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Circulating Smooth Muscle Cell Plasticity in the Development of Transplant Arteriosclerosis

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To date, chronic transplant dysfunction (CTD) is recognized as the major cause of long-term transplant loss (>1 year) after transplantation. CTD presents histologically with obliterated intragraft arteries as a result of intimal hyperplasia referred to as transplant arteriosclerosis (TA). Neointimal lesions predominantly consist of vascular smooth muscle cells (VSMCs) intermingled with some inflammatory cells. The pathogenesis of TA is believed to be multifactorial, and many risk factors have been identified. Because the precise pathogenetic mechanisms underlying TA are still largely unknown, adequate prevention and treatment protocols are not available. In this review, we discus the origin (donor vs recipient, bone marrow vs non–bone marrow) of neointimal endothelial cells (ECs) and VSMCs in TA lesions, which were formerly believed to be solely graft-derived. On the basis of the data obtained in both clinical and experimental transplantation, it appears that the process leading to TA is heterogeneous and that neointimal ECs and VSMCs can be recruited from different sources, possibly depending on the severity of vascular damage. These data suggest a significant role of host-derived circulating EC-VSMC progenitor cells, which may be partly bone marrow–derived. These circulating progenitor cells are potential targets for therapeutic intervention to ameliorate TA development or occlusive vascular disease in general.

Over recent decades, the short-term results of organ transplantation have significantly improved, and this is primarily because of the introduction of new, more effective, immunosuppressive agents. Especially the introduction of cyclosporine in the late 1970s resulted in a major improvement in short-term graft survival rates.[1] In addition to this, advances made in donor-organ preservation, surgical techniques, and human leukocyte antigen (HLA) tissue-typing assays contributed to the decreased morbidity and mortality after solid-organ transplantation. Despite the use of these new drugs and refined techniques and assays, however, clinical organ transplantation still has not achieved its goals as a long-term treatment for patients with end-stage organ failure. Since the late 1980s, a steady improvement in long-term graft survival has been observed only in renal allografts,[2] whereas this effect is less clear in other organs. Long-term success has thus steadily improved (in renal allografts) or remained at the same level as in the precyclosporine era, and no new drugs are currently available that can further extend graft survival time. To date, the development of so-called *chronic transplant dysfunction* (CTD) is considered as the major cause of allograft loss after the first posttransplantation year.[3] Clinically, CTD is defined as the progressive and irreversible loss of transplant function that manifests late in the posttransplant period (months to years after transplantation).[4] The incidence of CTD after transplantation depends on the type of organ grafted, indicating that differences in susceptibility to development of CTD exist. In liver allografts, the development of CTD is a relatively small problem (incidence, 3%-26%), whereas in kidney and cardiac allografts, the incidence reaches an incidence of >50% 5 years after transplantation. The highest incidence of CTD is observed in lung transplants with percentages of >70% 5 years after transplantation.[5-8] The deterioration of graft function is associated with a variety of organ-specific clinical parameters such as decreased glomerular filtration rate, increased plasma creatinine levels and protein-

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uria in renal allografts, and increased frequency of myocardial infarction, arrhythmias, and sudden death in cardiac allografts. CTD presents with decreased pulmonary function and increased levels of bilirubin and liver enzymes in blood in lung and liver transplants, respectively.[5,8,9] Besides functional deterioration, CTD is also characterized by graft-specific histologic abnormality.[9,10] Renal transplants develop glomerular sclerosis and tubular atrophy, whereas cardiac transplants with CTD present with parenchymal fibrosis. On the other hand, lung and liver transplants with CTD are characterized by the presence of obliterated bronchioli and degeneration of the bile ducts, respectively.[5,8-11] In addition to this graft-specific histologic abnormality, however, CTD also presents with a common histopathologic finding that is detected primarily in renal and cardiac allografts but that can also develop in liver and lung allografts (ie, graft arterial disease or transplant arteriosclerosis [TA]).[10-14]

Characteristics and Development of Transplant Arteriosclerosis

Concentric myointimal proliferation, resulting in the development of an occlusive neointima (NI) in the arterial structures of the graft, is the main characteristic of TA in solid-organ transplants.[4] Smooth muscle α-actin (SMA)–positive vascular smooth muscle cells (VSMCs) are the primary constituents of neointimal lesions; however, some infiltrating macrophages and T cells can also be detected. Other findings coinciding with TA include persisting inflammation (perivasculitis and endothelialitis), disruption of the internal elastic lamina, and thinning of the vascular media (loss of medial SMA-positive VSMCs).[5,11] Figure 1 shows typical examples of TA as can be observed after allogeneic aorta transplantation in rats. Aortic allografting in rats is commonly used to model clinical TA, and the vascular lesions show striking similarities with clinical TA. Although a causal relation between the clinical manifestation of CTD and the histologic presence of TA has still to be proven, progressive vessel occlusion leading to downstream ischemic tissue damage and disruptive fibrosis has generally been accepted as the main cause of CTD.[15]

