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Commentary

The physical integrity of endothelial cells (ECs) lining the blood vessels regulates the inflammatory response. Both innate immunity and inflammatory disorders hinge on the EC-neutrophil interaction. Neutrophil binding, rolling, and migrating along and between ECs is associated with vascular permeability. In this issue of the *JCI,* Owen-Woods et al. tracked neutrophils in vivo in venules of mouse striated muscle and revealed how endothelial permeability can affect neutrophil trafficking. Strikingly, many neutrophils that migrated between EC junctions were able to rejoin the blood circulation. Further, the chemokine and neutrophil chemoattractant, CXCL1, drove this reverse transendothelial migration (rTEM). This paradigm-shifting study provides a mechanism for distal organ damage as well as an explanation for sepsis-associated acute respiratory distress syndrome.

Leaking chemokines confuse neutrophils

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The physical integrity of endothelial cells (ECs) lining the blood vessels regulates the inflammatory response. Both innate immunity and inflammatory disorders hinge on the EC-neutrophil interaction. Neutrophil binding, rolling, and migrating along and between ECs is associated with vascular permeability. In this issue of the *JCI*, Owen-Woods et al. tracked neutrophils in vivo in venules of mouse striated muscle and revealed how endothelial permeability can affect neutrophil trafficking. Strikingly, many neutrophils that migrated between EC junctions were able to rejoin the blood circulation. Further, the chemokine and neutrophil chemoattractant, CXCL1, drove this reverse transendothelial migration (rTEM). This paradigm-shifting study provides a mechanism for distal organ damage as well as an explanation for sepsis-associated acute respiratory distress syndrome.

Tracking neutrophils through subendothelial space

Invading pathogens or sterile tissue injury initiate inflammatory reactions that result in increased blood flow (redness) and vascular permeability (swelling), which allows plasma proteins like antibodies and complement factors to reach the extravascular space. Neutrophils begin to roll on the activated endothelial cells. Chemokines from macrophages, dendritic cells, endothelial cells, epithelial cells, and stromal cells induce neutrophil arrest and transmigration, resulting in their rapid local accumulation (1, 2). The relationship between permeability and neutrophil extravasation has been debated for a century (3). Initially, vascular leakiness was hypothesized to promote neutrophil extravasation, but studies found that the two events are regulated independently (3). Another hypothesis was that transmigrating neutrophils themselves cause increased permeability by breaching the endothelial layer. Recent studies demonstrated that the endothelial cells tightly seal around the transmigrating neutrophils, preventing leakiness during this event (3, 4). However, neutrophils adhered to the vessel wall can increase vascular permeability via stimulating the endothelial cells through TNF release (5) and other mechanisms (6).

In this issue of the *JCI*, Owen-Woods and colleagues report a surprising and paradigm-changing discovery: increased vascular permeability actually acts against neutrophil extravasation via diverting the neutrophils from the subendothelial space back into the vessel lumen (7). Normally, extravasating neutrophils are thought to follow a positive chemokine gradient, with more chemokine in the extravascular space than in the vessel lumen. Chemokines released by the tissue and immune cells are thought to diffuse toward the vessel wall. Endothelial cells can bind some chemokines and present them on their apical surface into the vessel lumen. Other chemokines remain unbound. Owen-Woods et al. show that increased vascular permeability causes the chemokine CXCL1 to diffuse into the vessel lumen, resulting in a detectable increase in CXCL1 plasma concentration. The increased plasma CXCL1 is sufficient to initiate neutrophil reverse transendothelial migration (rTEM) back into the lumen.

The authors applied cutting-edge intravital confocal microscopy of the cremaster muscle microvasculature. In this model the cremaster muscle was exteriorized, opened, and spread out for imaging while being superfused with a physiological buffer. This widely used model allows administration of compounds in the superfusion buffer while simultaneously observing transendothelial diffusion of tracer molecules and migration of neutrophils (8). Owen-Woods et al. developed a brilliant approach to label those neutrophils that visited the subendothelial space and then returned to the vessel lumen. They labeled the circulating neutrophils by injecting biotinylated anti-Ly6G antibody intravenously and superfused the exteriorized cremaster muscle with fluorescently labeled streptavidin that is retained in the extravascular space, presumably because it is too large (~55 kDa) to diffuse through a leaky vessel wall against the stream of extravasating blood plasma. As the biotinylated neutrophils entered the subendothelial space, they bound streptavidin and became fluorescently labeled. With this elegant method, the authors tracked the fate of rTEM neutrophils, which first accumulated in the lungs and later in the bone marrow. rTEM neutrophil counts in the lung correlated with leakiness of the lung vasculature, suggesting that rTEM neutrophils caused distal organ damage. Distal organ damage in response to injury, for example ischemia followed by reperfusion, has been known for decades, but this study offers a convincing mechanism by which this occurs.

