Pathology of the Macro- and Microcirculation of the Kidney

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This overview follows the various sections of the vascular tree, starting with the extra renal arteries and going as far as the peritubular capillaries. The main focus is on non-inflammatory changes of the vascular system, which are worthy of closer attention in view of increasing life expectancy. Thrombi and emboli, especially ateroembolisms, are only briefly mentioned.

Extra-renal artery
The most important changes are atherosclerosis of the Arteria renalis, fibromuscular dysplasia and aneurysms.

The atherosclerosis of the arteria renalis is typically limited to the proximal third of the artery. The morphological characteristics, complications (thrombosis, emboli, aneurysm) as well as the etiology and pathogenesis are the same as in atherosclerosis of the aorta. The frequency is age dependent and it is more common in males. Stenosing atherosclerosis was seen in 5% of cases in unselected autopsies over 65 years of age. In cases with critical stenosis (over 80%) of the arteria renalis, 90% had arteriosclerosis. The changes in the ipsilateral kidney vary with age. Especially in younger patients, a pure ischemic injury will be seen. The presence of a contracted kidney with smooth surface is typical. The contraction of the kidney affects the cortex in particular; normally the weight ratio cortex to medulla is 3:2, with an ischemic contracted kidney this may reach 1:10. Histologically, closely spaced glomeruli and atrophic tubuli of the “endocrine type” (thin or barely visible basement membranes) are seen. In unselected autopsy cases an “ischemic contracted kidney” is seen in about 1%, and in patients with a history of hypertension in 5%.

The second most important change of the extra renal arteries is fibromuscular dysplasia. In contrast to atherosclerosis, these changes dominate in earlier age groups (under 40 in men and women). Depending on the extent of involvement of the vessel wall, an intimal, medial or adventitial form can be differentiated. The medial dysplasia is the most frequent. It is typically bilateral and other vascular regions (carotid and cerebral arteries) may be affected as well. The pathogenesis is still unclear, it may be inherited as well as acquired. Morphologically, the most frequent form, medial dysplasia, reveals ridges of collagen in longitudinal sections, followed by areas with atrophic media and loss of the Lamina elastica. As a result the typical angiographic picture with stenoses and aneurysms is seen.

The third important pathology of the extra renal artery is the aneurysm. The frequency is estimated to be about 0.1% in unselected autopsy cases. Most
aneurysms can be ascribed to atherosclerosis, whereby sack-like aneurysms are more frequent than fusiform types. In general they are small (< 2cms in diameter). As with all aneurysms, they can calcify. Dissecting aneurysms, resulting from idiopathic medial necrosis of the aorta, atherosclerosis or from a trauma are rare.

**Intra-renal artery**
The most important changes seen in the intra-renal artery are intima fibrosis with elastosis (fibro-elastosis = arteriosclerosis), intima fibrosis without elastosis and vasculitis.

Practically all adult renal arteries, from the Arteria renalis up to and including the Arteria arcuata and less frequently the interlobular arteries, exhibit variable degrees of fibro-elastosis. Morphologically, matrix, collagen and sparse myofibroblasts are seen between concentric layers of elastic lamellae in the widened, cell poor intima. The media can be normal, but often is atrophic. Hypertrophy is rarely present. The width of the intima increases with age. Excessive intimal fibrosis is seen in patients with risk factors for atherosclerosis and in contracted kidneys. Of interest in such cases is the lack of typical arteromas with foam cells, cholesterol crystals, areas of necrosis and calcification in the widened intima. The elastic lamellae stain as elastin, but are said to consist of abnormal collagen (pseudo-elastin). However, the only stain suitable for revealing elastosis is an elastin stain, e.g. the Verhoeff van Giesen elastin stain. The pathogenesis is still unclear. The endothelium appears normal, as far as this can be judged by light microscopy. Fibrin deposition is not seen. However, the close correlation between fibro-elastosis and the degree of general atherosclerosis on the one hand, and arteriolar hyalinosis on the other, strongly suggest that a similar, if less pronounced, endothelial lesion is the cause of the intimal fibrosis. This restructuring of the vessels leads to hardening of the arteries. The constriction of the vessel lumen due to intimal fibrosis is not trivial, but rather an important cause of ischemic cortical scarring. Fibro-elastosis probably is the most neglected diagnosis in both tumor nephrectomies and in renal biopsies.

The **intimal fibrosis without elastosis** is quite different to fibro-elastosis. As known from general pathology, elastin formation is not seen in organisation or granulation tissue, nor in inflammation. This means that an elastin stain is necessary for differentiation between the two forms of intimal fibrosis. This phenomena - lack of elastin formation in scarred tissue – is not specific for the kidney and occurs in skin scars as well.

These areas of intimal fibrosis are frequently focal and excentric. The structure varies with the age of the lesion. They usually consist of collagen without sclerosis and contain only a few myofibroblasts and even fewer inflammatory cells. Intimal fibrosis without elastin is seen in the course of vasculitis (in transplantation in cases of endarteritis) and in the organisation of emboli and thrombi. However, when contracted kidneys are investigated, for example as a result of vascular rejection or malignant hypertension after long term dialysis, a fibro-
elastosis is found in practically all arteries. This indicates that, following a variable
time lapse, elastin formation is seen in areas of intimal fibrosis that primarily do
not contain elastin.

Arteriole
The next stopping point on our trip through the vascular tree is the arteriole. This is
just as frequently the site of pathology as the arteries. The main lesions in the
arterioles are arteriolosclerosis and lesions in connection with a thrombotic
microangiography. Vasculitis may affect the arterioles, either in isolation or
together with other arteries.

The arteriolosclerosis (arteriolar hyalinosis) is seen predominantly in the afferent
arteriole, it may however also affect the efferent arteriole. Arteriolosclerosis of the
efferent arteriole may only be diagnosed when both the afferent and efferent
arterioles of a single glomerulus can be seen in the same section.
Arteriolar hyalinosis owes its name to the deposition of hyaline (glassy) material. It
begins with an eccentric accumulation of PAS positive material beneath the
endothelium. Later on, these deposits can expand circumferentially. As the degree
of severity increases, an increase in basement membrane-like material in the
neighbourhood of the hyaline deposits and between the smooth muscle cells is
seen. As long as the changes are eccentric, the media remains unaffected. In case
of circumferential deposits, the media atrophies and then the smooth muscle cells
form only a thin band around the outer side of the vessels. Finally, the whole
arterial wall seems to be replaced by hyaline material (at least as seen by light
microscopy). This replacement process is often accompanied by dilatation of the
vessels and is very common in diabetic nephropathy.
The hyaline deposits can stretch proximally into the interlobular arteries. With
severe hypertension, the glomeruli may be affected too (focal segmental
glomerulosclerosis, decompensated hypertension). Even though rarely seen in
biopsies, the arteriosclerosis may regress completely.
Immunohistology shows that the PAS positive glassy material contains IgM and
complement components (C5-9, C3, C1q, C4). Lipids (lipoproteins) may also be
present in large quantities as well. Arteriolosclerosis occurs in scarred tissue, in
advanced age and diabetes mellitus. It is however best correlated with hypertension
in contrast with arteriolosclerosis in other organs (spleen, mesentery).
Experimental studies have shown that these hyaline deposits do not occur at
random along the arterioles, but primarily at dilated segments of the vessels
following contraction rings. In post stenotic sections endothelial damage may result
from turbulent blood flow, which promotes insudation of plasma components into
the vessel walls. Arteriosclerosis of the efferent arterioles is typically found in
diabetes mellitus, but is not proof of diabetes.

