Abstract—To date, clinical solid-organ transplantation has not achieved its goals as a long-term treatment for patients with end-stage organ failure. Development of so-called chronic transplant dysfunction (CTD) is now recognized as the predominant cause of allograft loss long term (after the first postoperative year) after transplantation. CTD has the remarkable histological feature that the luminal areas of intragraft arteries become obliterated, predominantly with vascular smooth muscle cells (VSMCs) intermingled with some inflammatory cells (transplant arteriosclerosis, or TA). The development of TA is a multifactorial process, and many risk factors have been identified. However, the precise pathogenetic mechanisms leading to TA are largely unknown and, as a result, adequate prevention and treatment protocols are still lacking. This review discusses the origin (donor versus recipient, bone marrow versus nonbone marrow) of the VSMCs in TA lesions. Poorly controlled influx and subsequent proliferative behavior of these VSMCs are considered to be critical elements in the development of TA. Available data show heterogeneity when analyzing the origin of neointimal VSMCs in various transplant models and species, indicating the existence of multiple sites of origin. Based on these findings, a model considering plasticity of VSMC origin in TA in relation to severity and extent of graft damage is proposed. (Arterioscler Thromb Vasc Biol. 2003;23:380-387.)

Key Words: chronic transplant dysfunction • transplantation • transplant arteriosclerosis • origin • vascular smooth muscle cells

Solid-Organ Transplantation and Chronic Transplant Dysfunction (CTD)

Since the late 1950s, transplantation of solid organs has become an increasingly successful therapy for patients suffering from end-stage organ failure. The short-term results of organ transplantation have significantly improved over recent decades. This improvement is primarily because of the introduction of new, more effective immunosuppressive agents. Moreover, improved histocompatibility leukocyte antigen tissue-typing assays and surgical techniques as well as advances made in donor organ preservation contributed to the decreased morbidity and mortality after solid-organ transplantation. Despite the use of these new drugs, however, it has become clear that clinical transplantation has not achieved its goals as a long-term treatment. Although a steady improvement in the long-term survival of renal grafts since the late 1980s has been described,1 this effect is less clear in other organs. Depending on the organ grafted, long-term success has steadily improved or remained at the same level as in the pre cyclosporin A era and so far, no new drugs are available that can further extend graft survival time. CTD is now recognized as the primary cause of allograft loss after the first year after transplantation.2

CTD can be defined clinically as the progressive irreversible loss of graft function that occurs late in the post-transplant period (months to years after transplantation).3 The incidence of CTD after transplantation depends on the type of organ grafted and varies from 3% in liver allografts to >70% in lung allografts 5 years after transplantation.4–6 The clinical presentation of CTD generally implies deterioration of graft function and is associated with a variety of organ-specific clinical parameters.7 In kidney transplants, CTD is characterized among others by decreased glomerular filtration rate, increased levels of plasma creatinin, and proteinuria. CTD in...
Transplant Arteriosclerosis (TA)

TA is characterized by vascular lesions in the graft that consist of concentric myointimal proliferation, resulting in the development of an occlusive neointima (NI) in the arterial structures of the graft. The NI primarily consists of smooth muscle α-actin (SMA)-positive vascular smooth muscle cells (VSMCs; Figure 1), which are believed to be derived from the vessel media. This progressive blood vessel occlusion could lead to downstream ischemic tissue damage and disruptive fibrosis and has therefore generally been accepted as the main cause of progressive deterioration in graft function (CTD). Other findings coinciding with TA include a persistent focal perivascular inflammation (perivasculitis), endothelial swelling and inflammation (endothelialitis), disruption of the internal elastic lamina, thinning of the vascular media (loss of medial SMA-positive VSMCs), foam cell accumulation in the NI, and presence of macrophages and T cells in the neointimal lesion. In contrast with ordinary atherosclerosis, which is usually focal and eccentric, the common form of TA is concentric and generalized.

The presence of persistent perivascular inflammation in TA suggests that alloresponse-mediated injury of the graft vessels is the prime cause of TA development. However, the etiology of TA remains poorly defined. The pathogenesis of TA seems to be multifactorial, and although alloreactivity of the host against the graft appears to be the most important factor contributing to the development of TA, alloantigen-independent factors also seem to be associated with the pathogenesis of TA.