The pathomechanism of sepsis

The mechanism discovered by Owen-Woods and colleagues may also contribute to the pathomechanism of sepsis. Based on the latest consensus definition, sepsis is diagnosed when proven or suspected bacterial or fungal infection and organ dysfunction are present (9, 10). For organ

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dysfunction assessment, the performance of the cardiovascular system, lungs, blood coagulation system, liver, kidneys, and central nervous system are scored (Sequential Organ Failure Assessment [SOFA] score) (10). Sepsis can progress into septic shock, a condition with even higher mortality. This happens when vascular permeability increases to the extent that the immense blood plasma extravasation results in blood pressure drop and consequential tissue hypoperfusion and hypoxia (10, 11). The most frequent directly sepsis-related causes of death are unresponsive hypotension and respiratory failure (12). The underlying cause of these clinical symptoms is a dysregulated immune response to infection. Pathogen- and damage-associated molecular patterns trigger proinflammatory responses from immune and endothelial cells, vascular permeability increases, and leukocyte recruitment to the infected tissue begins. However, at the same time immunosuppressive mechanisms (such as endotoxin tolerance of innate immune cells, lymphocyte apoptosis and exhaustion) are initiated (11). These mechanisms lead to delayed pathogen clearance due to immune paralysis and host damage due to the persistent proinflammatory state (11).

The mechanism described by Owen-Woods could exacerbate sepsis. The increased neutrophil rTEM rate could contribute to delayed pathogen clearance, because fewer neutrophils will engage with the infected site (increased permeability diverted 20% of the TEM neutrophils). Quick eradication of the pathogen is crucial in sepsis treatment. Every hour delay in antibiotic administration was found to increase progression to septic shock by 8% (13). As shown in the current publication, rTEM neutrophils contribute to lung dysfunction as they accumulate around leaky vessels, a sign of acute respiratory distress syndrome (ARDS). Diagnostic criteria for ARDS are sudden onset of hypo-oxygenation and lung edema that is not explained by cardiac failure (14). The underlying cause of these symptoms is disruption of the alveolar-capillary barrier.

The contribution of neutrophils to ARDS development is well recognized but incompletely understood (15). The recruited neutrophils become activated and release various proinflammatory and proteolytic compounds, which will result in an immense increase in vascular permeability. ARDS can result from lung infection by inhaled pathogens, when neutrophil recruitment is initiated by the pathogen-activated resident macrophages via chemokine release (16), and after extrapulmonary infection (17). The mechanisms of neutrophil recruitment differ greatly between organs. In the lung capillaries physical entrapment of the circulating neutrophils plays a dominant role, and the involvement of selectins and integrins is minor (1). Owen-Woods et al. found that rTEM neutrophils showed increased β1 and β2 integrin and ICAM-1 expression on the cell surface, but it is unknown whether rTEM neutrophil accumulation in the lung is a consequence of upregulation of these adhesion molecules.

Human relevance

Owen-Woods and colleagues made their discovery in mice, whose immune system is known to differ from that of humans (18). Nevertheless, the human relevance of these findings is supported by the documented presence of increased vascular permeability (19), blood cytokine storm (increased IL-1β, IL-6, IL-8, IL-10, TNF, and CCL-2) (20, 21), and neutrophil accumulation in ARDS (16). Additionally, in vitro experiments showed that human neutrophils following rTEM upregulate ICAM-1 expression similarly to the mouse neutrophils (22, 23).

This paradigm-changing study (7) raises several new questions. Does increased vascular permeability induce rTEM in every organ? Will all of these rTEM neutrophils home into the lung? Can rTEM neutrophil accumulation in the lung protect against secondary lung infection? Further studies are necessary to help us understand the relevance of this permeability-dependent rTEM mechanism for human diseases.

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