1. ABSTRACT

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is characterised by lung oedema due to increased permeability of the alveolar-capillary barrier and subsequent impairment of arterial oxygenation. Lung oedema and endothelial and epithelial injury are accompanied by an influx of neutrophils into the interstitium and alveolar space. Hence, the activation and recruitment of polymorphonuclear neutrophils (PMNs) are thought to play key roles in the progression of ALI/ARDS. Neutrophils, which have anti-microbial activity, are the first cells to be recruited to the site of inflammation. This review focuses on the mechanisms of neutrophil activity in patients with ALIs with respect to attachment, recruitment, adhesion, migration, activation, release of damage mediators, and apoptosis via PMNs.

2. INTRODUCTION

Acute lung injury (ALI) is a diffuse injury of alveolar or capillary endothelial cells resulting in pulmonary interstitial and alveolar oedema, followed by acute hypoxic respiratory failure caused by direct or indirect factors other than cardiac factors. ALI is characterised by reduced lung volume, decreased lung compliance, ventilation-perfusion mismatch (increased dead space), and progressive hypoxemia and respiratory distress. ALI can progress to acute respiratory distress syndrome (ARDS) (1). A haemodynamic feature of ALI/ARDS is pulmonary artery hypertension, which is one of the crucial factors promoting alveolar and interstitial oedema. ALI/ARDS is caused not only by lung disease, but also by extra-pulmonary diseases with pulmonary symptoms as major manifestations. ALI/ARDS, initiated by

pulmonary/extra-pulmonary or infected/non-infected factors, can activate inflammatory cells to release inflammatory mediators leading to other organ dysfunction, resulting in multiple organ dysfunction syndrome (2,3,4). The activation of multiple inflammatory cells can initiate excessive or uncontrolled inflammation, which is defined as systemic inflammatory response syndrome (SIRS). SIRS is an excessive stress response of the body during the process of body repair and survival. Activation of inflammatory cells and release of inflammatory mediators can injure the body as well as initiate an inflammatory cascade. Within the inflammatory cascade, inflammatory mediators can activate additional inflammatory cells to release inflammatory mediators and cytokines and to enhance and amplify damage signals, thus resulting in serious injuries to the body.

In recent years, it has been accepted that ALI is an uncontrolled pulmonary inflammation mediated by various inflammatory cells, such as polymorphonuclear neutrophils (PMNs) and alveolar macrophages (AMs) (3). Among those cells, PMNs reflect excessive inflammation, and excessive PMN accumulation and activation in the lungs are essential features of ALI (3,5). PMNs are recruited by the pulmonary microvascular endothelium. After adhesion and activation, they migrate into lung tissues from the microvessels, as well as release inflammatory mediators and cytokines (e.g. proteolytic enzymes and reactive oxygen species), which can cause diffuse lung injury and result in ALI (6,7). Although a few patients with ALI and neutropenia have been described, the pathogenesis is unclear, and it may be related to the monocyte/macrophage system (8); most cases of ALI have been demonstrated to be dependent on PMNs. Therefore, in this paper, we review the mechanism of ALI with respect to attachment, recruitment, adhesion, migration, activation, release of damage mediators, and apoptosis via PMNs.

3. PMN ATTACHMENT AND RECRUITMENT

In the early stage of ALI, PMNs are recruited to attach to the pulmonary capillaries, after which they then migrate into alveolar spaces where they are activated to release cytotoxic substances (oxygen free radicals, lipid mediators, and proteases), resulting in injury to alveolar epithelial cells and capillary endothelial cells. The recruitment of PMNs into areas of inflammation is a complex process involving PMN tethering, capturing, rolling, activation, and firm adhesion. Inflammatory mediators are involved in each step. The recruitment of PMNs in pulmonary microvessels is one of the earliest events in patients with ALIs (9), which first appears as a change in cell biomechanics and kinetics. As the diameter of pulmonary capillaries is less than that of PMNs, PMNs can only emigrate out of capillaries by deformation and elongation. However, because the corresponding receptors on the surfaces of PMNs are stimulated by inflammatory mediators and intracellular signalling molecules (Ca^{2+}) are activated, the cytoskeleton of PMNs redistributes, which leads to PMN stiffening and a decrease in deformation, resulting in a large number of PMNs gathering in the capillary and a reduction in the number of PMNs in the peripheral blood of patients with ALIs (10). In humans, PMNs pool in the bone marrow, circulation, and tissues. When inflammatory mediators enter the circulation, the bone marrow is stimulated to release PMNs (10). It has been demonstrated that PMNs can be released from the bone marrow 7–10 min after complement fragments are injected into the body (11), indicating that PMN recruitment in pulmonary microvessels is due to a change in mature PMN biomechanics and that many immature PMNs migrate into the circulation, as the deformation of immature PMNs is flawed. However, continuous recruitment of PMNs depends on PMN adhesion to endothelial cells.

