Angiogenesis and Endothelial Cell Repair in Renal Disease and Allograft Rejection

Marlies E.J. Reinders,*† Ton J. Rabelink,‡ and David M. Briscoe*

*Transplant Research Center, Division of Nephrology, Department of Medicine, Children’s Hospital, and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts; and †Department of Nephrology and Hypertension, University Medical Center Leiden, Leiden, The Netherlands

This review discusses the concept that the turnover and replacement of endothelial cells is a major mechanism in the maintenance of vascular integrity within the kidney. CD133⁺CD34⁺KDR⁺ endothelial cell progenitor cells emigrate from the bone marrow and differentiate into CD34⁺KDR⁺ expressing cells, which are present in high numbers within the circulation. These progenitor cells are available for recruitment into normal or inflamed tissues to facilitate endothelial cell repair. In several forms of renal disease, proinflammatory insults mediate oxidative stress, senescence, and sloughing of endothelial cells. A lack of growth factors or an inefficient recruitment of endothelial cell progenitors results in hypoxic tissue injury and accelerates the process of chronic renal failure. Augmentation of vascular repair by the provision of growth factors such as vascular endothelial growth factor or by the transfer of progenitor cells directly into the kidney can be protective and prevent ongoing interstitial damage. In allografts, persistent injury results in excessive turnover of graft vascular endothelial cells. Moreover, chronic damage elicits a response that is associated with the recruitment of both leukocytes and endothelial cell progenitors, facilitating an overlapping process of inflammation and angiogenesis. Because the angiogenesis reaction itself is proinflammatory, this process becomes self-sustaining. Collectively, these data indicate that angiogenesis and endothelial cell turnover are important in renal inflammatory processes and allograft rejection. Manipulation of the response may have therapeutic implications to protect against injury and chronic disease processes.


Angiogenesis is well established to be a characteristic component of immune inflammation and has been shown to be of pathologic significance in ischemic and chronic inflammatory diseases, including diabetes, retinopathy, atherosclerosis, allograft rejection, coronary artery disease, and myocardial infarction (1–13). Moreover, several acute and chronic renal diseases, including ischemic nephropathy, glomerulonephritis, and interstitial nephritis, were found recently to be associated with angiogenesis, and there is interest in the concept that manipulation of this response can attenuate the disease process (9,14–19). In the course of acute inflammation, leukocytes and platelets induce and/or deliver angiogenesis factors into the local site, mediate the proliferation of local endothelial cells, and/or facilitate the recruitment of endothelial progenitor cells (EPC) (20–22). In this circumstance, the resolution of the acute response coincides with a resolution of the healing angiogenesis response. In contrast, in chronic inflammation, in which tissue destruction and mononuclear cell infiltration are dominant, the persistent delivery and local expression of angiogenesis factors can serve to sustain the angiogenesis response (4,12,23–26). In its normal guise, angiogenesis is thought to facilitate the repair of injured tissues and to restore oxygenation. In diseases such as glomerulonephritis, ischemic nephropathy, and tubulointerstitial fibrosis and in the aging process, accelerated attrition of the microvasculature as a result of inefficient delivery of angiogenesis factors and/or EPC results in ongoing and persistent hypoxia, which can result in further tissue destruction (9,27–29). In this circumstance, it has been demonstrated that delivery of angiogenesis factors and the augmentation of the angiogenesis response can be therapeutic to promote recovery. Conversely, in chronic diseases such as atherosclerosis and chronic allograft vasculopathy (CAV), persistent angiogenesis promotes the ongoing recruitment of inflammatory cells, which in turn sustains the angiogenesis reaction (3,5,8,24,30). In this scenario, it has been proposed that antiangiogenesis therapy can attenuate disease and is therapeutic. Collectively, these observations suggest that the process of angiogenesis is interrelated with acute and chronic inflammatory disease processes and that manipulation of the reaction may have therapeutic implications.