The etiology of TA is poorly defined, although the presence of persistent perivascular inflammation suggests that immune-mediated damage caused by the alloreactive response of the host against the graft vasculature (vascular rejection) is the prime cause of TA development. However, alloantigen-independent factors (eg, ischemia-reperfusion injury and viral infections [especially cytomegalovirus]) also seem to be associated with the pathogenesis of TA.[8,16] The precise pathophysiologic mechanism underlying the development of TA is still unknown, but the "response-to-injury" paradigm applicable to atherosclerosis and proposed by Ross et al[17] has been widely accepted for the development of TA despite discrepancies in histologic abnormality between ordinary atherosclerosis (focal, eccentric) and TA (generalized, concentric). This paradigm proposes that endothelial cells (ECs) along the graft arterial system become damaged and activated by transplant-related trauma (alloantigen-dependent and -independent) and the ongoing perivascular inflammatory response. The intragraft ECs subsequently initiate a remodeling process that is coordinated by growth factors (eg, platelet-derived growth factors [PDGFs] and fibroblast growth factors) and proinflammatory cytokines produced by the ECs themselves, as well as medial VSMCs and leukocytes. This cascade of events eventually results in migration of medial VSMCs into the subendothelial space, followed by local replication and formation of the neointimal lesion.[5,11]

Donor Versus Recipient Origin of Neointimal ECs and VSMCs

As described earlier, the "response-to-injury" paradigm implies that graft-derived medial VSMCs of damaged intragraft arteries migrate from the media into the subendothelial space just beneath the endothelial cell layer, followed by local proliferation.[10] In response to cytokines, growth factors, and other inflammatory mediators produced by infiltrating leukocytes and graft ECs, the phenotype of medial VSMCs is transformed from "contractile" to "synthetic," enabling the medial VSMCs to migrate and replicate.[17] According to this concept, neointimal VSMCs in TA originate from graft tissue (ie, the media of the intragraft arteries) and, therefore, should be donor-derived. Until recently, however, few data were available on the true origin of neointimal VSMCs in solid-organ transplants.

In contrast to the origin of neointimal VSMCs, the origin of intragraft ECs has been studied for quite a long time, and this work was initiated by Woodruff[18] and Medawar[19] in the early 1960s. They proposed that replacement of graft endothelium with host-derived ECs (ie, graft adaptation) im-

