Kidney International, Vol. 62 (2002), pp. 1539–1549

PERSPECTIVES IN BASIC SCIENCE

Microvascular endothelial injury and dysfunction during ischemic acute renal failure

TIMOTHY A. SUTTON, CHARLES J. FISHER, and BRUCE A. MOLITORIS

Division of Nephrology, Department of Medicine, and the Indiana Center for Biological Microscopy, Indiana University School of Medicine, Indianapolis, Indiana; Abbott Laboratories, Chicago, Illinois; and the Roudebush VA Medical Center, Indianapolis, Indiana, USA

Microvascular endothelial injury and dysfunction during ischemic acute renal failure. The pathophysiology of ischemic acute renal failure (ARF) appears to involve a complex interplay between renal hemodynamics, tubular injury, and inflammatory processes. While the current paradigm of the pathophysiology of ischemic ARF invokes both sublethal and lethal tubular injury as being of paramount importance to diminished renal function, a growing body of evidence supports the contribution of altered renal vascular function in potentially initiating and subsequently extending the initial tubular injury. We propose that the "extension phase" of ischemic ARF involves alterations in renal perfusion, continued hypoxia, and inflammatory processes that all contribute to continued tubular cell injury. Vascular endothelial cell injury and dysfunction play a vital part in this extension phase. In the constitutive state the endothelium regulates migration of inflammatory cells into tissue, vascular tone and perfusion, vasopermeability, and prevents coagulation. Upon injury, the endothelial cell loses its ability to regulate these functions. This loss of regulatory function can have a subsequent detrimental impact upon renal function. Vascular congestion, edema formation, diminished blood flow, and infiltration of inflammatory cells have been documented in the corticomedullary junction of the kidney, but linking their genesis to vascular endothelial injury and dysfunction has been difficult. However, new investigative approaches, including multiphoton microscopy and the Tie2-GFP mouse, have been developed that will further our understanding of the roles endothelial injury and dysfunction play in the pathophysiology of ischemic ARF. This knowledge should provide new diagnostic and therapeutic approaches to ischemic ARF.

Renal ischemia is the leading cause of acute renal failure (ARF) and delayed graft function. Over the past 10 to 15 years, important advances have been made in establishing the roles that altered vascular reactivity, tubular epithelial cell injury, and inflammation play in the pathogenesis of ischemic ARF. Emphasis has been placed on tubular epithelial cell injury during and immediately following the ischemic event. Events occurring at the cellular level have been identified and a much more complete understanding of how these cellular events result in cellular and organ dysfunction has been established. Although the renal tubular epithelial cell injury that initially occurs during an ischemic event undoubtedly plays a central role in ischemic ARF, there is growing evidence that additional mechanisms, including renal vascular endothelial (VE) injury and dysfunction, play an important part in extending renal tubular epithelial injury and thus contribute to the ongoing pathogenesis of ischemic ARF. Therefore, we propose the addition of an "extension phase" to the current paradigm of ischemic ARF during which renal VE injury and dysfunction play an important role. The contribution that VE injury and dysfunction make to the functional derangements observed during and following ischemic injury in other organ systems has become increasingly appreciated [1–3]. Differences may very well exist between endothelial cells in vascular beds of different organs as well as in different vascular beds of the same organ [4]. However, we feel that data from other organs in conjunction with what sparse information exists regarding alterations in the renal vasculature during ischemic ARF now indicate it is time to reevaluate the potential role that immediate and delayed injury to endothelial cells during and following ischemic injury plays in the pathophysiology of ischemic ARF. Therefore, the purpose of this review is to summarize what is known about endothelial injury/dysfunction during and following ischemia in the kidney, to present data on endothelial injury and dysfunction during ischemia from other organs in an attempt to infer possibilities for the role of the endothelium in ischemic ARF, to place these data into a unifying hypothesis regarding immediate and delayed epithelial cellular and organ dysfunction, and to demonstrate how newly available models will allow for progress not hitherto possible. Finally, we wish to indi-

Key words: endothelium, acute renal failure, ischemia, kidney disease, inflammation, coagulation.

Received for publication March 1, 2002
and in revised form May 1, 2002
Accepted for publication June 21, 2002

cate how therapy of ARF should take into account the pathophysiology of endothelial dysfunction.

VASCULAR ENDOTHELIUM AS AN ORGAN

Endothelial cells form an essentially contiguous barrier throughout the vessels that course through every organ. The endothelium exercises a variety of very specific tasks and insomuch functions as a highly specialized organ. The concept of the endothelium as an organ has become more widely appreciated [5, 6] given the recent recognition of the consequences of endothelial injury and dysfunction in a range of disease states such as sepsis, hemolytic uremic syndrome (HUS)/thrombotic thrombocytopenic purpura (TTP), diabetes, and hypertension. The vascular endothelium regulates vascular permeability and modulates vasomotor, inflammatory, and hemostatic responses. Impairment of these vital endothelial cell functions during and following renal ischemia can contribute to the impairment of renal perfusion, continued renal hypoxia, and the subsequent epithelial cell injury and diminution in the glomerular filtration rate (GFR) that are the hallmarks of ARF.

CLINICAL PHASES OF ACUTE RENAL FAILURE

Clinically, ischemic ARF has classically been divided into the "Initiation," "Maintenance" and "Recovery" phases. To this paradigm we would like to add an "Extension" phase; this article explains the importance of this phase in the pathophysiology of ARF and how ischemic ARF should be approached clinically. Recent studies now allow a direct relationship to be drawn between the clinical phases and the cellular phases of ischemic ARF (Fig. 1). Prerenal azotemia, which often precedes the initiation of ischemic ARF and may be part of a continuum with the "initiation phase" of ischemic ARF, occurs with reduced renal blood flow (RBF) and is associated with reduced organ function (decreased GFR). Cellular integrity is maintained during prerenal azotemia through vascular and cellular adaptive responses.

Initiation phase. The "initiation phase" of ischemic ARF occurs when RBF decreases to a level resulting in severe cellular adenosine 5′-triphosphate (ATP) depletion, which in turn leads to acute cell injury and dysfunction. Renal tubular epithelial cell injury is a key feature of the initiation phase. Renal ischemia in vivo rapidly induces a number of structural and functional alterations in renal proximal tubular epithelial cells that are directly related spatially and temporally with disruption of the normal framework of filamentous actin (F-actin) in the cell [7–9]. The extent of these alterations depends upon the severity and duration of ischemic injury. Although these alterations usually fall short of being lethal to the cell, they do disrupt the ability of renal tubular epithelial cells and renal vascular endothelial cells to maintain normal renal function. Additionally, ischemic injury to vascular smooth muscles cells and endothelial cells during the initiation phase may contribute to the structural abnormalities observed in the renal vasculature during ischemic ARF [10–12].

Recent evidence now indicates that "activation" of epithelial and possibly endothelial cells during the early initiation phase results in the up-regulation of a variety of chemokines and cytokines including but not limited to interleukin (IL)-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-α (TNF-α) [13, 14], which are instrumental in initiating the inflammatory cascade. For example, early up-regulation and release of TNF-α and activation of nuclear factor-κB (NF-κB) has been observed in an animal model of renal ischemic injury [15]. The cellular localization of these phenomena within the kidney is just beginning to be elucidated [16]. As these and other events resulting in cellular alterations are not well identified morphologically, they have been difficult to evaluate using standard methodologies. Therefore, what role these early cellular responses play in further worsening renal perfusion remains to be determined.

