Postischemic microvasculopathy and endothelial progenitor cell-based therapy in ischemic AKI: update and perspectives

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Postischemic microvasculopathy and endothelial progenitor cell-based therapy in ischemic AKI: update and perspectives. Am J Physiol Renal Physiol 311: F382–F394, 2016. First published May 18, 2016; doi:10.1152/ajprenal.00232.2016.—Acute kidney injury (AKI) dramatically increases morbidity and mortality rates of hospitalized patients. Incidences have been increased in recent years. The most frequent cause is transient renal hypoperfusion or ischemia which induces significant tubular cell dysfunction/damage. In addition, two further events take place: interstitial inflammation and microvasculopathy (MV). The latter evolves within minutes to hours postsepsis and may result in permanent deterioration of the peritubular capillary network, ultimately increasing the risk for chronic kidney disease (CKD) in the long term. In recent years, our understanding of the molecular/cellular processes responsible for acute and sustained microvasculopathy has increasingly been expanded. The methodical approaches for visualizing impaired peritubular blood flow and increased vascular permeability have been optimized, even allowing the depiction of tissue abnormalities in a three-dimensional manner. In addition, endothelial dysfunction, a hallmark of MV, has increasingly been recognized as an inducer of both vascular malfunction and interstitial inflammation. In this regard, so-called regulated necrosis of the endothelium could potentially play a role in postischemic inflammation. Endothelial progenitor cells (EPCs), represented by at least two major subpopulations, have been shown to promote vascular repair in experimental AKI, not only in the short but also in the long term. The discussion about the true biology of the cells continues. It has been proposed that early EPCs are most likely myelomonocytic in nature, and thus they may simply be termed proangiogenic cells (PACs). Nevertheless, they reliably protect certain types of tissues/organs from ischemia-induced damage but also in the long term. The most frequent cause (60%) of AKI in the hospital is transient renal hypoperfusion or ischemia with three fundamentally important pathophysiological consequences (29): 1) tubular cell dysfunction/damage, 2) interstitial inflammation, and 3) peritubular microvasculopathy. Particularly the two latter events may significantly prolong the process of renal recovery (29).

In recent years, cell-based therapeutic strategies have been applied in several AKI animal models. Quite promising results were acquired with mesenchymal stem cells (MSCs) which promote recovery of kidney function/structure in an indirect manner (77, 96, 100, 161). Other investigators used induced pluripotent stem cells (iPSCs) (91, 110), or adipose-derived stem cells (169). In 2006, endothelial progenitor cells (EPCs) were established as a new therapeutic measure in experimental AKI (113). Since then, several studies evaluated the exact role of EPCs in this situation.

The aim of the present review article is to summarize the current literature on postischemic MV and EPC-mediated renal repair.

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ISCHEMIC ACUTE KIDNEY INJURY: DEFINITIONS, EPIDEMIOLOGY, AND OUTCOME

The term “acute kidney injury” (AKI) encompasses both acute kidney damage without functional impairment and organ damage with a subsequent decline in kidney excretory function. The KDIGO (Kidney Disease: Improving Global Outcomes) criteria published in 2012 differentiate between these two phenomena (186). In Europe, as compared with the United States, “acute renal failure” (ARF) is commonly used to describe organ excretory failure due to hypoperfusion, or drug toxicity, or whatever the causes may be.

The exact clinical definition of AKI is being adapted on a regular basis with the latest criteria provided by the KDIGO guidelines in 2012 (68). According to these, AKI can be diagnosed if the following criteria are fulfilled: 1) a serum creatinine increase of greater than 0.3 mg/dl within 48 h, or 2) a 1.5-fold serum creatinine increase within 7 days (as compared with a known or suspected baseline value), and/or 3) a reduction in urine output to less than 0.5 ml·kg⁻¹·day⁻¹ for at least 6 h. It may however not be forgotten that serum creatinine is a poor parameter of renal function. Its concentration begins to rise late in AKI, that is if 60% of kidney function is being lost (39). New diagnostic markers are permanently being evaluated but shall not be reviewed in detail at the moment (40, 41). It needs to be mentioned that AKI markers do either reflect functional impairment (and/or) tissue damage, even if the latter does not result in any GFR reduction. Thus candidates such as KIM-1, or IL-18, or others may increase despite that the kidney still remains able to excrete water, solutes, and toxins (186). Figure 1, which has been published as part of the latest KDIGO section on “AKI Definition” (186) illustrates this relationship.