Despite discrepancies in histopathology between ordinary atherosclerosis and TA, the “response-to-injury” paradigm applicable to atherosclerosis and originally proposed by Ross has been accepted widely for the development of TA. This paradigm proposes that transplant-related trauma (alloantigen dependent and independent) causes activation and damage of endothelial cells (ECs) along the graft arterial system. The damaged and activated endothelium subsequently initiates a generalized remodeling process that is coordinated by proinflammatory cytokines and growth factors produced by the activated ECs themselves as well as vessel wall parenchymal cells and inflammatory cells. Moreover, the immune response characterized by perivascular inflammation induces further low-grade damage to the vascular endothelium. This cascade of events eventually results in replication of VSMCs in the vascular wall, influx of VSMCs into the subendothelial space (intima), and generation of the neointimal lesion.

Origin of VSMCs in TA

Current thinking on the process of TA, as described above, holds that in response to cytokines, growth factors, and other inflammatory mediators produced by inflammatory cells and damaged/activated graft endothelium, donor-derived medial VSMCs of affected arteries start to proliferate and migrate from the media into the subendothelial space just beneath the EC layer. During this process, they transform their phenotype from “contractile” to “synthetic” and become capable of replication. According to this concept, neointimal VSMCs in TA originate from graft tissue and therefore should be donor derived (Figure 2).

If neointimal VSMCs do indeed originate from the donor, they should be demonstrably graft derived. As long ago as the early 1960s, Woodruff and Medawar proposed that replace-
Table 1. Origin of Intimal Cells in Various Models

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Organ</th>
<th>Cell Type</th>
<th>Cell Source</th>
<th>Prevalence, %</th>
<th>Identification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Allo Tx in sex-mismatched combinations</td>
<td>Heart</td>
<td>VSMC, EC</td>
<td>Recipient-type</td>
<td>5 to 10</td>
<td>Y-probe/SMA</td>
<td>27</td>
</tr>
<tr>
<td>Human</td>
<td>Allo Tx in sex-mismatched combinations</td>
<td>Heart</td>
<td>VSMC</td>
<td>Recipient-type</td>
<td>&gt;95</td>
<td>Y-PCR/SMA</td>
<td>21,22</td>
</tr>
<tr>
<td>Human</td>
<td>Allo Tx in sex-mismatched combinations</td>
<td>Heart</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>&gt;95</td>
<td>Y-PCR/SMA</td>
<td>22</td>
</tr>
<tr>
<td>Human</td>
<td>Allo Tx in sex-mismatched combinations</td>
<td>Kidney</td>
<td>VSMC</td>
<td>Recipient-type</td>
<td>80 to 90</td>
<td>Y-probe/SMA</td>
<td>29</td>
</tr>
<tr>
<td>Rat</td>
<td>Allo Tx</td>
<td>Femoral artery</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>100</td>
<td>MHC I</td>
<td>32</td>
</tr>
<tr>
<td>Rat</td>
<td>Allo Tx in sex-mismatched rats</td>
<td>Heart</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>&gt;95</td>
<td>MHC I/HIS52</td>
<td>26</td>
</tr>
<tr>
<td>Rat</td>
<td>Allo Tx in sex-mismatched rats</td>
<td>Aorta</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>&gt;95</td>
<td>MHC I/HIS52</td>
<td>26</td>
</tr>
<tr>
<td>Rat</td>
<td>Allo Tx in BM chimeric rats</td>
<td>Aorta</td>
<td>EC</td>
<td>Recipient non-BM-derived</td>
<td>5</td>
<td>MHC I/HIS52</td>
<td>26</td>
</tr>
<tr>
<td>Rat</td>
<td>Allo Tx in BM chimeric rats</td>
<td>Aorta</td>
<td>EC</td>
<td>Recipient non-BM-derived</td>
<td>&gt;95</td>
<td>MHC I/SMA</td>
<td>Present study</td>
</tr>
<tr>
<td>Mouse</td>
<td>Allo Tx after anti-CD4 and anti-CD8 treatment</td>
<td>Heart</td>
<td>mVSMC, EC</td>
<td>Donor-derived</td>
<td>ND</td>
<td>MHC II</td>
<td>33</td>
</tr>
<tr>
<td>Mouse</td>
<td>Allo Tx in sex-mismatched/ BM chimeric mice</td>
<td>Aorta</td>
<td>VSMC</td>
<td>Recipient non-BM-derived</td>
<td>&gt;95</td>
<td>Y-probe/SMA</td>
<td>34</td>
</tr>
<tr>
<td>Mouse</td>
<td>Vein Tx in sex-mismatched/BM chimeric/Tg mice</td>
<td>Vein</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>~88</td>
<td>LacZ/GFP/SMA</td>
<td>36,38</td>
</tr>
<tr>
<td>Mouse</td>
<td>Allo Tx in BM chimeric/Tg mice</td>
<td>Heart</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>~82</td>
<td>LacZ/Y-probe/SMA</td>
<td>35</td>
</tr>
<tr>
<td>Mouse</td>
<td>Allo Tx in sex mismatched/Tg mice</td>
<td>Heart</td>
<td>VSMC</td>
<td>Majority</td>
<td>LacZ/Y-probe/SMA</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Allo Tx in BM chimeric/Tg mice</td>
<td>Aorta</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>&gt;95</td>
<td>LacZ/SMA</td>
<td>37</td>
</tr>
<tr>
<td>Mouse</td>
<td>Induced heart ischemia</td>
<td>Heart</td>
<td>VSMC</td>
<td>lin− c-kit− BM cells</td>
<td>ND</td>
<td>GFP/SMA</td>
<td>62</td>
</tr>
<tr>
<td>Mouse</td>
<td>Injury induced Ni formation in sex-mismatched BM chimeric mice</td>
<td>Iliac artery</td>
<td>VSMC</td>
<td>BM-derived</td>
<td>~50</td>
<td>Y-probe/SMA</td>
<td>65</td>
</tr>
</tbody>
</table>