In the case of arteriolonecrosis (today we usually speak of malignant
nephrosclerosis or TMA with malignant hypertension) it is less the arteriole but
rather the interlobular artery that is mainly involved. Proximal vessel involvement
can reach as far as the lobar arteries and distal involvement may reach the
glomeruli.

The typical lesions are thrombi, fibrinoid necroses, mucoid expansion of the intima
and onion skin-like changes of the vessel walls. Initially severe endothelial and
myocyte injury is present. Vessels that are particularly severely affected may, in
part, lack an endothelial lining. This, on the one hand, promotes thrombi formation
and, on the other hand, favours the insudation of plasma components and fibrin
into the vessel wall producing medial necrosis (fibrinoid necrosis), which may
extend into the perivascular connective tissue. The arteries often reveal a mucoid
widening of the intima, superimposed on an underlying intimal fibrosis. Within the
mucoid intima, a few proliferated myofibroblasts are seen, which may later arrange
themselves in a circular pattern and produce collagen and elastin (pseudoelastin).
This then results in an onion skin-like structure of the arteries. The changes in the
arteries do not differ from those seen in TMA of any other etiology. Depending on
the severity of disease, infarction may occur. The cause of the lesion is malignant
hypertension, which is usually a complication of an underlying but inadequately or
untreated hypertension.

Hyaline deposits of PAS positive material are not solely seen in arteriolosclerosis,
but also occur in thrombotic microangiography and in particular are especially
typical for calcineurine-inhibitor arteriolopathy. Here, in contrast to
arteriolosclerosis, the hyaline material is not primarily found subendothelially but
rather in the region of the media or even on the outside of the arteriole. This arises
since individual endothelial and muscle cells die (become necrotic) and knot-like
protein deposits are formed by plasma insudation in the empty basement membrane
“bags”. Finally the arteriolar wall is transformed into a ring of pearls form,
consisting of hyaline material. Even though the lesions resemble one another in
advanced stages, knot-like deposits should not be classified as hyalinosis. They
should be interpreted as arteriolopathy accompanying a late stage of TMA or
calcineurine-inhibitor arteriolopathy, which must be considered as a special,
abortive form of TMA. In the case of calcineurine-inhibitor arteriolopathy,
following dose reduction, regression of hyaline deposits and reconstruction of the
vessels is seen.

Capillary
The last stopping point in the vessel landscape is the capillary. In the past
pathologists have concentrated mainly on the glomerular capillaries. In connection
with the capillaritis of humoral rejection, the peritubular capillaries have attracted
renewed attention.

In this connection we can learn something from a disease that was never common
in the USA, but in Switzerland was responsible for more than 30% of end stage
renal disease till 1980: the Phenacetin-Kidney (incorrectly termed analgesic
nephropathy).
This latter disease is essentially due to injury of the peritubular capillaries in the kidney and the mucosal capillaries of the descending urinary tract (capillary sclerosis). By light microscopy the capillaries exhibit massive thickening of the vessel walls. The capillaries are lined by endothelium; in advanced stages this endothelial lining is lost. The electron microscope reveals that the capillary wall consists of concentric basement membrane layers separated by deposits of cell debris. These changes are induced by toxic intermediary products of phenacetin. The multi-layered structure of the basement membrane is due to repeated episodes of epithelial necrosis and regeneration, whereby the regenerating epithelial cells form a new layer of basement membrane.

From this finding we can generalise that capillary endothelial injury can best be judged on the basis of changes in the basement membrane. Mild injury results only in subendothelial edema, whereas severe, widespread injury as a rule leads to endothelial regeneration and synthesis of new basement membrane. Similar phenomena are seen not only in peritubular capillaries, but equally well in the glomerular capillaries, and are recognised as TMA and/ or transplant glomerulopathy.

Multi-layering of the peritubular capillaries seen in humoral transplant rejection is thus a result of repetitive endothelial injury, probably with necrosis. Here, the endothelial lesions are not of a toxic nature, but result from an antigen-antibody reaction with subsequent complement activation.

**In summary,** it is true to say that virtually all blood vessel pathology of acquired diseases has a common final pathway of injury: Following primary endothelial damage and, depending on the severity, reparation with matrix formation, vessel wall thickening and possibly renal injury occurs. The type of morphological lesion varies, depending on the vessel caliber and the cells present i.e. endothelium, myofibroblasts, and inflammatory cells.
ENDOTHELIAL PATHOLOGY IN THROMBOTIC MICROANGIOPATHIES

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Introduction
Thrombotic microangiopathy is a term for a morphologic lesion characterized by platelet and fibrin thrombi involving the microvasculature. The morphologic lesions thus overlap between hemolytic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and the lesions seen in scleroderma and malignant hypertension. HUS/TTP typically involves glomeruli and smaller vessels, whereas scleroderma and malignant hypertension involve interlobular arteries and arterioles. Hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) share the morphologic lesion of thrombotic microangiopathy, characterized by platelet thrombi occluding the microvasculature. The HUS and TTP syndromes overlap clinically. Recent evidence indicates differing pathogenesis (see below).

Clinical Features
The clinical features of essential hypertension complicated by malignant hypertension, scleroderma and classic HUS vary markedly. However, these entities may all have fibrin in the glomeruli and arterioles and interlobular arteries in the kidney. In essential hypertension, the level of blood pressure does not directly predict degree of end organ damage: African-Americans have higher risk for more severe end organ damage at any level of blood pressure. In malignant hypertension, fibrinoid necrosis primarily involves arterioles and interlobular arteries, contrasting the typical glomerular dominance of fibrin thrombi in classic HUS.

Scleroderma is a multisystem disease that affects the skin, the gastrointestinal tract, the lung, the heart and the kidney involvement occurs in approximately 60-70%. Scleroderma renal crisis, manifest by malignant hypertension, acute renal failure, some even with infarcts, develops in approximately 20% of patients with systemic sclerosis. Age at onset of systemic sclerosis is 30-50 years, and females are affected more than males.