The two fundamental problems associated with AKI are its high incidence and the poor prognosis once it has been established. The incidence of the syndrome has continuously been increased over the past 20–25 years (40). Meanwhile, up to 18% of all hospitalized patients in Europe develop AKI during the stay at the hospital. In 2013, a meta-analysis of more than 300 studies reported average world incidences of AKI of even more than 30% in adults (150). Important reasons might be aging of the population in general and growing multi-morbidity (13). The highest incidences of AKI occur in septic patients. Zeng and colleagues (183) performed a retrospective cohort study, including 31,970 hospitalized patients. According to the KDIGO criteria, incidences of AKI were sepsis, 68.4%; mechanical ventilation, 63.9%; hematopoietic stem cell transplantation, 55.9%; and cardiac surgery, 52.2% (183). The same study revealed dramatic differences in mortality rates, depending on the severity of AKI [according to the AKIN criteria (31)]; overall in-hospital mortality rate, 2.2%; no AKI, 0.6%; AKIN stage I, 5.3%; AKIN stage II, 13.4%; and AKIN stage III, 35.4%. In addition, AKI does not only increase the short- but also the long-term mortality with the highest rates in AKIN stage III (145). The worldwide AKI mortality has slightly been decreased over time, especially in countries with higher average income (150). Nevertheless, AKI remains a fundamental problem in patients undergoing in-clinic treatment, and new methods/strategies for early detection of renal dysfunction and more efficient treatment are urgently needed.

ISCHEMIC AKI: CURRENT PATHOPHYSIOLOGICAL CONCEPTS

The most frequent cause of AKI is transient renal hypoperfusion, resulting from various conditions such as heart failure, fluid losses, and sepsis (13). Three pathophysiological consequences evolve, augmenting each other by various cellular/molecular processes. 1) The function and structure of the tubular epithelium are severely affected. Tubular cell necrosis, although not the predominant mechanism of tubular cell damage (29), may occur (86, 88), particularly in severe ischemic AKI [acute tubular necrosis (ATN) (29)]. 2) The kidney is affected by interstitial inflammation resulting from ischemia-induced activation of virtually all components of the innate and acquired immune system (115). 3) The third consequence is microvasculopathy (MV), preferably occurring within the peritubular compartment. MV potentially worsens kidney function and structure in the short and long term. The respective processes involved will be discussed later. The multitude of cellular events triggering and perpetuating tubular malfunction and inflammation however shall not be reviewed in full detail in the current manuscript. The following sections will nevertheless provide a summary of the most important aspects.

Tubular cell dysfunction and damage. The tubular oxygen sensitivity differs between the respective segments. The proximal tubule is considered as the most sensitive part to oxygen deprivation or nephrotoxic substances (16). Cell death primarily manifests within the S3 segment of the proximal tubule (164). In addition, certain AKI models showed severe affection of the thick ascending limb as well, since this segment is metabolically highly active (81). The limited glycolytic activity of the kidney is responsible for dramatic decreases in tubular ATP concentrations (67, 165). Decreases of cellular ATP occur under hypoxia/ischemia and certain nephrotoxic agents (58, 74, 95, 106, 149). Studies by Kribben et al. showed significant protection of isolated proximal tubular cells by extracellularly administered ATP. Further analysis revealed these effects to be mediated by P(2) receptors (73). Mitochondrial dysfunction, responsible for decreased ATP generation, has extensively been studied in AKI disease models and in
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Pathophysiological and Inflammatory Aspects in AKI