Extension phase. The proposed "extension phase" is ushered in by two major events: continued hypoxia following the initial ischemic event and an inflammatory response. Both events are more pronounced in the corticomedullary junction (CMJ), or outer medullary region, of the kidney. Documentation of severely reduced blood flow, stasis and accumulation of red and white blood cells (RBC, WBC) has been historically noted; however, the epithelial ramifications of these events have only recently been uncovered [13, 17]. It is during this phase that renal vascular endothelial cell damage likely plays a key role in the continued ischemia of the renal tubular epithelium as well as the inflammatory response observed with ischemic ARF. During this phase cells continue to undergo injury and death, with both necrosis and apoptosis being present predominantly in the outer medulla [18]. In contrast, the proximal tubule cells in the outer cortex, where blood flow has returned to near normal levels, actually undergo cellular repair and improve morphologically during this phase. As cellular injury continues in the CMJ region during the extension phase, the GFR continues to fall. There is continued production and release of chemokines and cytokines that further enhance the inflammatory cascade. Interrupting the amplification of this inflammatory cascade may have therapeutic implications. For example, inhibition of TNF-α has been shown to limit the decrease in GFR occurring in the renal artery clamp model [19]. Although the extension phase is probably the most likely phase for therapeutic intervention in ischemic ARF, there is a short therapeutic window of opportunity. Based on animal models of renal ischemia,

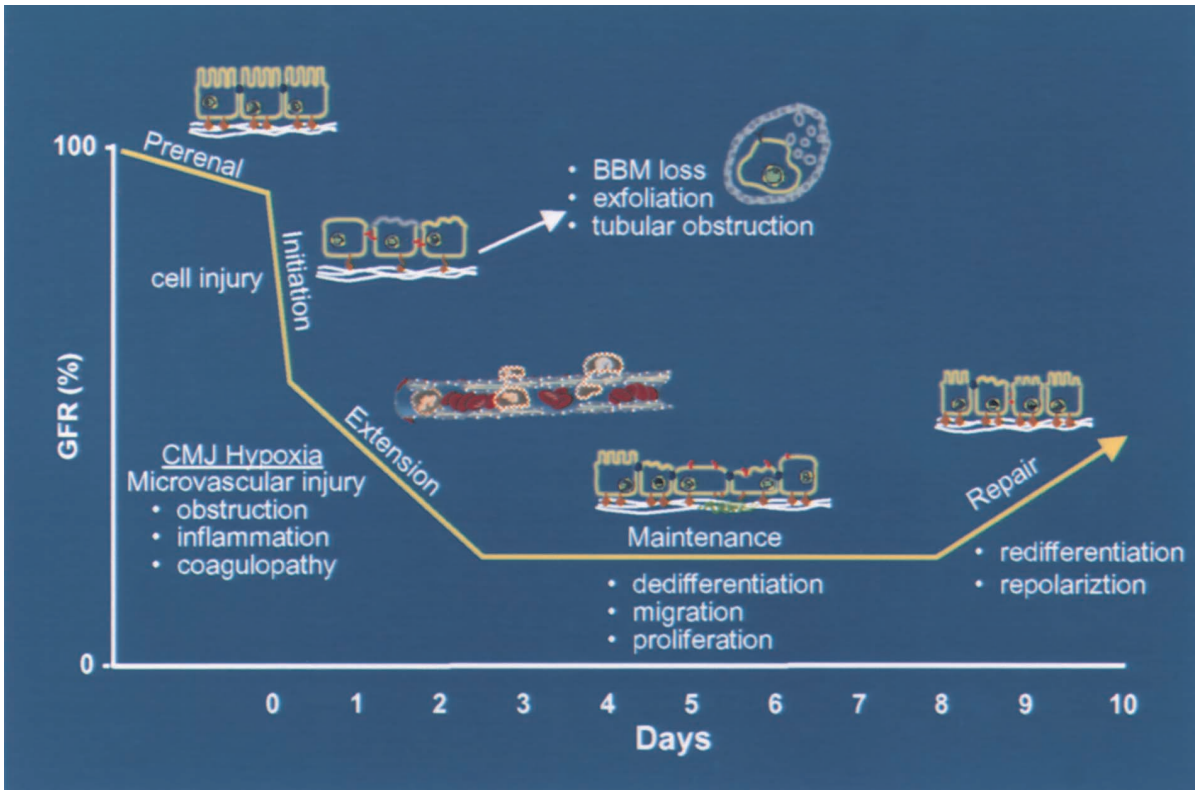
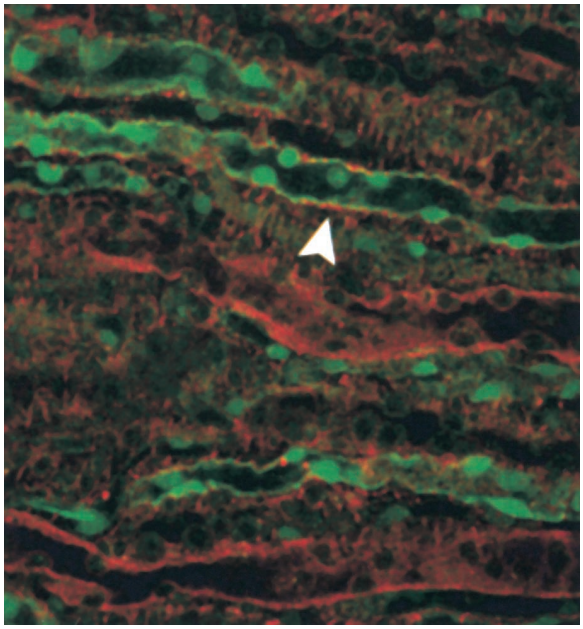


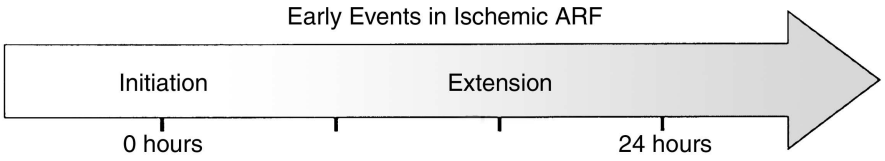
Fig. 1. Relationship between the clinical phases and the cellular phases of ischemic acute renal failure (ARF), and the temporal impact on organ function as represented by the glomerular filtration rate (GFR). Prerenal azotemia exists when a reduction in renal blood flow causes a reduction in GFR. A variety of cellular and vascular adaptations maintain renal epithelial cell integrity during this phase. The initiation phase occurs when a further reduction in renal blood flow results in cellular injury, particularly the renal tubular epithelial cells, and a continued decline in GFR. Vascular and inflammatory processes that contribute to further cell injury and a further decline in GFR usher in the proposed extension phase. During the maintenance phase, GFR reaches a stable nadir as cellular repair processes are initiated in order to maintain and re-establish organ integrity. The recovery phase is marked by a return of normal cell and organ function that results in an improvement in GFR.



inflammatory cell infiltration in the outer medullary region of the kidney is significant by 24 hours following ischemia [20–22], although leukocytes may begin to migrate in as early as two hours after ischemia [23]. Figure 2 summarizes the proposed cellular interplay involved in the pathophysiology of the initiation and extension phases during ischemic ARF.

Maintenance and recovery phases. During the clinical phase known as "maintenance," cells undergo repair, migration, apoptosis and proliferation in an attempt to re-establish and maintain cellular and tubule integrity. The GFR is stable albeit at a level determined by the severity of the initial event. This cellular repair and reorganization phase results in slowly improving cellular function and sets the stage for improvement in organ

Fig. 3. Localization of vascular endothelium in the Tie2-GFP mouse. Confocal image of the corticomedullary region of the mouse kidney. The GFP-labeled endothelium of the microvasculature is easily identifiable (arrowhead). Sections were also stained with rhodamine-phalloidin to label actin structures (red).



