Research progress of NETs-mediated endothelial cell dysfunction in sepsis

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Abstract: Neutrophil extracellular traps (NETs), a network of DNA, histone, myeloperoxidase, and other particles released by activated neutrophils, can effectively capture circulating pathogens. Inappropriate neutrophil activation and NETs release in sepsis induce endothelial cells to turn to an inflammatory cascade, degrade the glycocalyx on the surface of endothelial cells, and increase endothelial cell permeability, leading to microcirculation disorders, tissue hypoperfusion and life-threatening organ dysfunction in the later stages of sepsis. This article summarizes the formation of NETs and elaborates the mechanism of nets-mediated endothelial cell dysfunction in sepsis, so as to provide a new direction for targeted therapy of sepsis.

1. Introduction

Sepsis is a life-threatening organ dysfunction caused by an imbalanced response of the body to infection, and it is a clinical syndrome with high morbidity and mortality¹. Neutrophils play an important role in sepsis, mainly through three pathways: phagocytosis, degranulation and release of neutrophil extracellular traps (neutrophil extracellular traps, NETs)². NETs are extracellular reticular nuclei or mitochondrial DNA structures with bactericidal activity composed of histones, cytoplasmic proteins, and granule proteins, which can neutralize and kill bacteria, fungi, and viruses, thereby inhibiting the spread of pathogens. However, inappropriate release of NETs can induce inflammation and organ damage, promoting the development of sepsis. During sepsis, neutrophils release cytokines, reactive oxygen species, and NETs in blood vessels, leading to vasculitis³. Activated endothelial cells increase glycolysis, further promoting inflammation and oxidative stress. The direct destructive effect of NETs components, accompanied by the inflammatory environment and oxidative stress, will degrade the glycocalyx on the surface of endothelial cells and increase the permeability of endothelial cells through connection breakage, high expression of adhesion molecules, apoptosis, etc., Disruption of the endothelial barrier increases microvascular leakage, leading to vascular hypotension, tissue edema, and organ failure in sepsis⁴.
2. Formation and function of NETs

2.1 Formation of NETs

In 2004, Brinkmann et al first reported the release of NETs from neutrophils\[5\]. Activated neutrophils release antibacterial agents such as histones (H1, H2A, H2B, H3, H4), myeloperoxidase (MPO), and neutrophil elastase (NE) to the outside of the cell. Protein-modified deoxyribonucleic acid (DNA) networks, or NETs. The process of forming NETs by neutrophils is called NETosis, which mainly includes three ways: ① Suicidal NETosis. It takes several hours for this classic pathway to occur. After being stimulated by interleukin-8, LPS or phorbol ester, neutrophils are activated by protein kinase C (PKC) / mitogen-activated protein kinase (MEK) / fibrosarcoma protein (Raf) / Reduced nicotinamide adenine dinucleotide phosphate (NADPH) activated by the extracellular signal-regulated kinase (ERK) pathway promotes the generation of ROS, leading to increased calcium influx and deamination of peptidyl arginine Enzyme 4 (PAD4) activation and histone citrullination. NADPH facilitates the transfer of NE from cytoplasmic granules to the nucleus, where cleaving histones causes chromatin breakdown \[6\]. MPO promotes the decomposition of chromatin and nuclear membrane, and the mixing of particles in NET vacuoles to form NETs. Mature NETs are squeezed due to the rupture of neutrophil outer membrane, and finally released to the outside of the cell, thereby eliminating pathogens. ② Survival NETosis. Neutrophils are also able to release NETs in a ROS-independent pathway. The formation of NETs is mediated by CD11a, TLR4, TLR2, and complement C3, and they pass through the cytoplasm in the form of vesicles and fuse with the outer membrane, and are transported out of the cell, this Type NETosis has nothing to do with ROS and the process is more rapid. ③ Neutrophils can also release survival NETosis\[7\]. Derived from mitochondrial DNA (mtDNA), mtDNAs are similar to plasmid DNA and do not contain histones. After transporting nuclear DNA and mtDNA out of the cell, neutrophils still have effector functions such as chemotaxis, recruitment, and phagocytosis.

2.2 Two sides of NETs

NETs play a key role in fighting infection. IL-37 is an antibacterial protein externalized on NETs, which improves the development of sepsis by preventing the release of pro-inflammatory factors\[8\]. Excessive release of NETs can also promote inflammation, and its constituents, reticulated DNA as damage-associated molecular patterns (DAMPs), can stimulate mouse splenocytes and macrophages to secrete tumor necrosis factor (TNF-α) and IL-1β, causing tissue Organ damage. Extracellular histones can also act as DAMPs to activate immune cells through Toll-like receptor and Nod-like receptor signaling pathways, resulting in increased IL-6, IL-10 and TNF-α, thereby causing organ damage\[9\].