In vitro (9, 17, 43, 82, 146). The loss of renal brush border (LOBB) is one of the earliest morphological findings in ischemic AKI. It becomes apparent within 10–15 min and results from cytoskeletal breakdown (164). Other morphological abnormalities are the loss of cell polarity, partly resulting from destabilization of tight junctions-dependent cell-cell interactions (97), and detachment of tubular cells from the basement membrane. The latter ensues from basolateral-to-apical integrin redistribution, subsequently followed by integrin-mediated cell clumping within the tubular lumen (47). Administration of cyclized RGD peptides, which act as antagonists of integrin binding motifs, has been shown to prevent cell clumping and to improve renal function postischemia (105). Tubular cell dysfunction and damage in AKI significantly result from the generation of reactive oxygen species including superoxide anion, hydrogen peroxide, and hydroxyl radical. Further deleterious substances are nitrosative reactive products and peroxynitrite (103, 172). The kidney expresses several antioxidants (superoxide dismutase, catalase, glutathione S transferase) which neutralize reactive oxygen species under physiological conditions. Such defense mechanisms are affected in AKI (9). Tubular cells are being damaged by both apoptosis and necrosis. In some situations the whole renal cortex may become necrotic, especially in postpartum AKI (107). Apoptosis may be defined as “programmed” cell death, either induced by extrinsic and/or intrinsic signals whereas necrosis is historically viewed as a more passive process secondary to the loss of cellular energy sources. Both events occur simultaneously in AKI: Schumer and colleagues (141) demonstrated apoptosis and necrosis to occur in a dose-dependent manner. The respective pathways that induce apoptosis have extensively been studied in the past and a detailed discussion of the topic is not intended. For more information we refer to the excellent literature by Basile (9), Linkermann (86, 88), and Devarajan (29). Another aspect that shall finally be outlined briefly is related to modulatory effects of certain minerals, and in particular related to iron, which has been shown to play a major role in the damage caused during AKI (166). One study analyzed catalytic activity of iron in the kidney after glycerol administration (7); another investigation evaluated the same parameter in ischemia reperfusion injury (6). Tissue contents were increased in both situations. These and further studies revealed that iron is involved in reactive oxygen species (ROS)-mediated nephrotoxicity (6–8, 142). As a matter of fact, iron neutralization/removal using different approaches including the substances dimethylthiourea and sodium benzoate has been shown to significantly protect the kidney from acute damage (143). Interestingly, so-called heavy chain ferritin (FH) in the proximal tubule has been proven to protect from unilateral ureteral obstruction-induced kidney injury. In this situation, FHPT−/− mice displayed more severe inflammation and aggravated interstitial fibrosis (15). In addition, these animals were also more susceptible to cisplatin- and rhabdomyolysis-associated AKI (180).

Inflammation. Renal ischemia activates virtually all components of the innate and acquired immune system (115). Inflammation results from both endothelial and tubular cell dysfunction. Certain cytokines have been shown to induce and perpetuate the inflammatory process, such as IL-1, -6, -8, TGF-β, TNF-α, and MCP-1 (monocyte chemoattractant protein-1) (69, 129). Subsequently, different immune cells are mobilized and home to the kidney with earliest signs of cell infiltration in the outer medullary vasa recta capillaries. Increased endothelial expression of ICAM-1 and P-/E-selectin enables the cells to adhere to the vascular wall (45, 65, 99). Especially neutrophil engraftment may be prevented by inhibition of P-selectin and ICAM-1 (46, 66, 104, 127, 128). The exact consequences of neutrophil infiltration remain unclear since some studies showed protective effects of neutrophil depletion, while others did not (9, 66, 127). T cells in contrast are most likely significantly involved in modulating tissue damage in AKI. Nu/nu-mice, incapable of generating CD4 and CD8 positive T cells, showed higher ischemia resistance (20). Burne and colleagues demonstrated that by selective repletion of CD4+ T cells in this model, renal function substantially deteriorated (20). Similar results were observed in Rag-1 knockout mice (28). Regulatory (CD4+/CD25+) T cells however presumably act renoprotectively. In a model of cisplatin-induced AKI, Lee et al. (79) showed increased survival, reduced tubular epithelial cell damage, and decreased tissue levels of TNF-α after administration of CD4+/CD25+ T cells. Kinsey et al. (71) also suggested a protective role for regulatory T cells in AKI. B-cell depletion may only in part protect from ischemia-induced kidney damage, although neutrophil and T-cell infiltrates were not diminished in one study (21). Interestingly, ischemia susceptibility could be reestablished by serum transfer from wild-type animals. Transfer of B cells in contrast was not associated with decreased ischemia tolerance. Whether these effects were exclusively related to antibodies remains unclear (21, 115). Natural killer T-cells (NKT cells) represent a subset of the T cell family of lymphocytes. They express T cell surface markers and they are positive for NK1.1, also known as NKR-P1.9 (natural killer cell receptor P1.9) (70, 136). The T-cell receptor of NKT cells recognize glycolipids presented by the class I-like molecule CD1d (70). NKT cells produce numerous cytokines in response to antigen recognition which amplifies the activity of dendritic cells, conventional T cells, regulatory T cells, other NKT cells, and B cells, respectively (70). Li and colleagues (83) investigated NKT cells in murine ischemic AKI. After 30 min of ischemia NKT cells and neutrophils substantially accumulated in the kidney, accompanied by elevated IFN-γ tissue levels. NKT cell depletion lowered IFN-γ producing neutrophils in the kidney and protected mice from AKI (reviewed in 83 and 115). Another cell population critically modulating the inflammatory response are macrophages. They infiltrate the injured kidney within 1 h of ischemia-reperfusion (9). Injured endothelial cells express CX3CL1 (fractalkine) which acts as a potent chemoattractant molecule. By blocking CX3CL1, AKI severity can be decreased whereas adoptive transfer of macrophages restores ischemia sensitivity (84, 109). Finally, the complement system, an essential element of the innate immune system, is activated by ischemia. Complement factor B deficiency has been shown to mitigate functional and morphological injury in ischemic AKI. In addition, neutrophil infiltration was decreased (160).