In this review, we discuss the intriguing mechanistic and functional interrelationship between endothelial cell repair mechanisms and the angiogenesis reaction in renal inflammatory diseases and allograft rejection. We discuss several processes and mechanisms by which the EPC can be recruited into local sites to facilitate repair. Moreover, we identify some diseases in which the expression of angiogenesis factors and the angiogenesis reaction are lacking and others in which the persistent expression of growth factors and the angiogenesis reaction may in itself be of pathologic significance.
Mechanisms of Endothelial Cell Repair

The integrity of the vascular endothelium is critical for the health of an organ and is determined by the balance between endothelial turnover and repair. After an inflammatory insult, damaged endothelial cells slough into the circulation, and replacement occurs via the induced proliferation of neighboring endothelial cells and/or by the recruitment of EPC from the circulation. In recent years, it has become evident that an important source of endothelial cells for repair comes from the circulation and that “vascular health” is dependent on an ample supply of these cell types. In a recent study (31), it was found that levels of EPC in the circulation are indicative of risk for vascular disease. Patients with the highest numbers of circulating EPC were least at risk for coronary artery disease, suggesting that circulating EPC levels and the maintenance of vascular integrity clearly are associated and may be of major clinical relevance (32). However, it is important to note that, at present, our understanding is by association, and more research will be needed to understand the mechanism. For instance, several studies have found that this pattern is not true for dialysis patients, in whom several insults (hypertension, diabetes, oxidative stress, etc.) are associated with an increase in circulating EPC and an increased risk for acute cardiovascular events (33). Also, nitric oxide (NO) is known to mediate the release of EPC from bone marrow (34), and there is a well-established reduction in the bioavailability of NO in association with atherosclerosis. Therefore, reduced circulating EPC in association with atherosclerosis simply may be reflective of a common factor (e.g., NO) and/or suggest more complex interactions with proatherogenic mediators.

Growing evidence suggests that the bone marrow is a rich source of immature EPC and that bone marrow–derived EPC circulate constantly in the blood, albeit in low numbers. Circulating EPC can be recruited into vascular beds to maintain normal physiologic homeostasis/repair. They also may contribute to immune angiogenesis in the setting of chronic inflammation (see below), and several studies have indicated that they play an important role in the formation of new blood vessels in ischemia-reperfusion (35).

Populations of EPC express different cell surface receptors, which vary according to their stage of maturation. The receptors CD34 and the vascular endothelial cell growth factor receptor-2 (VEGFR-2), called KDR, were used initially to characterize circulating EPC (36), but additional studies have determined that another molecule, CD133, also is present on EPC at an earlier stage in their development (37,38). Therefore, although progenitor cells express CD133 at their earliest stage of maturation, it is lost in the course of maturation, whereas the expression of CD34 and KDR persists. Also, it has been found that CD14 is expressed on some immature CD34+/KDR+ EPC (39), whereas other molecules identify endothelial cells at both early and later stages of maturation. These include VE-cadherin, von Willebrand factor, CD31, and Ulex (36–40). Together, these observations suggest that EPC are a heterogeneous population of cells that circulate in the blood at different stages of maturation, each with the potential for differentiation into mature endothelial cells.

Regardless of maturation or cell surface phenotype, all EPC have the capacity to be recruited into sites of injury to maintain vascular integrity (Figure 1). Moreover, it is proposed that the predominant cell type that mediates repair is the most numerous EPC within the circulation (the CD14+/CD34+/KDR+ EPC),...
Development of Angiogenesis in Immune Inflammation

Although hypoxia is the main physiologic stimulus for the formation of new capillaries and for the recruitment of EPC, it also has been shown that endothelial cells proliferate and that angiogenesis is prominent in association with inflammation, even in the absence of hypoxia. Moreover, several studies have demonstrated that the angiogenesis reaction and inflammatory processes are interactive and interrelated (3,4,24). Some proinflammatory cytokines have angiogenic properties, and some potent angiogenesis factors, such as VEGF, have proinflammatory properties (Figure 2). In their original studies to define the ability of leukocytes to induce angiogenesis, Sidky and Auerbach (21,22) observed that the direct local infusion of spleen cells into the skin of nude mice resulted in the formation of characteristic neovessels that showed tortuosity and loop formation, similar to the angiogenesis observed in tumors. The angiogenesis reaction was reproducible, occurred within 3 to 6 d, and was dose dependent, showing a linear correlation between the number of induced vessels and the dose of injected spleen cells. The molecular basis for these observations now are understood and in part involves the secretion of several proangiogenic mediators, including VEGF (4,30,49), TNF-α (25), TGF-β, and NO (50). It is interesting that some of these factors have been found to function in part by stimulating VEGF production, suggesting that VEGF may be a common mediator for the initiation of leukocyte-induced angiogenesis (51–53).

