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Review

Molecular mechanisms in lipopolysaccharide-induced pulmonary endothelial barrier dysfunction

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ABSTRACT

The confluent pulmonary endothelium plays an important role as a semi-permeable barrier between the vascular space of blood vessels and the underlying tissues, and it contributes to the maintenance of circulatory fluid homeostasis. Pulmonary endothelial barrier dysfunction is a pivotal early step in the development of a variety of high mortality diseases, such as acute lung injury (ALI). Endothelium barrier dysfunction in response to inflammatory or infectious mediators, including lipopolysaccharide (LPS), is accompanied by invertible cell deformation and interendothelial gap formation. However, specific pharmacological therapies aiming at ameliorating pulmonary endothelial barrier function in patients are still lacking. A full understanding of the fundamental mechanisms that are involved in the regulation of pulmonary endothelial permeability is essential for the development of barrier protective therapeutic strategies. Therefore, this review summarizes several important molecular mechanisms involved in LPS-induced changes in pulmonary endothelial barrier function. As for barrier-disruption, the activation of myosin light chain kinase (MLCK), RhoA and tyrosine kinases; increase of calcium influx; and apoptosis of the endothelium lead to an elevation of lung endothelial permeability. Additionally, the activation of Rac1, Cdc42, protease activated receptor 1 (PAR1) and adenosine receptors (ARs), as well as the increase of cyclic AMP and sphingosine-1-phosphate (S1P) content, protect against LPS-induced lung endothelial barrier dysfunction. Furthermore, current regulatory factors and strategies against the development of LPS-induced lung endothelial hyper-permeability are discussed.

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1. Introduction

Acute lung injury (ALI) is a clinical syndrome characterized by impairment in gas exchange and/or lung mechanics that causes hypoxemia and increased work to breathe [1]. Lung injuries caused by inflammatory mediators can lead to pathophysiological syndromes such as ALI and severe pneumonia. Despite recent therapeutic advances, these conditions still have high (30–40%) rates of patient mortality [2–4]. Dysfunction of the endothelial barrier results in increased permeability, protein-rich fluid extravasation and lung edema, which is a common feature of ALI and acute respiratory distress syndrome (ARDS; a more severe form of ALI) [5].

Among the endogenous and exogenous agents that cause endothelial barrier dysfunction, lipopolysaccharide (LPS, endotoxin) is widely studied [6]. The majority of studies are focusing on pulmonary endothelial permeability use with LPS to stimulate the dysfunction. It comprises the majority of the outer wall of Gram-negative bacteria, binds to Toll-like receptor 4 (TLR4) and activates a variety of signaling pathways [7,8]. In addition, it activates macrophages, neutrophils, dendritic cells and other cell types that induce inflammation, oxidative stress and endothelial damage [9]. Exposure to LPS leads to endothelial barrier dysfunction and increased endothelial permeability.

For patients with ALI or ARDS, mechanical ventilation is a necessary and life-saving treatment but may also delay the inflammatory response and further enhance pulmonary endothelial barrier dysfunction. Specific pharmacological therapies that aim to improve pulmonary endothelial barrier function in patients with severe lung edema are absent [10]. Developing therapies that protect the integrity of the barrier and stabilize the gas exchange is a real matter of concern.

The knowledge of the mechanisms of pulmonary endothelial barrier dysfunction has greatly increased; the vast amount of new information has not yet been well summarized. Elucidating how pulmonary endothelial barrier permeability changes, is vital for researchers to understand the mechanisms of action. Importantly, no single mechanism explains all endothelial hyper-permeability. The selective control of barrier dysfunction requires either the inhibition of factors that increase endothelial permeability or the interference with intracellular activation mechanisms in endothelial cells leading to vascular hyper-permeability. Therefore, we attempted to provide an overview of recent insights into the molecular mechanisms in LPS-induced pulmonary endothelial barrier dysfunction. With this expectation, new insights in the mechanisms underlying hyper-permeability could offer potential novel targets and strategies for pharmacological intervention.