3. NETs-mediated endothelial dysfunction

3.1 Thrombosis

The components of NETs, reticulated DNA and histones, provide a scaffold to recruit erythrocytes, platelets, leukocytes, and plasma proteins, forming a positive feedback loop of blood cell adhesion, thereby increasing thrombus formation\[10\]. Studies by Von Brühl et al. have shown that NETs promote the interaction between factor XII (FXII) and neutrophils, activate the NF-κB signaling pathway in endothelial cells, induce synergy between histone H4, and initiate endogenous Coagulation pathways. The glycocalyx covering the luminal side of the vascular endothelium is extremely important for maintaining the antithrombotic effect in the vascular lumen. Once the
glycocalyx is destroyed by NETs, cell adhesion molecule-1 (ICAM-1), E-selectin and other adhesion molecules are exposed. In the exfoliated endothelium, interact with reticulated DNA, accelerate the aggregation of red blood cells and platelets, and promote thrombus formation. Thrombosis induced by NETs can lead to organ ischemic injury and disseminated intravascular coagulation (DIC)\textsuperscript{[11]}, DIC occurs more frequently in late sepsis and is associated with multiple organ failure and septic shock.

3.2 Inflammatory response

In the early stage of sepsis, a variety of pro-inflammatory factors and growth factors are released in large numbers, and endothelial cells maintain a proliferative state characterized by high levels of glycolysis\textsuperscript{[12]}. Lactate, the end product of glycolysis, activates the NF-κB signaling pathway by promoting the phosphorylation and degradation of the NF-κB inhibitor IκB-α. Activated NF-κB in turn enhances the proinflammatory response of endothelial cells. Pathogens such as TLRs can also recognize receptors to drive endothelial cells toward proinflammatory and proangiogenic responses. Studies have shown that NETs markers are elevated in patients with pulmonary arterial hypertension. NETs increased the expression of vascular cell adhesion molecule-1 (VCAM-1) and platelet endothelial cell adhesion molecule-1 (PECAM-1) and the secretion of IL-6, IL-8 and vascular endothelial growth factor (VEGF) through the activation of MPO/ H\textsubscript{2}O\textsubscript{2} dependent TLR4/NF-κB signaling pathway. Thus, it induces pro-inflammatory and pro-angiogenic responses in human pulmonary artery endothelial cells\textsuperscript{[13]}. NETs can also induce macrophages characterized by the release of inflammatory factors such as IL-1, IL-6, IL-8, and TNF. Macrophages secrete a variety of pro-angiogenic factors such as VEGF and fibroblast growth factors (FGFs). Among them, VEGF, as one of the important growth factors, can promote glycolysis by increasing the expression levels of glucose transporter 1 (GLUT1), fructose-2, 6-bisphosphatase 3 (PFKFB3) and lactate dehydrogenase-A (LDH-A), and the enhancement of glycolysis promotes NF-κB-driven inflammatory response of endothelial cells. Increased PFKFB3 activity stimulates ICAM-1 and VCAM-1 expression through NF-κB signaling pathway in the acute lung injury model, which further induces the aggregation of neutrophils and macrophages, thereby promoting the proinflammatory response of endothelial cells\textsuperscript{[14]}.

3.3 Increased permeability

The glycocalyx, which covers the surface of endothelial cells, plays an important role in pathophysiological activities and is composed of glycosaminoglycans (GAGs), proteoglycans, and glycoproteins\textsuperscript{[15]}. NETs can destroy the glycocalyx of endothelial cells, leading to increased endothelial permeability, exposure of endothelial cell adhesion molecules, further triggering inflammatory cascade reaction and adhesion of leukocytes and platelets, resulting in inflammatory disorders, microcirculatory blood Impaired blood flow, tissue hypoperfusion, and life-threatening organ failure. The glycocalyx also contains extracellular superoxide dismutase (SOD3), which protects endothelial cells from oxidative stress\textsuperscript{[16]}.

3.3.1 Connectivity breakage leads to increased permeability

The integrity of the endothelial barrier is mainly composed of adherent junctions (AJ) and tight junctions (TJ). The adherens junction with vascular endothelial cadherin (VE-Cadherin) as the core is crucial in maintaining the integrity of the endothelial barrier, and maintains the integrity of the junction by linking the actin cytoskeleton into a complex through catenin\textsuperscript{[17]}. In the NETs-induced inflammatory response, the cAMP/Rac1 signaling pathway is inhibited, stimulating Src and Pyk2
kinases, both of which lead to VE-Cadherin phosphorylation and endocytosis, increasing endothelial gaps between them, resulting in increased permeability of the endothelial barrier\[^{18}\]. TJs are anchored to the actin cytoskeleton by tight junction proteins (ZO). In sepsis, the inflammatory factor TNF-\(\alpha\) can destroy the tight junction protein at the junction of endothelial cells by activating the NF-\(\kappa\)B pathway, and ROS during the release of NETs can cause the rearrangement of intercellular junctions, thereby promoting its dissociation from ZO and increasing endothelial Cell Permeability\[^{19}\].