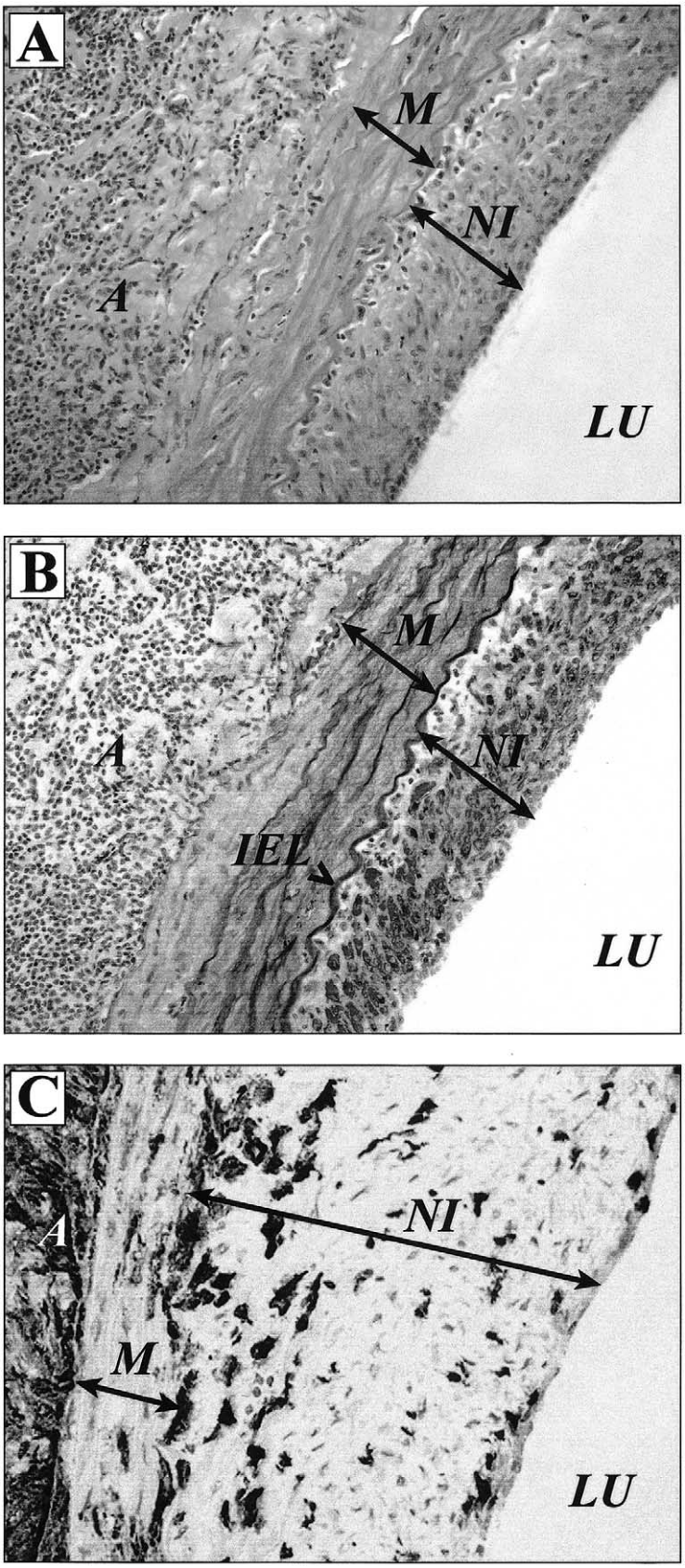

Figure 1. Histopathologic characteristics of TA in rat aortic allografts. Allografts were transplanted from Brown Norway to Fisher344 rats (**A** and **B**, explanted 8 weeks after transplantation) and from Brown Norway to Lewis rats (**C**, explanted 4 weeks after transplantation). **A**, Hematoxylin and eosin staining of an allograft showing severe perivascular inflammation in the adventitia, media necrosis (absence of nuclei in the media), and neointima formation. (Original magnification ×200.) **B**, Serial section of **A** after elastin (Lawson) and SMA double staining showing absence of α-actin–positive cells in the media. The NI contains high numbers of α-actin–positive cells. (Original magnification ×200.) **C**, Macrophage staining showing massive infiltration of the adventitia with macrophages. Also, the NI contains considerable numbers of infiltrating macrophages. (Original magnification ×400.) Abbreviations: A, adventitia; IEL, internal elastic lamina; LU, lumen; M, media.

Table 1. Origin of ECs in Rejection and TA

Reference	*Abnormality*	*Model*	*Species*	*Cell type/Origin/ Frequency*	*Detection technique*
Lagaaij et al[22]	Rejection	(Sex-mismatched) renal allograft	Human	ECs/recipient/ 0%->33%*	MHC class I IHC, ABO blood group antigen IHC, and X- and Y-chromosome–specific ISH
Xu et al[29]	Rejection	(Sex-mismatched) renal allograft	Rat	ECs/recipient/ 0%-30%†	X-chromosome ISH combined with vWF IHC
Yousem et al[20]	Rejection/TA	(Sex-mismatched) lung allograft	Human	ECs/donor/100%	Y-chromosome ISH
Sedmak et al[23]	Rejection/TA	Renal allograft	Human	ECs/recipient (3/13)‡/ 5%-20%	ABO blood group antigen IHC
Hruban et al[21]	TA	(Sex-mismatched) cardiac allograft	Human	ECs/donor/>95%	Y-chromosome–specific ISH
O'Connell et al[24]	TA	Cardiac allograft	Human	ECs/recipient (10/34)†/ up to 100%	ABO blood group antigen–specific IHC
Quaini et al[25]	TA	(Sex-mismatched) cardiac allograft	Human	ECs/recipient/42%	Y-chromosome FISH/vWF IF
Plissonnier et al[30]	TA	Aorta allograft	Rat	ECs/recipient/100%	MHC class II IHC
Hasegawa et al[31]	TA	Cardiac allograft	Mouse	ECs/donor/100%	MHC class II IHC
Hillebrands et al[26,27]	TA	(Sex-mismatched) cardiac allograft	Rat	ECs/donor/>95%	HIS52 (ECs)/MHC class I IHC
Hillebrands et al[27]	TA	(Sex-mismatched) aorta allograft	Rat	ECs/recipient/>95%	HIS52 (ECs)/MHC class I IHC
Hillebrands et al[28]	TA	Aorta allograft in BM-chimeric hosts	Rat	ECs/recipient/>95%	HIS52 (ECs) and MHC class I IF

Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization; vWF, von Willebrand factor; FISH, fluorescent in situ hybridization; IF, immunofluorescence; HIS52, monoclonal antibody reactive with rat ECs.

*Percent recipient-derived ECs correlated with the severity of rejection.

†Trend observed that the percent recipient-derived ECs correlates with the severity of rejection.