The pathophysiological importance of AKI-associated inflammation may not be underestimated. Recently, Muly and colleagues (102) extensively reviewed potential therapeutic approaches for modulating inflammatory events including oxidative stress and reactive oxygen species-related necroinflammation, regulated cell death-related necroinflammation, immunoregulatory lipid mediators, cytokines and cytokine signaling.
hemokines and chemokine signaling, neutrophils and neutrophils extracellular traps (NETs), and others. Taken together, “inflammation in AKI” should increasingly be recognized as a separate area in the field.

HEMODYNAMIC CHANGES AND MICROVASCULOPATHY IN AKI

After renal reperfusion the kidneys remain affected for minutes to hours, even if the initial cause of ischemia has been eliminated. This results from increased vasoconstriction and from endothelial cell dysfunction (ED), respectively. In addition, ED promotes interstitial inflammation. Finally, structural alterations of the microvasculature may ensue, substantially increasing the risk for CKD in the long term. These certain aspects shall be discussed separately.

Ischemic AKI was originally termed as “vasomotor nephropathy” since very early studies showed impaired postischemic reperfusion to be associated with higher renal vascular resistance (RVR) (53, 60). Several mechanisms have been identified to elevate RVR, including stimulation of the sympathetic nervous system, release of vasoconstrictive mediators, increased production of reactive oxygen species, and inflammation. Prolonged postischemic hypoperfusion predominantly occurs within the outer medulla, an area with low oxygen concentrations even under physiological conditions (9, 132). From a clinical perspective ongoing renal hypoperfusion may be reflected by the so-called extension phase of AKI (99).

Mechanisms that increase RVR. Increased activity of the sympathetic nervous system has been shown to impair renal blood flow (RBF) in experimental AKI. Studies by Conger and colleagues (26) demonstrated that intra-arterial (renal artery) norepinephrine injections reduce RBF, subsequently followed by AKI. Elevated levels of blood norepinephrine have been detected in the renal vein following acute renal ischemia (44). Renal denervation on the other hand improved RBF and protected from ischemia-induced kidney damage (44). Finally, one investigation showed that the substance clonidine augments RBF if applied in a rat model of ischemic AKI (147). Besides direct effects on the renal vasculature, sympathetic nervous activity also stimulates production/secretion of renin which results in elevated levels of ANG II. Nevertheless, the exact role of ANG II in possibly perpetuating renal tissue damage in AKI remains unclear (9).

Increases in RVR are significantly induced by circulating mediators of different origin. Endothelin (ET), which interacts with ET-A and -B receptors, has been shown to increase in septic patients (systemic) and in a rat model of cold ischemic storage (locally: peritubular endothelial cells) (125, 173). Experiments performed in rats showed AKI-protective effects of ET antagonists (22), while the exact role in humans remains controversial. So far, one study showed aggravation of radiocontrast nephropathy by ET antagonists (167). Other mediators involved in regulating the RBF are arachidonic acid-derived substrates and platelet activating factor. During renal ischemia, vasodilatory prostaglandin E2 (prostacyclin) maintains renal perfusion, and the administration of cyclooxygenase 2 inhibitors can further reduce renal perfusion and the GFR (170). PAF in contrast stimulates constriction of afferent arterioles and PAF antagonists can ameliorate AKI under experimental conditions (93). Finally, RBF may significantly be affected by the formation of microthrombi postischemia. Microthrombogenesis has been reported in ischemia-reperfusion injury (IRI) and in kidney transplant models (35, 151).

Vascular function and RVR may in addition significantly be compromised by reactive oxygen species. Renal superoxide production amplifies renal vasoconstrictor responses and the reactivity of ANG II in both the renal cortex and the medulla (185). Oxidative stress also augments vasoconstrictive effects of adenosine (24). Superoxide, by converting NO to peroxynitrite, may compromise medullary perfusion, and these effects have been shown to normalize under the administration of antioxidants (185).

