
Kidney Endothelial Dysfunction: Ischemia, Localized Infections and Sepsis

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Abstract

Endothelial cells play a key role in initiating and propagating the inflammatory response seen in ischemia, infections and sepsis. Situated in a key position between the epithelial cells and white blood cells (WBC), they interact and respond to signals from both cell types. Microvascular endothelial cells within the kidney mediate coagulation, WBC attachment, WBC migration into the interstitium, microvascular flow rates and permeability. Low regeneration potential and endothelial-mesenchymal transformation lead to fibrosis and subsequent microvascular dropout. This last event is in large part responsible for a chronic reduction in regional perfusion, subsequent increased vulnerability to recurrent acute kidney injury, and acceleration of chronic kidney disease progression to end-stage renal disease. Glomerular endothelial dysfunction may lead to preglomerular shunting of blood flow allowing kidney blood flow to remain close to normal while resulting in a reduction in glomerular filtration rate.

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Under physiologic conditions, the cells within the kidney function in an integrated, synergistic and 4-D fashion to perform a number of finely tuned and interactive processes resulting in a complexity of cell-cell interactions and tissue heterogeneity second only to the brain. The kidney has one of the richest and most diversified endothelial populations found within any organ. While receiving approximately 20–25% of the cardiac output, the renal endothelium experiences unparalleled environmental extremes in oxygenation and osmolality. For example, endothelial cells in the outer cortex are exposed to a normal osmolality and oxygen tension where those in the inner medullary region are exposed to an osmolality of up to 1,200 mOsm and an oxygenation content down to

approximately 20–30 mm Hg. Glomerular endothelial cells are fenestrated as are certain peritubular microvascular endothelial cells.

The kidney endothelial cell is positioned in a strategic position either mediating filtration across the glomerulus or in direct contact with epithelial cells, whose apical membrane face the external milieu, and white blood cells (WBC) that monitor the internal milieu. Crosstalk between these three cell types occurs across and through endothelial cells, and all three cell types, as well as resident dendritic cells, are involved with the inflammatory response within the tissue. Endothelial cells also regulate the coagulation cascade as part of the response to injury and inflammation.

When blood flow diminishes in the kidney, regardless of whether ischemia occurs due to a macrovascular systemic or a microvascular focal event, the result remains the same. Adaptation to a reduction in blood flow can only occur to a certain point and then, when delivery of oxygen and metabolic substrates is inadequate, cellular ATP drops and cellular injury results leading to organ dysfunction. The purpose of this review is to identify, organize and define interactions between the different types of cells and processes involved in the pathophysiology of endothelial dysfunction and acute kidney injury (AKI); detail how bacterial infections mediate endothelial changes through proximal tubule signals; delineate how preglomerular vascular shunting can in part explain near normal total renal blood flow and yet a reduced glomerular filtration rate (GFR); and outline an approach that facilitates patient care and development of therapies.

Lessons Learned from Isolated Tubular Bacterial Infections

In a novel series of intravital 2-photon studies, the laboratory of Agneta Richter-Dahlfors demonstrated how *Escherichia coli* infections isolated to one proximal tubule lumen could dramatically alter endothelial function in a site-specific manner [1–3]. Utilizing a fluorescent cytopathologic strain of *E. coli* and micro-puncture delivery to the early proximal tubule (PT), these investigators were able to record in four dimensions (3-D plus time) bacterial attachment to PT cell apical membranes and the resulting local responses in peritubular blood flow and WBC recruitment. Rapidly, within 1–2 h following PT intraluminal bacterial injection and attachment to PT cells, and before there was disruption of the epithelial lining at 5–8 h, there was a marked reduction in peritubular red blood cell flow rates surrounding only the infected tubule. Neutrophils started accumulating at 2–3 h and continued to grow in number, and endothelial permeability increased. These responses were markedly delayed when an identical strain of *E. coli* lacking only one virulence factor was used [1]. Utilizing Clark-type microelectrodes within the lumen of infected tubules in vivo, oxygen tension (PO_2) decreased within 1 h of bacterial injection and was 0 mm Hg within 3.5–4.0 h [3]. This decrease in O_2 tension was not due to bacterial O_2 utilization.