Event	Ischemia	Vascular congestion with hypoxia	Inflammation
Site	Entire kidney	Cortical medullary junction	Cortical medullary junction
Cells Affected	Epithelial cells • especially PTC S_1–S_3	Epithelial cells • PTC-S_3 • TAL	Epithelial cells • PTC-S_3 • TAL
	Vascular smooth muscle cells		
	Endothelial • large and small vessels	Endothelial cells • small vessel	Endothelial cells • small vessels

Fig. 2. Early events in ischemic ARF. The initial ischemic insult results in morphological and functional alterations in the renal tubules and the renal vasculature. Following the initial ischemic insult, further alterations in the renal vascular endothelium of the cortico-medullary junction contribute to inflammation and vascular congestion. These processes are proposed to extend the initial injury to the renal tubules.

function. Blood flow returns toward normal and epithelial cells establish intracellular and intercellular homeostasis. During the "recovery phase" cellular differentiation continues, epithelial polarity is re-established, and normal cellular and organ function returns [8, 24, 25]. Thus, renal function can be directly related to the cycle of cell injury and recovery. There is very little known about endothelial cell events during these two phases. However, from studies in other organs, repair of the endothelium is of key importance to overall recovery.

ISCHEMIC ACUTE RENAL FAILURE

Alterations in renal perfusion

A decrease in renal blood flow is of critical importance in initiating the pathophysiology of ischemic ARF. Under physiological conditions, the oxygen tension of the kidney decreases as one moves from the outer cortex to the inner medulla [26]. Interestingly, studies have provided some evidence that regional alterations in renal blood flow persist after the initial ischemic event. These regional alterations can play an important role in the extension phase of renal ischemic injury. During reperfusion following an ischemic insult, a reduction in total renal blood flow of 40% to 50% of normal has been reported in both animal models of ischemic ARF and in human ischemic ARF [27]. Studies have demonstrated that a persistent reduction in renal blood flow significantly contributes to the diminished GFR observed in human renal allografts following ischemic ARF [28]. These persistent perfusion deficits have been demonstrated to be of greater magnitude in the outer medulla than in the outer cortex or inner medulla in an animal model of ischemic ARF [29, 30].

Mechanisms involved in the alteration of renal perfusion following ischemic injury are incompletely understood. An imbalance between mediators of renal vasoconstriction and renal vasodilation has been proposed to play a role in animal models of ischemic ARF. In support of this observation, antagonists to endogenous vasoconstrictors have been shown to ameliorate renal ischemic injury in animal models [31–34]. The role various vasoreactive mediators may play in controlling renal vascular tone following ischemic injury has been the subject of a recent review [35].

Congestion of the renal microcirculation, especially in the peritubular capillaries of the outer medullary region (vasa recta), contributes to deficits in renal perfusion. Accumulation of red blood cells and leukocytes in the outer medulla has been demonstrated in animal models of ischemic ARF as well as in human ischemic ARF [29, 36–38]. This medullary congestion has been proposed to shunt blood flow away from the outer medulla, resulting in continued hypoxia and cellular injury in this area. Experimental maneuvers to diminish trapping of red blood cells and leukocyte attachment in the renal microcirculation have been demonstrated to improve morphological and functional aspects of renal injury in animal models of ischemic ARF [34, 39–42].

Ischemic injury to endothelial cells and subsequent cell swelling has been demonstrated to contribute to vascular obstruction in other organs [43]. Given the susceptibility to hypoxia in the outer medulla, endothelial ischemic injury and cell swelling may be a plausible mechanism contributing to the congestion observed in this region. Lastly, activation of the coagulation pathway via a damaged endothelium may negatively impact the rheologic properties of the blood, and play a role in stasis and decreased perfusion in the CMJ. The potential importance of activation of the coagulation pathway in the pathogen-

esis of ischemic ARF is highlighted by studies that demonstrate pretreatment with anticoagulants have a beneficial effect in animal models of ischemic ARF [44–47], although this is tempered somewhat by opposing studies that have demonstrated no benefit [48, 49]. This difference in outcomes may reflect a more complex interplay of the coagulation pathway with other pathways, such as the inflammatory cascade, than just serving as an isolated thrombotic pathway.

Morphology of vascular injury

The morphologic changes of the renal vasculature described to date during ischemic ARF in animal models and in humans have been subtle and limited to the smooth muscle layer in renal arterioles [10–12]. Interestingly, no consistent morphologic changes of the renal vascular endothelium in ischemic ARF have been reported. In part this may be due to sampling bias in human ischemic ARF (that is, predominantly cortical vs. medullary tissue on biopsy) and difficulty in visualizing the endothelium of potentially affected microvasculature in animal models. Although no consistent morphologic alterations have been ascribed to the endothelium of the renal vasculature, evidence of endothelial dysfunction and injury in other organ systems as a result of ischemic injury lends credence to the concept that endothelial dysfunction and injury play an important role in ischemic ARF [1, 50]. Separation of endothelial tight junctions, loss of endothelial attachment to the basement membrane, endothelial blebbing, and endothelial necrosis have been described in the cerebral and coronary vasculature following ischemic injury [51, 52]. In patients experiencing septic shock, a condition that shares many pathological derangements with ischemic injury [53–55] and is often a concomitant condition in human ischemic ARF [56], detached, circulating endothelial cells have been documented [57]. Potential functional consequences of these morphological alterations include altered vascular reactivity, increased vascular permeability, increased leukocyte adherence and extravasation, and altered coagulation due to loss of normal endothelial function and/or barrier. Furthermore, circulating activated endothelial cells could potentially contribute to distant organ effects attributed to leukocytes such as pulmonary dysfunction following ischemic ARF [58].

Functional aspects of endothelial injury

Alterations in endothelial permeability. Increased peritubular capillary permeability has been documented as a consequence of ischemic ARF in animal models [41]. Two general mechanisms can account for increased endothelial permeability during ischemic injury: increased paracellular permeability and/or increased transcellular permeability [3]. While most evidence favors increased paracellular transport as the pathway for movement of fluid and most solutes, recent evidence has made it apparent that transcellular movement of large molecules and importantly albumin may play an important role in endothelial permeability [3].

Specialized cellular junctions similar to those in epithelial cells maintain endothelial cell-cell contacts. Cadherin-containing adherens junctions are ubiquitous between endothelial cells throughout the vasculature [59]. Tight junctions are more prominent in "tight" vascular beds such as between the endothelial cells of the cerebral vasculature forming the blood-brain barrier, whereas they are sparse and simplified in "leakier" vascular beds such as post-capillary venules of many organ vascular beds [59]. Recent studies highlighting the differences in the molecular composition of junctional complexes in various vascular beds, including those within the kidney, provide insight into the functional differences of the cellular junctions in these vascular beds [60–63]. Classic desmosomes are not present in endothelial cells [64]. In general, these various specialized cellular junctions are not as organized spatially or morphologically in endothelial cells as they are in epithelial cells. There is essentially no in vivo information on the effect of ischemic injury on the function and organization of these intercellular junctions between endothelial cells in the renal vasculature. Furthermore, very few in vivo data exist on this subject in other vascular beds. However, disruption of endothelial adherens junctions in vivo by the use of an inhibitory antibody to VE cadherin (cadherin-5) has been demonstrated to induce gaps between endothelial cells, increase endothelial permeability, and promote the accumulation of inflammatory cells in coronary and pulmonary vascular beds [65]. This finding demonstrates the importance of endothelial cell-cell junctions in maintaining the integrity of the endothelial permeability barrier.

Much of the current knowledge regarding the mechanisms regulating endothelial cell-cell interaction during ischemic and oxidant injury has come from in vitro models utilizing cultured endothelial cells. Increased endothelial permeability and intercellular gap formation has been demonstrated with ATP depletion as a model of ischemic injury and with H_2O_2 as a model of oxidant-mediated reperfusion injury [66–68]. Increased endothelial permeability in these models has been associated with the redistribution of occludin and its dissociation from zona occludens 1-antigen (ZO-1) in endothelial tight junctions as well as dissociation and internalization of VE cadherin from endothelial adherens junctions [67, 69, 70].