*Percent of neointimal cells.

Fit-1 indicates α-mouse-EC Mab; GFP, Green Fluorescent Protein reporter gene construct; HISS2, α-rat-EC Mab; mVSMC, medial-VSMC; Tg mice, transgenic mice; Tx, transplantation; Y-PCR, male Y-chromosome specific polymerase chain reaction; Y-probe, male Y-chromosome specific probe; ND, not determined.

Table 1 continued

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Organ</th>
<th>Cell Type</th>
<th>Cell Source</th>
<th>Prevalence, %</th>
<th>Identification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Allo Tx in BM chimeric/Tg mice</td>
<td>Aorta</td>
<td>VSMC</td>
<td>Recipient-derived</td>
<td>&gt;95</td>
<td>LacZ/SMA</td>
<td>37</td>
</tr>
<tr>
<td>Mouse</td>
<td>Induced heart ischemia</td>
<td>Heart</td>
<td>EC</td>
<td>BM-derived SP cells</td>
<td>3.3</td>
<td>LacZ/Flt-1</td>
<td>64</td>
</tr>
<tr>
<td>Mouse</td>
<td>Injury induced Ni formation in sex-mismatched BM chimeric mice</td>
<td>Iliac artery</td>
<td>VSMC</td>
<td>BM-derived</td>
<td>~50</td>
<td>Y-probe/SMA</td>
<td>65</td>
</tr>
</tbody>
</table>

*Percent of neointimal cells.

Fit-1 indicates α-mouse-EC Mab; GFP, Green Fluorescent Protein reporter gene construct; HISS2, α-rat-EC Mab; mVSMC, medial-VSMC; Tg mice, transgenic mice; Tx, transplantation; Y-PCR, male Y-chromosome specific polymerase chain reaction; Y-probe, male Y-chromosome specific probe; ND, not determined.