‡Number of individuals with positive results out of total number of subjects.

proves late graft function and might be the reason why long-term allograft survivors experience relatively few rejection episodes. In both clinical studies[20-25] and studies performed in experimental transplant models in mice and rats,[26-31] several groups have now analyzed the origin (graft vs recipient) of intragraft ECs (summarized in Table 1). Both graft[20,21,26,31] and (partly) recipient[22-25,27-30] origins of the intragraft ECs have been reported in these studies, indicating heterogeneity of the underlying process. Whether and to what extent donor endothelium will be replaced with recipient-derived ECs seems to be related to the severity of donor EC damage; more severe damage will result in a higher percentage of EC replacement.[22,29]

Only 1 decade ago, Hruban et al[21] reported for the first time about the origin of neointimal VSMCs in human organ transplants, and since then several studies have been described with clinical and experimental models used to determine neointimal VSMC origin (summarized in Table 2). When studying sex-mismatched human cardiac transplant patients (female graft, male host) and using Y-chromosome–specific in situ hybridization, Hruban et al[21] did not detect signs of hybridization in cardiac myocytes or VSMCs or in >95% of ECs. These results exclude significant replacement of transplanted cardiac tissues with host-derived cells and indicate a donor origin of neointimal VSMCs. Using the same technique, Glaser et al[32] recently found 5% to 10% of neointimal VSMCs in medium and small coronary arteries to be of recipient origin.[32] On the other hand, high levels (60%) of recipient VSMC chimerism in cardiac allografts was reported by Quaini et al.[25] However, in the latter study, the level of VSMC chimerism was only determined in the undamaged myocardium relatively shortly after transplantation (median, 53 days), and areas with neointimal thickening were excluded. In the study by Glaser et al,[32] the origin of specifically neointimal VSMCs was determined long after transplantation (median, 5.1 years). Although a role of host chimerism of VSMCs in the microvasculature (without TA) cannot be excluded, it seems from human sex-mismatched cardiac transplants that recipient cells contribute only marginally, if at all, to the expansion of neointimal VSMCs after transplantation. In clinical renal transplantation, however, Grimm et al[33] recently showed

Table 2. Origin of VSMCs (Donor vs Recipient) in TA

Reference	*Abnormality*	*Model*	*Species*	*Cell type/Origin/ Frequency*	*Detection technique*
Hruban et al[21]	TA	(Sex-mismatched) cardiac allograft	Human	niVSMCs/donor/ 100%	Y-chromosome–specific ISH
Quaini et al[25]	TA	(Sex-mismatched) cardiac allograft	Human	mVSMCs/recipient/ 60%	Y-chromosome FISH/SMA IF
Glaser et al[32]	TA	(Sex-mismatched) cardiac allograft	Human	niVSMCs/recipient/ 5%-10%	Y-chromosome FISH/SMA IF
Grimm et al[33]	TA	(Sex-mismatched) renal allograft	Human	niVSMCs/recipient/ 80%-90%	Y-chromosome FISH/SMA IF
Plissonnier et al[30]	TA	Aorta allograft	Rat	niVSMCs/recipient/ 100%	Alloantisera on isolated neointimal cells (flow cytometry)
Brazelton et al[36]	TA	Femoral artery allograft	Rat	niVSMCs/recipient/ 100%	MHC class I IHC
Hillebrands et al[26,27]	TA	(Sex-mismatched) cardiac allograft	Rat	niVSMCs/recipient/ >95%	SMA IHC/Y-chromosome SC-PCR
Hillebrands et al[27]	TA	(Sex-mismatched) aorta allograft	Rat	niVSMCs/recipient/ >95%	SMA IHC/Y-chromosome SC-PCR
Johnson et al[37]	TA	Aorta allograft	Rat	niVSMCs/recipient/ 100%	MHC class I PCR on isolated neointimal cells
Saiura et al[41]	TA	(Sex-mismatched) cardiac allograft in Laz$^+$ hosts	Mouse	niVSMCs/recipient/ 86%	Y-chromosome ISH or LacZ expression
Sata et al[42]	TA	(Sex-mismatched) cardiac allograft in Laz$^+$ BM chimera	Mouse	niVSMCs/recipient/ majority	Y-chromosome ISH or LacZ expression/SMA IHC

Abbreviations: niVSMCs, neointimal vascular smooth muscle cells; ISH, in situ hybridization; mVSMCs, medial vascular smooth muscle cells; FISH, fluorescent in situ hybridization; IF, immunofluorescence; IHC, immunohistochemistry; SC-PCR, single-cell polymerase chain reaction; PCR, polymerase chain reaction.

that the majority (80%-90%) of the neointimal VSMCs are recipient-derived.[33] This observation suggests that organ-specific differences might influence the establishment of host neointimal VSMC chimerism, but this remains to be elucidated. From the few clinical data available so far, one can conclude that indications both supporting and discounting a major contribution of recipient-derived VSMCs in the process of NI formation exist.