Endothelial cell dysfunction (ED). Initial studies related to ED in AKI were published in 1972 (42): rats undergoing renal artery clamping showed swelling of all cellular elements in the kidney, followed by sustained renal hypoperfusion. Such “no-reflow,” which was in part also attributable to endothelial cell swelling, could effectively be treated by the injection of hypertonic mannitol solution. It remained unaffected by an equivalent expansion of the extracellular fluid volume with either isotonic saline or isotonic mannitol (78). Later, studies by Goligorsky’s group revealed that peritubular perfusion is compromised within a few minutes after unclamping with some capillaries showing even patterns of retrograde blood flow (175). Meanwhile, a lot of progress has been made in the field of intravital microscopy of the kidney, not only in terms of analyzing the peritubular perfusion. In this regard, particularly the studies by Castrop and colleagues have significantly expanded our knowledge (137–139). In addition, Torres and colleagues lately used multiphoton microscopy for analyzing kidney morphology in a three-dimensional manner (162). Nevertheless, the mentioned study evaluated chronic and not acute consequences of (cisplatin-induced) kidney injury. Sutton’s group on the other hand evaluated capillary integrity postischemia by using rhodamine-labeled dextran, showing increased endothelial permeability (152–154). The essential role of impaired endothelial stability in perpetuating ischemia-associated kidney dysfunction was recently highlighted by Kümper’s group (135). The synthetic Tie-2 agonist peptide vasculotide stabilized the vasculature and ameliorated experimental AKI. These and other investigations confirm significant endothelial dysfunction to occur after acute ischemia. In 2002, Brodsky and colleagues already “utilized” these alterations for therapeutic purposes. Rats were prevented from AKI by systemic injections of mature human endothelial cells (HUVECs) (18). An essential property of the cells used for therapy was the ability to constitutively express the NOS3 (nitric oxide synthase 3) gene. Endothelial NOS3 is particularly expressed within the renal medulla. Its role in regulating renal perfusion was shown by studies of Conger et al. (27): renal ischemia induced impaired endothelial NOS function as reflected by a loss of vasodilatory responses to acetylcholine and bradykinin. In addition, NOS3 knockout mice showed higher degrees of endotoxin-induced kidney damage compared with controls (168). Taken together, these data indicate a crucial role for NOS3-mediated NO production as vasoprotective mechanism in AKI.

Another hallmark of ED in AKI is the ability of endothelial cells to perpetuate interstitial inflammation. The exact roles of inflammatory cells in AKI still need to be elucidated more in detail. However, leukocytes contribute to vascular congestion
by interacting with endothelial ICAM-1 and P-/E-selectin. The latter are being upregulated in response to ischemia (45, 65, 98). Leukocytes have been shown to adhere to the endothelium within several hours after reperfusion (64, 144). Besides interacting with leukocytes in a direct manner, endothelial cells also provide signals for recruiting white blood cells to sites of ischemia-induced tissue damage [production of CX3CL1: fractalkine (12)]. Some new aspects related to cell death-associated inflammation shall be outlined briefly since they may also apply to ED in AKI. For many years, exclusively apoptosis has been suggested to occur in a regulated manner while necrosis has been proposed as a more passive event responsible for cell death. With the identification of so-called regulated necrosis (RN) (87), our understanding of the balance between tissue regeneration and degradation in diseases has fundamentally been modified. RN may ensue in either a protein kinase-dependent manner (necroptosis) or may result from increased mitochondrial permeability (87). Necroptosis has been shown to occasionally involve the maturation of certain proinflammatory cytokines (pyroptosis). This has been documented in macrophages, tubular epithelial cells, and neurons, respectively (2, 25, 30, 72, 94, 176). Pyroptosis is thought to critically stimulate the inflammatory response (87). Receptor-interacting protein kinase 3 (RIPK3) is an essential inducer of necroptosis, which has been shown to cause organ failure following stroke, myocardial infarction, and renal ischemia/reperfusion injury (88). Interestingly, the highest level of RIPK3 expression has been documented in glomerular endothelial rather than in tubular epithelial cells (85). One may hypothesize that endothelial necroptosis induces and perpetuates postschismic kidney inflammation as well. Nevertheless, further studies are needed to specifically address the role of ED-induced inflammation in the context of regulated necrosis and particularly pyroptosis.

All of the above-mentioned effects take place within minutes to hours after the ischemic insult. They partly contribute to renal repair but they also further aggravate alterations of tissue structure. Nevertheless, vascular abnormalities also occur in the long term. Such sustained microvasculopathy is critical for the long-term prognosis of kidney excretory function and structure.