HUS/TTP overlap clinically. TTP is more common in adults, and is characterized by fever, bleeding, hemolytic anemia, renal failure and neurologic impairment. Classic diarrhea-associated HUS is most common in infants and small children, and is characterized by acute renal failure, non-immune hemolytic anemia and thrombocytopenia. The renal manifestations at presentation include hematuria and low grade proteinuria with elevated creatinine in severe cases. Intravascular hemolysis is evident by increased bilirubin and LDH, reticulocytosis and low haptoglobin. Of note, thrombocytopenia may not be present by the time a renal biopsy is performed, especially in the transplant setting, where these manifestations of injuries in the kidney may be dysynchronous with peripheral blood manifestations.

Pathologic Features
Malignant hypertension/scleroderma
Microscopically, there is fibrinoid necrosis of afferent arterioles. Interlobular arteries show intimal thickening, proliferation of endothelial cells, and edema. RBC fragments are often present within the injured vessel wall, and there may be vessel wall necrosis and/or fibrin thrombi within vessels. Glomeruli may show ischemic collapse, or fibrinoid necrosis. In chronic injury, there is reduplication of the elastic internal lamina, so-called onion skin pattern. Tubules may show degeneration and even necrosis, especially in scleroderma crisis. Tubulointerstitial fibrosis develops with chronic injury. There are no immune complexes, and EM shows only increased lucency of the lamina rara interna, similar to chronic TMA (see below).

Thus, the pathologic appearance of scleroderma overlaps with malignant hypertension and thrombotic microangiopathy. Idiopathic malignant hypertension tends to involve smaller vessels, i.e. afferent arterioles, whereas scleroderma renal crisis may more often extend to interlobular size and larger
vessels, and TMA typically involves primarily glomeruli. However, distinction of scleroderma and malignant hypertension solely on morphological grounds is not often feasible, and clinicopathologic correlation is required for specific diagnosis.

**HUS/TTP**

Fibrin and platelet thrombi are present, primarily in the glomeruli. Fibrin is best visualized on hematoxylin and eosin or silver stains. Lesions may extend to arterioles, with some overlap with progressive malignant hypertension and scleroderma, where arteriolar and even larger vessel involvement occurs. At early stages, the glomeruli show thickened capillary walls due to fibrillar lucent material in the lamina rara interna of the basement membrane. This may result in a double contour basement membrane by light microscopy silver stains. Mesangiolysis occurs frequently, a subtle lesion, which may be overlooked. Mesangial areas seem to "unravel", resulting in very long, sausage-shaped capillary loops due to the loss of mesangial integrity and coalescence of adjoining loops.

In infants and young children, thrombotic lesions predominate. In older children and adults, varied lesions occur. Many glomeruli may show only ischemic changes with corrugation of the glomerular basement membrane and retraction and collapse of the glomerular tuft. Segmental necrosis may be seen with rare well-developed fibrin thrombi. In severe cases, cortical necrosis with necrosis of tubules and glomeruli may occur. Secondary changes late in the course include glomerular sclerosis, either segmental or global. Reduplication of the glomerular basement membrane may occur in the late phase due to organization following endothelial injury. Arterioles and arteries, when involved, show thrombosis and sometimes necrosis of the vessel wall, with intimal swelling, mucoid change and later intimal proliferation. Fragmentation of red blood cells within the vessel wall may also be seen. Tubular and interstitial changes are proportional to the degree of glomerular changes.

Immunofluorescence studies show no immunoglobulin deposits. Complement and IgM may be present in sclerotic areas. Fibrin and fibrinogen are present in glomeruli and arterioles.

Electron microscopy reveals increased lucency of the lamina rara interna due to accumulation of fluffy material in the subendothelial space, a sign of endothelial injury. Endothelial cells are frequently swollen and detachment may be seen by electron microscopy. Mesangiolysis is a prominent finding in early phases. In the subacute phase, the increased lucency of the lamina rara interna is in part correlated to breakdown of coagulation products. This zone contains breakdown products of fibrin, laminin and fibronectin.

**Pathogenesis**

**Arterionephrosclerosis/malignant hypertension**

Arterionephrosclerosis lesions do not correlate directly with level of systolic blood pressure. It is possible that underlying microvascular disease causes the hypertension and the renal disease in susceptible patients. Underlying causes include possible genetic and structural components, such as decreased nephron number, perhaps linked to low birth weight, even in normal African Americans versus Caucasians. Most recently, polymorphisms in nonmuscle myosin heavy chain (MYH9) have been linked to excess risk of sclerosis in hypertension and HIVAN in African Americans. This protein is expressed in podocytes, and platelets, and appears to be important for actin cytoskeleton in podocyte foot processes. Malignant hypertension is postulated to develop if usual hypertension remains untreated with direct hemodynamic injury causing endothelial injury and fibrinoid necrosis.

**Scleroderma**

The pathogenesis of scleroderma is probably immune with unknown inciting events. Autoantibodies are often present, including anti-topoisomerase I, anticientromere, anti-RNA polymerase, each present in 25%, and only one of these positive in any one patient. Imbalance of vasodilators (e.g. nitric oxide, vasodilatory neuropeptides such as calcitonin gene-related peptide, substance P) and vasoconstrictors (e.g. endothelin-1, serotonin, TXA2) has been described in scleroderma patients. Endothelial injury and impaired vasculogenesis are thought to play key roles in renal scleroderma. Early lesions include endothelial cell apoptosis with large gaps between endothelial cells, loss of integrity of the endothelial lining, and vacuolization of endothelial-cell cytoplasm, with proliferation of pericytes and vascular smooth muscle cells, with later increase in fibroblasts in the interstitium. In addition, arterioles show several basal lamina-like layers, perivascular infiltrates of mononuclear immune cells, obliterator microvascular lesions, and later rarefaction of capillaries. Fibrosis is likely contributed to by increased endothelin-1, TGF-beta, and PDGF, among other growth factors.
HUS/TTP

Numerous etiologies for HUS/TTP exist. We will review pathogenesis of classic, D+ and atypical HUS, infection and drug-related mechanism of injury.

**Classic, D+ HUS.** Recent increased understanding has allowed some separation of most common etiologies underlying HUS and TTP, although much overlap remains. Typical diarrhea associated (D+) HUS is caused by shiga toxin from pathogenic strains of Escherichia coli 0157:H7. Verotoxin was associated with ~90% of cases of HUS in children in North America and Europe. Undercooked hamburger meat is most closely associated with such outbreaks in North America, pointing to cattle as an important reservoir for the implicated strain. Undercooked hamburger meat is most closely associated with such outbreaks in North America, pointing to cattle as an important reservoir for the implicated strain. In addition, this E. coli strain can be transmitted from person-to-person, and outbreaks associated with swallowing contaminated lake water, or ingestion of contaminated fruit or vegetables or cider have occurred.