The origin of intimal cells in various models is summarized in Table 1. Data indicating a donor origin of neointimal VSMCs have been reported by Hruban et al. Using Y chromosome-specific fluorescence in situ hybridization on cardiac tissue of two sex-mismatched human cardiac transplant patients, no sign of hybridization was observed in cardiac myocytes, VSMCs, and >95% of ECs, thus excluding significant replacement of transplanted cardiac tissues with host derived cells. Glaser et al recently extended these studies and found in orthotopic human cardiac transplantation in sex-mismatched combinations approximately 5 to 10% of VSMCs to be of recipient origin. All cardiac allografts analyzed showed histopathological signs indicative of TA, and the majority of host-derived VSMCs were identified in medium and small coronary arteries. Using specific antibodies against SMA and factor VIII (to discriminate between VSMCs and ECs, respectively), Quaini et al recently reported about the presence of high levels of chimerism of VSMCs (60%) and ECs (42%) in human cardiac allografts transplanted in sex-mismatched combinations. Some major differences between both studies might explain the different outcome described in both articles. Quaini et al restricted their analysis to coronary vessels with normal structure, and areas with neointimal thickening were excluded because the only objective was to elucidate the role of chimerism in the undamaged myocardium. Moreover, the median graft survival...
time in their study subjects was relatively short (53 days). However, Glaser et al\textsuperscript{27} in their study focused specifically on the origin of VSMCs in neointimal lesions in human cardiac allografts in which the median survival time was 5.1 years.

It seems thus from these studies in human sex-mismatched transplants that recipient cells contribute only marginally if at all to the expansion of neointimal VSMCs after transplantation. A role of host chimerism of VSMCs in the microvasculature (without TA) in determining graft function/survival cannot be excluded, but this lies beyond the scope of this review and will not be further discussed.

In contrast with human cardiac allografting, however, Grimm and colleagues\textsuperscript{29} showed that approximately 35\% of SMA-positive neointimal cells in sex-mismatched renal grafts undergoing chronic rejection displayed recipient-type characteristics. With a hybridization efficiency of 40\%, this suggests that actually 80\% to 90\% of neointimal SMA-positive cells were of recipient origin. However, analysis of reciprocal combinations clearly demonstrated also the existence of a persistent population of donor type cells. Obviously both indications for a minimal and for a major contribution of recipient cells in neointimal VSMCs hyperplasia can be derived from these human studies. Whether organ-specific differences in this process exist, which might explain the observed differences between the cardiac and renal transplants, remains to be elucidated and will be further discussed below.

The question on the origin of neointimal VSMCs in TA has recently also raised considerable interest in experimental transplantation. Stump et al\textsuperscript{30} already in 1963 demonstrated the existence of blood-borne VSMC precursors in experiments in which they implanted Dacron hubs in the aorta of young pigs. These Dacron hubs became covered with ECs and VSMC-like cells originating from cells in the blood. Moreover, implantation of biodegradable synthetic vascular grafts in rats showed development of new vascular wall structures, including a neomedia (with SMA-positive VSMCs).\textsuperscript{31} VSMCs on the Dacron hubs as well as in the synthetic vascular grafts must by definition have been host derived. Indeed, when Brazzelton et al\textsuperscript{32} analyzed the origin of neointimal SMCs in allografted rat femoral artery segments using monoclonal antibodies specifically directed against donor or recipient major histocompatibility complex (MHC) class I antigens, all mesenchymal cells in the NI, of which approximately 50\% expressed SMA, were found to be recipient derived. Using a Y chromosome–specific single-cell polymerase chain reaction on microdissected nuclei of SMA-positive neointimal VSMCs, thereby excluding the risk of sample contamination with infiltrating recipient-derived inflammatory cells, we showed that virtually all of the neointimal VSMCs in rat aortic and cardiac allografts are of recipient and no longer of donor origin.\textsuperscript{21,22} These findings have independently been confirmed in a variety of mouse studies using MHC class II antigens,\textsuperscript{33} Y chromosome–specific probes,\textsuperscript{34,35} and LacZ\textsuperscript{35–38} and/or green fluorescent protein\textsuperscript{36,38} as reporter genes to define the origin of the neointimal cells (summarized in Table 1).