Furthermore, whereas products of activated monocyte/macrophages originally were reported to be most potent in their ability to induce angiogenesis (25,50,54–56), it now is known that activated T cells also are a major source of angiogenesis factors (57–59) and that they stimulate a profound VEGF-inducible angiogenesis reaction (53,60). Consistent with these observations, the expression of angiogenesis growth factors typically is found in tissues with enhanced inflammation. It therefore is not surprising that angiogenesis frequently is associated with inflammatory conditions and that angiogenesis and inflammation induce overlapping and interactive processes.

Another important observation is that angiogenic reactions (even in tumors) typically are associated with inflammatory infiltrates. Using videomicroscopy, Jain’s group (61–63) has defined this interrelationship elegantly, and they reported that neovessels are “sticky” and elicit rolling and stable arrest interactions with leukocytes. It now is established that neovessels at sites facilitate the recruitment of leukocytes, in part via distinct molecular receptor–ligand interactions, such as those that are mediated by adhesion molecules and chemokines (62,64,65). Also, some angiogenesis factors, most notably VEGF, can function as a major proinflammatory cytokine (30). VEGF induces the expression of the adhesion molecules E-selectin, intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 and the chemokines IL-8 and monocyte chemoattractant protein-1 on endothelial cells. Moreover, VEGF is directly chemoattractant for monocytes and can promote inflammation via its potent effect to enhance vascular permeability (30,62,64,66,67). In our own studies that have addressed the proinflammatory function of VEGF, we also identified its ability to augment IFN-γ-inducible expression of the T cell chemoattractant chemokine IP-10 and IP-10–dependent leukocyte recruitment (68). Collectively, these observations indicate that there is a clear molecular basis for the interrelationship between angiogenesis and inflammation and that each process cannot be considered in isolation. Moreover, it is likely that VEGF represents a potent intermediary between each of these reactions.

Exposure of endothelial cells to adverse inflammatory conditions increases endothelial cell apoptosis and turnover. For example, in renal transplant patients, several studies have shown increased levels of CD146 necrotic endothelial cells (69) (discussed in more detail below). Although mature endothelial cell and angiogenesis to divide and facilitate endothelial repair and angiogenesis in vivo (41).

A clinically important extension of these observations is that the systemic or local tissue administration of bone marrow or EPC into patients after an inflammatory reaction can be therapeutic to prevent tissue destruction/scarring and promote healing. In experimental animals, the transfer of EPC after limb or cardiac ischemia has been established to be therapeutic (35,44–48). Early clinical trials in humans have hinted that patients with cardiac disease might benefit from the local administration of enriched bone marrow–derived EPC after acute myocardial infarction (44–46). Furthermore, as is discussed next, in experimental kidney diseases, including ischemic nephropathy, interstitial nephritis, and glomerulonephritis, the provision of EPC or angiogenesis factor(s) can promote neoangiogenesis and can attenuate the severity of disease.

Figure 2. Central role for vascular endothelial growth factor (VEGF) in immune-mediated angiogenesis. VEGF is well established as an angiogenesis factor via its direct effects on endothelial cells. It also has potent proinflammatory properties, suggesting that it also may elicit angiogenesis via the recruitment of circulating leukocytes.
cells have the capacity to proliferate and replace dying cells, chronic exposure to an inflammatory milieu and the associated oxidative stress have been shown to lead to premature replicative senescence and limit this form of endothelial repair. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells (70). Eventually, endothelial cell death and shedding may lead to disturbances of the endothelial monolayer, necessitating endothelial cell repair. Therefore, in the setting of chronic inflammation, several factors elicit damage, and disease may reflect in part a balance between the degree to which loss occurs and the ability of EPC to facilitate repair and maintain tissue homeostasis. Hypoxia, VEGF, basic fibroblast growth factor, angiopoietin-1, placental growth factor, and stromal cell–derived growth factor-1 all have been shown to induce the mobilization and recruitment of EPC into the local site (20,41,43,71). Furthermore, local EPC release growth factors, including VEGF, G-CSF, and GM-CSF, which are known to be chemotactic for EPC and facilitate additional migration of EPC into the site. Therefore, once effective repair mechanisms occur, chronic disease also may be related to amplification loops that serve to maintain the local angiogenic microenvironment.