The mature verotoxin has alpha and beta subunits. The A subunit has ricin-like N-glycosidase activity and the B subunits form a multimer responsible for binding to membrane glycolipids, globotriaosyl ceramide (Gb3), globotetraosyl ceramide and a blood group glycolipid Ag P, that comprise the shiga toxin receptors. The translocated intimin receptor (Tir) is inserted into the plasma membrane of the host enterocyte, and E. coli is anchored to the cells, followed by bloody diarrhea in nearly all patients. The alpha unit is cleaved and taken up by endocytosis, inactivating 60S ribosomes, thereby causing cell death. The Gb3 receptor for verotoxin is highly expressed in human kidney, perhaps underlying the susceptibility of the kidney to this toxin. However, Gb3 levels were not different in normal children vs adults, so the excess risk of children for D+ HUS cannot be simply explained by overexpression of Gb3.

In response to shiga toxin, fractalkine (FKN), a CX3C transmembrane chemokine, acts as an adhesion counterreceptor on endothelial cells and a chemoattractant. This induction plays an essential role in promoting leukocyte-endothelial cell interaction and may contribute to renal microvascular dysfunction. Whether this pathway is particularly upregulated in children remains to be determined. The microvascular endothelium is log orders more sensitive to the toxin than large vessel endothelium. The kidney endothelium is particularly sensitive to the toxin. Toxin exposure causes a proadhesive and prothrombotic change in the microendothelium, even at doses below those resulting in cell death, mediated through effects on protein synthesis. Particularly interesting is the stabilization by toxin of endothelin-1 mRNA, a transcript that is normally labile. A verotoxin-response element in the 3'-UTR of the ET-1 gene allows toxin to stabilize ET-1 mRNA interaction with polyribosomes.

**Atypical HUS/complement/vWF factor dysregulation.** Atypical, diarrhea negative (D-) HUS may often be due to abnormalities in complement dysregulation or abnormalities in von Willebrand factor function. Complement activation is normally controlled by complex interplay of various membrane-bound and soluble factors, such as factor I, which normally dissociates activated C3b. When these regulatory factors are dysfunctional, vast excess of C3b is deposited by amplification. Inheritance has variable penetrance and may be autosomal recessive or dominant. Frequency of mutations of complement regulatory proteins in atypical HUS w=varies, with complement factor H most common (CFH, 20-30%). Other mutations include CFHR1/3 (6%), complement factor I (CFI, 4-10%) or B (CFB (1-2%) , C3 (5-10%), thrombomodulin (THBD, 5%) or membrane cofactor protein, MCP (10-15%). Prognosis varies according to the mutation involved, with best prognosis with MCP mutations, and worse with complement factor H mutations, with mutations of CFI and C3 intermediate.

TTP patients often have abnormalities of the vWF-cleaving protease ADAMTS13 (a member of the "a disintegrin and metalloprotease with thrombospondin type 1 repeats" family of zinc metalloproteases). Absence of ADAMTS 13 results in large von Willebrand factor multimers, which promote platelet aggregation and thrombosis. Patients may have inherited deficiency of ADAMTS13, usually inherited in an autosomal recessive manner, or secondary acquired inhibitors with antibodies to ADAMTS13, which may be triggered, by drugs or infection.

**HIV Infection.** Thrombotic microangiopathy may also occur in other infections including HIV. This complication is a common cause of acute renal failure in HIV positive patients. The pathogenesis is poorly understood. Direct infection of renal endothelial cells with HIV has not been shown, and the renal microvasculature lacks expression of CD4 and other coreceptors such as CCR5 and CXCR4 that mediate HIV infection in leukocytes. Some data suggest that HIV variants and peptide subunits can cause apoptosis in microvascular endothelial cells in vitro. The HIV envelope protein gd120 can induce expression of the procoagulant tissue factor in human arterial smooth muscle cells. However, these mechanisms have not been shown to be operative in vivo. Macaque monkeys infected with HIV -2 developed thrombotic microangiopathy with widespread apoptosis of endothelial cells with TUNEL positivity, an injury pattern referred to as tunelosis.
Drugs. Numerous drugs have also been associated with development of thrombotic microangiopathy including calcineurin inhibitors, antagonists of vascular endothelial growth factor (VEGF), anti-platelet drugs and chemotherapeutic drugs.

Glomerular cell interaction and TMA: anti-VEGF antibodies. Injury of the mesangium with dissolution of matrix and loss of cells is a characteristic finding seen early on in many thrombotic microangiopathies and may result in microaneurysms. This injury pattern is frequently also seen in diabetic nephropathy, illustrating the interplay of glomerular cells in producing the final phenotype of injury. Interestingly, in diabetic nephropathy, local increased red blood cell fragmentation was associated with increased PAI-1, further pointing to local microvascular injury.

The podocyte is crucial for maintaining endothelial cell survival. Elegant studies by the group of Quaggin have pointed to the necessity of having the correct balance of VEGF, produced by podocytes, to optimize endothelial cell survival. This principle is illustrated by the occurrence of thrombotic microangiopathy in a small subset of patients receiving cancer therapy with antagonism of VEGF, such as bevacizumab. Local deficiency of VEGF induced in adult mice by podocyte knockout of VEGF resulted in thrombotic microangiopathy. These results illustrate the importance of the podocyte for maintenance of normal glomerular integrity. The fenestrated glomerular endothelium is dependent upon induction of VEGF expression. Loss of VEGF does not allow formation of the normal fenestrated phenotype, a key component of the normally functioning microvasculature in the glomerulus. Importance of the VEGF pathway for endothelial injury is also seen in preeclampsia, where increased soluble receptor for VEGF, sFlt (soluble fms-like tyrosine kinase) is observed, likely arising from ischemic injury arising in the placenta. The increased levels of sflt bind and inactivate VEGF and placental growth factor, with resulting endothelial injury in the glomerulus. Rodents with excess sFlt develop features of preeclampsia, including the classic endotheliosis lesion, with swollen endothelial cells and a bloodless appearance of the glomerulus.

Other drugs- chemotherapy, calcineurin inhibitors, anti-platelet drugs. Other chemotherapeutic drugs also associated with thrombotic microangiopathy, including mitomycin and gemcitabine. A peculiar feature of mitomycin is the long delay after exposure until appearance of thrombotic microangiopathy, perhaps resulting from the dose dependency of injury and the slow turnover of glomerular endothelial cells. These drugs are thought to involve direct toxicity to endothelial cells. Similar mechanisms are thought to play a role with calcineurin inhibitors, which may also injure the media if given in high doses, resulting in the nodular insudation extending to the media and hyalinosis characteristic of calcineurin inhibitor toxicity. Anti-platelet agents such as clopidogrel and ticlopidine also directly activate and are toxic to endothelial cells. Acute immune-mediated injury may also occur with some anti-platelet agents with antibodies directed to platelets, such as can occur with quinine. Rarely, anti-platelet agents may also result in antibodies produced against ADAMTS13. Thus in sum, the mechanisms of drug induced injury are diverse, including endothelial toxicity, immune mechanisms with some patients developing auto-antibodies, and perturbation of the podocyte-endothelium interaction that is key for glomerular homeostasis.