Primary cultures of PT cells showed increased oxygen consumption and release of proinflammatory cytokines including TNF- α , IL-1 β and IL-6 when exposed to the bacteria. These same cytokines were increased post-PT lumen injection of bacteria into kidney tissue by PCR techniques 5 h later. These authors also noted an important role for coagulation in the process of bacterial isolation. If infected rats were treated with heparin, there was a marked delay in red blood cell flow rate deterioration surrounding the infected PT with subsequent sepsis and widespread dissemination of bacteria throughout the body. This consequence was not encountered in nonheparin-treated infected rats. These data imply there is a rapid signal sent from the PT cell once bacterial attachment to the apical membrane occurs, resulting in endothelial cell alterations leading to activation of the clotting cascade, WBC recruitment and endothelial dysfunction. This crosstalk has important implications regarding uptake and processing of bacterial products, such as lipopolysaccharide, by PT cells [4]. These data imply that injury to PT cells leads to localized effects on the microcirculation. A general scheme for PT, WBC and endothelial cell interaction is shown in figure 1.

Endothelial Dysfunction

Endothelial cells contribute to vascular tone, regulation of blood flow to local tissue beds, modulation of coagulation and inflammation, and vascular permeability. Both ischemia and sepsis have profound direct and indirect effects on the renal endothelium, resulting in microvascular dysfunction leading to continued ischemic conditions and further injury following the initial insult, especially in the outer stripe of the kidney [5, 6]. Histopathologically, this is seen as vascular congestion, edema formation, diminished microvascular blood flow, and margination and adherence of inflammatory cells to endothelial cells, leading to the extension phase of ischemic AKI [5]. It is important to realize that while a marked decrease in total kidney perfusion results in global ischemia, a fall in the regional perfusion can result in continuing ischemic injury to this region with minimal effects on global perfusion. However, the complexity in vascular beds within the kidney makes interpretation of total kidney blood flow challenging following ischemic or septic injury.

Vascular Tone

Conger et al. [7, 8] were among the first to demonstrate that postischemic rat kidneys cannot autoregulate blood flow even when total renal blood flow had returned to baseline values up to 1 week after injury. Goligorsky et al. [9] found that the constrictor response could be blocked by Ca²⁺ antagonists, and demonstrated loss of normal endothelial nitric oxide synthase (eNOS) function was due to a loss of vasodilator responses to acetylcholine and bradykinin.

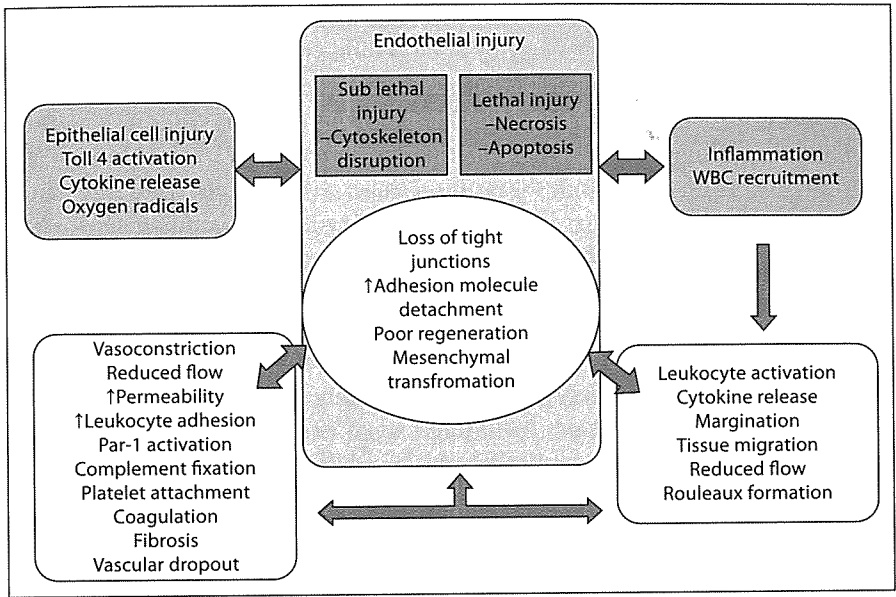


Fig. 1. Events in endothelial cell activation and injury. Ischemia, infection or sepsis cause upregulation and expression of genes encoding for various cell surface proteins such as E- and P-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and reduced thrombomodulin. Activated leukocytes adhere to endothelial cells through these adhesion molecules. Endothelial injury increases the production of endothelin-1 and decreases eNOS which serve to induce vasoconstriction and platelet aggregation leading to a hypercoagulable state in the microvasculature. The combination of leukocyte adhesion and activation, platelet aggregation, and endothelial injury serves as the platform for vascular congestion of the kidney microvasculature. Defects increase permeability between endothelial cells as a result of tight and adherens junctional alterations. The close proximity and crosstalk of the epithelial PT cells with the microvascular endothelial cells, via release of cytokines and chemokines, further enhance ongoing inflammation. Dendritic cells are also thought to play a major role in this inflammatory cascade and serve as a mediator between the endothelial and epithelial cells.