Also, in experimental transplantation, the question on the origin of neointimal VSMCs in TA has recently raised considerable interest, and to date much effort has been put forth in identifying the role of circulating (progenitor) cells in the development of vascular lesions. The first indication that the blood contains a population of EC and VSMC precursors was described in 1963 by Stump et al,[34] who showed that Dacron polyester hubs implanted in the aorta of young pigs became covered with (host-derived) ECs and VSMC-like cells that originated from cells in the blood. Also, in the development of new vascular wall structures (including a neomedia with SMA-positive VSMCs) in biodegradable synthetic vascular grafts after implantation in rats, the VSMCs are by definition host-derived.[35] These data indicate that host-derived, possibly blood-borne ECs and VSMCs can repopulate synthetic implants and raise the question whether these cells are also involved in the development of TA after allogeneic transplantation. When Plissonnier et al[30] analyzed the origin of isolated neointimal cells obtained from rat aortic allografts using alloantisera and fluorescence-activated cell sorter analysis, these cells were indeed found to be of recipient origin. In a similar study, Brazelton et al[36] determined the origin of neointimal cells in allografted rat femoral artery segments using antibodies directed against donor or recipient major histocompatibility complex (MHC) class I antigens and showed all mesenchymal cells in the neointimal lesion (containing ~50% SMA-positive cells) to be recipient-derived. Because neointimal lesions are known to contain considerable numbers of (recipient-derived) inflammatory cells[15] (see also Figure 1, *C*), we wanted to exclude the risk of sample contamination with infiltrating recipient-derived inflammatory cells when determining the origin of neointimal VSMCs. Therefore we performed Y-chromosome–specific, single-cell polymerase chain reaction on microdissected nuclei of SMA-positive neointimal VSMCs and showed that virtually all of the neointimal VSMCs in rat aortic and cardiac allografts are of recipient origin.[26,27] A major contribution of host-

derived neointimal VSMCs has independently been confirmed in a variety of rat and mouse studies. In an aortic transplant model in rats, Johnson et al[37] showed all neointimal cells to be of recipient origin using MHC class I haplotype-specific PCR analysis. Using the same transplant model, Religa et al[38] showed only 25% to 35% of the noninflammatory cells (and presumably VSMCs) to be of host origin. Similar results were reported in mouse aortic[39,40] and cardiac[41-43] transplant models in which the origin of neointimal cells was defined by means of Y-chromosome–specific probes (in situ hybridization) or detection of β-galactosidase (LacZ)/green fluorescent protein (GFP) transgene expression.

From studies performed in experimental transplant models, there is, thus, now compelling evidence that neointimal VSMCs are frequently (in the majority) derived from recipient cells and not from donor cells, which is in sharp contrast with the current paradigm that predicts a major role for medial VSMCs in the process of TA development.[5,10,11] It should, however, be noted that rodent studies have especially provided high percentages of donor VSMC replacement after transplantation, whereas in most human studies no or only low percentages of recipient-type VSMCs could be identified. This could be explained by the fact that in most of the previously described experimental animal models immunosuppressive treatment is not used or is only minimally used, and this contrasts to the situation in clinical human transplantation.[1] Insufficient immunosuppression will probably lead to complete medial necrosis, which is indeed quite often observed in experimental transplantation,[27,30,44-47] whereas in human allografts medial VSMC cellularity frequently remains unchanged.[47] If viable medial VSMCs remain available (eg, the human situation), one can imagine that such medial cells can provide a source of neointimal VSMCs, whereas in the case of complete destruction of medial VSMCs (eg, the rodent situation) other resources by definition are required. However, also in our model of TA after cardiac transplantation in immunomodulated rats, a small rim of presumably donor-derived medial VSMCs remained even in almost completely occluded coronary arteries,[48] but, nevertheless, virtually all neointimal VSMCs were found to be of recipient origin.[26,27]

Moreover, the positioning of the transplanted tissue may influence the outcome of the process. It is conceivable that local cells preferentially colonize an interposition graft because they can invade transmurally or via pannuslike growth from the sides. Finally, rodent models and human transplantation differ at the level of pre-existing vascular disease that can be found in the majority of human donor material and that may provide the basis of further outgrowth of VSMCs during the subsequent development of a neointimal lesion after transplantation.[49] In animal tissues used for transplantation, such lesions are rarely found. Taken together, experimental transplantation indicates an important contribution of recipient-derived cells in NI formation in TA, although the possibility of medial or intimal VSMC contribution to TA development cannot be excluded.

Bone Marrow Versus Non–Bone Marrow Origin of ECs and VSMCs

Because host-derived ECs and VSMCs can play an important role in the development of neointimal lesions, the question arises as to the anatomic origin of these cells. With regard to ECs, it was believed for many years that vasculogenesis (ie, blood vessel formation through local differentiation of primitive endothelial precursors [angioblasts]) occurred only during embryonic development.[50,51] On the other hand, blood vessel formation in postnatal life was considered to occur only through sprouting of new vascular structures from existing vessels, a process referred to as angiogenesis.[50,52] However, recent data indicate that endothelial stem cells, which are possibly involved in repair processes after EC injury, also exist in adult life.[53,54] In addition to the bone marrow (BM) compartment,[52]Asahara et al[55] showed for the first time that human peripheral blood also contains a population of putative EC progenitor cells that can give rise to mature ECs. These BM-derived endothelial progenitor cells and circulating endothelial progenitor cells in the peripheral blood (CEPs) have properties similar to those of embryonal angioblasts. Early endothelial progenitor cells in the BM or immediately after their migration into the circulation are $CD34^+$ $CD133^+$ vascular endothelial growth factor receptor 2 $(VEGFR\text{-}2)^+$, whereas CEPs are $CD34^+$ $VEGFR\text{-}2^+$ $CD31^+$ vascular/endothelial (VE)–$cadherin^+$. They lose CD133 expression and start to express von Willebrand factor once they mature.[51,53,54,56] CEPs have been shown to contribute to the formation of blood vessels,[55,57] and numbers of CEPs increase after ischemia or after granulocyte-macrophage colony-stimulating factor treatment.[58] Moreover, Reyes et al[59] recently identified a $CD34^-$ $VE\text{-}cadherin^-$ $CD133^+$ $VEGFR\text{-}2^+$ multipotent adult progenitor cell in postnatal hu-