*Sustained microvasculopathy.* Postischemic vascular rarefaction [loss of peritubular capillaries (PTC)] is presumably the most important long-term consequence related to sustained microvascular dysfunction. The mechanisms responsible for PTC have been analyzed in the past. First, endothelial cells may undergo ischemia-induced apoptosis. This phenomenon has been described in the liver but not in renal IRI. Nevertheless, studies published by Sutton’s group showed endothelial apoptosis in response to CD95 ligand infusion (54). A second process that possibly contributes to PTC is endothelial-to-mesenchymal transdifferentiation (EndoMT). Studies by Goligorsky’s and Kaluri’s groups revealed EndoMT to perpetuate interstitial fibrosis in chronic heart and kidney disease models (108, 181, 182). By acquiring a mesenchymal phenotype, endothelial cells functionally/structurally disintegrate from vessels and PTC ensues. Similar observations were made by Basile et al. and by our own studies (10, 120). It may be argued whether PTC and EndoMT are interacting with each other in a bidirectional manner. Recently our own experiments suggest a dynamic cascade of postschismic PTC with aggravated EndoMT, the latter possibly being induced by activation of the endothelial cilium (119). Endothelial cilia have been shown to stabilize endothelial cell properties under pathological conditions (32, 33).

The loss of peritubular capillaries is an irreversible phenomenon which indicates a limited self-renewal capacity of mature endothelial cells in the kidney. Transforming-growth factor-beta (TGF-β) has been shown to negatively regulate endothelial proliferation and to augment EndoMT (174). Tissue levels of TGF-β are elevated postischemia, and neutralization of the cytokine has been documented to preserve vascular structure in rats (11, 148). Other inhibitors of endothelial cell proliferation are angiotatin, endostatin, ADAMTS-1, sFLT, arrestin, and casatin (reviewed in 12).

Figure 2 summarizes the most important pathophysiological determinants of postschismic microvasculopathy.

ENDOTHELIAL PROGENITOR CELLS

The field of EPC research was “founded” in 1997. The first investigation that introduced the term “endothelial progenitor cells” was published by Asahara and colleagues (3). In this landmark study, CD34+ cells, isolated from human umbilical vein blood, were cultured under pro-endothelial growth conditions. A few (5–7) days later the cells displayed phenotypic characteristics of mature endothelial cells and in addition they did express certain endothelium-restricted cell surface markers. Systemic cell administration in immunoincompetent nude rats, suffering from hindlimb ischemia, promoted vascular and tissue recovery in a significant manner. Microscopic analysis revealed sporadic incorporation of injected cells into the microvascular endothelium, subsequently followed by faster postschismic reperfusion. Since then, approximately 10,000 articles have been published on the topic. Nevertheless, the discussion about origin and biological properties of EPCs continues until now. It has come to attention that EPCs are most likely represented by at least two major subpopulations, although even this particular concept has been questioned in 2011 (134). If one assumes the existence of two subpopulations, the first is represented by cells that express both hematopoietic and endothelial cell surface antigens. In the literature, they are described by different terms including “colony-forming unit-endothelial cells” (CFU-ECs) and “early endothelial progenitor/outgrowth cells” (eEPCs/eEOCs) (12, 37, 49, 111, 117). The heterogeneous terminology results from the methods being used for cell isolation, propagation, and quantification. Originally, CD34+ cells, isolated by immunomagnetic beads technique, were seeded on fibronectin-coated dishes which led to the formation of spindle-shaped cells within 3 days. At day 7 (early EPCs/eEOCs) some attached cells expressed CD34, CD31, Flk-1, Tie-2, or E-selectin (3). In addition, they were able take up the lectin Ulex Europeaus Agglutinin-1 (UEA-1) and fluorescence-labeled acetylated low-density lipoprotein (acLDL). Especially the double-positive staining for UEA-1 and acLDL was initially suggested as an EPC-specific attribute. The problem with such an approach lies in the fact that many other blood-derived cells and even platelets do interact with UEA-1 as well (1, 5, 140). In order to eliminate false positive signals, another approach included replating of nonadherent cells after 24–48 h with the emerging colonies (day 7) termed as CFU-ECs (CFU-Hill) (51, 57). Finally, cytometric
analysis has been established to measure circulating EPCs from the blood. The respective markers widely used in the past (and presently) are CD34, CD133, and Flk-1 (KDR in humans) (124). Investigations published in 2010 revealed that such cells are positive for CD45 as well, a leukocyte antigen that is expressed on the majority of nucleated circulating blood cells (36). Therefore, none of the reported cell populations could be identified as true progenitors of endothelial cells so far. Thus Richardson and Yoder proposed that the term EPCs may not be used in these situations but suggested to define the cells as “proangiogenic hematopoietic stem and/or progenitor cells” or simply as “Proangiogenic cells” (PACs) (134).