Collectively, all of these findings indicate that the recruitment of EPC into local tissue sites can facilitate endothelial repair and vasculogenesis. The mechanisms just discussed describe how EPC might be recruited into sites of inflammation. Furthermore, because CD34+ cells also have the capacity to differentiate into macrophages and dendritic cells after treatment with specific cytokines (72,73), the inflammatory milieu may determine whether a CD34+ stem cell matures into either an EPC or a dendritic cell. For instance, treatment of CD34+ cells with GM-CSF and IL-4 promotes their differentiation into dendritic cells (72,73), whereas the presence of VEGF and other endothelial cell growth factors in cell culture medium enhances differentiation into mature endothelial cells (36,37,74). Some recent observations indicated that purified CD34+KDR+EPC can differentiate into dendritic cells (T.J.R., unpublished observations, 2005). Therefore, the overlap between angiogenesis and inflammation may be more complex and inasmuch as the type of inflammation or tissue environment may be a major determinant of the response.

**Endothelial Cell Progenitors and Angiogenesis in Renal Inflammatory Disease**

The development of tubulointerstitial fibrosis is characteristic of chronic renal disease, and inhibition of its progression has been proposed to be of major importance in the preservation of renal function. In both experimental animal models and in humans, it has been shown that there is significant loss of peritubular capillaries as well as defective capillary repair in association with the development of interstitial fibrosis (75–77). Even in the normal aging process, it has been shown that the loss of glomerular endothelium is associated with progressive renal impairment (27,77). It is important to note that the angiogenic growth factor VEGF plays an important role in maintaining glomerular integrity under normal physiologic conditions. VEGF is expressed by podocytes, tubular epithelial cells, and endothelial cells (78–81), and increases in VEGF expression are well established to be functional for renal vasculogenesis and renal development in the embryo (82–84). In addition, in experimental models, it has been shown that postnatal glomerular capillary function is under strict control by VEGF (79,85). When intraglomerular VEGF levels decrease in a transgenic mouse, capillary endothelial cells swell, capillary loops collapse, and proteinuria develops (79,86). Moreover, it has been found thatconditional VEGF isoform knockout mice have impaired glomerular filtration (82). Consistent with these observations, when VEGF function is inhibited in vivo with sFlt-1 (the soluble form of VEGFR-1) or with anti-VEGF antibodies, proteinuria develops, and it has been found that this process is associated with rapid glomerular endothelial cell detachment and downregulation of nephrin, a key epithelial protein in the glomerular filtration apparatus (87,88). Furthermore, the treatment of pregnant rats with sFlt-1 has been found to result in hypertension, proteinuria, and glomerular endotheliosis, the classic lesion of preeclampsia (89). Therefore, decreases in local intrarenal concentrations of VEGF by VEGF antagonists under normal conditions will disrupt glomerular integrity and the glomerular permselective properties.

In models of aging-associated renal disease, the loss of expression of the angiogenesis factor VEGF in podocytes and tubules has been found to correlate with a reduction in the number of proliferating endothelial cells (27). It is proposed that the loss of the intrarenal vasculature results in impaired delivery of oxygen and nutrients to the tubules, which in turn results in chronic ischemia and cell death. Several regions of the medulla normally exist in some degree of hypoxia and low oxygen tension; therefore, any defect in the vasculature will disrupt the high metabolic demands of the tubular epithelial cell and may promote cell death. Therefore, the peritubular capillary network of vessels plays a major role in the maintenance of renal function and hemodynamics. The progressive loss of intraglomerular endothelial cells and/or peritubular capillaries may be a primary factor in the development of chronic renal disease (9,15,88).