Lupus anticoagulant/anti-phospholipid antibody. TMA may occur in patients with SLE, often but not always associated with anti-cardiolipin or anti-phospholipid antibodies. Antiphospholipid syndrome also occurs in patients without SLE. The morphology of the TMA is not unique, but coexistence of TMA with immune complex disease with features of lupus nephritis should suggest possible APL as an etiology. Isolated APL syndrome in the kidney results in a vascular nephropathy characterized by small vessel vaso-occlusive lesions associated with fibrous intimal hyperplasia of interlobular arteries, recanalizing thrombi in arteries and arterioles with resulting focal sharply demarcated cortical atrophy. Glomerular and arteriolar TMA lesions may also occur.

Rejection. Acute humoral rejection may also have endothelial injury and fibrinoid necrosis of arteries, most often with positive C4d as a marker of antibody binding to the peritubular capillaries, the primary target of this injury. In a study of platelet aggregation in various transplant biopsies, intracapillary platelet activation was found related to injury from many causes and was not specific for antibody-mediated rejection.

Animal models. Further insight and understanding of mechanisms in HUS/TTP have been hampered by a lack of suitable animal models. Radiation injury in the rodent may produce some features mimicking thrombotic microangiopathy, with early endothelial injury, fibrin thrombi followed by organization of lesions and increased scarring. This progressive renal injury was sensitive to inhibition of angiotensin with associated marked decrease in plasminogen activator inhibitor-1 (PAI-1). However, in a mouse model of HUS with intraperitoneal injection of shiga toxin- II and lipopolysaccharide, PAI-1 deficiency did not modify
morbidity or mortality, but was associated with increased inflammation and increased expression of TNF-alpha. Of note, this model is limited in that there mainly is tubular injury with minimal fibrin thrombi, although peripheral blood hemolysis and thrombocytopenia was observed, and acute renal failure developed.

In vitro studies of human glomerular endothelial cells support that local renin angiotensin system activation may be important in promoting injury. Cells exposed to angiotensin showed increased tissue factor activity when they also were injured with TNF-alpha and shiga toxin 1, and with less injury when treated with either angiotensin receptor blocker or angiotensin converting enzyme inhibitor. Recently, a novel mouse model of HUS was reported. Injury was induced by selective renal arterial perfusion with the lectin concanavalin A (Con A) followed by specific anti-Con A antibody. Glomerular and peritubular capillary microvascular thrombosis developed with renal failure and presence of schistocytes. Although the initial pathogenic insult differs from that typically seen in humans, this model could prove useful in establishing secondary mechanisms of injury.

Clinicopathologic Correlations and Prognosis of HUS/TTP lesions
Histological distribution of lesions may have some prognostic significance (see below). Age has a major impact on prognosis. Mortality of TTP in adults was nearly 100% before advent of plasma therapy. Children have a much more benign course, with less than 10% mortality even when only symptomatic treatment was given. Improved survival in the last ten years is associated with use of a combination of anti-platelet agents and plasmapheresis. In some series, plasma exchange has resulted in better prognosis than plasma infusion, but the results are not clear-cut. HUS accounts for about half of cases of acute renal failure in HIV patients, and has a poor outcome.

Long-term follow-up 10 years after HUS has shown decrease in GFR in half of patients. Histological distribution of lesions may have some prognostic significance. Degree of histologic damage rather than initial clinical severity was the best predictor of long-term prognosis in HUS. Predominantly glomerular involvement has a better outcome than larger vessel involvement. Glomerular predominant injury is the most frequent pattern of injury in children. Hypertension is more frequent with larger vessel, rather than glomerular, injury. Poor prognosis was predicted by cortical necrosis or thrombotic microangiopathy involving >50% of glomeruli at time of presentation. Segmental sclerosis was associated with decreased GFR long term. Recurrence in the transplant is very common in familial forms of HUS, and is most often associated with graft loss, except for patients with MCP mutation, as this factor is produced in the kidney, and the normal transplant may thus provide sufficient factor to normalize function. Other complement regulatory factor mutations may require liver transplant for cure. Initial levels of serum plasminogen activator inhibitor-1 (PAI-1) in patients with HUS also correlated with worse long-term outcome, perhaps because high PAI-1 promotes thrombosis and also inhibits matrix breakdown.

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THE TRANSCRIPTOME OF ENDOTHELIAL PATHOLOGY:
RELEVANCE TO ANTIBODY-MEDIATED MICROCIRCULATION INJURY

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Introduction

In solid organ transplantation, two forms of allograft rejection have been recognized: T-cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR). The destructive power of alloantibody was first recognized in 1960s as hyperacute rejection due to preformed donor specific antibody (DSA) at time of transplantation (1). However, the problem of ABMR post transplantation remained unrecognized for decades. In early 1990s, Halloran et al. (2;3) identified cases of acute rejection of kidney allografts that were associated with anti-HLA DSA. These rejections were more severe than TCMR, were resistant to therapy, and were associated with endothelial injury in the microcirculation and vasculitis (2-4). This observation was then followed by discovery of C4d (a split product of component C4) staining in allograft tissues as evidence of antibody acting on endothelium, which linked antibodies to graft pathology (5;6). After another decade, late graft losses and histology of chronic graft injury were related to C4d and DSA (7-10).

ABMR has been demonstrated in kidney, heart, pancreas and lung allografts, and is not restricted to presensitized patients (11-18). Acute C4d+ ABMR has since been reported to occur in about 7% of conventional kidney transplant recipients and in 20–30% of biopsies with acute rejection, and in 20-48% of positive cross-match patients (14;19-21).

Diagnosis of ABMR currently requires the simultaneous presence of diffuse C4d staining, DSA, and histopathologic evidence of tissue injury (22;23). C4d+ ABMR can occur early or late post transplant and can be acute or chronic. The morphology of acute ABMR in kidney include peritubular capillaritis; glomerulitis; fibrin thrombi; infarction; and occasionally fibrinoid necrosis of arteries or acute tubular injury (4;22;24). Features of chronic C4d+ ABMR are variable, but include transplant glomerulopathy (TG) (7;10;23;25), multilayering of the peritubular capillary basement membrane, and scarring of parenchymal tissue (7;10;11;23). However, more than 50% of TG with alloantibodies are C4d-negative (10;26), suggesting that C4d is insensitive for chronic ABMR. Thus true incidence of chronic ABMR remains unknown.