There is thus now compelling evidence from experimental studies that VSMCs in neointimal lesions after transplantation are frequently and in 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.\textsuperscript{5,9,10} This finding is actually not surprising in view of the fact that existing medial VSMCs and associated contractility of the graft completely disappear before (day 28 after transplantation) vascular remodeling starts in rat aorta allografts.\textsuperscript{39} At that time NI formation was restricted to the edges of the graft, suggesting ingrowth of VSMC precursors from the anastomosis rather than migration of VSMCs from the graft media. Also, Rossmann et al\textsuperscript{40} studied vascular changes in rat aortic allografts at various times after transplantation. They observed severe destruction of the allograft media, as indicated by karyolysis of the cells, depletion of SMA, and focal calcification. Subsequently, intimal thickening occurred as a result of proliferation of longitudinally oriented SMA-positive myointimal cells. No signs of migration or proliferation of medial VSMCs were observed. Similar results were reported by others.\textsuperscript{41–43}

Although recipient cells thus appear to contribute significantly to neointimal VSMCs hyperplasia in TA, it should, however, be noted that especially rodent studies have 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 (summarized in Table 1). One of the most striking differences between experimental murine grafts and human organ transplants displaying TA is that medial cell destruction in rodent allografts leads to complete medial necrosis,\textsuperscript{22,39–43} whereas in human allografts medial VSMC cellularity frequently remains unchanged.\textsuperscript{42} This is probably because of the fact that immunosuppressive treatment is not or only minimally used in most experimental animal models that have been used to study the origin of VSMCs in TA and this contrasts the situation in clinical human transplantation.\textsuperscript{44} One can imagine that when viable medial VSMCs remain available (eg, the human situation) such medial cells can provide a source of neointimal VSMCs, whereas in the case of complete destruction of medial VSMCs (eg, the murine situation) by definition other resources are required. Also the positioning of the transplanted tissue may influence the outcome of the process. It is probably easier for local cells to colonize an interposition graft, because they can invade transmurally or via pannus-like growth from the sides. In this respect, it would be interesting to study the origin of the neointimal VSMCs in a model described by Mennander et al\textsuperscript{42}, in which the adventitia of rat aorta grafts is exposed to starch before syngeneic transplantation. In contrast with regular syngeneic grafts, these starch-pretreated syngeneic transplants develop an inflammatory reaction and display extensive intimal thickening at the site of inflammation whereas medial VSMCs remain preserved. Under these circumstances also a contribution of medial VSMCs to intimal hyperplasia could well be envisaged in this rodent model. However, our model of vascular transplant arteriosclerosis after cardiac allotransplantation in immunomodulated rats also showed a small rim of presumably donor-derived medial VSMCs remained even in almost completely occluded coronary arteries.\textsuperscript{45} Nevertheless, virtually all neointimal VSMCs were found to be of recipient origin in
Figure 3. Origin of neointimal VSMCs after aortic allografting in BM-chimeric rats. Confocal laser scanning microscopy of VSMC origin after Dark-Agouty (DA) aortic allografting in LEW$_{1u}$-BN chimeric rats using the following monoclonal antibodies: A, MN4-916 (DA MHC class I, red); ASM-1 (vascular SMA, green), and DAPI (nuclei, blue). Arrow indicates remaining donor (DA) class I MHC-positive VSMCs in the media. B, OX27 (BN MHC class I, green); RECA-1 (ECs, red), and DAPI (nuclei, blue). Arrow indicates recipient (BN) non-BM-derived class I MHC-positive VSMCs in the neointima. Insert represents higher magnification of these cells. Abbreviations: lu, lumen; m, tunica media; ni, neointima.

such vessels. Similar findings were obtained using aortic allografting in bone marrow (BM)-chimeric rats (this report). As shown in Figure 3A all of the neointimal VSMCs are not of donor-type, even in the presence of remaining donor-type medial VSMCs (red). The absence of SMA staining (green) in these medial VSMCs, however, may indicate severe damage and loss of viability of those cells. Another difference between rodent models and human transplantation is the presence of existing vascular lesions in the majority of human donor material, which may provide the basis of further outgrowth of VSMCs during the subsequent development of a post-transplant NI lesion. Such lesions are rarely found in animal tissues used for transplantation. Taken together, although strong experimental evidence exists favoring recipient involvement, the possibility of medial or intimal VSMC contribution to NI development in TA cannot be omitted.