2. Pulmonary endothelial barrier

The inner walls of pulmonary microvessels are covered with a confluent endothelial cell (EC) monolayer. This delicate monolayer ensures effective and rapid gas exchange between alveolar and vascular lumens [7,10]. An important physiological function of this vascular barrier is to minimize the leakage of plasma proteins and blood cells into the pulmonary interstitium and prevent life-threatening alveolar flooding at normal vascular pressures [11]. Stability of the barrier is highly dependent on the adhesion between endothelial cells, firm attachment of endothelial cells to the underlying basement membrane, and shape of the endothelial cells, all of which are dependent on the cellular cytoskeleton [12,13].

On one hand, the complex network of the cytoskeleton is critical for EC barrier regulation. Endothelial barrier integrity is maintained by the precisely regulated balance between actomyosin contractile forces and adhesive EC–EC and EC-matrix tethering forces [14]. Disruption of either intact actin or the microtubule network leads to the formation of paracellular gaps and an increase in permeability.

On the other hand, adjacent endothelial cells are closely connected to each other by interendothelial junctions (IEJs). There are three main junctional complexes: adherens junctions (AJs), tight junctions (TJs) and gap junctions (GJs) [15]. Under physiologic circumstances, the permeability across the endothelial cell monolayer involves transcellular, paracellular or the combination of both pathways, maintaining the circulatory fluid homeostasis [16]. Unlike the vesicular-mediated transcellular route, the more widely studied paracellular route depends on the formation of gaps in the formerly intact endothelial monolayer. Current research states that paracellular endothelial hyperpermeability is induced, on one hand, by the generation of the centripetal contractile forces and, on the other hand, by the destruction of junctional integrity, provided mostly by adherens and tight junctions [13,16].

3. Pulmonary endothelial barrier-disruptive mechanisms

Different mechanisms involved in LPS-induced pulmonary barrier dysfunction lead to diverse effects on endothelial cells. As shown in Fig. 1, the increase in EC cytoskeleton contraction, disruption of EC junctions and decrease in the number of EC are the main three causes of LPS-induced barrier disruption.

3.1. MLCK activation

Barrier-disruptive contractility involves the formation of cell-crossing stress fibers, and barrier-maintaining intercellular junctions require strong anchoring to the cortical actomyosin structures [17]. Both endothelial contractility and the maintenance of junctional organization depend on actomyosin filaments. The condition of actomyosin in endothelium is regulated by myosin light chain (MLC) phosphorylation because an elevated level of phospho-MLC is essential to activate the actomyosin ATPase and induce filament formation. The level of phospho-MLC, which is controlled by phosphorylation and dephosphorylation, mainly depends on the activity of two enzymes: MLC kinase (MLCK) and Rho kinase (ROCK). Both of these enzymes can directly phosphorylate MLC *in vitro* and *in vivo* [17,18]. A basal level MLCK activity is required to maintain physiological microvascular permeability. In contrast, pathologically increased MLCK activity induces microvascular barrier dysfunction. Conversely, MLC dephosphorylation by MLC phosphatase (MLCP) decreases actomyosin contractility, which relaxes the actin cytoskeleton and reduces paracellular permeability. Additionally, both MLCK and ROCK-dependent pathways can be modulated by the activity of protein kinase A (PKA), which is known to decrease the MLC phosphorylation level [19,20].

In regard to the lung, the role of MLCK in regulating pulmonary endothelial permeability is unpredictable. There are at least three MLCK genes: MYLK1, MYLK2 and MYLK3. In adult cells, MYLK1 is prominently expressed in nonmuscular lineages, including ECs, epithelial cells, and neutrophils. LPS activates MLCK in ECs and causes EC contraction, resulting in barrier dysfunction and endothelial hyper-permeability. Interestingly, no significant differences are observed in LPS-mediated pulmonary microvascular hyper-permeability between knockout (KO, deletion of MYLK1) and wild type (WT) mice [21]. This finding suggests that MLCK may not be critical in the upregulation of endothelial

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