man BM and showed in vitro differentiation into $CD34^+$ angioblasts and in vivo differentiation into ECs.[59] Thus there is now compelling evidence that BM-derived precursor cells can contribute to the formation of blood vessels, and recent data indicate that adult BM also keeps the potential to differentiate into many other different tissues, including hepatocytes, neurons, muscle cells, and cardiomyocytes.[60,61] This process is referred to as *adult stem cell plasticity* (ie, differentiation of adult stem cells into a whole series of other progeny, once relocated and appropriately stimulated).[62-64] Because of this plasticity, transplantation of adult BM cells might be a feasible strategy to restore organ function in different diseases (eg, myocardial infarction).[54,62] The efficacy of this procedure has been tested in both clinical and experimental models of cardiac infarction, and it was analyzed whether transplantation of adult BM stem cells indeed leads to improvement of myocardial performance.[60] Injection of BM cells directly into the infarcted myocardium in rats promoted angiogenesis with some of the new BM-derived capillaries and was associated with improved cardiac function.[65] Injection of purified GFP-expressing Lin^- $c\text{-}kit^+$ cells into infarcted myocardium resulted in the generation of new BM-derived myocytes, as well as ECs and SMA-positive VSMCs.[66,67] Similar results were reported by Kocher et al,[68] who injected granulocyte colony-stimulating factor–mobilized circulating human angioblast precursors in infarcted rat hearts.[68]

That BM-derived progenitors also contribute to improved cardiac function in clinical myocardial infarction was recently shown in studies in which autologous mononuclear BM cells[69] or purified autologous $CD133^+$ BM cells[70] were injected directly into the ischemic myocardial tissue. Finally, by use of an ischemia model in mice, it has been shown that infusion of so-called BM-derived side-population cells ($CD34^{-/low}$ $c\text{-}kit^+$ $Sca\text{-}1^+$) into lethally irradiated mice results in engraftment (3.3%) of ECs, indicating circulatory capacities and differentiation into ECs of such cells.[71]

These data indicate that BM-derived adult stem cells have the capability to migrate and differentiate into both cardiac myocytes and vascular cells. In a restenosis model in mice, in which the development of neointimal lesions is similar to that of TA, it has been shown that in these lesions ~50% of the neointimal SMA-positive VSMCs originate from the BM compartment.[72,73] Taken together, these studies convincingly show that primitive BM cells can circulate through the body and contribute to vascular remodeling of tissue damage (ischemia, mechanical damage), giving rise to both ECs and SMA-positive VSMCs. Several groups have now extended these studies into the field of organ transplantation and addressed the issue of whether BM-derived progenitor cells are also involved in the development of neointimal lesions in TA (summarized in Table 3). Using LacZ-transgenic BM chimeric mice in an aortic allotransplant model, Shimizu et al[40] found that, although all neointimal VSMCs were exclusively host-derived, only ~11% of these cells were of BM origin. The majority of the neointimal VSMCs was apparently derived from radioresistant host, non-BM cells that seeded the graft[40], and similar findings were reported by Li et al.[39] Also in the allogeneic aorta transplant model, using BM-chimeric recipient rats, we recently showed that the host-derived neointimal ECs are primarily derived from a non-BM source.[28] Moreover, the neointimal VSMCs, which were previously shown to be of host origin,[27] were predominantly, if not completely, non–BM-derived.[74] On the other hand, by using transgenic BM-chimeric recipient mice in a cardiac TA transplant model, Sata et al[43] showed that the BM gives rise to most (~82%) neointimal cells.[43] However, because these authors did not perform transgene/SMA double staining in this specific experiment, it cannot be deduced what proportion of those cells are actually SMA-positive VSMCs.