Some chronic renal disease processes that are associated with endothelial cell loss and peritubular capillary dropout can be attenuated by the augmentation of angiogenesis or by the transfer of EPC into the kidney. Johnson’s group (9,15,16,27) performed an extensive analysis of the degree to which intraglomerular endothelial cell and peritubular capillary loss is a component of renal insufficiency. Using the remnant kidney model, this group reported that, after injury, there is an initial early angiogenic response with increases in the proliferation of peritubular and glomerular endothelial cells (15). In addition, early after injury, there is an increase in the expression of VEGF (mainly in tubules and glomerular podocytes), but this increase in VEGF expression is transient. A subsequent decrease in VEGF expression is associated with a decrease in endothelial cell proliferation, and it was found that the degree of glomerular and peritubular capillary loss correlates with the severity of glomerulosclerosis, interstitial fibrosis, and tubular atrophy.
Within the glomerulus can result in glomerulosclerosis and a number of diseases, such as diabetic nephropathy, have been associated with progressive renal failure to facilitate glomerular endothelial cell proliferation is of importance in the repair of capillaries (29) and that the administration of VEGF promotes capillary repair and prevents global sclerosis and subsequent chronic renal failure (28). Moreover, blockade of VEGF with a VEGF antagonist inhibited capillary repair and enhanced the progression of renal impairment after the induction of mesangio proliferative nephritis (87). Also in another study, the administration of VEGF as an interruption protocol after injury was already established within the kidney resulted in an increase in peritubular capillary density and improved renal function and was associated with less fibrosis (90). Collectively, all of these studies have demonstrated that progressive capillary loss may be a primary factor in the development of chronic renal insufficiency and that the administration of VEGF can attenuate both the degree of capillary loss and the degree of interstitial disease/renal damage.

Although the systemic administration of VEGF has been shown to mobilize endothelial cell stem cells (91,92), none of these reports addressed whether this is an underlying mechanism by which VEGF therapy improves renal function after injury. Uchimura et al. (93) assessed whether the administration of bone marrow mononuclear cells could limit glomerular endothelial cell injury in the anti-Thy-1.1 model of acute glomerulonephritis, it was shown that glomerular endothelial cell proliferation is of importance in the repair of capillaries (29) and that the administration of VEGF promotes capillary repair and prevents global sclerosis and subsequent chronic renal failure (28). Moreover, blockade of VEGF with a VEGF antagonist inhibited capillary repair and enhanced the progression of renal impairment after the induction of mesangio proliferative nephritis (87). Also in another study, the administration of VEGF as an interruption protocol after injury was already established within the kidney resulted in an increase in peritubular capillary density and improved renal function and was associated with less fibrosis (90). Collectively, all of these studies have demonstrated that progressive capillary loss may be a primary factor in the development of chronic renal insufficiency and that the administration of VEGF can attenuate both the degree of capillary loss and the degree of interstitial disease/renal damage.

Endothelial cell loss and dysregulated repair also have been found to be of pathophysiologic significance in anti–glomerular basement membrane models of glomerulonephritis and chronic renal dysfunction. In the well-established anti-Thy-1.1 model of acute glomerulonephritis, it was shown that glomerular endothelial cell proliferation is of importance in the repair of capillaries (29) and that the administration of VEGF promotes capillary repair and prevents global sclerosis and subsequent chronic renal failure (28). Moreover, blockade of VEGF with a VEGF antagonist inhibited capillary repair and enhanced the progression of renal impairment after the induction of mesangio proliferative nephritis (87). Also in another study, the administration of VEGF as an interruption protocol after injury was already established within the kidney resulted in an increase in peritubular capillary density and improved renal function and was associated with less fibrosis (90). Collectively, all of these studies have demonstrated that progressive capillary loss may be a primary factor in the development of chronic renal insufficiency and that the administration of VEGF can attenuate both the degree of capillary loss and the degree of interstitial disease/renal damage.

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Nevertheless, it is important to note that some kidney diseases, such as diabetic nephropathy, have been associated with high levels of VEGF and that forced overexpression of VEGF within the glomerulus can result in glomerulosclerosis and a histologic pattern that is similar to that seen in HIV nephropathy (79,96). Therefore, the augmentation of peritubular capillary repair and the preservation of angiogenesis by VEGF may not be a uniform mechanism to prevent chronic renal injury. For instance, high levels of glucose are widely known to stimulate VEGF expression, and the proangiogenic growth factors IGF-1 and TGF-β are known to be expressed in early diabetic nephropathy. Moreover, treatment of animals with anti-VEGF antibodies has been shown to improve early renal dysfunction in a model of experimental diabetes (97); and inhibition of angiogenesis with the selective antiangiogenesis agents tumstatin (98) and endostatin (10) has been shown to attenuate glomerular hypertrophy, hyperfiltration, and proteinuria that are associated with this disease. We suggest that further studies will be necessary to understand the interrelationship and balance between too little angiogenesis factor expression, which results in capillary loss and chronic hypoxia-inducible damage, or too much angiogenesis factor expression, which may result in glomerular disease, hypertension, and vascular permeability. Nevertheless, the concept that angiogenesis and EPC can be used as therapeutic agents to prevent chronic renal disease is novel and exciting and has great potential once they reach the clinic.
that govern the recruitment and integration of EPC into endothelial cell beds all are present within allografts (100,101).

