CHAPTER 89

Endothelial Dysfunction of the Kidney in Sepsis

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OBJECTIVES

This chapter will:
1. Describe the role of the endothelium dysfunction during sepsis and its interplay with the local environment.
2. Explain how endothelium damage translates into a loss of kidney function: sepsis-induced acute kidney injury.
3. Summarize the therapies targeting renal endothelial cells in sepsis-induced acute kidney injury.

Endothelial dysfunction and microcirculation impairment are recognized hallmarks of sepsis-related organ failure.[1–7] Many experimental and clinical studies have emphasized the central role of endothelial cell (EC) activation and dysfunction in promoting the coagulation cascade, leukocyte adherence, vascular barrier compromise, hemodynamic collapse, and vascular hyporesponsiveness. These processes contribute to organ failure with an increased risk of morbidity and mortality for the patient. Among the organ failures observed in septic patients, acute kidney injury (AKI) is one of the most frequent. Sepsis-induced AKI may be associated with mortality rates above 50%.[8–11] In fact, the kidney exhibits a high sensitivity to microcirculatory alterations, especially heterogeneity, and to tissue hypoxia, two main phenomena that occur during septic shock.[12–15]

This chapter provides an overview of sepsis-related endothelial dysfunction with a particular focus on the kidney. We discuss the features of healthy and injured septic vascular endothelium of the kidney, the interplay between ECs and blood cells, and interactions among a wide range of molecules. Moreover, we describe how septic insult to endothelium can lead to a loss of kidney function.

ROLE OF RENAL ENDOTHELIUM IN FUNCTION

Endothelial Cells

The ECs form the interface between the content of the inner lumen of vessels and the surrounding environment, comprising the vascular smooth muscle cells (VSMCs), the interstitial space and the parenchymal cells that are responsible for organ function.[16] The ECs constitute a monolayer, lining the interior of all blood vessels. This surface is estimated to comprise approximately 10^{13} cells, covering 4000 to 7000 m^2. ECs generally have a thickness of approximately 0.5 µm and are 100 µm long by 10 µm wide.

The endothelium is remarkably heterogeneous in structure and function. The arrangement of ECs may differ significantly from one organ to the next, from juxtaposed arrangements to overlapping arrangements. This heterogeneous distribution also may vary within the same organ; in the kidney, for instance, various EC arrangements grant different permeability properties. The EC lining contains pores and fenestrations to ensure partial permeability and to transport molecules to the underlying cells and basal membrane. The kidney and intestines exhibit the highest permeability. ECs are linked together by transcellular components, including gap junctions for electrical communication for upstream vascular regulation and intercellular tight junctions for maintaining vascular barriers.[6,17] Previously considered a passive barrier, it is now apparent that ECs play a crucial role in the regulation of vasomotor tone, hemostasis, immunologic functions, and the secretion of molecules by sensing through mechanotransducers, which subsequently initiate transcellular and intracellular signaling and activation.

Glomerular and Peritubular Endothelium

Glomerular endothelial cells are unusually thin; around capillary loops, they have a cell thickness of approximately 50 to 150 nm, whereas in other locations, this thickness is approximately 500 nm.[18] ECs in the glomerulus present large fenestrated areas constituting 20% to 50% of the entire endothelial surface.[19] These fenestrations are typically 60 to 70 nm in diameter but, unlike renal peritubular ECs, do not seem to possess a thin (3–5 nm) diaphragm.[20,21] In general, these fenestrations act as a sieving barrier to control the production of urine in the glomerulus, filtering plasma because of hydrostatic pressure.[22] The kidney has one of the richest and most diverse EC populations found within any organ. The microcirculation of the kidney presents two specialized capillary beds connected in series: the glomerular capillary bed in the cortex for plasma filtration and the peritubular capillary bed, which forms the vasa recta responsible for electrolyte reabsorption in the outer and inner medulla. Thus the arrangement of ECs and the permeability of the endothelium differ for these two microcirculations. Glomerular microcirculation functions via continuous and fenestrated endothelium with no diaphragm, whereas it is more continuous and nonfenestrated in the descending vasa recta in peritubular microcirculation.[23,24]

The endothelium must face and resist extreme physiologic conditions, such as large changes in oxygenation and osmolality. Indeed, ECs in the cortex are exposed to almost normal oxygen partial pressure and osmolality, whereas those in medullar microcirculation function in an osmolarity of up to 1200 mosmol/L^{-1} and a PO_2 as low as 20 mm Hg.[23,25,26] The microvascular arrangement has a specialized structure in the medulla. The vasa recta, connected in series with the juxtaglomerular microvasculature, surround the peritubular cells in the outer and inner medulla and are responsible for solute exchange. The ECs of these microvessels are exposed to countercurrent oxygen exchange,

resulting in a gradient of decreasing oxygen tension (to approximately 10 mm Hg).[26,27] Thus their functions differ considerably along the tubule. In addition, ECs are affected and injured by ischemia injury differently.[28]