Selective inhibition, depletion or deletion of inducible NOS have clearly shown renoprotective effects during ischemia [10, 11]. The relative decrease in eNOS, secondary to endothelial dysfunction and damage, may result in a loss of anti-thrombogenic properties of the endothelium, leading to increased susceptibility to microvascular thrombosis [9]. Administration of L-arginine, NO-donor molsidomine or the eNOS cofactor tetrahydrobiopterin can preserve medullary perfusion and attenuate AKI induced by ischemia/reperfusion (I/R). Conversely, the administration of N-nitro-L-arginine methyl ester, an NO blocker, has been reported to aggravate the course of AKI following I/R injury. Although clearly important, these pharmacological studies continue to assess the contribution of eNOS impairment in the overall course of reduced renal function following I/R

[12, 13]. Hence, in ischemic AKI, there is an imbalance of eNOS and inducible NOS leading to tissue injury likely by several different pathways.

Permeability

The endothelial barrier serves to separate the inner space of the blood vessel from the surrounding tissue and to control the exchange of cells and substances between the two. This barrier is diminished during ischemia and sepsis, leading to a loss of integrity [14]. Both transcellular and paracellular pathways participate, the latter being a major contributor to the inflammation-induced barrier dysfunction. Sutton et al. [6, 15] have studied the role of endothelial cells in AKI in a series of experiments utilizing fluorescent dextrans and 2-photon intravital imaging. The increased microvascular permeability observed in AKI is likely a combination of several factors including loss of endothelial monolayer integrity, alterations in the actin cytoskeleton, breakdown of perivascular matrix, alterations of endothelial cell contacts, upregulated leukocyte-endothelial interactions and severe alterations in the integrity of the adherens junctions of the renal microvasculature [6]. In vivo 2-photon imaging demonstrated a loss of capillary barrier function as early as 4 h of reperfusion with maximal effects seen 24 h after injury.

The breakdown of barrier function may also be due to matrix metalloproteinase-2 or -9 activation, and this upregulation is temporally correlated with an increase in microvascular permeability [5, 15]. In addition, minocycline, a broad-based matrix metalloproteinase inhibitor, and the gelatinase-specific inhibitor ABT-518 both ameliorated the increase in microvascular permeability in this model.

Coagulation

Endothelial cells play a central role in coagulation regulation via interaction with protein C through the endothelial cell protein C receptor and thrombomodulin. Protein C is activated by thrombin-mediated cleavage and the rate of this reaction is further augmented 1,000-fold when thrombin binds to the endothelial cell-surface receptor protein thrombomodulin. The activation rate of protein C is further increased approximately 10-fold when the endothelial cell protein C receptor binds protein C and presents it to the thrombin:thrombomodulin complex. Then, activated protein C essentially has antithrombotic actions, profibrinolytic properties and participates in numerous anti-inflammatory and cytoprotective pathways to restore normal homeostasis [16]. It has also been shown to be an agonist of protease activated receptor-1 [17].

During an inflammatory response, many of the natural anticoagulants including protein C are consumed along with downregulation of the endothelial cell protein C receptor and thrombomodulin expression, which decreases the anticoagulant and anti-inflammatory effects of the protein C pathway. Damaged endothelial cells undergo apoptosis and this further contributes to

amplifying the coagulation cascade because the disrupted endothelium provides a procoagulant surface [18]. There is also expression of the tissue factor and von Willebrand factor, as well as interaction with platelets [19]. Activation of the inflammation and coagulation pathways continue to cycle with little control, leading to enhanced microvascular coagulation and further endothelial cell dysfunction. Ultimately, microvascular function is compromised resulting in disseminated intravascular coagulation and microvascular thrombosis, decreased local tissue perfusion and organ dysfunction/failure. It has shown that both pre-treatment and postinjury treatment with soluble thrombomodulin attenuates renal injury by minimizing vascular permeability defects with improvement in capillary renal blood flow [20].