Several studies have demonstrated that recipient EPC do migrate into human allografts and have the ability to differentiate into endothelial cells (102,103). After human cardiac transplantation, this process occurs rapidly and is extensive. Quaini et al. (104) reported that after a median of 53 d, as many as 20% of donor vascular endothelial cells are replaced by recipient endothelial cells and that this process can begin as early as day 4 after transplantation. These observations suggest that after early ischemia-reperfusion injury, acute rejection, or other insults, recipient EPC are most efficient to repair damaged vessels. In time, it is proposed that the endothelium within the graft becomes “chimeric,” consisting of endothelial cells that are derived from both the donor and the recipient. Lagaaaij et al. (105) examined the replacement of damaged donor peritubular capillary endothelium in human renal allografts. Similar to the Quaini study, they found that donor endothelial cells are replaced by recipient endothelial cells, but they noted that this repair mechanism was most prominent after acute vascular rejection (105). Another study, by Grimm et al. (106), noted that recipient cells infiltrated renal allografts in the course of chronic rejection. Collectively, all of these studies indicate that circulating recipient EPC may be recruited into renal allografts and that this repair process is most evident after microvascular destruction that occurs in association with acute rejection and in the course of the chronic rejection process (102,103,105,106).

It is not yet known whether endothelial chimerism within allografts is a contributing factor for further allograft injury. A growing body of literature indicates that mobilization of EPC may be beneficial to allograft function by maintaining graft acceptance and limiting downstream hypoxic tissue injury. However, it also is possible that this reparative compensation and chimerism of endothelial cells might be a factor in the development of chronic rejection by altering the immunologic properties of the graft. For instance, Heeger’s (107) group noted that recipient T cells can recognize allogeneic peptides that are processed and presented by recipient endothelial cells lining the graft in a manner similar to the indirect pathway of allorecognition. This results in cell lysis and therefore ongoing damage (107). Because the indirect pathway of allorecognition is thought to be dominant in chronic allograft rejection (108), this may suggest that chronic replacement of donor endothelial cells by recipient endothelial cells will facilitate immunologic injury. Therefore, it will be important to understand how excessive turnover and perhaps neoangiogenesis, although potentially protective after acute injury, may be of pathophysiologic significance for ongoing injury and the development of chronic allograft rejection.

Angiogenesis and Chronic Allograft Rejection

Chronic allograft rejection is associated with the expression of adhesion molecules, chemokines, and growth factors such as VEGF (101,109–111) that have the capacity to mediate EPC recruitment. Therefore, one could argue that excessive angiogenesis could occur in the setting of chronic allograft rejection (24). Indeed, as discussed above, neovascularization is established to occur within allografts, and recipient endothelial cells may represent as many as 20% of the total number of endothelial cells within a failed allograft (104). In our own studies, we have evaluated the process of recipient angiogenesis/reendothelialization using a humanized mouse (huSCID) model. The SCID mouse is permissive for the transplantation of human skin and for the adoptive transfer of human peripheral blood leukocytes. In our studies, human foreskin was transplanted onto SCID mice; after engraftment, peripheral blood leukocytes that were derived by plasmapheresis of human donors were adoptively transferred by intraperitoneal injection into the mouse. Seven days after transfer, the human leukocytes were evident within the human skin graft but not in the mouse skin (30,52,60). In our analysis, we found that there was a marked local human angiogenesis response in these skins at early times in the course of infiltration (Figure 3). The angiogenesis reaction was quantified by both videomicroscopy and immunohistochemistry and was found to be temporally and spatially associated with the leukocytic infiltrates (60). Furthermore, the angiogenesis response seemed to precede the development of vasculitis and microvascular destruction in association with fulminant rejection. Therefore, local tissue hypoxia likely was not the primary stimulus for initiating the angiogenesis that occurred early in these allografts. Rather, the angiogenesis reaction likely was initiated by leukocytes as is typical in leukocyte-induced angiogenesis.