Despite these controversial observations, PACs are capable of promoting vascular repair under both physiological and pathological conditions. Countless experimental studies successfully utilized PACs for systemic treatment of ischemic diseases such as heart, cerebrovascular, peripheral artery, and kidney disease (4, 38, 59, 61–63, 113, 118, 120, 184). The cells predominantly act by indirect rather than by direct mechanisms. Two processes are fundamentally important. First, they modulate the perivascular microenvironment by secreting paracrine factors in an orchestrated manner (37, 49). Pula et al. (126) performed mass spectrometry analysis of the CFU-EC secretome and identified 272 candidate proteins, of which 124 were also found in eEPCs [no preplating performed; e.g., matrix metalloproteinase-9; interleukin-8; macrophage migration inhibitory factor; different cathepsins and protease inhibitors; the S100 proteins A11, A8, and A4; plasminogen activator inhibitor-2; and apolipoprotein E; finally thymidine phosphorylase, a potent proangiogenic and prosurvival factor] [reviewed by Goligorsky and Salven (48)]. The second mechanism has been identified by a series of elegant experiments published by Cantaluppi and colleagues (23). They showed that the cells are capable of secreting vasomodulatory microvesicles which contain certain micro-RNA molecules (miR-126 and -296). A third mechanism of PAC-endothelial cell crosstalk was introduced by studies of Goligorsky’s group: by establishing distinct microtubular organelles, so-called...
nanotubes, PACs may communicate with mature endothelial cells in a direct manner. Nanotubes mediate a direct transfer of cellular constituents such as the von Willebrand factor and others (177).

The second EPC population is represented by so-called “endothelial colony-forming cells” (ECFCs). They can be isolated from peripheral blood as well, but in contrast to PACs they do not display any hematopoietic properties (12, 101, 179). ECFCs are cultured from peripheral mononuclear cells on either collagen type-1- or fibronectin-coated dishes. After a short period of preplating, nonadherent cells are discarded and adherent cells are cultured for another 14–21 days. The late appearance in culture explains the alternative terminology as “late endothelial progenitor/outgrowth cells” (lEPC/lEOCs) (52). Other studies defined the cells as “blood outgrowth endothelial cells” (BOECs) (90). ECFCs do not act paracrine by modulating the perivascular milieu but by incorporating into the endothelial layer of small blood vessels. It has therefore been suggested that ECFCs are in fact mature endothelial cells, shed from blood vessels in the bone marrow or from other sites (56). One significant difference between mature endothelial cells and ECFCs is the faster in vitro proliferation of the latter (90).

The exact role of endogenous PACs and ECFCs in the process of vascular self-repair remains unclear. An intriguing hypothesis has been proposed by Ingram et al., related to the collaborative interplay between ECFCs and PACs in vivo. It has been suggested that PACs may create a proangiogenic microenvironment, thereby recruiting ECFCs to sites of vascular damage. Thus the two populations may interact very dynamically and support each other in maintaining vascular homeostasis (56). Figure 3 summarizes procedures for isolation and culturing of the different EPC subpopulations (adapted from 52). Figure 3 also shows patterns of cell surface antigens in the respective groups.

In the following sections, the term eEPCs will be used for PACs; ECFCs will exclusively be used if this particular subpopulation is being discussed.

THERAPEUTIC ADMINISTRATION OF EEPC AND ECFC IN AKI

Since the first description by Asahara et al. (3) numerous experimental studies evaluated the therapeutic efficacy of eEPCs in ischemic diseases. Meanwhile, the cells are administered in human trials as well, mostly in ischemic heart and peripheral artery disease. In 2002, Brodsky and colleagues published a manuscript showing that rats may be prevented from AKI by systemic injections of mature human endothelial cells (HUVECs) (18). Microscopic analysis showed the in-
jected cells to be incorporated in the peritubular and glomerular capillary network. First investigations about the role of eEPCs in ischemic AKI were not performed until 2006 (113): FVB/NJ mice were subjected to bilateral renal ischemia for 25 min. This measure induced robust endogenous mobilization of CD34+/Flk-1+ cells into the spleen, without any cell increase in the circulation. Ischemic preconditioning in contrast depleted the cells from both spleen and blood. Nevertheless, kidneys were now infiltrated by c-Kit+/Tie-2+ cells (eEPCs). eEPC-enriched mononuclear cells, isolated from the medullopapillary areas, significantly protected recipient animals from AKI. This study showed that eEPCs act renoprotectively and may be used for therapeutic purposes. Since then, a number of our own studies were related to strategies that may agonize syngeneic murine eEPCs in AKI. Several mediators were identified, helpful to increase the renoprotective competence of the cells. Among those were the substance 8-O-cAMP (116), the hormone melatonin (112), angiopoietin-1 and -2 (111, 117), and bone morphogenetic protein-5 (120). Later studies showed that eEPCs are capable of protecting the kidney in chronic disease models as well (118, 120). Although the mentioned studies demonstrated the cells as an effective tool in AKI (and CKD), eEPCs were always applied in a systemic manner (intravenously). Ratliff and Goligorsky discussed certain problems that may arise in such a situation (131). In general, only ~3% of systemically delivered cells home to the kidney and engraft. A significant amount is being trapped in the lungs (75, 80, 89, 171). Second, cells may undergo programmed cell death before entering the renal circulation (131). The third problem results from insufficient cellular integrin activation due to the lack of specific activators such as cRGD (34) if the cells are applied in an aqueous solution (131). Therefore, the authors suggested (and published) an alternative strategy for delivering EPC-mediated effects in AKI. Hyaluronic acid-based hydrogels (HA-HG), enriched with embryonic EPCs, were implanted either superficially into ears or subcapsularly into kidneys. Postischemic kidney function was significantly improved in both situations (130). Such an approach was superior to intravenous injection even in terms of preventing the microvascular architecture (130). These investigations also indicated that the kidney can be prevented from ischemic damage by transferring the cellular mechanisms by which eEPCs act in vertebrate organisms: the effects of HA hydrogels was comparable in animals with versus without enzymatic HG-HA digestion.