Microcirculation endothelium is the main target of ABMR

Although alloantibodies play major roles in rejection, the precise mechanisms by which alloantibodies cause allograft injury, dysfunction, and loss are not well understood. On the other hand, it is well recognized that the endothelium in microcirculation is the main target of ABMR (16;27-29). Antibody ligation to HLA or blood antigens expressed on the endothelium can initiate activation of the complement system, recruitment of leukocytes (causing vascular
inflammation), and facilitation of NK-cell-mediated or monocyte/macrophage-mediated cytotoxicity. Together, these reactions to alloantibodies can result in endothelial damage, loss of vascular integrity, and increased coagulation (19;30-33). Antibodies and complement components can alter gene expression in endothelial cells or inflammatory cells via Fc receptors and complement receptors, which further remodel the lesions of ABMR that compromise graft function.

The current diagnostic criterion of C4d staining does not identify many kidneys that are failing due to ABMR

Detecting which renal transplants are at risk for antibody-mediated deterioration is an unmet need. Anti-HLA antibodies develop in 25% of renal transplant recipients and are associated with increased graft failure (34), but have low predictive value for graft loss.

ABMR is a major cause of late kidney transplant failure (35). Data from two major transplant centres independently indicated that most late kidney transplant losses have a specific etiology that is not idiopathic fibrosis or drug toxicity (35;36). We recently studied phenotypes of late failed grafts (after 1 year) in a prospective study of 173 conventional kidney transplants (35). In this study, the Banff diagnostic categories (23;37;38) (the current gold standard for the assessment of kidney transplant biopsies) could not explain many of the graft losses. Conversely, if histologic changes in microcirculation and presence of anti-HLA alloantibodies were used to define ABMR (regardless of the presence of C4d), 63% of the late kidney failures were attributed to antibody-mediated microcirculation injury, of which many were C4d-negative and thus were missed by the current Banff diagnostic criteria, suggesting that detection of this phenotype requires new diagnostic criteria.

Microarrays of kidney allograft biopsies decipher molecular burden of ABMR:

Microarrays of prospective and unselected kidney transplant biopsies that sampled the entire population of renal transplants with clinical difficulties, provided novel insights in understanding the molecular phenotype of ABMR in the allograft tissue when compared with other diseases (39;40).

a. Increased endothelial gene expression

Recognizing the key role of endothelial changes in ABMR, we hypothesized that altered expression of endothelial genes in biopsies from patients with alloantibody would identify kidneys incurring antibody-mediated damage. In the Transplant Transcriptome Project at the University of Alberta, we studied 173 consecutive renal transplant biopsies for acute or chronic renal dysfunction and/or proteinuria. A study of the literature identified 119 endothelial-associated transcripts (ENDATs), and we used microarrays to determine expression of these transcripts (40). Mean ENDAT expression was increased in all rejection cases; however, their expression was selectively higher in C4d+ ABMR biopsies than in TCMR. ENDAT scores correlated with DSA and histopathologic lesions of ABMR, but not lesions of TCMR.
Many individual ENDATs were increased C4d+ ABMR and predicted future graft loss. The expression of VWF, EDN1, CAV1 (caveolin 1), CDH5 (cadherin 5), CDH13 (cadherin 13), PALMD, PECAM1, SELE, FY (Duffy blood group), RHOJ, SOX7, SOX18, THBD (thrombomodulin), and MALL (BENE; interacts with CAV1) was higher in ABMR than in TCMR biopsies (40). The increased expression of endothelial genes in ABMR with microarrays was confirmed by RT-PCR.

The biology underlying increased ENDAT expression could include a number of processes, including endothelial activation, repair, and/or angiogenesis. For example, seven ENDATs elevated in C4d+ ABMR (VWF, MCAM, CDH5, SELE, PECAM1, CD34, CAV1) are associated with endothelial activation and endothelial cell-cell interactions (30;41). KLF4 (Kruppel-like factor 4), another ENDAT elevated in ABMR, is a regulator of endothelial activation and has anti-inflammatory and anti-thrombotic effects on endothelium (42). SOX7 and SOX18, other endothelial transcripts that increased in ABMR, play redundant roles in endothelial differentiation and some members of SOX family i.e. SOX18 are involved in postnatal angiogenesis in mice (43).

**b. Increased Ifn-γ effects**

Interferon-gamma (Ifn-γ), presumably from antigen-triggered effector T cells, plays a major role in allograft rejection. Both ABMR and TCMR biopsies showed high expression of Ifn-γ induced transcripts i.e. CXCL11, CXCL9, CCL5 (RANTES) (39;40). Ifn-γ induces expression of many genes in the donor tissue and in host infiltrating cells, inducing chemokines such as Cxcl9, Cxcl10, CXL11 and MHC class Ia (HLA-A, HLA-B), class Ib (e.g. HLA-E), and class II (HLA-DP, -DQ, and -DR) (44;45).

Ifn-γ secreted by T cells and/or NK cells is essential for induction of class I and class II antigens on endothelium (45). Increased major histocompatibility antigens on endothelium by Ifn-γ, increases antibody ligation to endothelium and thus makes antibodies more effective in inducing graft inflammation and injury.

**c. Leukocytes, platelets, and Fc Receptors are involved in antibody-mediated graft injury**

Microvascular inflammation (capillaritis, glomerulitis) is the typical histopathological feature of ABMR and is associated with DSA (46). Leukocyte recruitment to the allograft capillaries can be mediated by complement split products (C3a, C5a), activated endothelial cells, and activated platelets. There is recent evidence that complement-independent mechanisms can also trigger microvascular inflammation: Yamakuchi et al (47) showed that alloantibody against class I triggers endothelial exocytosis by the release of VWF and externalization of P-selectin in vitro in the absence of complement, but this requires cross-linking of two Fab portions of IgG. The same group also showed that alloantibody induces VWF expression and platelet adhesion to microvessels in mouse skin allografts (33). Released VWF is a prothrombotic molecule that can mediate platelet aggregation. P-selectin, whereas, is a cell adhesion molecule expressed on activated endothelium and platelets, that triggers the initial stages of leukocyte trafficking.
Antibody-dependent cellular cytotoxicity can damage/kill endothelial cells coated by alloantibodies. NK cells, monocytes/macrophages, and other leukocytes have surface receptors for the Fc portion of IgG. In particular, FcγRIII (CD16) binding to IgG causes release of cytotoxic granules (perforin, granzymes) from NK cells that in turn triggers apoptosis of the target cell (19;48).

**Endothelial gene expression studies led to discovery of C4d negative ABMR**

Kidneys with high intragraft expression of ENDATs and circulating antibody showed lesions of ABMR (capillaritis, glomerulitis, TG, and fibrosis/atrophy) and poor outcome (40).

Many of these active ABMR cases were missed: Only 40% of kidneys with high ENDAT expression and chronic ABMR or graft loss was diagnosed by C4d positivity. However, high ENDAT expression was not an indicator of graft damage or eventual graft loss in patients that lacked anti-HLA antibodies.

