Transplant Graft Vasculopathy
A Dark Side of Bone Marrow Stem Cells?
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“‘He hibernates in this world, and feeds on his own marrow.’” Henry David Thoreau, “A Week on the Concord and Merrimack Rivers: Sunday” (1849)

In his essay, Thoreau was romanticizing the survival skills of a poet, but his words transcend time and, according to the article by Hu et al1 in the present issue of Circulation, can be used to anthropomorphize the “life” of an atherosclerotic plaque. Their data indicate that in addition to the therapeutic possibilities of bone marrow–derived cells, which have ignited multiple recent clinical trials, the marrow may also be the source of cells for plaque neovascularation that propels this pathological process forward.

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Since the 1960s, transplantation of solid organs has become an increasingly successful therapy for patients suffering from end-stage organ failure. The greatest strides have been made in the short-term results of organ transplantation, due in large part to the introduction of effective immunosuppressive agents. Despite these successes, however, transplantation remains limited as a long-term treatment, the lone exception being renal transplantation.2 Chronic transplant dysfunction (CTD), which can be defined clinically as the progressive irreversible loss of graft function,3 is now recognized as the primary cause of allograft loss after the first year.4 CTD is associated with organ-specific histopathology but also features common histomorphological changes of vessels, most notably in kidney and heart transplants, a phenomenon that is referred to as transplant arteriosclerosis (TA).5

TA traditionally has been considered a chronic form of vascular rejection with concentric luminal narrowing accompanied by perivascular inflammation.6 The pathological findings include perivasculitis; endothelialitis; and infiltration of α-actin–positive vascular smooth muscle cells, macrophages, and T lymphocytes, culminating in the progressive formation of a neointima.7 The common form of TA is concentric and generalized, in contrast to native vessel atherosclerosis, which is usually focal and eccentric. Until recently, it was believed that TA was initiated by immune damage of the endothelium followed by smooth muscle cell migration and proliferation in the intima.8 TA therefore was considered to originate from grafted tissue and to be donor derived.

In the past several years, bone marrow–derived progenitor cells have been shown to have the potential to differentiate into endothelial cells9,10 or vascular smooth muscle cells11 and to participate in the formation of microvessels in ischemic tissue12,13 and of neointima and neointima in postangioplasty arteries.14 The notion that these cells represented a potential supply of vascular endothelium that could be recruited to improve perfusion of ischemic tissue has led to the initiation of trials that attempt to capitalize on this natural mechanism for augmenting blood vessel growth using bone marrow cells for therapeutic angiogenesis.15–18

More recently, however, several studies have illuminated another potential role of circulating and bone marrow–derived progenitors in vascular physiology. These studies have suggested that plaque endothelial cells and vascular smooth muscle cells derive from the recipient circulation and bone marrow in animal models of TA.11,19–21 However, although these prior studies documented a contribution of host cells, the quantification and cell source for endothelial replacement and plaque growth varied considerably (Table).25–28 Hu et al1 present data that challenge prior findings and are likely to generate controversy about the genesis of the luminal neointima and plaque microvasculature.

The authors employed several clever animal models, combined with challenging surgical models, to yield some striking histology. The Figure will help the understand the key findings. Four animal models were used, as shown. The authors used Tie-2–driven β-galactosidase expression to identify endothelial cells. β-Gal (expressed by the LacZ gene) is not normally expressed in mammals and yields a blue reaction product under specific staining conditions. Tie-2 is expressed almost exclusively in endothelial cells, The Tie2-LacZ mouse therefore exhibits β-gal expression (resulting in blue coloration) in cells of endothelial lineage. By transplanting aortas from wild-type mice (with no β-gal expression) into Tie2-LacZ mice, and vice versa, the authors show convincingly the striking and rapid replacement of donor endothelium by host cells in Figure 2 of their article. Then, by showing the even distribution of host cells, rather than migration from the anastomotic edge, the authors provide evidence of a circulating source of replacement cells (Figure 3 of their article). Finally, and perhaps most notably, the authors use the transplantation models depicted in our Figure to determine whether the cells contributing to endothelial replacement derive from the bone marrow. As shown in Figure 4 of their article, a non–bone marrow source appears to contribute the majority of cells to the neointima. The authors calculated that 35% of endothelial cells lining the
graft derived from the recipient bone marrow, although the comparison between panels b and c of Figure 4 would suggest a lesser contribution.