This new perspective was fundamentally supported by studies of Cantaluppi and colleagues (23). EPCs were shown to release micro-RNA containing microvesicles that mediate renoprotection in experimental AKI and chronic kidney disease in a cell-independent manner (14). Another, recently published investigation supports the concept of transferring the mere mechanism of EPC action as a sufficient measure to prevent AKI. Burger and colleagues employed ECFCs instead of eEPCs (19). The exclusive administration of ECFC-derived exosomes (40- to 100-nm diameter as opposed to microparticles with 100- to 1,000-nm diameter) directly to postischemic mice mitigated acute renal injury, as assessed by blood creatinine and tubular damage markers.

In summary, eEPCs (and ECFCs) have meanwhile been established as reliable therapeutic tools in ischemic AKI. In addition, it has become evident that the cells must not necessarily be transferred to recipient organisms in order to mediate protective effects.

**Perspectives**

Although early EPCs (and ECFCs) significantly protect from ischemic AKI in the short- and mid-term, any transfer of an EPC-based strategy into the clinical situation may be associated with a number of problems. The problems to be discussed may also be of relevance for other approaches that utilize cell-based strategies. They may even apply to the administration of so-called induced pluripotent stem cells (iPS). iPS have meanwhile been used for generating whole kidneys including tubules, glomeruli, and vessels, thus offering new perspectives in regenerative medicine which most likely will revolutionize the whole field (76, 92, 155–159). In this context it has to be mentioned that iPS have also been administered for therapeutic purposes in ischemia-reperfusion injury. Postischemic subcapsular administration of iPS resulted in preservation of kidney function, reduced tubular dilation/cell necrosis, and diminished interstitial fibrosis (163). Current and future studies will help to clarify the exact role of iPS in AKI-treatment regimens. The first problem related to any kind of cell therapy is general in nature: it is more or less impossible to predict AKI. The currently available markers usually (begin to) increase at the moment when renal function/structure are severely affected. Under optimal circumstances, cells (iPS, PACs, ECFCs, MSCs, or other cell-types) should be administered shortly before or at least at the time when renal ischemia occurs. The second problem is related to the time needed for cell isolation and propagation. It usually takes between 5 and 7 days for culturing eEPCs from the blood. Thus AKI should be diagnosed almost a week in advance. The third problem is to ensure immunological compatibility between cell donor and recipient. In order to prevent eEPC/ECFC (or other cell) rejection, the cells should be administered in an autologous setting. Nevertheless, numerous diseases have been shown to decrease the mere quality of eEPCs (paracrinic and vasculogenic activity, cell survival). Among those are noninflammatory and inflammatory (micro)vascular diseases such as sepsis, systemic lupus erythematosus, Wegener’s disease, and diabetes (121–123, 178).

We therefore assume that it will not become possible to administer eEPCs/ECFCs or other cell types in AKI patients in the near future. However, the cellular mechanisms by which the cells act within the perivascular microenvironment may be utilized for therapeutic purposes. As pointed out earlier, eEPCs communicate via two essential mechanisms: secretion of vasomodulatory microvesicles (23) and release of humoral factors that modulate endothelial function in a paracrinic manner (37, 49, 114, 133). Particularly the latter are of great interest in terms of establishing new therapeutic tools. We currently expand our studies to a more detailed analysis of the eEPC secretome under both native and stimulated conditions. Proteomic analysis will help to characterize the secretome and to potentially identify molecules that may be used in the management of human AKI. Hopefully, these and other investigations will help to substantially improve incidences and outcomes of human AKI in the near future.
REFERENCES