Both leukocytes and endothelial cells are dynamically involved in the process of adherence of leukocytes to the vascular endothelium. Leukocyte activation including expression of cell surface adhesion molecules and release of cytokines require signals through chemokines circulating in the bloodstream, or through direct contact with the endothelium.

Rolling leukocytes can be activated by chemoattractants such as complement C5a and platelet-activating factor. Once activated, leukocyte integrins bind to endothelial ligands to promote firm adhesion, with β_2 -integrin (CD18) being most important. These interactions with the endothelium are mediated through endothelial adhesion molecules that are upregulated during ischemic conditions [21]. Singbartl and Ley [21] have found that platelet P-selectin and not endothelial P-selectin was the main determinant in neutrophil-mediated ischemic renal injury. There is also significant protection from both ischemic injury and mortality by blockade of the shared ligand to all three selectins (E-, P- and L-selectin) which seems to be dependent on the presence of a key fucosyl sugar on the selectin ligand [22, 23]. In a CLP model of septic azotemia, mice gene-deficient for E-selectin or P-selectin, or both, were completely protective.

Long-Term Effects of Endothelial Cell Injury

Recent evidence indicates injury to endothelial cells may have long-term chronic disease implications. Basile [24] documented significant reductions in blood-vessel density following acute ischemic injury leading to the phenomenon of vascular dropout. This was verified by Horbelt et al. [25] who found a drop in the vascular density of almost 45% at 4 weeks after ischemic insult. Endothelial cells undergo mesenchymal transformation and lack regenerative potential [26]. Furthermore, vascular endothelial growth factor (VEGF-121), which is downregulated after injury, minimized vascular dropout, but had no effect on the proliferative potential of endothelial cells. These data imply VEGF-121 functions to limit apoptosis and prevent endothelial to mesenchymal transformation, and the resulting fibrosis and microvascular dropout. The important role for endothelial cells after injury in fibrosis is in agreement with data from three

different models of interstitial fibrosis where a significant number of interstitial fibroblasts were derived from endothelial cells [27]. Therefore, the lack of vascular endothelial repair could be due to lack of VEGF, as shown by experiments where administration of VEGF-121 preserved the microvascular density [28]. This reduction of the microvasculature density is thought to mediate increases in hypoxia-mediated hypoxia-inducible factor production and fibrosis, as well as alter proper hemodynamics, leading to hypertension. This may play a critical role in accelerating progression of chronic kidney disease following initial recovery from I/R-induced AKI [24, 29]. Vascular dropout may also predispose to recurrent ischemic events and AKI [30, 31].

Targets of Therapy – Role of Endothelial Progenitor Cells

There are several targets available to reduce the effect of endothelial cell injury as well as potentially minimize actual endothelial cell damage itself. The concept of restoration of vascular supply to damaged or ischemic organs for accelerating their regeneration is well established. One therapeutic strategy based on this concept is the delivery of angiogenic factors. The current view is that endothelial progenitor cells are a heterogeneous group, originating from hematopoietic stem cells or their angioblastic subpopulation and mesenchymal stem cells. In bone marrow, these cells are characterized by the combination of surface markers such as CD34, VEGF-R2 (Flk-1) and an early marker CD133; moreover, in the blood they may express markers of hematopoietic stem cells, c-kit and Sca-1. Upon further differentiation, these cells lose CD133 and acquire VE cadherin and von Willebrand factor [32]. Recent data also suggest that endothelial progenitor cells are mobilized after acute ischemic injury and are recruited to the ischemic kidney where they can ameliorate AKI through both paracrine effects as well as repair of the injured renal microvasculature [33].

White Blood Cells and Parenchymal Inflammation

Inflammation and recruitment of leukocytes during epithelial injury are now recognized as major mediators of all phases of endothelial and tubular cell injury. Inclusion of WBC endothelial interactions is beyond the scope of this review. However, figure 1 tries to emphasize the interdependent roles of WBC and endothelial and epithelial cells in mediating tissue injury. Early inflammation is classically characterized by margination of leukocytes to the activated vascular endothelium via interactions between selectins and ligands that allows firm adhesion, followed by transmigration. A number of potent mediators are generated by the injured proximal epithelial tubular cell, including proinflammatory cytokines such as TNF- α , IL-6, IL-1 β , MCP-1, IL-8, TGF- β and RANTES [34]. Toll-like receptor 2 (TLR2) has been shown to be an important mediator of endothelial ischemic injury, while TLR4 has been shown to play a similar role

in animal models of both ischemic and septic injury [35], especially in PT cells [36].