There is evidence that the interaction of endothelial cell-cell junctions with the actin cytoskeleton plays an important role in regulating endothelial paracellular transport. Transfection of a mutant VE cadherin lacking a portion of the cytoplasmic domain important for binding to β-catenin and linking the adherens junction to the actin cytoskeleton has been demonstrated to increase mono-

layer permeability as compared to cells transfected with wild-type VE cadherin [71]. ATP depletion of endothelial cell monolayers and exposure of endothelial monolayers to oxidants such H_2O_2 have both been demonstrated to alter the normal actin cytoskeleton of endothelial cells. ATP depletion has been demonstrated to disrupt the normal cortical and basal F-actin structures in endothelial cells [72–74]. As has been demonstrated in renal tubular epithelial cells during ATP depletion as well as the renal artery clamp model, F-actin structures associated with the surface membrane breakdown and F-actin aggregates appear dispersed throughout the cytoplasm [73]. Total cellular F-actin content increases during ATP depletion in many cell types in cell culture studies. Oxidant-mediated endothelial cell injury also has been demonstrated to disrupt the cortical actin band in cultured endothelial cells [75–77]. These same studies have demonstrated an increase in basal actin stress fibers during oxidant-mediated endothelial cell injury [75–77]. Interestingly, there is evidence suggesting that activation of endothelial contraction through actin stress fibers can increase endothelial paracellular permeability [78, 79]. Thus, not only can disruption of the actin cytoskeleton (especially the cortical actin band) increase endothelial paracellular permeability, but activation of contractile force through the actin cytoskeleton (tensegrity model) [80] can also increase endothelial paracellular permeability.

Numerous signaling pathways have been implicated in the regulation of the actin cytoskeleton including pathways involving the Rho family GTPases, phosphoinositides, tyrosine kinases, protein kinase C, and cAMP-dependent protein kinase [81–86]. The complexity and cross talk of the signaling pathways regulating the actin cytoskeleton in endothelial cells during ischemic injury has garnered increased appreciation in recent years. Much work is needed at the molecular level to elucidate the relative contributions of the signaling pathways involved.

Increased interstitial edema may contribute to further diminishing the compromised medullary blood flow by compressing peritubular capillaries [87]. Additionally, leakage of plasma from the vascular space through a leaky endothelium contributes to hemoconcentration that can lead to stasis and diminished perfusion in the CMJ as observed in other organs [43]. Hemoconcentration and stasis also increases the potential for endothelial-leukocyte interactions. Activated leukocytes can initiate an inflammatory cascade that leads to further endothelial cell injury and further dysfunction of the endothelial permeability barrier [88]. This may be particularly important in the medullary region as endothelial cells there, but not in the cortex, express surface markers important in lymphocyte activation [89]. While the interaction of activated leukocytes and the endothelium has been demonstrated to play a role in enhancing endothelial permeability [90–92], increased endothelial permeability during ischemia reperfusion-injury has been documented to occur also in the absence of leukocytes [93]. Therefore, the quantitative role of leukocytes in increasing endothelial permeability remains to be determined.

Alterations in endothelial-leukocyte interactions. As mentioned previously, the stasis of leukocytes observed in the outer renal medulla after reperfusion in human and animal ischemic ARF can potentially extend the injury incurred during ischemia. In addition to physically obstructing the renal microcirculation, activated leukocytes can initiate an inflammatory cascade that leads to endothelial dysfunction and alteration of the endothelial permeability barrier. The relative roles specific leukocytes play in the injury observed in models of ischemic ARF has been a point of uncertainty. A growing body of evidence suggests that T cells may play a pivotal part in this injury [22, 94–96], although a recent study adds to the controversy concerning the role of T cells in ischemic acute renal failure [97]. Regardless of the specific leukocyte type, activated leukocytes can release cytokines, proteases, and mediators of oxidant injury [88], which likely plays an important pathophysiological role in epithelial cell injury and subsequent organ dysfunction.

Endothelial-leukocyte interactions mediated through complementary adhesion molecules on endothelial cells and leukocytes play a key role in the local accumulation of leukocytes. The selectin family of adhesion molecules (L-selectin on leukocytes and P- and E-selectins on endothelial cells) and their carbohydrate-containing counter ligands initiate intermittent, low-affinity binding (rolling) between leukocytes and endothelial cells [98]. Members of the IgG superfamily of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) on endothelial cells and their counter β-integrin receptors on leukocytes, mediate a tighter binding that plays a role in leukocyte extravasation [99].

Ischemic injury has been demonstrated to increase expression of P- and E-selectin on the surface of endothelial cells [100, 101]. Rearrangements of the actin cytoskeleton are important in the rapid delivery of P-selectin from its storage in Weibel-Palade bodies to the surface of endothelial cells [50]. Increased expression of ICAM-1 by endothelial cells has been demonstrated also in vitro in response to oxidant injury [102]. The functional significance of these findings in ischemic ARF is underscored by evidence in animal models that inhibition of P- and E-selectin mediated binding of leukocytes [40] and inhibition of ICAM-1 mediated binding of leukocytes [20, 103] decreases renal injury. Endothelial cell injury and dysfunction additionally may contribute to the inflammatory response through loss of normal endothelial NO production [17].

Alterations in coagulation. Fibrin deposition in the microvasculature following ischemic injury has been noted in a variety of organs systems including the kidney [104–

107]. Although the constitutive state of endothelial cells is an anticoagulant state, injury and activation of endothelial cells can induce a procoagulant response. Whether or not this occurs during prerenal azotemia is unknown. As a result of endothelial injury, anticoagulant substances such as thrombomodulin are internalized or released in an inactive form [108]. Thrombomodulin acts as a molecular switch that converts thrombin from activating procoagulant pathways to preferentially activating anticoagulant pathways. Loss of membrane-bound thrombomodulin activity promotes the local generation of fibrin by thrombin and diminishes activation of protein C by thrombin [109]. Diminished activated protein C (aPC) results in the decreased formation of protein C/protein S complexes thus reducing the inactivation of factors Va and VIIIa and further promoting local thrombin generation [110]. The net result is a shift in the balance toward a procoagulant response. As a result of endothelial cell injury, the profibrinolytic properties of the endothelial cell are also diminished. Loss of tissue plasminogen-activator (tPA) secretion by the injured endothelium, release of plasminogen-activator inhibitor (PAI) from localized inflammatory cells [111] that can inhibit what little tPA is released, and loss of activated protein C inhibition of thrombin-activatable fibrinolysis inhibitor (TAFI) [109] can all contribute to diminished fibrinolysis. Furthermore, loss of NO production by injured endothelial cells also may contribute to an overall procoagulant state through loss of its inhibitory role on cytokine-induced expression of tissue factor [112]. While abnormalities in coagulation per se may have a deleterious role in ischemic ARF, recent studies have shed light on the relative importance the coagulation cascade plays as a mediator of inflammation in ischemic ARF [47, 113].

FUTURE DIRECTIONS AIMED AT THE EXTENSION PHASE

As mentioned previously, investigating the role that alterations in the renal vasculature play in the pathophysiology of ischemic renal injury during the extension phase has been difficult. Fortunately, new tools may provide avenues of fruitful investigation into this area. Spatially localized genomic approaches including direct in situ reverse transcription-polymerase chain reaction (RT-PCR) assays or real time PCR assays coupled with laser-capture microdissection have the potential to provide new insight into the complexity of gene expression in the renal vasculature during injury and recovery [114–117]. Similarly, progress in proteomics may provide powerful and complimentary information to genomic approaches [118, 119]. Functional deletion of proteins in a tissue specific manner or exploitation of tissue specific promoters may further our understanding of the mechanisms involved in renal injury and repair, as well as, provide a means to localize the cell types involved. For example, the transgenic mouse developed by Motoike et al that expresses the green fluorescent protein (GFP) under control of the endothelial-specific Tie2 promoter provides a tool to identify and visualize the endothelium of the renal vasculature (Fig. 3) [120].