The contribution of BM-derived SMA-positive VSMCs in the development of vascular lesions appears not to occur only in TA development. As mentioned earlier, neointimal lesions that develop after mechanical injury contain BM-derived SMA-positive VSMCs,[72,73] and this was recently confirmed by others.[38,43] Moreover, by using a mouse model for conventional atherosclerosis (apolipoprotein E $[ApoE]^{-/-}$ mice), Sata et al[43] showed that in atherosclerosis BM-derived SMA-positive VSMCs contribute to the process of atherosclerotic plaque formation. Also Hu et al[75] determined the contribution of BM-derived SMA-positive VSMCs in vein plaque formation after vein isografting in LacZ transgenic normal and BM-chimeric mice. They showed ~40% of the neointimal VSMCs to be recipient-derived, whereas ~60% remained donor (vein isograft)–derived. In contrast to Sata et al,[43] no BM-derived neointimal VSMCs were found in this model, thereby excluding the BM as a primary source of these cells.[75] Studying human coronary atherosclerosis in BM-transplant subjects, Caplice et al[76] recently showed for the first time that

Table 3. Contribution of the BM Compartment as a Source for ECs and VSMCs in the Development of TA, Restenosis, and Atherosclerosis

Reference	*Abnormality*	*Model*	*Species*	*Cell type/Origin/ Frequency*	*Detection Technique*
Hillebrands et al[28]	TA	Aorta allograft in BM chimeras	Rat	ECs/recipient non-BM/ >95% ECs/recipient BM/< 5%	HIS52 (ECs) and MHC class I IF
Hillebrands et al[74]	TA	Aorta allograft in BM chimeras	Rat	niVSMCs/recipient non-BM/>95%	SMA and MHC class I IF
Li et al[39]	TA	Aorta allograft	Mouse	niVSMCs/recipient non-BM/>95%	Y-chromosome ISH/ SMA IHC
Shimizu et al[40]	TA	Aorta allograft in Laz^+ BM chimeras	Mouse	niVSMCs/recipient/ >95% niVSMCs/ BM/11%	LacZ expression/SMA IHC
Religa et al[38]	TA	Aorta allograft in sex-mismatched BM chimeras	Rat	niVSMCs/recipient/ 64% niVSMCs/BM/ 24%-49%	Y-chromosome real-time PCR
Sata et al[43]	TA	Cardiac allograft in Laz^+ or GFP^+ BM chimeras	Mouse	Neointimal cells/recipient BM/ 83%	LacZ/GFP expression/ SMA IF
Han et al[72,73]	Restenosis	Mechanical injury of iliac artery in sex-mismatched BM chimeras	Mouse	Neointimal cells/BM/ ~50%	Y-chromosome ISH/ SMA IHC
Sata et al[43]	Restenosis	Mechanical injury of femoral artery in Laz^+ BM chimeras	Mouse	niVSMCs/BM/43%	LacZ expression/SMA IF
Religa et al[38]	Restenosis	Balloon injury of carotid artery in BM chimeras	Rat	niVSMCs/BM/7%-10%	Y-chromosome real-time PCR
Hu et al[75]	Atherosclerosis	Vein isograft in $LacZ^+$/sex-mismatched BM chimeras	Mouse	niVSMCs/donor/60% niVSMCs/recipient non-BM/40%	Y-chromosome ISH/ LacZ expression/SMA IHC
Sata et al[43]	Atherosclerosis	Laz^+ or GFP^+ BM chimeric $ApoE^{-/-}$ hosts	Mouse	Neointimal cells/BM/ 63%	LacZ/GFP expression
Caplice et al[76]	Atherosclerosis	Sex-mismatched BM-transplanted subjects	Human	niVSMCs/BM/~10%	Y-chromosome FISH/ SMA IF

Abbreviations: HIS52, monoclonal antibody reactive with rat ECs; IF, immunofluorescence; niVSMCs, neointimal vascular smooth muscle cells; ISH, in situ hybridization; IHC, immunohistochemistry; PCR, polymerase chain reaction.

in human atherosclerosis BM-derived VSMCs contribute to plaque formation, although the percentage is relatively low (~10%) as in TA. In summary, one can conclude that, although the BM can provide VSMCs found in neointimal lesions (in TA, restenosis, and atherosclerosis), other non-BM resources also definitely provide precursor cells. Such host non–BM-derived VSMC precursors must be radioresistant because most of these studies used irradiation to create BM-chimeric animals.[40]

Ingrowth of VSMCs into the neointimal lesions from the host side of the anastomosis cannot be excluded as a source of (host-derived) neointimal VSMCs in TA.[77] However, no indications for ingrowth of adjacent host medial VSMCs were found by Shimizu et al[40] when studying longitudinal sections from the anastomosis of mouse aortic allografts, including both host and donor tissue. Consequently, a blood-borne origin of VSMC precursors seems most likely, and recent data indeed suggest that the human peripheral blood contains a population of circulating VSMC precursor cells. Simper et al[78] showed that in vitro culture of human peripheral blood mononuclear cells in the presence of PDGF-enriched medium resulted in the generation of so-called smooth muscle outgrowth cells. Phenotypically, these in vitro generated smooth muscle outgrowth cells resemble VSMCs because they express a variety of VSMC markers such as SMA, myosin, and calponin. The question remains whether

such precursors adhere to the luminal side of the vessel or migrate from the adventitia through the vasa vasorum toward the subendothelial space. Although Shimizu et al[40] argued that sheer forces on the luminal side might prevent adhesion of cells, we showed that at the start of neointimal lesion development the first SMA-positive cells are found in a scattered pattern on the luminal side, suggesting direct entry from the lumen.[79] In line with this observation, Sasaki et al,[80] who studied human postmortem vein graft material from patients after coronary bypass grafting, showed that the process of NI formation started with the loss of endothelial cells, followed by the appearance of SMA-negative spindle-shaped cells at sites of injury, also suggesting a blood-borne origin of these cells.