Angiogenesis has been demonstrated within the intimal proliferating lesion characteristic of CAV. Atkinson et al. (112) evaluated the expression of endothelial cell markers in CAV lesions from failed human cardiac transplants. They found that most vessels (approximately 90%) had evidence of neovessels predominantly within the middle portion of the neointima. In addition, they found that these neovessels were activated inas-
much as they expressed adhesion molecules and MHC class II, suggesting that the angiogenesis response itself may promote leukocyte recruitment and activation (112). These observations are similar to those published by others in experimental models. Tanaka et al. (113) evaluated the intima of the transplanted aorta in a hypercholesterolemic rabbit model and found that it contained prominent microvessels compared with controls or animals that did not receive a transplant. They also noted that the increased capillary density was associated with T cell and monocyte infiltrates within the parenchyma of cardiac transplants (113). Using an established Lewis into Fisher rat model of chronic cardiac allograft rejection (8,114,115), we also found that angiogenesis was present in large CAV lesions, again in association with mononuclear infiltrates. Because the neovascular response itself is proinflammatory, we questioned whether it could sustain the growth of the intimal component of the CAV lesion. We treated recipients with TNP-470, a synthetic fumagillin derivative that is well established to inhibit endothelial cell proliferation in vitro and in vivo, and found that it interrupted the progression of CAV when given late. In contrast, it did not prevent its development when given in the immediate posttransplantation period (8), suggesting that angiogenesis is functional in the progression of CAV but is not associated with its initiation. Consistent with this interpretation, we also found that inhibition of angiogenesis with endostatin, a most selective antiangiogenesis agent, interrupts CAV development in an established murine model of CAV (M. Sho, A. Contreras, D.M.B., unpublished observations, 2005).

We interpret all of these data to suggest that the mediators that govern the recruitment of mononuclear cells into the intima of large vessels also facilitate the recruitment of EPC and therefore angiogenesis within the lesion. Moreover, EPC migrate to sites of vascular injury in response to T cell– and monocyte-derived cytokines, chemokines, and growth factors that are known to be present within the intimal lesion. Therefore, it is possible that chronic endothelial cell damage elicits a response that is associated with the recruitment of T cells, monocytes, and EPC. The recruitment of the EPC is physiologic to repair the disrupted vessel and to promote angiogenesis. Because the neovascular reaction is in itself proinflammatory, once present within the CAV lesion, the process will be self-sustaining. This explains why inhibition of angiogenesis in CAV (and perhaps in atherosclerosis) is therapeutic to limit disease progression.

Conclusions

An emerging body of literature has determined that circulating EPC maintain vascular integrity and are functional in the repair of damaged tissues. EPC are recruited into the kidney in the healing phase of renal inflammation, and a deficiency in this process can result in persistent cell death and the development of chronic disease. Early studies have indicated that augmentation of EPC-dependent repair by the provision of VEGF or by the direct transfer of EPC into the kidney can augment healing and can limit ongoing disease. In contrast, in allografts, ongoing inflammation facilitates the persistent recruitment of EPC that may result in a marked angiogenesis reaction, especially within the intimal proliferating lesion of large vessels with CAV. Because EPC are a heterogeneous population of cells, in future studies, it will be important to understand their nature and tissue selectivity and whether select growth factors, cytokines, and cell surface molecular interactions within tissues facilitate different differentiation patterns. To this end, we conclude by noting some interesting observations about the hormone erythropoietin, which is commonly used in patients with chronic renal failure. Among its biologic effects, erythropoietin is a potent physiologic stimulus for the mobilization of stem cells (116,117), including EPC (118), and it has been found to augment neovascularization in vitro and in vivo (118). Erythropoietin receptors are present on cultured human endothelial cells, and activation of postreceptor signaling elicits a protective antiapoptotic and promigratory angiogenic phenotype (118–120). In animal models, erythropoietin has been shown to stimulate angiogenesis in association with ischemia and acute inflammation (118), and in humans, it markedly increased the number of CD34+ circulating EPC (121). Therefore, the use of erythropoietin in humans with chronic renal disease or chronic allograft rejection might enable novel studies to be performed on the role and potential importance of mobilized EPC as a therapeutic agent.

Finally, we suggest that in future studies, it will be important to understand how excessive turnover and angiogenesis are protective after acute injury but are pathologic in chronic disease states, especially within the vasculature. Nevertheless, the growing literature indicates that angiogenesis and endothelial turnover are most important concepts for our understanding of renal disease and allograft rejection and that manipulation of the response will have major consequences for therapeutics in the future.

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