At first glance, these findings seem to contrast sharply with an article by Hillebrands et al, which showed less than 3% of endothelial cell replacement derived from host bone marrow. On closer inspection, however, the 2 studies differ in several aspects. Hillebrands et al studied a rat model and performed cross-sectional histology using confocal laser scanning microscopy to identify major histocompatibility complex class antigens of host bone marrow at 3 months. This is a laborious approach for quantification of endothelial composition with uncertain fidelity. In addition, these authors did not evaluate the contribution of non–bone marrow–derived circulating cells. In the present study by Hu et al, the mice were evaluated after 4 weeks using en face histology after x-gal staining. Strikingly, the contribution of non–bone marrow–derived circulating cells was much greater than that of the bone marrow–derived cells. Comparison of Figure 2, panels b and c, would lead one to the conclusion that the bone marrow component was actually far less than suggested in the quantification in Figure 2d and, to our eyes, doesn’t appear far off from Hillebrands’ estimate. The major finding, in our view, is that non–bone marrow–derived circulating cells appear to play a major role in establishing the neoendothelium, suggesting that other tissues harbor radiation-resistant endothelial progenitor cells (EPCs) that can enter the circulation and participate in endothelial regeneration.

Another key feature of TA, microvascular formation in the neointima, was also evaluated in the study by Hu et al. Normal arteries have a microvasculature that is restricted to the adventitia. As lipid-rich atherosclerotic lesions develop, a microvasculature is recruited to support the growing fatty streak. In transplanted organs, a more abundant microvasculature has been observed in the atherosclerotic lesions of allografted arteries than in those of the recipients’ own arteries. Hu et al, using the same models, observed not only neointimal microvascularization but also a major contribution (almost 100%) of bone marrow–derived EPCs to microvessel formation in the neointima, and also noted that microvessels appeared in allografts earlier than neointimal formation. Although the role of the neointimal microvascularization remains unclear, vasculogenesis, rather than angiogenesis, within the intima may be a crucial event for the development of TA.

Since the detection of EPCs in adult human peripheral blood in our laboratory in 1997, numerous studies have refined our understanding of the mechanisms by which these cells contribute to angiogenesis/vasculogenesis in ischemic disease. In many of these studies, EPCs have been shown to have a favorable impact, whereas few studies have suggested that EPCs could act as a promoter of disease. In the present issue of Circulation, Hu et al have shown not only a major contribution of EPCs to endothelial regeneration but also a major contribution to microvessel formation in the neointima, which may be permissive for or actually promote TA. These observations suggest that EPCs can act as a suppressor and a promoter of neointimal formation in TA simultaneously and
that the pathogenesis of TA, involving a variety of cells originating from both donor and recipient, is highly complex. By extrapolation, one also can imagine similar dueling roles for EPCs in the advent and progression of native atherosclerosis. The findings of Hu et al with regard to the pathogenesis of TA might suggest novel therapeutic concepts for TA prevention. A possible approach could include modulation of plaque angiogenesis/vasculogenesis by manipulation of specific mediators, eg, angiopoietin, vascular endothelial cell growth factor, and basic fibroblast growth factor. Indeed, a very recent publication has demonstrated that angiopoietin-1, a natural antagonist for angiopoietin-2, had an inhibitory effect for intimal hyperplasia in cardiac allograft arteriosclerosis.24 In addition, angiogenesis inhibitors—ie, thrombospondin, angiostatin, and endostatin—might also be effective for TA, although impairment of endothelial recovery might be an undesirable side effect of a systemically employed strategy. For this reason, local gene or drug delivery might represent a preferable method, made practical by the possibility of pretreatment of the donor organ before implantation.

Although better immunosuppressive medicine for CTD has been developed, TA is still a major cause of CTD. Integrative, multidisciplinary research into transplantation biology will undoubtedly contribute to improved understanding of CTD and better approaches to its prevention. The insights of Hu et al undoubtedly contribute to improved understanding of CTD. Multidisciplinary research into transplantation biology will hopefully lead to new therapeutic approaches to this major clinical problem.

References

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