TLR2 and TLR4 are constitutively expressed on renal epithelium, and their expression is enhanced following renal I/R injury. El-Achkar et al. [4] have shown that in the CLP rat model of sepsis, TLR4 expression increases markedly in all tubules (proximal and distal), glomeruli and the renal vasculature. Furthermore, they demonstrated that in sepsis there is a TLR4-dependent increase in the expression of proinflammatory mediator Cox-2, which was mostly restricted to cortical and medullary thick ascending loops of Henle which characteristically express and secrete Tamm-Horsfall protein [37]. Tamm-Horsfall protein may stabilize the outer medulla in the face of injury by decreasing inflammation, possibly through an effect on TLR4 [38]. Genetic deletion of either TLR2 or TLR4 protects from renal I/R injury [36, 39], thus indicating the prominent role TLR plays in AKI.

Dissociation of Renal Blood Flow and Glomerular Filtration

A conundrum often exists regarding the dissociation between total renal blood flow and GFR in states of AKI. Possible explanations include an obstructed tubule with luminal pressure exceeding filtration pressure, backleak of filtrate across injured epithelial cells and a reduction in K_f mediating a reduced permeability to filtration. In these situations, glomerular blood flow could proceed uninterrupted and net GFR would be reduced. However, we also know afferent arteriole vasoconstriction plays an important role in the pathophysiology of reduction in GFR during ischemic and septic AKI. This would not dissociate renal blood flow and GFR, but yet we know they are. So, how does one explain these observations?

Extensive anatomical studies using several techniques to visualize arteriole level vessels within the kidney, performed early on, clearly delineated glomerular pathways in the outer cortex (Ludwig's arteriole) and juxtamedullary cortex (vasa recta vera) [40, 41]. Using 2-photon microscopy techniques, we have begun to investigate the anatomical and functional existence of glomerular or Ludwig's vessel in the rat outer cortex (fig. 2). The existence of such afferent to efferent shut vessels is known to be common in the juxtamedullary cortex, but whether or not they connect to glomerular capillaries is unknown. Therefore, this vessel could divert flow around the glomerulus, allowing for dissociation of renal blood flow and afferent arteriole vasoconstriction-mediated reduced GFR. This would be important in maintaining cortical peritubular capillary flow in diseases involving obstruction of glomerular capillary flow.

In summary, AKI involves a complex interplay of a number of different types of cells. Epithelial cell injury mediates functional alterations in cases of direct failure of the cells to transport ions and molecules or indirect mediation of a

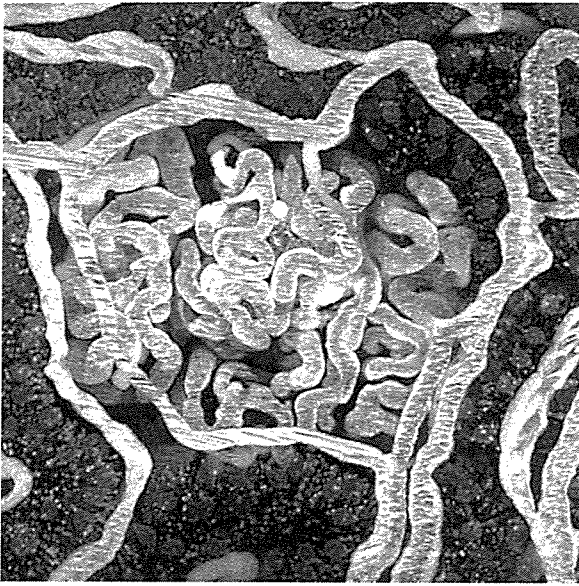


Fig. 2. Glomerular and peritubular microvascular connections. 3-D reconstruction of the outer portion of a surface glomeruli in a Munich-Wistar Fromter rat using 2-photon microscopy to acquire individual 1- μm planes. A 150-kDa fluorescent dextran provides the fluorescence detected only within the arteriole and capillary/venous microvasculature. Note the many connections between surface vessels and capillaries. Additional studies will be needed to interrelate the functional and anatomic aspects of these vessels and their importance in AKI.

fall in GFR. They also influence the function of endothelial cells by release of chemokines and cytokines and other soluble mediators. Endothelial and WBC interactions lead to continued hypoxia, inflammation and further epithelial cell injury and dysfunction. Although we try to study individual cell types in isolation, it is important to realize this does not occur in vivo where cell:cell interactions and autocrine and paracrine effects are essential components of tissue response.

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