We grouped patients according to increased ENDAT score (“E”), HLA antibody positivity (“A”), and C4d positivity (“C”). Of 161 kidneys, 13 were AEC, 37 AE, 21 E only, 50 A only and 40 with no AEC. AEC and AE kidneys were associated with increased TG, scarring and poor survival. Survival did not differ significantly between AEC and AE; thus; C4d positivity did not have an additional adverse prognostic impact over high ENDAT with antibodies. However, kidneys with only E or only A did not show increased TG, scarring or graft loss, compared to kidneys with no AEC (40). We conclude that ENDAT changes in renal transplants occur in rejection and in other forms of renal injury (infections, ischemic injury), but their impact on TG and graft loss is principally in patients with antibodies.

High ENDAT expression with the presence of antibodies predicts graft loss with higher sensitivity (77% vs. 31%) and slightly lower specificity (71% vs. 94%) than the presence of C4d. These results were validated in independent set of 82 kidney grafts. Therefore, high renal endothelial transcript expression in patients with circulating alloantibody is a proven indicator of active antibody-mediated allograft damage and poor graft outcome (40).

Most C4d negative ABMR biopsies demonstrated chronic ABMR pathology (TG). However, our recent analyses show that there are some C4d negative biopsies with DSA, high ENDAT expression, and acute ABMR lesions in the absence of TCMR or other pathology (unpublished data). Thus C4d negative acute ABMR probably occurs, and its incidence is yet to be determined. Supporting this point, 49% of sensitized kidney transplant patients developed subclinical C4d negative ABMR (capillaritis plus glomerulitis plus DSA) (versus 31% C4d+ ABMR) at 3-month protocol biopsies, who later developed increased fibrosis, capillaritis, TG, and low graft function at 1-year (49).

**Conclusion**

Microarrays of kidney allograft biopsies defined molecular features of ABMR and detected a new group of C4d negative active ABMR with endothelial transcript changes, chronic graft injury, and failure. Thus, complement-independent mechanisms also probably operate in
ABMR. Recent molecular data show that C4d staining has low sensitivity for detecting active ABMR. Measuring endothelial gene expression in biopsies from kidneys with alloantibody is a sensitive and specific method to diagnose ABMR and predict graft outcomes.

Reference List


Cardiovascular-renal diseases constitute a growing epidemic in the Western world. Thus far, therapeutic approaches to treat these conditions have focused on merely preventing cellular damage and slowing the progression of functional deterioration. Modern medicine has been challenged to develop ways to regenerate damaged, dysfunctional organ tissue. Within recent years, evolving research has challenged the classical dogma that terminally differentiated organs lack the capacity for cell turnover and regeneration. The recent discovery of endogenous repair mechanisms implicating hematopoietic stem and progenitor cells set off the concept of tissue repair. Stem cells (SC) possess the capability of self-renewal, transformation into dedicated progenitor cells, and differentiation into specialized progeny such as endothelial progenitor cells (EPC). In contrast to SC, progenitor cells are immature cells with a more limited differentiation potential. They proliferate for a finite number of cell divisions. By convention, EPC are characterized by hematopoietic SC markers such as CD34 or CD133 and expression of an endothelial surface marker (vascular endothelial growth factor receptor-2 or kdr, von Willebrand factor, VE cadherin, CD146), uptake of Dil-acetylated lipoprotein, and lectin binding. On the basis of surface markers, EPC can be quantified and isolated by flow cytometry. Because of their adhesion properties to fibronectin, EPC can be further grown in culture from unfractionated bone marrow using selectively enriched medium.
EPC that were originally isolated from the mononuclear cell fraction of peripheral blood have subsequently also been isolated from human umbilical cord blood as well as from mononuclear cell, CD34+ and CD133+ hematopoietic SC fraction derived from bone marrow. It seems that the bone marrow may not be the single source of EPC, because a CD14+/CD34−/CD133− mononuclear side population with angiogenic profile has been characterized in various tissues. Overall, controversy still exists regarding the identification and origin of EPC.

Mobilization and Homing of EPC
Whereas EPC levels remain low under normal conditions, ischemia and tissue injury are believed to be the predominant trigger for EPC to travel from the bone marrow niche. The release of EPC from bone marrow is regulated by a variety of growth factors, enzymes, ligands, and surface markers. Today, the concept of EPC mobilization envisions an ischemia-dependent secretion of proangiogenic cytokines as stromal cell–derived factor-1 (SDF-1) or vascular endothelial growth factor (VEGF) induced by hypoxia-inducible factor at the site of ischemia, which, in turn, are released to the circulation. Other EPC-mobilizing factors are placental growth factor, G-CSF, and GM-CSF. In the bone marrow activation of matrix metalloproteinase-9, which cleaves membrane-bound Kit ligand to a soluble Kit ligand, releases cKit+ EPC to the vascular zone of the bone marrow niche. Endothelial nitric oxide synthase–derived nitric oxide is a prerequisite for matrix metalloproteinase-9. We have recently shown that also the cleaved receptor for uPA is a migratory stimulus for EPC. This molecule seems to be released from neutrophils during an inflammatory response.

Tissue injury has also been described by Goligorsky et al. as an
important stimulus for EPC release. Acute elevation of uric acid may serve as a universal herald of tissue injury fast-acting endogenous mediator of EPC mobilization and renoprotection.

Hetero-, homo-, and autologous EPC are capable of entering sites of active neovascularization. Integrins and tissue-specific adhesion molecules are key elements for adhesion of hematopoietic SC. Angiogenic chemokines such as SDF-1 and VEGF do not merely function systemically to mobilize EPC from the remote bone marrow. These factors also attract circulating EPC locally to the ischemic tissue. SDF-1 overexpression enhances homing of EPC that are particularly sensitive to SDF-1. The SDF-1 receptors CXCR4 and CXCR7 play an essential, but differential, role in the therapeutic homing of human renal progenitor cells in ARF, with important implications for the development of stem cell-based therapies.

Rabelink and coworkers have recently shown that the phenotype of EPC can be influenced by the „milieu interieur“ of diabetic patients. In patients with diabetes and in diabetic animals, the number of circulating EPC is decreased, and functional parameters such as adhesion, migration, and the paracrine secretion of proangiogenic factors are impaired, likely as a consequence of hyperglycemia. They observed that bone marrow differentiation cultures for EPC, macrophages, or dendritic cells from hyperglycemic bone marrow yielded fewer EPC and more macrophages compared with control bone marrow. These changes were directly related to the hemoglobin A1C levels of the donor mice. bone marrow-derived dendritic cell numbers were not affected by hyperglycemia. The composition of the bone marrow was not altered; in particular, the numbers of CD31+/Ly6C+ cells, which serve as common progenitors for EPC, macrophages, and dendritic cells, were unaffected. In addition,
bone marrow-derived EPC from hyperglycemic mice were less angiogenic and more proinflammatory in regards to endocytosis, T-cell activation, and interleukin 12 production.