Interplay of Endothelium and Leukocytes

The inner lumen of ECs is exposed to blood flow, consisting of red blood cells, leukocytes, and plasma. In the physiologic state, endothelium–leukocyte interactions are limited. Five steps generally describe the interactions between ECs and leukocytes during inflammation, beginning with limited contact, then more prolonged contact, leukocyte rolling, strong adhesion, and finally transendothelial migration, a process referred to as diapedesis.[29] ECs regulate leukocyte trafficking between circulating blood and the surrounding tissue. When activated, the endothelium exhibits enhanced endothelium–leukocyte interactions that are secondary to increased expressions of cell adhesion molecules (CAMs), such as intercellular adhesion molecule selectins (E-selectin and L-selectin), ICAM-1, ICAM-2, vascular adhesion molecule (VCAM), and platelet endothelial cell adhesion molecule (PECAM).[6,30] The upregulation of CAMs promotes increased adhesion, rolling, and transmigration of circulating leukocytes. Many integrins also are involved in the adhesion of polymorphonuclear leukocytes and monocytes in the proximal tubule and serve as transcellular mechanotransducers.[31]

Regulation of Vascular Tone

An essential mechanism involved in the vasomotor tone underlying renal autoregulation is endothelium-dependent relaxation. Various physiologic stimuli, including myogenic, metabolic, and neurohormonal factors, lead to endothelium-mediated vascular smooth muscle (VSM) relaxation or constriction. Myogenic activation is mediated by shear stress induced by blood flow and translated into biochemical signals through mechanotransductors, inducing vasorelaxation by vasoactive compounds such as nitric oxide (NO).[4,5,17,30,32] ECs synthesize and release various enzymes or molecules (EDHF: endothelium-derived hyperpolarizing factor, NOS: nitric oxide synthase, SOD: superoxide dismutase, COX: cyclooxygenase, and CSE: cystathionine γ-lyase) that also produce various relaxing factors (NO, PGI_2, and H_2S). These factors then diffuse toward VSM cells to produce relaxation. Endothelial NOS (eNOS)-derived NO prevents vascular dysfunction via a direct vasodilatory effect, inhibiting platelet aggregation and leukocyte activation.[4]

Under physiologic conditions, the glomerular filtration rate (GFR) is regulated tightly by the renin-angiotensin-aldosterone system (RAAS). The RAAS system is activated by tubuloglomerular feedback (TGF), which acts as a negative feedback control mechanism driven by distal tubular fluid flow and sodium and chloride reabsorption.[25] TGF modulates the renal blood flow entering the glomerulus, subsequently controlling the hydrostatic pressure within Bowman's capsule by varying the tone of afferent and efferent arterioles via the aforementioned regulators.[26]

Endothelial Surface Layer: Role of the Glycocalyx

Among the essential components constituting of vascular barrier, the glycocalyx, a thin layer lining of the luminal membrane of ECs, protects the endothelium and regulates cellular and macromolecular traffic.[33–35] The glycocalyx contributes to the microvascular barrier permeability.[34] The glycocalyx is a 0.2- to 0.5-μm–thick negatively charged gel-like surface structure of proteoglycans with bound polysaccharide chains called glycosaminoglycans (GAGs), glycoproteins, and glycolipids.[33–35] It is thought to represent 20% of the intravascular volume.[33,36] This highly interactive scaffold is capable of sensing blood flow and facilitating protein interactions with their receptors or other proteins, and it houses many EC receptors and compounds important for maintaining hemostasis and homeostasis and providing antiinflammatory defense to the parenchymal cells.[31,34,35] The glycocalyx also exhibits anticoagulant properties[33] by means of negatively charged components.[34]

The morphology and composition of the glycocalyx layer vary from organ to organ and contribute to the heterogeneity observed in EC functions. Significant differences among vascular beds[37] and between fenestral and interfenestral regions of glomerular ECs were revealed in an analysis of the composition of the ECs in the glycocalyx.[38] For instance, the glycocalyx in the fenestrae region presents a higher ratio of heparan sulfates and hyaluronic acid to sialoproteins. The particular composition of the glycocalyx in the fenestrae is likely crucial for its sieving and permeability properties.[33]

Endothelium and Hemostasis

The interactions between ECs and the coagulation system are very complex. These interactions not only regulate pro- and anticoagulant patterns involving ECs, plasma-soluble factors, platelets, and leukocytes but also direct platelets and fibrin clotting to injured areas of the endothelium. ECs are like minifactories that synthesize and express molecules necessary to regulate hemostasis, with either anticoagulant or procoagulant activities. Thrombomodulin, endothelial protein C receptor (EPCR), heparan, tissue-type plasminogen activator (tPA), tissue factor pathway inhibitor (TFPI), eNOS, and CD39 are the main anticoagulant factors. Procoagulant factors include tissue factor (TF), von Willebrand factor (vWF), factor VIII, and plasminogen activator inhibitor type 1 (PAI-1). TF creates complexes with factor VIIa and then activates factors IX and X. Importantly, each of these molecules is expressed differentially across the vascular tree, arteries, capillaries, or veins. For every procoagulant response, there is a natural anticoagulant reaction, resulting in the finely tuned balance that is needed to control hemostasis.