4. PMN ADHESION

Cell adhesion molecules mediate the adhesion between PMNs and endothelial cells activated by the endothelium, in which steps include loose adherence and compact adhesion. Cell adhesion molecules are glycoproteins located on the cell surface, and these molecules are involved in binding with other cells or the extracellular matrix. Cell adhesion molecules include the cadherins, selectins, immunoglobulins, and integrins (12).

4.1. Selectin family

Selectins consist of 3 family members (L-selectin (leukocyte), P-selectin (platelet), and E-selectin (endothelial)). L-selectin is natively expressed on the surface of most PMNs and lymphocytes, but adhesion does not occur after activation. P-selectin is stored in granules called Weibel-Palade bodies and α-granules in non-activated platelets. P-selectin mediates the adhesion by platelets and leukocytes to endothelial cells. During inflammation, molecules such as histamine or thrombin activate endothelial cells, and P-selectin moves from an internal cell location to the endothelial cell surface. P-selectin and L-selectin co-mediate the loose adherence of PMNs to endothelial cells (13). E-selectin is expressed several hours after stimulation because it is not natively expressed or stored in endothelial cells, but is only synthesised and transported to the cell surface when endothelial cells are activated. Thus, endocytosis can reduce the expression of P-selectin and E-selectin (14).

4.2. Immunoglobulin superfamily

Members of the immunoglobulin superfamily include cell adhesion molecule-1, -2, and -3, intercellular adhesion molecule (ICAM)-1, -2, and -3, vascular cell adhesion molecule (VCAM)-1, platelet/endothelial cell adhesion molecule (PECAM), and mucosal addressin cell adhesion molecule (13). ICAM-1 is an important receptor of CD11b/CD18 that is natively expressed on the surface of endothelial cells. ICAM-1, which can be induced by lipopolysaccharide, human tumour necrosis factor (TNF), and interleukin (IL)-1 in conditions of inflammation, plays a key role in the mediation of PMN adhesion and activation (13).

4.3. Integrin family

Integrins are glycoproteins consisting of obligate heterodimers containing 2 distinct chains (α and β

subunits). Integrins integrate 2 cells through cytoskeletons or extracellular matrices (13). The receptor-binding site consists of extracellular regions with 2 subunits that can bind to different substrates such as ICAM (13). According to the subunits, integrins can be divided into 3 categories (β1, β2, and β3). β2 Integrin is most closely associated with PMN adhesion in the endothelium, and it mediates PMN adhesion in pulmonary microvessels and emigration into lung tissues. β2 Integrin consists of 3 subcategories (CD11a/CD18, CD11b/CD18, and CD11c/CD18), of which CD11b/CD18 is the most important subcategory, as it mediates the compact adhesion of PMNs to endothelial cells.

It is known that endothelial cell activation is crucial in the pathology of ALI/ARDS (14) because only activated endothelial cells can adhere to PMNs before PMNs participate in the inflammatory process. Many factors, such as bacterial toxins and other microbial products, cytokines, and haemodynamic disorders can activate endothelial cells.

5. PMN ACTIVATION AND EMIGRATION

PMNs are co-activated by pro-inflammatory agonists and the adhesion surface. The adhesion surface bears the integrin ligand ICAM-l. An integrin is crucial in the activation of PMNs, which must be stimulated and bind to ligands. Integrin is stimulated by the following 2 signal transduction methods: outside-to-inside and inside-to-outside (15).

After binding to ligands, activated integrins stimulate PMNs through a series of complex biological signalling processes. There are 3 signal transduction pathways. First, phosphorylation of the cytoskeleton linker protein forms a transmembrane integrin link with the actin cytoskeleton and binds to multiple signal molecules. Src tyrosine kinase is one such signal molecule, and is crucial to the generation of oxidants during the respiratory burst. Second, the Ras-Raf-MAPK pathway participates in cytokine expression and secretion, in which it is crucial that Src tyrosine kinase stimulates the transcription factor NF-κB. NF-κB regulates the expression of immediate early genes involved in the stress response, immune response, inflammation, and apoptosis, and it is also an upstream transcription factor of ICAM-1 (15,16). Activated NF-κB can regulate the expression of ICAM-1, IL-1, and TNF-α (17,18), which are released from PMNs, AMs, and endothelial cells after attacks by toxins or bacteria in lung tissues. As a result, the microcirculatory disturbances and pulmonary damage are aggravated. Third, PI3K-Akt signalling involves PMN activation and migration through activating NF-κB (Figure 1).

In summary, PMNs are stimulated by integrins to exhibit increased affinity. PMN function is activated when integrins bind to ligands and cluster (13). In addition, p38-MAPK phosphorylation is also required in the stimulation of PMNs.

