Transplant Graft Vasculopathy: An Emerging Target for Prevention and Treatment of Renal Allograft Dysfunction

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Maintenance of healthy endothelium is essential to vascular homeostasis, and preservation of endothelial cell function is critical for transplant allograft function. Damage of microvascular endothelial cells is now regarded as a characteristic feature of acute vascular rejection and chronic allograft nephropathy, which is an important predictor of graft loss and is often associated with transplant vasculopathy. In this review, we will discuss the role of microvascular endothelium, in renal allograft dysfunction, particularly as it relates to markers of endothelial dysfunction and endothelial repair mechanisms. We also discuss the potential for therapies targeting endothelial dysfunction and transplant graft vasculopathy.

Key Words: Endothelium, graft vasculopathy, circulating endothelial cell, hypoxia

The vascular endothelium is not a mere barrier between intravascular and interstitial compartments. Rather, the vascular endothelium plays an active role in the regulation of the hemodynamics, angiogenic vascular remodeling, and metabolic, synthetic, inflammatory, and antithrombogenic processes.[1] Endothelial cell dysfunction, initially introduced to describe impaired endothelium-dependent vasodilatation,[2] has been broadened to encompass disturbances in the barrier function of the vascular endothelium, impaired antithrombogenic properties, perturbed angiogenic capacity, inappropriate regulation of tonicity, proliferative capacity and migratory properties of vascular smooth muscle, and deterrent of neutrophils and monocytes from diapedesis. The pathophysiology of endothelial cell dysfunction is emerging as a hallmark of several highly prevalent cardiovascular and renal diseases,[3] including various spectrum of allograft nephropathy. In this review, we will discuss the role of the microvascular endothelium in terms of the development and aggravation of renal allograft dysfunction and the potential therapeutic modalities targeting transplant vasculopathy.

CHRONIC ISCHEMIA IN PROGRESSIVE RENAL DISEASE

Renal ischemia is regarded as one of the most important mechanisms of renal disease progression.[4] Ischemia may occur via several mechanisms, such as by intrarenal vasoconstriction, or via structural lesions that impair blood flow delivery to the tubules. The latter could result from arteriolar disease (such as in diabetes or hypertension), from intraglomerular lesions (such as in rapidly progressive glomerulonephritis) or from loss of the peritubular capillaries. Interstitial fibrosis itself may lead to local ischemia, by impairing the diffusion gradient from the capillary to the

tubule. This may be especially important for the tubules in the juxtamedullary region and outer medulla, since these tubules are normally in a borderline hypoxic state due to the countercurrent circulation and high oxygen demands of the medullary thick ascending tubules and the S3 segments of the proximal tubules.[5] Hence modest reductions in renal blood flow could lead to worsening hypoxia in this region.[6,7] In turn, hypoxia can induce tubular and interstitial cell injury and activation, proliferation, cytokine generation, and matrix synthesis associated with an increased expression of hypoxia- inducible factor-1 α (HIF-1 α).[8,9]

THE LOSS OF THE MICROVASCULATURE IN PROGRESSIVE RENAL DISEASE

The maintenance of the microvasculature would thus appear to be critical for the prevention of progressive renal disease. Maintenance of glomerular capillary number would help maintain glomerular filtration, whereas in the interstitium maintaining peritubular capillaries would be essential for providing oxygen and nutrition to the tubules and interstitial cells.

Morphometric studies in animal model of progressive renal disease have documented that the initial response to a fall in nephron number is a hypertrophic response in which the glomerular capillaries increase in both number and length.[10,11] Unfortunately, the proliferation is not sustained, and over time there is a progressive loss of the endothelium due to unchecked apoptosis. Indeed, the loss of glomerular endothelium correlates directly with the development of glomerulosclerosis.[12,13] A similar finding also occurs in the interstitium. Bohle noted that there was a loss of peritubular capillaries in progressive renal disease in man, and he posited an essential role for impaired blood flow in the etiology of the interstitial fibrosis.[14]

TRANSPLANT VASCULOPATHY IN RENAL ALLOGRAFT

Damage of microvascular endothelial cells is

also a characteristic feature of acute vascular rejection and chronic allograft nephropathy, which is an important predictor of graft loss.[15,16] One of the prevalent manifestations of chronic allograft nephropathy is salient vascular lesion, often called as transplant vasculopathy which consists fibrointimal thickening of arteries, breaks in the elastic layer and vessel wall infiltration with inflammatory cells.[17] The vascular narrowing with downstream ischemia is responsible for the chronic glomerular and tubulointerstitial lesions.