Under physiologic conditions, ECs inhibit blood coagulation via the activation of protein C, the expression of thrombomodulin and specific proteoglycans, and the release of TPA. TFPI controls the extrinsic pathway and regulates TF,[39] antithrombin III (ATIII)–heparan counterbalances the serine proteases in the cascade,[40] the thrombomodulin (TM)/protein C/protein S mechanism inactivates cofactors Va and VIIIa, and plasmin degrades preformed fibrin.[30] Plasma vWF binds and aggregates platelets by bridging platelet receptor platelet glycoprotein (GPIb-IX) to denudated endothelium. The cleaving of vWF is regulated by a disintegrin and metalloproteinase with thrombospondin motifs, also known as ADAMTS-13. Various components of the coagulation system directly signal within endothelium via protease-activated receptors (PAR1). This complex cascade and interplay among leukocytes, endothelium, and hemostasis is disturbed severely in sepsis and contributes to the pathogenesis of sepsis-induced organ failure.

ENDOTHELIAL CELLS AND THE PATHOPHYSIOLOGY OF SEPSIS

The host response to pathogens primarily determines how severe it inflammatory activation is promoted, contributing to endothelial injury. This sequence of events leads to microcirculation derangement, resulting in plugged microvessels, functional microcirculation shunting contributing to reduced O_2 extraction, and renal tissue hypoxemia. This heterogeneous flow generates microareas of ischemia, leading to functional failure of the kidney. The next step is characterized by organ dysfunction, which affects patient outcome. Therefore septic kidneys can become compromised because of several pathogenic events, including (1) endothelial function directly affecting peritubular epithelial cells causing AKI,[41] (2) glycocalyx shedding that alters the venal vascular barrier,[33] and (3) microcirculation heterogeneity and renal tissue hypoxia.[15]

Endothelial cell activation causes important structural changes in the glomerular and peritubular capillaries. The damage- or pathogen-associated molecular patterns (DAMPs/PAMPs) are key signals that trigger systemic reactions by priming, signaling, alerting, and guiding the immune system to fight pathogens.[42,43] The cause of endothelium impairment during sepsis has not been established clearly. DAMPs/PAMPs involved in EC dysfunction also can be recognized by several cellular types, causing indirect deleterious effects on EC functions. Thus ECs, leukocytes, platelets, and the endothelium surface simultaneously trigger cascades of mediators, causing massive physiologic changes. However, leukocyte–EC interactions are considered of specific importance. The cross-talk between ECs and leukocytes is mediated by CAMs, as described above, which enables leukocytes to adhere the endothelium. Because these molecules are embedded in the EC glycocalyx layer, such activation presumably is preceded by glycocalyx shedding because of inflammatory mediators (e.g., cytokines, reactive oxygen species, and nitrosative species).[6,13,17,33,44,45] An illustrative clinical setting that puts into perspective the contributing effect of each component in sepsis-induced endothelium dysfunction is the context of agranulocytosis/neutropenia or bone marrow–suppressed patients. These patients exhibit the most severe pattern of septic shock, although no leukocyte–EC interactions are present because no leukocytes exist. Moreover, many studies have analyzed endothelium dysfunction via perfusion with various fluids and molecules without the addition of leukocytes. Eliminating leukocytes could be considered a weakness of such studies or could emphasize the fact that leukocyte–EC interactions may not be a fundamental element contributing to endothelial dysfunction in renal dysfunction.

TNF-α exerts deleterious effects on renal ECs and plays a key role in AKI.[46] In the light of these findings, Xu et al. observed in TNF receptor 1 (TNFR1) knocked-out mice submitted to endotoxin challenge that the glomerular endothelial surface layer, endothelial cell fenestrae, GFR, and albuminuria were diminished. These results suggest that sepsis-induced endothelial dysfunction may be mediated by TNF-α activation of TNFR1.[46,47] In addition, Wu et al. demonstrated that mice lacking expression of ICAM-1 exhibited reduced AKI, leukocyte infiltration, and mortality in response to endotoxin.[48] ICAM-1 may not be crucial solely for leukocyte–EC interactions, although it may play a pivotal role in endotoxin-induced AKI. Mice lacking expressions of the two principal ligands for ICAM-1, ß1 integrin LFA-1 (CD11a/CD18), and Mac-1 (CD11b/ CD18), present on circulating leukocytes, were not protected against neutrophil infiltration nor endotoxin-induced AKI.[48] These findings suggest that sepsis involves multiples pathways leading to endothelial dysfunction and AKI.