Functional imaging techniques, including spinning-disc confocal fluorescence microscopy, 2-photon microscopy, and blood oxygenation level-dependent (BOLD) magnetic resonance imaging (MRI), are other potentially powerful investigational tools that have the promise of providing new insights into the in vivo functional alterations that occur during ischemic renal injury [121, 122]. For example, BOLD MRI is a noninvasive technique that takes advantage of the capacity of deoxyhemoglobin to act as an endogenous contrast agent. This technique has been utilized to examine regional alterations in oxygenation of the kidney in response to various physiological and pathophysiological perturbations such as diuretic and intravenous radiocontrast administration [123–125].

Recent advances made toward delineating the cellular mechanisms involved in ischemic ARF have not yet led to accepted therapeutic interventions that alter the natural course of ARF or improve the clinical outcome for ARF. Overall mortality for ARF remains between 40 and 50% in a general series of patients and 70 and 80% for patients in intensive care units [126]. Moreover, 1 to 2% of all patients and 30% of patients in the intensive care unit who survive an episode of ARF requiring dialytic therapy become dependent on long-term dialysis or renal transplantation [126, 127]. Some excellent reviews that outline potential therapeutic strategies for the treatment of ARF and that detail some of the barriers that need to be overcome in bringing effective therapies to fruition have been recently published [14, 88]. Needless to say, human ARF is heterogeneous in its pathophysiology. Consequently, combined therapies targeting more than one pathophysiological pathway may prove to be the most beneficial approach [14, 88]. It is also necessary to point out that differences exist between animal models of ARF and what has been documented to occur during human ARF. These differences and similarities have been recently discussed in two reviews and are beyond our scope [128, 129]. However, a major problem is the lack of clinical data, especially in the cortical-medullary junctional area, during the early phases—initiation and extension—of human ARF.

Therapies directed at processes during the extension phase including endothelial dysfunction/injury and its myriad of pathophysiological implications will have a role in this therapeutic approach. Promising approaches include targeting endothelial injury with agents such as lecithinized superoxide dismutase [130], targeting endothelial activation of the coagulation cascade with agents such as activated protein C (APC) [113], and targeting enhanced

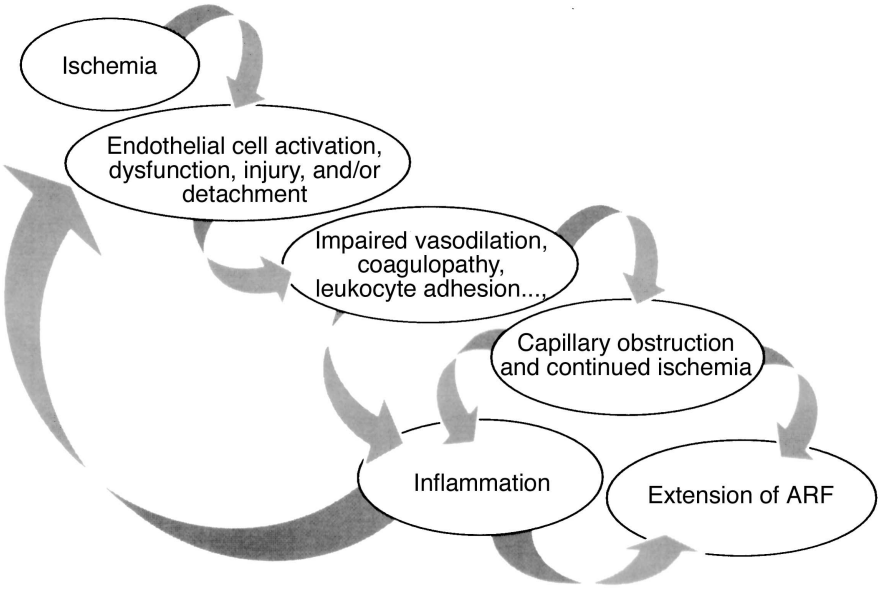


Fig. 4. Proposed contribution of vascular endothelial cell injury and dysfunction to injury during ischemic ARF. Altered coagulation, vascular tone, vasopermeability, and regulation of inflammatory cell adhesion and migration into tissue as a result of endothelial cell injury and dysfunction during ischemic ARF may contribute to continued hypoxia and inflammation especially in the corticomedullary region. Consequently, endothelial cell injury is proposed to extend the initial ischemic injury to the tubular epithelial cells in this region.

endothelial-leukocyte interactions with agents such as anti-B7-1 [95], anti-ICAM-1 antibodies [20], P-selectin antagonists [40], platelet activating factor (PAF) antagonists [34], adenosine 2A receptor agonists, phosphodiesterase type IV antagonists [131], and TNF-α binding protein [19]. This list is not meant to categorize these biomolecules into one particular mode of action as it clear that some have an impact on more than one pathophysiological pathway. For example, the beneficial effect of APC may be the result of its anti-inflammatory properties more than its anticoagulant or fibrinolytic properties [113]. Furthermore, if vector technology can reliably traverse the glomerular capillary network, the endothelium of the CMJ would be an accessible target for gene therapy. Although early recognition of renal injury and prompt intervention remain to be clinical challenges in this field, ameliorating endothelial dysfunction/injury may ultimately provide an important prong in the therapeutic armamentarium of ischemic ARF.

SUMMARY

A growing body of evidence lends support to the roles endothelial dysfunction and vascular injury play in overall renal injury during ischemic ARF. This may be especially important during the early events of ischemic ARF (Fig. 4). Furthermore, vascular injury and dysfunction resulting from acute ischemic injury may have long-term ramifications in regards to renal function even after apparent recovery from the initial insult [132]. Given that the endothelium is central to the myriad of biological processes performed by the microvasculature, endothelial injury and dysfunction is a crucial factor in the overall alteration of vascular function during both the initiation and extension phases. Further investigation into the mechanisms of endothelial injury and dysfunction during the extension phase should provide more insight into the pathophysiology of ischemic ARF and reveal additional as well as novel therapeutic interventions.

NOTE ADDED IN PROOF

Since acceptance of this manuscript in final form, important articles by Yamamoto et al (*Am J Physiol Renal Physiol* 282:F1150–F1155, 2002) and Brodsky et al (*Am J Physiol Renal Physiol* 282:F1140–F1149, 2002) have been published that give further evidence for the importance of vascular and endothelial dysfunction/injury in the pathophysiology of ischemic acute renal failure.

ACKNOWLEDGMENTS

This work was supported by the following grants: NIH/NIDDK (53465), NIDDK (41126), and VAMR grants to BAM and NIDDK (60621) and NKF of Indiana grants to TAS. The authors acknowledge Ms. Melanie Hosford and Mr. Ruben Sandoval for their technical expertise in the preparation of this manuscript and the accompanying figures.

Reprint requests to Timothy A. Sutton, M.D., Ph.D., Division of Nephrology, Indiana University School of Medicine, 1120 South Drive, Fesler Hall 115, Indianapolis, Indiana 46202, USA.
E-mail: tsutton2@iupui.edu

REFERENCES

1. Carden DL, Granger DN: Pathophysiology of ischaemia-reperfusion injury. *J Pathol* 190:255–266, 2000
2. Menger MD, Richter S, Yamauchi J, *et al*: Role of microcirculation in hepatic ischemia/reperfusion injury. *Hepatogastroenterology* 46(Suppl 2):1452–1457, 1999
3. Stevens T, Garcia JG, Shasby DM, *et al*: Mechanisms regulating endothelial cell barrier function. *Am J Physiol (Lung Cell Mol Physiol)* 279:L419–L422, 2000