### 3.3.2 Upregulation of adhesion molecules leading to increased permeability

The adhesion molecule ICAM-1 plays an important role in maintaining the integrity of the endothelial barrier, and its expression is significantly upregulated in the inflammatory response. Overexpression of ICAM-1 promotes phosphorylation of VE-Cadherin in NETs-induced inflammation, thereby disrupting endothelial permeability in a neutrophil-dependent manner. Sumagin et al proposed that in the absence of leukocytes, ICAM-1 overexpression promotes ROS production and increases endothelial permeability through a protein kinase C (PKC)-dependent signaling pathway\[^{20}\]. Studies have also shown that ICAM-1 signaling, in addition to playing a key role in regulating endothelial cell permeability, can also participate in the regulation of transcellular permeability such as vesicle transport.

### 3.3.3 Apoptosis leads to increased permeability

NETs have direct endothelial cell death induction and cytotoxicity mediated by histone and MPO. Under inflammatory conditions, NETs trigger the production of O\(^2\) and NO, increase the formation of peroxynitrite (ONOO\(^-\))\[^{21}\], induce increased mitochondrial outer membrane permeability, and efflux various pro-apoptotic signaling molecules. Peroxynitrite can also induce DNA damage and then activate the DNA repair enzyme PARP-1. When severe DNA damage occurs, excessive activation of PARP-1 can deplete NAD\(^+\) in the cell. NAD\(^+\) is a key factor in the glycolytic pathway and is the raw material for the formation of adenosine triphosphate (ATP). This leads to further endothelial dysfunction\[^{22}\].

### 4. Potential therapeutic targets for sepsis

#### 4.1 Inhibition of NETs formation

Peptidylarginine deiminase 4 (PAD4) is an enzyme involved in chromatin depolymerization\[^{23}\], which enhances reticulated DNA and Binding of histones and thus PAD4 can induce NETs formation, and overexpression of PAD4 leads to severe endothelial cell dysfunction by releasing NETs and inducing the expression of ICAM-1 and VACM-1 on endothelial cells. Martinod et al. found that PAD4-negative phenotype mice had a protective effect on LPS-induced septic shock, could effectively prevent the formation of NETs and improve the overall survival rate, and used the PAD4 inhibitor GSK484 to inhibit the formation of NETs to induce pulmonary thrombosis in mice. The formation was significantly reduced and the endothelial barrier function was clearly preserved\[^{24}\]. Reticular DNA is the skeleton structure of NETs. Deoxyribonuclease I (DNase1) can specifically act on the phosphodiester bonds of DNA, and block the positive feedback loop of thrombus formation by degrading the structural scaffold of NETs. In vivo and in vitro experiments, DNase1 also has a protective effect on sepsis endothelial cells.
4.2 Inhibition of glycolysis

Similar to tumor cells, activated endothelial cells in sepsis have active glycolysis[25]. High expression of fructose-2, 6-bisphosphatase 3 (PFKFB3) in tumor endothelial cells compared with normal endothelial cells indicates a stronger glycolytic ability, and the therapeutic concept of PFKFB3 inhibitors has been extended to sepsis patients. Furthermore, inhibition of glycolysis in endothelial cells prevents pathological angiogenesis during inflammation[26].

4.3 Inhibit free radical imbalance

In sepsis, the imbalance between the production and clearance of ROS is one of the main mechanisms of endothelial cell dysfunction. Glutathione (GSH) or the precursor of GSH, N-acetylcysteine (NAC), can significantly reduce endothelial cell death and ROS production in patients with septic shock[27]. Several clinical trials have shown that patients receiving NAC treatment have shorter ICU stays and improved sepsis severity scores. In addition to scavenging ROS, NAC attenuated the formation of NETs during bacterial infection, shortened ICU stay and improved severity scores. Albumin has antioxidant effects associated with its sulfhydryl groups that reduce circulation and tissue oxygen generation, and albumin-treated animals show reduced ROS and RNS generation, further preventing endothelial cell dysfunction. In addition, HSA can also inhibit the activation of NF-κB signaling pathway, thereby alleviating oxidative stress in vascular endothelial cells. Vitamin C can also be used in patients with sepsis due to its antioxidant effect. After clearing ROS and RNS, vitamin C is oxidized to ascorbic acid free radicals by inhibiting the expression of inducible nitric oxide synthase and improving the bioavailability of NO. degree to further inhibit the production of ROS and RNS[28].

5. Conclusions and Prospects

In sepsis, neutrophils and NETs constitute a powerful anti-inflammatory system. Simultaneously, inappropriate neutrophil activation and release of NETs switch endothelial cells from an anti-inflammatory, anticoagulant phenotype to a pro-inflammatory, procoagulant phenotype. In addition, NETs can degrade glycocalyx and increase endothelial cell permeability, and the disruption of endothelial cell barrier further promotes the progression of sepsis. To date, several potential therapeutic targets for endothelial cell dysfunction have been proposed, and although it is increasingly recognized that excessive release of NETs may be an important therapeutic target, studies are still inconclusive. Most inhibitors of NET formation are still in the preclinical state and further clinical trials are needed. Antiglycolytic therapy is mainly used to inhibit tumor growth, and the therapeutic dose and administration time for sepsis patients need further study.

References