Shedding or damaged glycocalyx expose CAMs, previously embedded in the glycocalyx and shielded from the leukocyte interaction. Under such conditions, many EC functions mediated by the glycocalyx are jeopardized.[17,33,44,49] Glycocalyx degradation contributes to an increase in vascular barrier permeability with subsequent albuminuria.[17,50–53] An upregulation of P-selectin, ICAM-1 and VCAM-1 also has been observed in the peritubular capillaries during sepsis.[48,54] Inflammatory mediators and reactive oxygen species released by leukocytes and ECs and upregulated to destroy pathogens may themselves harm the endothelium as an unwanted side effect. Clinical studies have demonstrated that increased excretion of glycocalyx degradation products in urine was associated with microalbuminuria.[51,52] This finding indicates that the glomerular basal membrane is damaged during sepsis as the result of the endothelial vascular barrier injury. At the tissue level, damage to the glycocalyx correlates with increased interstitial fluid and tissue edema.

Plasma leakage is a result of altered vascular barrier permeability. In addition to CAMs and cell-cell junction molecules, vascular endothelial growth factor (VEGF) and angiopoietin–Tie2 systems are involved critically in vascular barrier permeability compromise during sepsis.[55–60] High levels of VEGF have been shown in septic patients.[58–63] However, low VEGF concentrations were associated preferentially with more severe renal dysfunction.[63] Whereas VEGF and angiopoietin-2 promote microvascular permeability, angiopoeitin-1 (Ang1) acts as an antipermeability molecule and may protect against plasma leakage during sepsis.[64,65] In fact, Kim et al. showed that the administration of a variant of Ang1 (COMP-Ang1) in mice subjected to endotoxin-mitigated renal EC dysfunction and protected against AKI.[66]

The systemic inflammatory response associated with sepsis leads to activation of coagulation and disrupts hemostasis, promoting procoagulant and antifibrinolytic states.[67,68] Increasing evidence points to an extensive cross-talk between coagulation and inflammation during sepsis. After injury, ECs express tissue factor and initiate coagulation cascades that activate thrombin and deposit fibrin.[40,68] Tissue factor expression triggers the activation of coagulation. In sepsis, TF upregulation is counteracted insufficiently by TF inhibitors. In addition, the fibrinolytic pathway is altered with increased PAI-1 activity, resulting in an inhibition of fibrin removal.[67] The level of activated protein C is decreased, contributing to the formation of micro-clots/plugs in the microcirculation.[5] If uncorrected, this procoagulant state may cause thrombi formation and/or severe bleeding or hematomas as part of disseminated intravascular coagulation (DIC) syndrome. Bleeding is caused by the consumption and subsequent exhaustion of coagulation proteins and platelets because of the ongoing activation of the coagulation system.[67] In addition, platelets also may attach to ECs and are involved in EC activation by several mechanisms. The engagement of platelet GPIIb/IIIa upregulates CD40 ligand expression on the platelet membrane, stimulating endothelial cells to express adhesion molecules and TF.[69] Platelet P-selectin, not endothelial P-selectin, is key in the development of ischemic AKI.[70] Platelet consumption also may play an important role in patients with sepsis because of the ongoing generation of thrombin.[71] Platelets contribute to microvascular dysfunction and play a pivotal role in organ failure.[72,73] Finally, neutrophil extracellular traps (NETs) also contribute to microvascular impairment.[68] NETs are networks of chromatin filaments composed of histones

and DNA strands, decorated with proteins and lysosomal enzymes. They are released by neutrophils upon contact with infectious agents, inflammatory cytokines, or reactive oxygen species (ROS).[68]

A common end product of microvascular injury and the combined degradation arising from the glycocalyx, ECs, leukocytes, red blood cells, and platelets is the generation of microparticles (MPs).[74] MPs are cell membrane–derived particles that are 0.2 to 2 μm in diameter, promote coagulation and inflammation, and contribute to multiple-organ injury, including AKI.[75] MPs remotely promote widespread endothelial dysfunction.[76] Zafrani et al. showed a direct role of MPs in sepsis-induced AKI by using calpastatin, a calpain-specific inhibitor (calpain is essential in release of MPs).[77] Several procoagulants (TF and phosphatidylserine) are included in the outer leaflet of MPs perpetuating the prothrombotic state of sepsis.