Another vascular lesion found in renal allograft is related to the toxicity of calcineurin inhibitors, so called afferent arteriolopathy. These agents are also capable of causing thrombotic microangiopathy with characteristic endothelial damage. *In vivo*, cyclosporine A has been shown to impair nitric oxide synthesis,[18] induce production of superoxide,[19] and enhance vascular permeability.[20] Moreover, induction of endothelial apoptosis,[21] release of von Willebrand factor,[22] and production of endothelin[23] have been reported in response to cyclosporine A.

A single center study in 282 protocol biopsies at 3 months of follow up in stable grafts revealed that the presence of renal allograft vasculopathy in 21 patients (7.5%), which is a rather high incidence considering the early timing of protocol biopsies.[24] They also observed that long-term graft survival was significantly decreased in patients displaying renal allograft vasculopathy (41%) when compared to patients displaying chronic allograft nephropathy without vessel involvement (82%) or to patients not displaying chronic renal lesions in the protocol biopsy (95%; $p=0.001$). The observation that the presence or absence of vessel narrowing correlates with marked difference in long-term outcome supports the hypothesis that chronic allograft nephropathy with and without renal allograft vasculopathy may represent two different clinical conditions and that renal allograft vasculopathy in patients with stable renal function may have a significant deleterious impact on graft dysfunction.

Evidence for involvement of the complement system in transplant vascular disease is supported by the finding that the complement degradation product, C4d, is frequently found in peritubular capillary endothelium in acute humoral (vascular)

rejection as well as in some patients with chronic immunologic allograft rejection.[25] Furthermore, the development of this vascular lesion after the first year of renal transplantation clearly impacts on long-term prognosis of graft.[26]

Regardless of the pathophysiologic characteristics and clinical implications of vascular lesions in the renal allograft, endothelial dysfunction and damage are the prerequisite for further structural alterations in vessel walls including smooth muscle cells and perivascular tissue, and by reducing oxygen and nutrient delivery to the allograft kidney and may also lead to activation of hypoxia-related mechanisms of renal scarring.

ROLE OF MICROVASCULAR ENDOTHELIUM IN RENAL TRANSPLANT GRAFT

Endothelial cells have a pivotal role in transplant immunology in that they served as a barrier between graft tissue of donor origin and circulating immune cells of the recipient. During reperfusion, immunocompetent cells of the recipient first encounter donor epitopes, such as histocompatibility antigens, on donor endothelial cells. Maintenance of quiescent endothelium is also essential to prevent vascular leakage and thrombosis.

During acute vascular rejection, donor endothelial cells become the primary targets of apoptosis and necrosis of the immune attack. Endothelial damage is also an important feature of chronic allograft nephropathy, the single most common cause of long-term graft loss.[26] Furthermore, endothelial cells are the target of drug toxicity, particularly by calcineurin inhibitors.[27]

Microvascular endothelial cell loss in the kidney was also observed in hyperacute, acute cell-mediated, and acute vascular rejection as in native kidney disease.[28,29] Histological and molecular studies of renal allografts from patients with acute rejection show upregulation of the antiapoptotic genes in peritubular endothelial cells, smooth muscle and mononuclear cells, suggesting that apoptotic signals target the microvasculature during acute immunologic attack in allografts.[30] Along with the expressions of anti-apoptotic genes, the elaboration of growth factors such as

vascular endothelial growth factor (VEGF), the transient production of new vessels (pathologic neoangiogenesis), and the recruitment of endothelial precursor cells reflect the process of ongoing injury and repair in renal allograft. Therefore, the microvascular endothelium is not only a primary target of immune- and nonimmune-mediated injury, but it may also play an important role in acute inflammation by hastening capillary repair and preserving the endothelium to prevent further vascular injury.

Based upon the results of many contemporary studies showing that vascular endothelial cell loss accompanies renal allograft rejection and direct cellular injury, strategies designed to promote endothelial cell survival can be regarded as a new target to reduce graft dysfunction and loss.

CIRCULATING ENDOTHELIAL CELLS AND ENDOTHELIAL CELL CHIMERISM : MARKER OF ONGOING ENDOTHELIAL INJURY AND VASCULAR REPAIRER

Circulating endothelial cells have been recently evaluated as a marker of endothelial cell injury in various diseases such as sickle cell disease and vasculitis.[31] In renal transplant recipient, Woywodt et al. reported that patients with vascular rejection (72 ± 39 cells/mL) had significantly higher cell numbers than healthy controls (7 ± 5 cells/mL, $p < 0.01$), patients without rejection (24 ± 19 cells/mL, $p < 0.001$) or tubulointerstitial rejection (30 ± 23 cells/mL, $p < 0.02$). Among patients with vascular rejection, patients with severe intimal arteritis (Banff IIB) had higher numbers of circulating endothelial cells than those with mild intimal arteritis (Banff IIA). There was a significant negative correlation of cell numbers with time since transplantation in all patients after patients with vascular rejection had been excluded.[32]