Co-mediated adhesion molecules (ICAM-1), chemokines (IL-8), and activated PMNs can emigrate from pulmonary capillaries in a process termed leukocyte extravasation (8,9). There are 2 types of leukocyte extravasation: CD11/CD18-dependent and CD11/CD18-independent (1,9). However, only the CD11/CD18-dependent type is associated with NF-κB or ICAM-l. The mechanism underlying CD11/CD18-independent extravasation of leukocytes has not been confirmed. Whether CD11/CD18-independent extravasation of leukocytes is mediated by PECAM-l or VCAM-4 requires further study (9).

6. PMN RELEASE-INDUCED DAMAGE MEDIATORS AFTER STIMULATION

6.1. Reactive oxygen species

Reactive oxygen species are important inflammatory mediators that injure pulmonary tissues. Reactive oxygen species are produced and released when PMNs are activated (18). Reactive oxygen species can directly injure lung tissues via the oxidation of cytomembrane lipids as well as injure pulmonary capillary basement membranes, resulting in increased alveolar permeability, followed by pulmonary oedema and aggravated pulmonary injury (19). Furthermore, superoxide anion (O_2^-) reacts with NO free radicals to generate peroxynitrite ($ONOO^-$), which can directly injure lung tissues. When PMNs are stimulated, oxygen consumption is increased, and a large amount of unstable and highly reactive oxygen free radicals is produced. The reactive oxygen species promote PMNs and AMs to recruit and activate the inflammatory process, after which more reactive oxygen species are released, thus creating a vicious circle (the respiratory burst or so-called oxidative burst).

6.2. Neutrophil elastase (NE)

NE is the most crucial protease produced in PMNs. The physiologic functions of NE are as follows: plays an important role in the migration of PMNs; exerts anti-microbial effects, especially against anti-Gram–negative bacteria and their toxins; affect the expression of IL-2, IL-6, and IL-8 (4); influence tissue repair; and down-regulate inflammation (20). In general, NE digestion is limited in the cell; however, in pathologic cases, PMNs are over-activated, and NE spills out of the cell. NE outside cells can induce endothelial cells to produce chemokines, up-regulate IL-8 expression, and release and enhance PMN adhesion. Moreover, NE can damage the extracellular matrix and basement membrane, which leads to the destruction of the endothelium-vessel barrier, an increase in alveolar permeability, and pulmonary interstitial oedema. In addition, NE outside cells can combine with integrins (C3R; CD11b/CD18, Mac-1, and aMb2) and degrade ICAM-1, thus promoting the migration of inflammatory cells. Moreover, some studies have demonstrated that NE is the most significant protease in ALI (21,22).

6.3. Cytokines

Activated PMNs can produce and secrete pro-inflammatory and anti-inflammatory cytokines to regulate inflammation. In ALI, the dynamic balance between pro-inflammatory and anti-inflammatory cytokines is characterised by excessive pro-inflammatory