EPC and Regenerative Mechanisms
For a long time, the vascular endothelium was believed to be an inactive, inner lining of blood vessels. Repair of the endothelium was believed to occur by proliferation of endothelial cells from wound margin to center. However, in recent years, the endothelium has been identified as a highly active structure running from the endothelial layers of conduit vessels through into microcirculation. A novel perspective proposes an interaction of BMC and EPC with the endothelium to repair vasculature by angiogenic action. EPC have further been shown to promote repair of endothelium (reendothelialization) and postnatal formation of new capillaries (neovascularization).

Earlier, EPC were believed to affect vascular repair by differentiation into endothelial cells. Consistent with the idea of transdifferentiation, EPC historically were reported to incorporate vessel structure. However, several report showed no incorporation of BMC into growing vessels structure while BMC accumulated in perivascular areas. Therefore, the capability to promote vascular growth seems to be related to paracrine effects.

Homing of EPC to the injured tissue releases cytokines, chemokines, and growth factors. These secreted factors, in turn, affect the surrounding injured tissue in a paracrine manner. bone marrow-derived mononuclear cells have been reported to release multiple angiogenic cytokines such as VEGF, angiopoietins, and fibroblast growth factor. Because progenitor cells might serve more as a "cytokine factory" rather than the building material, the external EPC application potentially boosts
endogenous repair mechanisms after tissue injury. This indicates that cell therapy seems more favorable than a single-gene therapy.

EPC and renal disease
EPC correlate inversely with risk factors of arteriosclerosis; age, smoking, hypercholesterolemia, and diabetes lower EPC number and function. Manifest coronary artery disease, angina pectoris, and myocardial infarction have a robust impact on EPC mobilization. EPC levels are also inversely related to the severity of congestive heart failure.

We have shown that the number of EPCs is significantly reduced in patients with advanced renal failure as compared with age- and gender-matched healthy subjects. Moreover, impaired renal function and uraemia result in +AH4-30% decrease of circulating EPCs. In addition, these EPCs are also malfunctioning. In patients with chronic kidney disease (CKD) stage V receiving haemodialysis therapy, a low level of circulating CD34 cells, i.e. a population including also EPCs, was shown to be an independent predictor for both prevalent cardiovascular events and all-cause mortality. In addition, we found a significant correlation between CD34+ HPCs and EPCs both in healthy subjects as well as in renal patients. Taken together, these findings point to a problem of differentiation of precursor cells to EPCs or to reduced mobilization of EPCs from the bone marrow, or both, in uremia. The former assumption is supported by the observation of a significant inhibitory effect of uremic serum on the differentiation capacity of EPCs in vitro. Several factors present in patients with CKD may have a negative impact on EPC number and function: hypertension, dyslipidaemia, glucose intolerance (even in the absence of frank diabetes), micro-inflammation and increased C-reactive protein (which has a direct effect on EPC number
and activity), oxidative stress, low levels of erythropoietin (EPO), known and yet unidentified uraemic toxins etc. The latter is particularly intriguing, since we and others could show that initiation of haemodialysis therapy or successful kidney transplantation improves EPC number and function. Interestingly, kidney graft function directly determines EPC number. Conversely, support or replacement of renal function seems to be accompanied by recovery of EPC biology. Within 12 mo after kidney transplantation, uremia-induced EPC function was restored in a small sample population, although EPC numbers remained unchanged. In line with this finding, already nocturnal hemodialysis improved impaired EPC levels and function in patients with end-stage renal disease, whereas in the conventional hemodialysis group, EPC biology was impaired.

Therapeutic strategies with EPC

EPC seem to support repairing mechanisms in kidneys of endothelial, mesangial, and tubular structures. Transplantation of EPC expanded from a muscle SC pool, locally engrafted, and improved renal function in an acute renal ischemia rat model. Some animal studies provide evidence that EPC contribute to glomerular capillary repair. In humans, acceptor-derived EPC have been localized in kidney allografts, replacing donor endothelial cells and potentially repairing transplant-related vascular injury. In a glomerulonephritis rat model, injection of bone marrow-derived mononuclear cells into the renal artery improved
endothelial injury and mesangial cell activation. Overall, these findings strongly advocate that EPC can also play a significant role in renal repair and present an exciting target for regeneration in renal disease.

The application of EPC seems to improve tissue perfusion and function after ischemia. After the initial flurry of early clinical studies, it is time to reconsider its real potential. In order to advance regenerative medicine, deeper knowledge of the underlying mechanism in this treatment is mandatory. The disappointing regional retention of transplanted cells at this time, for example, needs improvement. In the clinical setting, cell type, dosage, and timing of application need further refinement. Even though the clinical application seemed to be safe over a span of months, long-term follow-ups for adverse events, morbidity, mortality, and quality of life are essential before final safety conclusions can be drawn. Overall, conducting large-scale, double-blinded, randomized, controlled studies is the only way to find the answers to remaining questions and ensure patient safety and improved outcomes.

Pharmacological modulation of EPC

Instead of cell therapy with EPCs (i.e. progenitor cell transplantation) that is still hampered by many technical and regulatory obstacles, pharmacological stimulation and/or modulation of EPCs might be more promising in terms of clinical practicability. It is known that physiological mobilization of EPCs from their niches such as the bone marrow can be triggered by mechanical injury and ischaemic stress via generation of hypoxia-inducible factor-1 regulated release of VEGF, EPO, SDF-1 and GM-CSF. With respect to the possibility of their pharmacological
mobilization, various drugs like statins, glitazones or insulin [have been shown to stimulate EPCs. The angiotensin-converting enzyme inhibitors ramipril and enalapril were shown to increase EPC levels both in experimental studies and in patients, probably by interfering with the CD26/dipeptidylpeptidase IV system. Similar findings were shown for angiotensin receptor blockers like olmesartan, irbesartan, losartan or telmisartan. The EPO receptor, which main function is to stimulate the proliferation and the differentiation of erythroid precursor cells, is also present on endothelial cells, suggesting a common ontogenesis for the haematopoietic and endothelial lineage. Both rHuEPO as well as its longer lasting derivate darbepoetin alpha significantly enhance the number and functional properties of EPC via the activation of the intracellular Akt protein kinase pathway. Interestingly, this effect was already observed with a dose of 30 IU/kg per week and less, i.e. a dose which does not induce erythropoiesis. Finally, some drugs such as immunosuppressive agents may also negatively affect EPCs since their number was reduced by >40% with addition of prednisolone or cyclosporine A in concentrations corresponding to the usual daily therapeutic doses into the cell culture media.
References