The above-described events generate oxidative and nitrosative stress. Because of tissue hypoxia caused by microvascular dysfunction during sepsis, parenchymal and endothelial cells can switch from aerobic to anaerobic respiration, producing toxic byproducts, such as ROS. Reactive nitrogen species (RNS) derived from NO and ROS, such as superoxide (O_2^-), are produced in large amounts during sepsis. The upregulated production of superoxide arises from (1) the reactions catalyzed by NAPDH oxidase present in leukocytes and endothelial cells, (2) the conversion of xanthine dehydrogenase to xanthine oxidase, and (3) the uncoupling of NOS in conditions of tetrahydrobiopterin deficiency.[78] In an anaerobic state, ROS are produced aggressively by the mitochondria, resulting in more cell damage and endothelial cell dysfunction. RNS and ROS alter EC functions and cause lipid peroxidation, in addition to exerting antimicrobiotic action. Noiri et al. demonstrated that L-N^6-(1-iminoethyl) lysine hydrochloride (L-NIL) administration, an inhibitor of inducible NO synthase (iNOS), resulted in amelioration of renal dysfunction and reduced nitrotyrosine formation, lipid peroxidation, and DNA damage.[79] This suggests that peroxynitrite, rather than superoxide anions, is responsible for renal cell damage. In a model of renal ischemia/reperfusion (IR) injury, L-NIL also prevented ischemic-induced renal microvascular hypoxia, indicating that renal IR induced early iNOS-dependent microvascular hypoxia by disrupting the balance between the microvascular oxygen supply and consumption.[80] Conversely, *N*-nitro-L-arginine methyl ester (L-NAME), an NO blocker, has been shown to aggravate the course of AKI after IR injury.[81]

In contrast to many other organs, iNOS is expressed constitutively in mouse and human renal tubule cells[82] and may contribute to changes in renal hemodynamics and a reduction in the GFR. The generation of iNOS results in two pathogenic actions: (1) the inhibition of eNOS-derived NO generation and (2) the contribution of ROS to the formation of peroxynitrite. Depletion of essential cofactors necessary for eNOS activity, such as tetrahydrobiopterin, uncouples eNOS and results in the generation of superoxide anion and reduced NO production, a process referred to as eNOS uncoupling. Langenberg et al. demonstrated in a sheep model of sepsis-induced AKI that all subtypes of NOS were highly expressed in the renal cortex but not in the renal medulla.[83] They hypothesized that overexpression of NOS isoforms in the cortex may lead to intrarenal shunting. This may induce a lack of blood flow in the medulla, promoting medullar hypoxia. Vascular hyporesponsiveness is one of the final pathogenic effects in EC injury and further contributes to microcirculation disorders.[4] However, vasodilation, microcirculation alterations, and tissue hypoxia are heterogeneous during septic shock, and the kidney is no exception.[1,15]

TRANSLATION OF ENDOTHELIUM DAMAGE INTO A LOSS OF KIDNEY FUNCTION

As described above, endothelial dysfunction is a consequence of a series of simultaneous or consecutive pathogenic events mediated by toxic compounds produced in large quantities, including IL-6, IL-8, TNF-α, vWF fragments, plasma-free hemoglobin, MPs, ROS, and RNS (Fig. 89.1). It previously was thought that histologic damages were present in critically ill patients suffering from AKI. Lerolle et al. revealed fibrin deposition in the glomeruli and signs of acute tubular injury and leukocyte activation, primarily monocytes, in kidney biopsies taken from 19 patients with AKI within 30 minutes postmortem.[84] However, these results must be interpreted with caution, because the patients were enrolled after having AKI for 2 days with severe hypoperfusion, which could be responsible for the observed renal alterations and not related solely to AKI (causing irreversible damages). Indeed, in experimental studies, no cellular changes have been found, which could account for the marked reduction in glomerular filtration as observed in AKI, supporting the notion that early septic acute kidney injury represents a functional disturbance within the kidney.[85,86] This concept is in line with the need to identify physiologic biomarkers of AKI instead of pharmacologic markers of cellular damage.[87] These results suggest that AKI may be prevented or at least controlled via pharmacologic treatments because the insult does not induce irremediable histologic damages.

Because different microcirculations coexist in the kidney, sepsis-induced disturbances of the microvascular bed may take different forms. Thus albuminuria may be related more to glomerular microcirculation EC glycocalyx degradation, inadequate Na^+ reabsorption, and ineffective TGF because of the peritubular microcirculation. All of these contribute to decreases in the GFR. Rodent models of sepsis-induced AKI suggest that cortical peritubular microvasculature is among the first structures injured.[88–91] The interactions among various factors contributing to the development of renal failure implicates inflammatory activation characterized by leukocyte-EC interactions. These interactions paired with the imbalance of O_2 homeostasis, involving the delicate balance among O_2, NO, and ROS, and inflammation causes renal microcirculatory dysfunction. These events represent the mechanism of renal function failure.