Lagaaij et al. examined the replacement of damaged peritubular capillary endothelium by recipient endothelial cells (endothelial chimerism) in renal allografts. They reported that an increased number of recipient-derived endothelial cells correlated with more poorly functioning grafts due to prior vascular rejection.[33] These host endo-

thelial cells may be recruited from endothelial stem cells (angioblast) in the circulation. However, it was not clear what the consequences and clinical implications of this endothelial cell replacement are. Although Lagaaij et al. implied it was not beneficial, there is no compelling reason to believe that the endothelial cell replacement per se is harmful. It seems more likely that harmful vascular rejection may only create an opportunity for innocuous or beneficial endothelial-cell replacement possibly by reac- tivating development-related gene programs to replace damaged endothelium. Therefore, allograft endothelial cell chimerism potentially enhances long-term graft survival. Recent insights suggest that the injured endothelial monolayer is regenerated by circulating bone marrow derived endothelial progenitor cells, which accelerates reendothelialization and limits further vascular damage.[34] However, risk factors for various vascular diseases such as age, diabetes or immunosuppression reduce the number and functional activity of these circulating endothelial progenitor cells and endothelial chimerism, thus limiting the regenerative capacity. The impairment of stem/ progenitor cells by risk factors may contribute to transplant vasculopathy as well as atherogenesis and atherosclerotic disease progression.

Renal vascular repair mediated by circulating endothelial cells is not unique to transplant allografts. For example, renal endothelial injury is the defining lesion in thrombotic microangiopathy, and human endothelial cell precursors derived from bone marrow were shown in the damaged renal microvasculature.[35] Ischemic limb, damaged coronary vessels and cardiac allografts also show progenitor cell-mediated reendothelilaization.[36-38]

POTENTIAL THERAPEUTIC POSSIBILITIES TARGETING RENAL MICROVASCULATURE IN ALLOGRAFT

Considering that renal microvascular dysfunction and/or loss contributes to allograft dysfunction, then direct angiogenic therapies using growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietin or other agents that simulate endo-

genous production of endothelial cell growth factors may have the potential to prolong transplant allograft function by mobilizing endothelial precursor cells, upregulating antiapopotic endothelial cell genes, and preserving graft microvasulature.[39,40] Another protein, survivin, a member of the inhibitors of apoptosis protein (IAP) family, provides cytoprotection in endothelial cells in which enhanced expression occurs during endothelial cell proliferation and angiogenesis whereas survivin expression is down-regulated in quiescent human cells.[41]

Immunosuppressive therapy with potential benefit for endothelial function should be considered. Currently, rapamycin (maintenance of angiostasis) or FTY720 (activation of endothelial cell survival signal transduction pathway) can be potential candidates.[42,43] Supplemental use of growth factors such as recombinant human erythropoietin (rHuEpo) that may stimulate VEGF production by renal residential cells and delay the progression of allograft loss.[44] Direct angiogeneic effect of rHuEpo was also reported.[45] Interventions that promote mobilizing endothelial progenitors to initiate chimerism (eg, HMG Co-A reductase inhibitor, GM-CSF) and medications that reduce endothelial injury and pathologic vascular remodeling (eg, angiotensin converting enzyme inhibitor, angiotensin II receptor blockade) or inhibit microvascular vasoconstriction are another option.[46,47] Millan et al. recently reported the beneficial effect of infusions of repair-promoting cells at the time of transplantation and during vascular graft rejection.[48]

Not only the treatment targeting renal microvascular changes, but identification of genetic vascular risk profiles (eg, ACE polymorphism, eNOS polymorphism) before transplantation and at the time of initial presentation with chronic renal dysfunction is also important for identifying the high risk patients and proper management of renal allograft.[49,50]

CONCLUSION

A well-functioning vascular endothelium with structural integrity of renal graft is essential for the maintenance of immediate and long-term sur-

vival of the transplant kidney. The vascular endothelium manifests intrinsic cytoprotective properties and elaborates signals that direct self-repair. To improve the survival of graft kidney, adequate immunosuppression with supportive management including blood pressure control and modification of other metabolic risk factors is critical. However, given the importance of transplant graft vasculopathy on both short- and long-term prognosis of the allograft, identification of ongoing endothelial cell activation and injury such as periodic measurement of peripheral blood circulating endothelial cells and an attempt of challenging new strategies for appropriate repair in allograft should be seriously considered.

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