Circulating VSMC precursors might also originate from other sources. Bucala et al[81] have described a population of non–BM-derived fibroblast-like cells in the peripheral blood (so-called fibrocytes) that are specifically recruited from the blood to wounded areas. As has been suggested by Gittenberger-de Groot et al,[82] another possible VSMC source could be ECs that transdifferentiate into VSMCs, and De Ruiter et al[83] indeed showed that embryonic endothelial cells can differentiate into SMA-expressing cells in vivo and in vitro. So far, it is unknown whether transdifferentiation of ECs is a unique property of embryonic ECs or is also shared by adult ECs. Also, transdifferentiation of VSMCs into ECs should be mentioned in this respect. Noncontractile intimal (epithelioid) smooth muscle cells with morphologic resemblance to endothelial cells, lacking most VSMC-associated proteins, can transform into media-like VSMCs and generate capillary tubes in vitro consisting of ECs and VSMCs.[83]

Moreover, smooth muscle cells from various locations in the vessel wall display extensive heterogeneity,[82,85-87] and one should realize that VSMCs at different locations may have different embryonic origins.[82] VSMCs in the coronary arteries, for instance, are derived from the epicardial lining and are, therefore, of mesodermal origin, whereas VSMCs of the aortic arch are of neuroectodermal origin. Furthermore, VSMCs in the descending aorta, tissue extensively used for the study of TA, are considered to originate predominantly from the local mesenchyme. This could well mean that the origin of VSMC precursors in TA also depends on the tissue studied.

VSMC Progenitor Plasticity

Because different anatomic origins of neointimal VSMCs have been stated to exist, we hypothesize that VSMC precursors are not a single entity but can be recruited from a variety of resources.[74] Depending on the severity and duration of vessel damage and the critical need for vessel repair, VSMC precursors will be recruited from different anatomic origins varying from the damaged vessel wall itself to the BM compartment.[74] So, in the case of limited superficial vessel damage with a remaining vascular structure, medial VSMCs themselves will probably provide sufficient repair potential, and in this case the neointimal cells will be donor-derived. More severe vascular damage, including medial VSMC damage, over a limited period of time might signal ingrowth of VSMCs from adjacent (host) vessels. Severe but time-restricted vessel damage, including full disruption of medial VSMC layers, will lead to recruitment from non-BM sources, whereas similar damage over a prolonged period of time will probably need additional VSMC precursor recruitment from the BM. In line with this hypothesis, Han et al[72] reported recently that BM-derived SMA-positive neointimal VSMCs in restenosis were found only after severe vascular damage and not in arteries with minimal damage.[72] Moreover, also in regeneration of infarcted myocardium, tissue damage appeared to be the major determinant required for the transdifferentiation of primitive BM cells into ECs and VSMCs.[66,67,88] One can imagine that, in clinical transplantation, probably the entire spectrum of VSMC precursor derivation occurs, perhaps even in one and the same patient depending on the severity of damage throughout the tissue.

Conclusions

In contrast to the paradigm that neointimal VSMCs are derived from the graft vascular wall, recent data indicate that host-derived VSMCs also contribute to NI formation. These host-derived VSMCs can originate from different anatomic locations, and, probably depending on the duration and severity of the vascular damage, VSMC progenitor cells from different locations will be recruited to the site of damage. Especially, circulating VSMC progenitor cells, which contain both BM-derived and non–BM-derived populations, appear to play an important role in NI formation. The contribution of BM-derived VSMCs is not unique for TA because in other vas-

cular diseases such as restenosis and atherosclerosis BM-derived VSMCs can be detected in the vascular lesions. The identification of circulating BM-derived and non–BM-derived VSMCs in TA and vascular lesions in general might also have implications for future treatment strategies. Whereas previous (rather unsuccessful) therapies primarily focused on medial VSMC proliferation and migration, future strategies should possibly focus on targeting circulating VSMC progenitors to treat or ameliorate vascular disease. Therefore further research should focus on identifying the recruitment-homing factors involved in trafficking VSMC progenitor cells to the damaged vascular wall. It is possible that factors which are present among the cytokines and chemokines produced by the ongoing inflammatory process, as well as by damaged ECs and mesenchymal cells, provide signals essential for homing of the progenitor cells to the damaged site. These factors might subsequently be used as targets for intervention, resulting in decreased VSMC recruitment and proliferation and in amelioration of vascular disease.

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