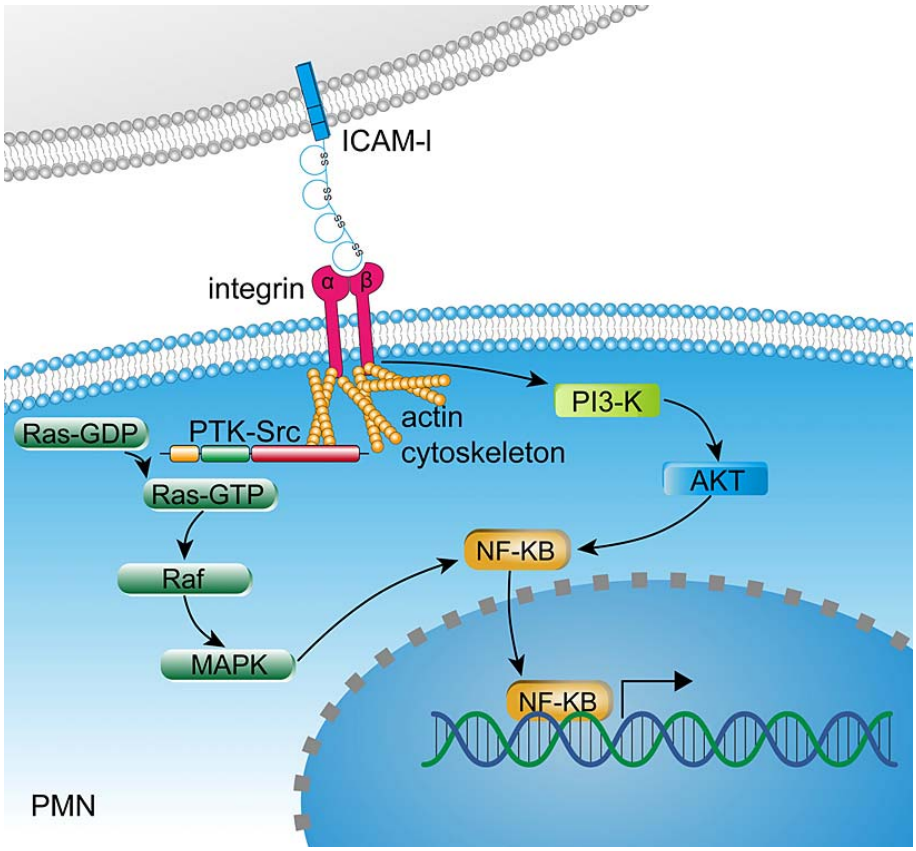

Figure 1. PMN activation. PMNs are co-activated by pro-inflammatory agonists and the adhesion surface. The adhesion surface bears the integrin ligand, ICAM-l. An integrin is crucial in the activation of PMNs, which must be stimulated and bind to ligands. After binding to ligands, activated integrins stimulate PMNs through three signal transduction pathways. First, the cytoskeleton linker protein phosphorylation makes a transmembrane integrin link with the actin cytoskeleton and binds to many signal molecules. Src tyrosine kinase is one such signal molecules and is significant in generation of oxidants during the respiratory burst. Second, the Ras-Raf-MAPK pathway participates in cytokine expression and secretion, in which it is crucial that Src tyrosine kinase stimulates transcription factor NF-κB. Third, PI3K-Akt signaling involves PMN activation and migration through activating NF-κB.

cytokine and insufficient anti-inflammatory cytokine production, which is a cause of uncontrolled inflammation (3).

7. PMN APOPTOSIS

PMNs can be removed by the process of necrosis or apoptosis. During necrosis, cytomembrane permeability is increased or the cell is broken, resulting in intracellular toxin overflow and damage to lung tissues. By contrast, PMNs do not injure lung tissues during apoptosis because PMNs are engulfed by macrophages before the cytomembrane is broken and the intracellular toxins do not spill out of the cell. Thus, apoptosis is appropriate in the removal of PMNs. Appropriate apoptosis of PMNs can reduce lung tissue injury. A previous study demonstrated that PMN apoptosis is delayed in ALI, and inflammation is extended.

PMN apoptosis delay is associated with the following factors. First, inflammatory cytokines inhibit PMN apoptosis. Second, PMN apoptosis is controlled by the Bc1-2 protein family. Members of the Bc1-2 protein family include anti-apoptotic proteins (Bcl-2, Bc1-XL, Mc1-1, and A1/Bfl-1) and apoptotic precursor proteins (Bax, Bak, Bad, Bik, and Bid) that regulate PMN apoptosis through caspase-3. Third, IL-8 immune complexes can delay PMN apoptosis through the up-regulation of anti-apoptotic protein expression, down-regulation of apoptotic precursor protein expression, and inhibition of the activity of caspase-3 and -9. Fourth, myeloperoxidase delays PMN apoptosis through the ERK and Akt pathways. Fifth, NF-κB delays PMN apoptosis under conditions of endotoxin- or ischaemia-reperfusion-induced ALI (23). Apoptosis delay is a benefit to PMN recruitment in lung tissues and permits sufficient time for the biological effects of PMN (24).

8. CONCLUSION

PMNs are required in the elimination of pathogens. A large number of PMNs are recruited and activated in pulmonary tissues when the inflammatory

cascade is activated, thus becoming a lethal event. PMNs play a key role in initiating inflammation and ALI. In addition, the influence of PMNs is usually limited to the location of infection or trauma. PMNs deform during emigration from vessels. However, PMN deformation is decreased in conditions of sepsis or severe trauma, and recruitment to pulmonary vessels results in increased permeability of the pulmonary vasculature and extensive damage of interstitial lung tissues.

In conclusion, ALI mediated by PMNs begins with PMN adhesion to pulmonary capillaries, migration, and recruitment to the site of inflammation, followed by activation. In this complex process, excessive recruitment of PMNs in pulmonary tissues is crucial in initiating ALI.

9. ACKNOWLEDGEMENTS

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Abbreviations: ALI: acute lung injury, ARDS: acute respiratory distress syndrome, PMN: polymorphonuclear neutrophil, PAH: pulmonary artery hypertension, MODS: multiple organ dysfunction syndrome, SIRS: systemic inflammatory response syndrome, Ams: alveolar macrophages, IgSF: immunoglobulin superfamily, ICAM: inter-cellular adhesion molecule, VCAM: vascular cell adhesion molecule, PECAM: platelet/endothelial cell adhesion molecule, MadCAM: mucosal addressin cell adhesion molecule, LPS: lipopolysaccharide, TNF: tumor necrosis factor, IL: interleukin

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