Role of Kidney Hypoxia

The final common pathway of the pathophysiologic response to sepsis, as discussed above, leads to tissue hypoxia.[15] EC damage is associated with microcirculation impairment followed by tissue hypoperfusion and hypoxia. Lack of oxygen to parenchymal cells directly causes loss of organ function, especially in tubular cells of the kidney.[15] Increasing evidence has demonstrated that a loss of renal oxygenation in heterogeneous areas occurs during sepsis and resuscitation.[92–94] Therefore the role of hypoxia may have been overlooked previously, assuming that renal blood flow (RBF) is increased during the early phase of sepsis such that oxygen delivery is maintained or even increased. However, a thorough literature review emphasized the high heterogeneity of RBF changes during sepsis that depends on the time of insult, the model of sepsis-induced AKI, and the monitoring tools and analyses used in the experiments.[95] Alterations in the microvascular perfusion distribution and a mismatch between kidney tissue oxygen demand

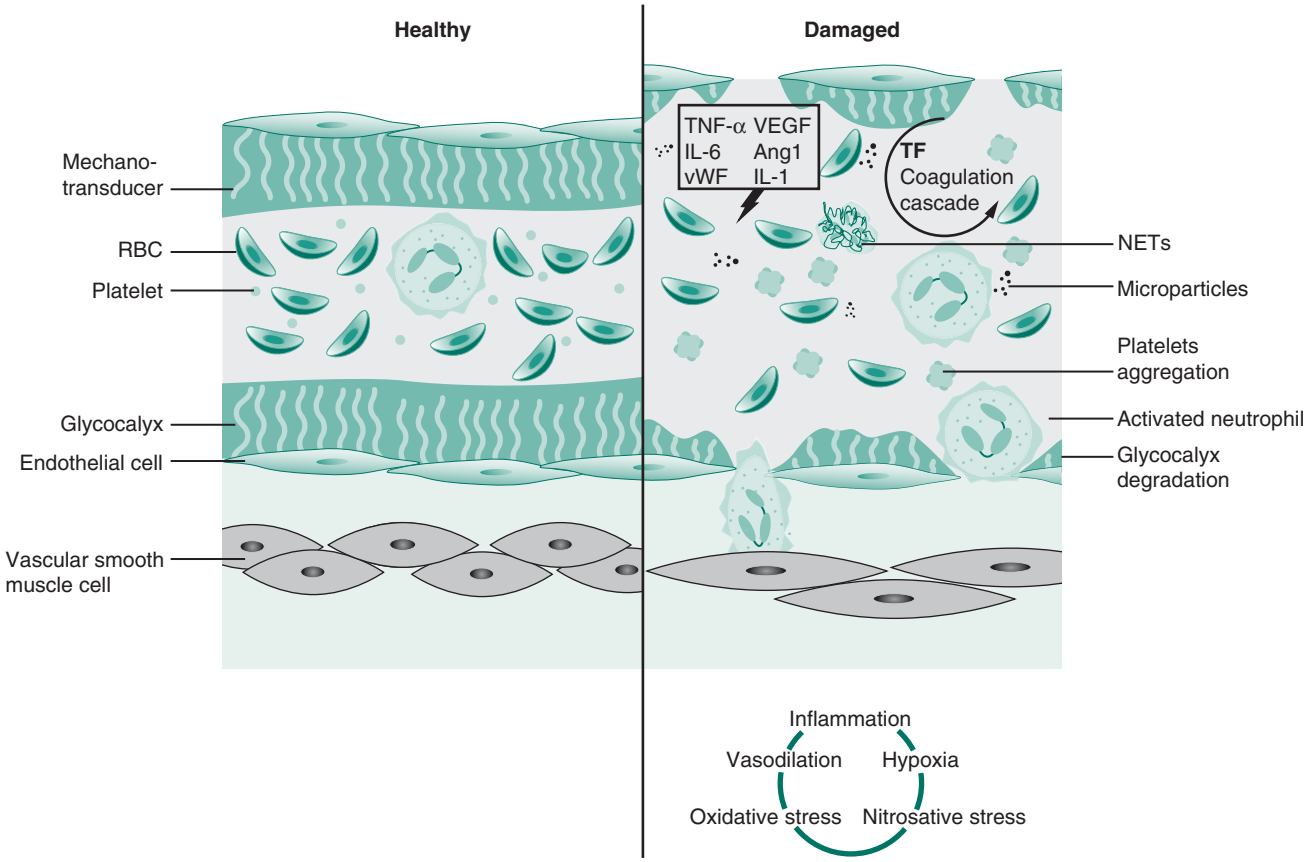

FIGURE 89.1 General overview of endothelium dysfunction occurring during sepsis. The region of interest is focused on arterioles where vascular smooth muscle cells are present. *Ang1,* Angiopoietin 1; *IL-,* interleukin; *NETs,* neutrophil extracellular traps; *RBC,* red blood cell, *TF,* tissue factor; *TNF-α,* tumor necrosis factor-alpha; *VEGF*, vascular endothelium growth factor, *vWF,* von Willebrand factor.

and delivery occur, especially in the kidney cortex during the early phase of insult. Functional shunting of O_2 transport occurs, resulting in small areas of hypoxia.[96,97]