4. Stevens T, Rosenberg R, Aird W, *et al*: NHLBI workshop report: Endothelial cell phenotypes in heart, lung, and blood diseases. *Am J Physiol (Cell Physiol)* 281:C1422–C1433, 2001
5. Goligorsky MS: Endothelial cell dysfunction and nitric oxide synthase. *Kidney Int* 58:1360–1376, 2000
6. Hack CE, Zeerleder S: The endothelium in sepsis: Source of and a target for inflammation. *Crit Care Med* 29(Suppl):S21–S27, 2001
7. Siegel NJ, Devarajan P, Van Why S: Renal cell injury: Metabolic and structural alterations. *Pediatr Res* 36:129–136, 1994
8. Thadhani R, Pascual M, Bonventre JV: Acute renal failure. *N Engl J Med* 334:1448–1460, 1996
9. Ashworth SL, Molitoris BA: Pathophysiology and functional significance of apical membrane disruption during ischemia. *Curr Opin Nephrol Hypertens* 8:449–458, 1999
10. Matthys E, Patton MK, Osgood RW, *et al*: Alterations in vascular function and morphology in acute ischemic renal failure. *Kidney Int* 23:717–724, 1983
11. Terry BE, Jones DB, Mueller CB: Experimental ischemic renal arterial necrosis with resolution. *Am J Pathol* 58:69–83, 1970
12. Kwon O, Phillips CL, Molitoris BA: Ischemia induces alterations of actin filaments in renal vascular smooth muscle cells. *Am J Physiol (Renal Physiol)* 282:F1012–F1019, 2002
13. Rabb H, O'Meara YM, Maderna P, *et al*: Leukocytes, cell adhesion molecules and ischemic acute renal failure. *Kidney Int* 51:1463–1468, 1997
14. Star RA: Treatment of acute renal failure. *Kidney Int* 54:1817–1831, 1998
15. Donnahoo KK, Meldrum DR, Shenkar R, *et al*: Early renal ischemia, with or without reperfusion, activates NFkappaB and increases TNF-alpha bioactivity in the kidney. *J Urol* 163:1328–1332, 2000
16. Donnahoo KK, Meng X, Ao L, *et al*: Differential cellular immunolocalization of renal tumour necrosis factor-alpha production during ischaemia versus endotoxaemia. *Immunology* 102:53–58, 2001
17. Linas S, Whittenburg D, Repine JE: Nitric oxide prevents neutrophil-mediated acute renal failure. *Am J Physiol* 272:F48–F54, 1997
18. Kelly KJ, Plotkin Z, Dagher PC: Guanosine supplementation reduces apoptosis and protects renal function in the setting of ischemic injury. *J Clin Invest* 108:1291–1298, 2001
19. Donnahoo KK, Meng X, Ayala A, *et al*: Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. *Am J Physiol* 277:R922–R929, 1999
20. Kelly KJ, Williams WW Jr, Colvin RB, *et al*: Antibody to intercellular adhesion molecule 1 protects the kidney against ischemic injury. *Proc Natl Acad Sci USA* 91:812–816, 1994
21. Rabb H, Mendiola CC, Saba SR, *et al*: Antibodies to ICAM-1 protect kidneys in severe ischemic reperfusion injury. *Biochem Biophys Res Commun* 211:67–73, 1995
22. Ysebaert DK, De Greef KE, Vercauteren SR, *et al*: Identification and kinetics of leukocytes after severe ischaemia/reperfusion renal injury. *Nephrol Dial Transplant* 15:1562–1574, 2000
23. Willinger CC, Schramek H, Pfaller K, *et al*: Tissue distribution of neutrophils in postischemic acute renal failure. *Virchows Arch B Cell Pathol Incl Mol Pathol* 62:237–243, 1992
24. Harris RC: Growth factors and cytokines in acute renal failure. *Adv Ren Replace Ther* 4:43–53, 1997
25. Schena FP: Role of growth factors in acute renal failure. *Kidney Int* 53(Suppl 66):S11–S15, 1998
26. Brezis M, Rosen S: Hypoxia of the renal medulla–Its implications for disease. *N Engl J Med* 332:647–655, 1995
27. Lieberthal W: Biology of acute renal failure: Therapeutic implications. *Kidney Int* 52:1102–1115, 1997
28. Alejandro V, Scandling JD Jr, Sibley RK, *et al*: Mechanisms of filtration failure during postischemic injury of the human kidney. A study of the reperfused renal allograft. *J Clin Invest* 95:820–831, 1995
29. Olof P, Hellberg A, Kallskog O, *et al*: Red cell trapping and postischemic renal blood flow. Differences between the cortex, outer and inner medulla. *Kidney Int* 40:625–631, 1991
30. Mason J, Torhorst J, Welsch J: Role of the medullary perfusion defect in the pathogenesis of ischemic renal failure. *Kidney Int* 26:283–293, 1984
31. Kramer HJ, Mohaupt MG, Pinoli F, *et al*: Effects of thromboxane A2 receptor blockade on oliguric ischemic acute renal failure in conscious rats. *J Am Soc Nephrol* 4:50–57, 1993
32. Chan L, Chittinandana A, Shapiro JI, *et al*: Effect of an endothelin-receptor antagonist on ischemic acute renal failure. *Am J Physiol* 266:F135–138, 1994
33. Gellai M, Jugus M, Fletcher T, *et al*: Reversal of postischemic acute renal failure with a selective endothelin A receptor antagonist in the rat. *J Clin Invest* 93:900–906, 1994
34. Kelly KJ, Tolkoff-Rubin NE, Rubin RH, *et al*: An oral platelet-activating factor antagonist, Ro-24–4736, protects the rat kidney from ischemic injury. *Am J Physiol* 271:F1061–F1067, 1996
35. Conger J: Vascular Alterations in ARF: Roles in initiation and maintenance, in *Acute Renal Failure: A Companion to Brenner and Rector's The Kidney*, edited by Molitoris BA, Finn WF, Philadelphia, Saunders, 2001, p 535
36. Hellberg PO, Kallskog O, Wolgast M: Nephron function in the early phase of ischemic renal failure. Significance of erythrocyte trapping. *Kidney Int* 38:432–439, 1990
37. Mason J, Welsch J, Torhorst J: The contribution of vascular obstruction to the functional defect that follows renal ischemia. *Kidney Int* 31:65–71, 1987
38. Solez K, Kramer EC, Fox JA, *et al*: Medullary plasma flow and intravascular leukocyte accumulation in acute renal failure. *Kidney Int* 6:24–37, 1974
39. Rabb H, Mendiola CC, Dietz J, *et al*: Role of CD11a and CD11b in ischemic acute renal failure in rats. *Am J Physiol* 267:F1052–F1058, 1994
40. Takada M, Nadeau KC, Shaw GD, *et al*: The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand. *J Clin Invest* 99:2682–2690, 1997
41. Hellberg PO, Kallskog OT, Ojteg G, *et al*: Peritubular capillary permeability and intravascular RBC aggregation after ischemia: Effects of neutrophils. *Am J Physiol* 258:F1018–F1025, 1990
42. Hellberg PO, Bayati A, Kallskog O, *et al*: Red cell trapping after ischemia and long-term kidney damage. Influence of hematocrit. *Kidney Int* 37:1240–1247, 1990
43. Vollmar B, Glasz J, Leiderer R, *et al*: Hepatic microcirculatory perfusion failure is a determinant of liver dysfunction in warm ischemia-reperfusion. *Am J Pathol* 145:1421–1431, 1994
44. Druid H, Enestrom S, Rammer L: Effect of anticoagulation upon nephron obstruction in experimental acute ischaemic renal failure. A morphological study. *Int J Exp Pathol* 79:55–66, 1998
45. Druid H, Rammer L: Protective effect on postischemic renal edema by anticoagulation. *Nephron* 60:319–323, 1992
46. Druid H, Rammer L: Reduction of renal edema by heparin in postischemic renal damage. *Nephron* 51:89–94, 1989
47. Ozden A, Sarioglu A, Demirkan NC, *et al*: Antithrombin III reduces renal ischemia-reperfusion injury in rats. *Res Exp Med (Berl)* 200:195–203, 2001
48. Wolgast M, Karlberg L, Kallskog O, *et al*: Hemodynamic alterations in ischaemic acute renal failure. *Nephron* 31:301–303, 1982
49. Losonczy G Jr: Intrarenal blood coagulation induced by ischemia in rats. Heparin and thrombocytopenia does not prevent the intracortical fibrin formation. *Thromb Res* 34:87–92, 1984
50. Lum H, Roebuck KA: Oxidant stress and endothelial cell dysfunction. *Am J Physiol (Cell Physiol)* 280:C719–C741, 2001
51. VanBenthuysen KM, McMurtry IF, Horwitz LD: Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. *J Clin Invest* 79:265–274, 1987
52. Pomfy M, Huska J: The state of the microcirculatory bed after total ischaemia of the brain. An experimental ultrastructural study. *Funct Dev Morphol* 2:253–258, 1992
53. van der Poll T, van Deventer SJ: Cytokines and anticytokines in the pathogenesis of sepsis. *Infect Dis Clin North Am* 13:413–426, 1999
54. Parent C, Eichacker PQ: Neutrophil and endothelial cell interac-