Oxygen consumption by the kidney is related predominantly and directly to Na reabsorption (~80%).[98–101] Indeed, the oxygen requirement of the kidney is determined primarily by the ATP production needed for Na/K pump function on the basal side of thick ascending limb cells. Microcirculatory dysfunction can severely limit the ability of circulation to provide adequate oxygen for fueling oxidative phosphorylation for the production of ATP and can directly impair the function of the Na/K ATPase pump.[102] Numerous studies have demonstrated oxygen impairments in the kidney even during the early phase of sepsis.[94,103,104] The unique arrangement of the renal microvasculature, serially organized, with vasa recta emerging from the efferent arterioles of the juxtamedullary glomeruli, makes the maintenance of the medullary perfusion highly depend on adequate cortical perfusion. O_2 shunting occurs between the descending and ascending vasa recta and contributes to the high sensitivities of the medulla and corticomedullary junction to decreased O_2 supply.[23,26,27,92,105] Thus decreased O_2 availability reduces solute and electrolyte exchange, leading to further activation of the TGF to maintain GFR constant. Johannes et al. showed in an endotoxemia rat model that cortical microcirculatory PO_2 was preserved despite hypotension and a drop in renal arterial flow.[94] Interestingly, despite fluid resuscitation in this model, which corrected the mean arterial pressure and increased RBF, the renal cortical microvascular oxygenation paradoxically worsened and VO_2/T_{Na+} increased.[94] Evans et al. suggested that arteriovenous shunts may represent an adaptive mechanism for preventing hyperoxia and the overproduction of ROS because of the high renal perfusion needed to sustain the GFR and for limiting oxygen debt in the entire kidney.[27]

The role of hypoxia-induced factor α (HIFα) activation and regulation in the kidney during sepsis is a subject of intense investigation.[83,92,106–108] HIFα may play a crucial role in protecting the kidney during IR injury.[28] Studies have shown that the expressions of HIFα genes and mRNA is upregulated during sepsis-induced AKI in cecal ligation puncture and endotoxemia models of insult.[83,107] HIFα is an O_2-sensitive transcription factor that accumulates and binds to the key sequence of the erythropoietin (EPO) gene, the hypoxia-responsive element (HRE), and activates transcription of EPO when oxygen tensions decrease. HIFα possesses two isoforms, HIF-1α and HIF-2α, which are expressed in a cell-type fashion: HIF-1α is expressed in tubular cells, and HIF-2α is expressed in interstitial and endothelial cells.[109] In a multi-insult rat model of AKI, Rosenberger et al. showed that HIF-1α was more expressed in collecting ducts than in the medullary thick ascending limb (mTAL) of the loop of Henle.[109] The limited activation of HIF-1α in the mTAL may explain the susceptibility of these cells to injury.

The activation of TGF via the renin-angiotensin aldosterone system (RAAS) is part of the response to sepsis insult. Increased levels of angiotensin-2 (Ang-2) leads to a

reduction in GFR by causing vasoconstriction of the afferent and efferent arterioles in the glomerulus. The activation of TGF then decreases hydrostatic pressure in the glomerulus and reduces the GFR.[43] Regional reductions in microcirculatory flow cause heterogeneous regional microischemia, resulting in impaired cortical and medullar μPO_2 and contributing to the recruitment of shunts and a reduction in renal oxygen extraction.[92]

Renal Tissue Edema

The primary cause of renal edema is thought to be initiated by the degradation of the EC glycocalyx. The actors involved in glycocalyx shedding are thought to be ROS, such as hydroxyl anions, hydrogen peroxide, peroxynitrite, and superoxide, or other molecules/enzymes, including heparanase or tumor necrosis factor-α (TNF-α).[6,110,111] Indeed, antioxidant enzyme treatments (catalase or superoxide dismutase) experimentally dampened the shedding products of the endothelium and maintained vascular barrier integrity.[45] The final result of loss-of-barrier function is interstitial edema. Edema and, more generally, fluid overload after fluid resuscitation of septic patients, is a well-recognized contributor to organ dysfunction. The use of fluids to treat AKI is a controversial topic, because the amount[112–114] and type of fluid are currently under debate. The kidney is contained in a noncompliant capsule, increasing the harmfulness of increased interstitial renal pressure secondary to fluid overload.[114] Because of glycocalyx degradation and EC dysjunction, increases in vascular barrier permeability in the glomerular and peritubular microcirculations occur.[86,112,115] Subsequent fluid accumulation in renal tissue causes a rise in interstitial pressure, increases the pressure gradient across the glomerular capillary, and may result in microcirculatory flow impairment and capillary collapse. In a large cohort of critically ill, septic patients presenting AKI, fluid overload was a major contributor of AKI.[116] During renal IR in a murine model, intrasubcapsular pressure increased because of tissue edema, possibly increasing renal vein pressure, and was correlated with considerably reduced tubular excretion rates and renal perfusion.[117] These considerations suggest that fluid resuscitation during sepsis must be guided carefully to avoid fluid overload and further impairment of renal microcirculation.[112,114] Vascular congestion also has been noted as a cause of AKI.[118]