tions in sepsis. The role of adhesion molecules. *Infect Dis Clin North Am* 13:427–447, 1999
55. Symeonides S, Balk RA: Nitric oxide in the pathogenesis of sepsis. *Infect Dis Clin North Am* 13:449–463, 1999
56. Solez K, Morel-Maroger L, Sraer JD: The morphology of "acute tubular necrosis" in man: Analysis of 57 renal biopsies and a comparison with the glycerol model. *Medicine (Baltimore)* 58:362–376, 1979
57. Mutunga M, Fulton B, Bullock R, *et al*: Circulating endothelial cells in patients with septic shock. *Am J Respir Crit Care Med* 163:195–200, 2001
58. Kramer AA, Postler G, Salhab KF, *et al*: Renal ischemia/reperfusion leads to macrophage-mediated increase in pulmonary vascular permeability. *Kidney Int* 55:2362–2367, 1999
59. Vestweber D: Molecular mechanisms that control endothelial cell contacts. *J Pathol* 190:281–291, 2000
60. Kurihara H, Anderson JM, Farquhar MG: Diversity among tight junctions in rat kidney: Glomerular slit diaphragms and endothelial junctions express only one isoform of the tight junction protein ZO-1. *Proc Natl Acad Sci USA* 89:7075–7079, 1992
61. Fina L, Molgaard HV, Robertson D, *et al*: Expression of the CD34 gene in vascular endothelial cells. *Blood* 75:2417–2426, 1990
62. Aurrand-Lions M, Johnson-Leger C, Wong C, *et al*: Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members. *Blood* 98:3699–3707, 2001
63. Morita K, Sasaki H, Furuse M, *et al*: Endothelial claudin: Claudin-5/TMVCF constitutes tight junction strands in endothelial cells. *J Cell Biol* 147:185–194, 1999
64. Schmelz M, Franke WW: Complexus adhaerentes, a new group of desmoplakin-containing junctions in endothelial cells: The syndesmos connecting retothelial cells of lymph nodes. *Eur J Cell Biol* 61:274–289, 1993
65. Corada M, Mariotti M, Thurston G, *et al*: Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. *Proc Natl Acad Sci USA* 96:9815–9820, 1999
66. Noll T, Muhs A, Besselmann M, *et al*: Initiation of hyperpermeability in energy-depleted coronary endothelial monolayers. *Am J Physiol* 268:H1462–H1470, 1995
67. Kevil CG, Oshima T, Alexander B, *et al*: H(2)O(2)-mediated permeability: Role of MAPK and occludin. *Am J Physiol (Cell Physiol)* 279:C21–C30, 2000
68. Ochoa L, Waypa G, Mahoney JR Jr, *et al*: Contrasting effects of hypochlorous acid and hydrogen peroxide on endothelial permeability: Prevention with cAMP drugs. *Am J Respir Crit Care Med* 156:1247–1255, 1997
69. Kevil CG, Ohno N, Gute DC, *et al*: Role of cadherin internalization in hydrogen peroxide-mediated endothelial permeability. *Free Radic Biol Med* 24:1015–1022, 1998
70. Alexander JS, Jackson SA, Chaney E, *et al*: The role of cadherin endocytosis in endothelial barrier regulation: Involvement of protein kinase C and actin-cadherin interactions. *Inflammation* 22: 419–433, 1998
71. Navarro P, Caveda L, Breviario F, *et al*: Catenin-dependent and -independent functions of vascular endothelial cadherin. *J Biol Chem* 270:30965–30972, 1995
72. Watanabe H, Kuhne W, Spahr R, *et al*: Macromolecule permeability of coronary and aortic endothelial monolayers under energy depletion. *Am J Physiol* 260:H1344–H1352, 1991
73. Kuhne W, Besselmann M, Noll T, *et al*: Disintegration of cytoskeletal structure of actin filaments in energy- depleted endothelial cells. *Am J Physiol* 264:H1599–H1608, 1993
74. Hinshaw DB, Armstrong BC, Beals TF, *et al*: A cellular model of endothelial cell ischemia. *J Surg Res* 44:527–537, 1988
75. Lum H, Barr DA, Shaffer JR, *et al*: Reoxygenation of endothelial cells increases permeability by oxidant-dependent mechanisms. *Circ Res* 70:991–998, 1992
76. Zhao Y, Davis HW: Hydrogen peroxide-induced cytoskeletal rearrangement in cultured pulmonary endothelial cells. *J Cell Physiol* 174:370–379, 1998
77. Hinshaw DB, Burger JM, Armstrong BC, *et al*: Mechanism of endothelial cell shape change in oxidant injury. *J Surg Res* 46:339–349, 1989
78. Garcia JG, Davis HW, Patterson CE: Regulation of endothelial cell gap formation and barrier dysfunction: Role of myosin light chain phosphorylation. *J Cell Physiol* 163:510–522, 1995
79. Goeckeler ZM, Wysolmerski RB: Myosin light chain kinase-regulated endothelial cell contraction: The relationship between isometric tension, actin polymerization, and myosin phosphorylation. *J Cell Biol* 130:613–627, 1995
80. Ingber DE: Tensegrity: The architectural basis of cellular mechanotransduction. *Annu Rev Physiol* 59:575–599, 1997
81. Bishop AL, Hall A: Rho GTPases and their effector proteins. *Biochem J* 348(Pt 2):241–255, 2000
82. Diviani D, Scott JD: AKAP signaling complexes at the cytoskeleton. *J Cell Sci* 114:1431–1437, 2001
83. Carpenter CL: Actin cytoskeleton and cell signaling. *Crit Care Med* 28:N94–N99, 2000
84. Keenan C, Kelleher D: Protein kinase C and the cytoskeleton. *Cell Signal* 10:225–232, 1998
85. Parsons JT, Martin KH, Slack JK, *et al*: Focal adhesion kinase: A regulator of focal adhesion dynamics and cell movement. *Oncogene* 19:5606–5613, 2000
86. Takenawa T, Itoh T, Fukami K: Regulation of phosphatidylinositol 4,5-bisphosphate levels and its roles in cytoskeletal re-organization and malignant transformation. *Chem Phys Lipids* 98:13–22, 1999
87. Klingebiel T, von Gise H, Bohle A: Morphometric studies on acute renal failure in humans during the oligoanuric and polyuric phases. *Clin Nephrol* 20:1–10, 1983
88. Kelly KJ, Molitoris BA: Acute renal failure in the new millennium: Time to consider combination therapy. *Semin Nephrol* 20:4–19, 2000
89. Kinjo T, Takashi M, Miyake K, *et al*: Phenotypic heterogeneity of vascular endothelial cells in the human kidney. *Cell Tissue Res* 256:27–34, 1989
90. Demertzis S, Langer F, Graeter T, *et al*: Amelioration of lung reperfusion injury by L- and E-selectin blockade. *Eur J Cardiothorac Surg* 16:174–180, 1999
91. Toda K, Kayano K, Karimova A, *et al*: Antisense intercellular adhesion molecule-1 (ICAM-1) oligodeoxyribonucleotide delivered during organ preservation inhibits posttransplant ICAM-1 expression and reduces primary lung isograft failure. *Circ Res* 86:166–174, 2000
92. Hallahan DE, Virudachalam S: Intercellular adhesion molecule 1 knockout abrogates radiation induced pulmonary inflammation. *Proc Natl Acad Sci USA* 94:6432–6437, 1997
93. Sakuma T, Takahashi K, Ohya N, *et al*: Ischemia-reperfusion lung injury in rabbits: Mechanisms of injury and protection. *Am J Physiol* 276:L137–L145, 1999
94. Burne MJ, Daniels F, El Ghandour A, *et al*: Identification of the CD4(+) T cell as a major pathogenic factor in ischemic acute renal failure. *J Clin Invest* 108:1283–1290, 2001
95. De Greef KE, Ysebaert DK, Dauwe S, *et al*: Anti-B7–1 blocks mononuclear cell adherence in vasa recta after ischemia. *Kidney Int* 60:1415–1427, 2001
96. Rabb H, Daniels F, O'Donnell M, *et al*: Pathophysiological role of T lymphocytes in renal ischemia-reperfusion injury in mice. *Am J Physiol (Renal Physiol)* 279:F525–F531, 2000
97. Park P, Haas M, Cunningham PN, *et al*: Injury in renal ischemia-reperfusion is independent from immunoglobulins and T lymphocytes. *Am J Physiol (Renal Physiol)* 282:F352–F357, 2002
98. McEver RP: Selectins. *Curr Opin Immunol* 6:75–84, 1994
99. Smith CW, Rothlein R, Hughes BJ, *et al*: Recognition of an endothelial determinant for CD 18-dependent human neutrophil adherence and transendothelial migration. *J Clin Invest* 82:1746–1756, 1988
100. Eppihimer MJ, Russell J, Anderson DC, *et al*: Modulation of P-selectin expression in the postischemic intestinal microvasculature. *Am J Physiol* 273:G1326–G1332, 1997
101. Molitoris BA, Marrs J: The role of cell adhesion molecules in ischemic acute renal failure. *Am J Med* 106:583–592, 1999
102. Ichikawa H, Flores S, Kvietys PR, *et al*: Molecular mechanisms of anoxia/reoxygenation-induced neutrophil adherence to cultured endothelial cells. *Circ Res* 81:922–931, 1997
103. Kelly KJ, Williams WW Jr, Colvin RB, *et al*: Intercellular

adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest* 97:1056–1063, 1996
104. del Zoppo GJ: Microvascular responses to cerebral ischemia/inflammation. *Ann N Y Acad Sci* 823:132–147, 1997
105. Enestrom S, Druid H, Rammer L: Fibrin deposition in the kidney in post-ischaemic renal damage. *Br J Exp Pathol* 69:387–394, 1988
106. Gando S, Kameue T, Nanzaki S, *et al*: Massive fibrin formation with consecutive impairment of fibrinolysis in patients with out-of-hospital cardiac arrest. *Thromb Haemost* 77:278–282, 1997
107. Losonczy G Jr: Early postischaemic renal fibrin deposition and reduction of glomerular filtration rate in the rat: Effect of the defibrinating agent Arwin. *Acta Physiol Hung* 66:183–187, 1985
108. Rapaport SI, Rao LV: Initiation and regulation of tissue factor-dependent blood coagulation. *Arterioscler Thromb* 12:1111–1121, 1992
109. Faust SN, Heyderman RS, Levin M: Coagulation in severe sepsis: A central role for thrombomodulin and activated protein C. *Crit Care Med* 29:S62–S67, 2001 (discussion pp S67–S68)
110. Esmon CT: Protein C anticoagulant pathway and its role in controlling microvascular thrombosis and inflammation. *Crit Care Med* 29:S48–S51, 2001 (discussion pp 51–42)
111. ten Cate H: Pathophysiology of disseminated intravascular coagulation in sepsis. *Crit Care Med* 28:S9–S11, 2000
112. Yang Y, Loscalzo J: Regulation of tissue factor expression in human microvascular endothelial cells by nitric oxide. *Circulation* 101:2144–2148, 2000
113. Mizutani A, Okajima K, Uchiba M, *et al*: Activated protein C reduces ischemia/reperfusion-induced renal injury in rats by inhibiting leukocyte activation. *Blood* 95:3781–3787, 2000
114. Kohda Y, Murakami H, Moe OW, *et al*: Analysis of segmental renal gene expression by laser capture microdissection. *Kidney Int* 57:321–331, 2000
115. Kher R, Bacallao R: Direct in situ reverse transcriptase-polymerase chain reaction. *Am J Physiol (Cell Physiol)* 281:C726–C732, 2001
116. Nagasawa Y, Takenaka M, Matsuoka Y, *et al*: Quantitation of mRNA expression in glomeruli using laser-manipulated microdissection and laser pressure catapulting. *Kidney Int* 57:717–723, 2000
117. Grone HJ, Cohen CD, Grone E, *et al*: Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing human kidney. *J Am Soc Nephrol* 13:957–967, 2002
118. Witzmann FA, Fultz CD, Grant RA, *et al*: Regional protein alterations in rat kidneys induced by lead exposure. *Electrophoresis* 20:943–951, 1999
119. Banks RE, Dunn MJ, Forbes MA, et al: The potential use of laser capture microdissection to selectively obtain distinct populations of cells for proteomic analysis–preliminary findings. *Electrophoresis* 20:689–700, 1999
120. Motoike T, Loughna S, Perens E, *et al*: Universal GFP reporter for the study of vascular development. *Genesis* 28:75–81, 2000
121. Grego S, Cantillana V, Salmon ED: Microtubule treadmilling in vitro investigated by fluorescence speckle and confocal microscopy. *Biophys J* 81:66–78, 2001
122. Prasad PV, Edelman RR, Epstein FH: Noninvasive evaluation of intrarenal oxygenation with BOLD MRI. *Circulation* 94:3271–3275, 1996
123. Prasad PV, Epstein FH: Changes in renal medullary pO_2 during water diuresis as evaluated by blood oxygenation level-dependent magnetic resonance imaging: Effects of aging and cyclooxygenase inhibition. *Kidney Int* 55:294–298, 1999
124. Prasad PV, Priatna A, Spokes K, *et al*: Changes in intrarenal oxygenation as evaluated by BOLD MRI in a rat kidney model for radiocontrast nephropathy. *J Magn Reson Imaging* 13:744–747, 2001
125. Epstein FH, Prasad P: Effects of furosemide on medullary oxygenation in younger and older subjects. *Kidney Int* 57:2080–2083, 2000
126. Liano F, Pascual J: Outcomes in acute renal failure. *Semin Nephrol* 18:541–550, 1998
127. Chertow GM, Christiansen CL, Cleary PD, *et al*: Prognostic stratification in critically ill patients with acute renal failure requiring dialysis. *Arch Intern Med* 155:1505–1511, 1995
128. Rosen S, Heyman SN: Difficulties in understanding human "acute tubular necrosis": Limited data and flawed animal models. *Kidney Int* 60:1220–1224, 2001
129. Lieberthal W, Nigam SK: Acute renal failure. II. Experimental models of acute renal failure: Imperfect but indispensable. *Am J Physiol (Renal Physiol)* 278:F1–F12, 2000
130. Koo DD, Welsh KI, West NE, *et al*: Endothelial cell protection against ischemia/reperfusion injury by lecithinized superoxide dismutase. *Kidney Int* 60:786–796, 2001
131. Okusa MD, Linden J, Huang L, *et al*: Enhanced protection from renal ischemia: Reperfusion injury with A(2A)-adenosine receptor activation and PDE 4 inhibition. *Kidney Int* 59:2114–2125, 2001
132. Basile DP, Donohoe D, Roethe K, *et al*: Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. *Am J Physiol (Renal Physiol)* 281:F887–F899, 2001