THERAPIES TARGETING RENAL ECS IN SEPSIS-INDUCED ACUTE KIDNEY INJURY

Targeting Endothelial Cells

More than 100 randomized clinical trials have tested the hypothesis of modulating the host response to sepsis, many dedicated to studying target receptors on or in ECs or blocking/modulating molecules known to promote EC injury.[119] Unfortunately, most studies failed to demonstrate any benefits on outcome. Matejovic et al. analyzed all ongoing experimental and clinical studies, aiming to improve renal microcirculation hemodynamics during sepsis, either in the glomerular or in the peritubular microvascular bed, with promising results that require confirmation.[120] There is a growing body of evidence suggesting benefits of using corticosteroids during septic shock.[121] However, the specific action of glucocorticoids on ECs in the kidney is addressed incompletely. Selective iNOS inhibition for the treatment of sepsis-induced AKI also has been suggested as a potential EC-targeted therapeutic strategy, outlining the importance of this enzyme as a therapeutic target.[122] However, specific iNOS inhibitors are not currently in use in clinical practice, although antiinflammatory drugs such as steroids are potent inhibitors of iNOS. Holthoff et al. investigated the effects of rolipram, a selective phosphodiesterase 4 inhibitor, and resveratrol, a polyphenol vasodilator that is also capable of scavenging reactive nitrogen species, in a rodent model of sepsis-induced AKI induced by CLP.[90,123] Both treatments improved renal microcirculation, reduced vascular permeability, and improved renal function. In a retrospective clinical study, the administration of ulinastatin, a urinary trypsin inhibitor that possesses a variety of antiinflammatory effects, was found to be associated with a lower incidence of AKI after cardiopulmonary bypass surgery.[124] The authors concluded that the favorable outcome observed may be related to less EC-damaging cytokines that are often present in AKI. Another recent review listed the novel repair targets in ECs during AKI.[125] However, these therapies often focused on one target, whereas cellular and tissue repair are more complex processes involving hundreds of pathways.

New and interesting approaches incorporate cell-based therapies, such as mesenchymal stem cells[126–128] and endothelial progenitor cells (EPCs),[129,130] which may be more efficient given the complex mechanisms of injury. Cellular therapies aim to use cells that are capable of modulating the global inflammatory response by secreting large quantities of proinflammatory and antiinflammatory agents and other types of molecules. The many molecules that can be secreted cells can target a variety of receptors, better preventing/limiting sepsis-induced organ damage, restoring microcirculation, and promoting parenchymal function than a single drug may do. These cell-based therapies also are believed to speed the recovery of renal function and healing. This approach is the object of intense research and several ongoing projects.[126,129] Several authors have demonstrated potential interest of cell-based therapies for treatment of acute renal ischemia/reperfusion injury in rats using EPCs derived from either bone marrow or Wharton's jelly of human umbilical cords.[127,130,131] However, the reported data regarding EPCs during sepsis are inconsistent. Some authors found that an increased number of EPCs in critically ill patients was associated positively with better outcomes,[132,133] suggesting a positive effect, whereas others reported the opposite.[134]

Impact of Fluid Resuscitation on Endothelial Cells

Fluid resuscitation is the cornerstone of the management of sepsis-induced AKI. The choice of fluid used during the resuscitation process has been debated extensively. Briefly, current evidence suggests that fluid resuscitation should not further harm ECs or aggravate tissue edema, with the goal to not further worsen kidney function.[112,114] However, the optimal fluid and optimal dose that should be administered to a specific patient remains a source of debate, and to what extent fluid therapy promotes microcirculatory flow and preserves EC functions in the kidney is unknown.[135] Data regarding the specific action of fluids on ECs in the kidney are scarce. To date, although there is a growing body of evidence regarding the importance of the glycocalyx in vascular barrier permeability, it is not clear if restoring or preserving the glycocalyx layer from shedding influences renal outcome or decreases the occurrence of sepsis-induced AKI.

CONCLUSION

ECs play a key role in the protection of the kidney from injury and functional failure. New monitoring techniques that aim to directly provide information regarding renal ECs and microcirculatory function may facilitate goal-directed strategies for identifying and treating AKI.

Key Points

1. Two different types of microcirculatory compartments (tubular and glomerular) exist in the kidney. Therefore the injury to the kidney can occur at different levels, compromising the kidney function at distinct levels.
2. Kidney hypoxia and microcirculatory alterations play a central role in the pathophysiology of sepsis-induced kidney injury.
3. Fluid resuscitation may have deleterious effects on the renal microcirculation irrespective of the type of fluid.
4. Therapies aimed at restoring endothelial cells function, renal microcirculation, and renal tissue oxygenation are needed to prevent the occurrence or treat sepsis-induced acute kidney injury.

Key References

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