Anatomical Origin of Recipient Derived VSMCs in TA: Circulating Stem Cells?

The use of experimental animal models obviously also allows to address questions on the anatomical origin of the recipient cells involved in neointimal VSMC expansion.

For many years it was believed that vasculogenesis, the process in which new blood vessels are formed through the local differentiation of primitive endothelial precursors (e.g., angioblasts) only occurred during embryogenesis. In postnatal life the formation of new blood vessels was considered to occur only through angiogenesis, e.g., sprouting of new vascular structures from existing vessels. However, recent studies have shown that endothelial stem cells also exist in adult life. EC precursors have been isolated from the bone marrow and from peripheral blood and can contribute to the formation of blood vessels. Circulating numbers of EC precursors increase following ischemia or after GM-CSF treatment and are CD34$^+$ Flk-1$^+$ AC133$^-$, $^{47,48}$ and CD34$^-$ Flk-1$^+$ AC133$^+$, $^{47,50}$ Reyes et al recently identified a CD34$^+$ vascular endothelial cadherin$^+$ AC133$^+$ Flk-1$^+$ multipotent adult progenitor cell in postnatal human bone marrow. When cultured in vitro with the appropriate growth factors these cells differentiated into CD34$^+$ angioblasts. In vivo multipotent adult progenitor cell can differentiate into ECs. Thus, BM-derived primitive precursor cells can contribute to the formation of blood vessels. However, they contribute not only to blood vessel formation. Although previously BM stem cells were considered to be predominantly hematopoietic precursor cells, a variety of reports (reviewed in the references$^{56,57}$) have now shown that adult BM cells still hold the potential to differentiate into many different tissues, including liver, neurons, muscle cells, cardiomyocytes, and so on. Gradually, the concept of adult stem cell plasticity has developed, for example, adult stem cells, which were until now assumed to be committed to a fixed range of progeny, can switch, once relocated and appropriately stimulated, to a whole series of other progeny. Based on these studies transplantation of BM cells has been used as a therapeutic procedure both in clinical and experimental models of infarction and heart failure to see whether this would lead to improvement of myocardial performance. Direct injection of infarcted myocardium with BM cells promoted angiogenesis, with some of the new capillaries derived from BM. Injection of purified Lin$^-$ c-kit$^+$ cells resulted in the generation of new BM-derived ECs and SMA-positive VSMCs. Similar results were obtained after injection of G-CSF mobilized circulating human angioblast precursors in rat hearts after infarction. Strikingly, functional improvement in these studies resulted from the generation of new human capillaries, not through the generation of new myocytes. Infusion of LacZ-marked BM-derived stem cells, for example, side-population (CD34$^{47}$ c-kit$^{low}$ Sca-1$^+$) cells, into lethally irradiated mice followed by ischemia induction resulted in 3.3% endothelial engraftment, indicating circulatory capacities of such cells. Similarly, analysis of VSMC-like cells in irradiated sex-mismatched BM-reconstituted Ly5-congenic mice in damage-induced neointimal lesions showed that approximately 50% of the neointimal SMA-positive VSMCs are of male, and thus of BM origin.$^{55,56}$ These studies convincingly show that primitive BM cells can circulate through the body and contribute to vascular remodeling on tissue damage, giving rise to both ECs and VSMCs. It is therefore not surprising that several groups have addressed the issue of BM involvement in the process of NI development and VSMC hyperplasia.

Shimizu et al found a mouse aortic allo-transplant model using bone marrow chimeric LacZ-transgenic mice that, although all neointimal VSMCs were exclusively host derived, approximately 11% of these cells were of BM origin, the remainder being derived from radio-resistant host non-BM cells that can seed the graft. Similar findings were reported by Li et al Using BM-chimeric rats, allowing discrimination between BM and non-BM-derived cells, we also recently showed that the host-derived ECs in advanced neointimal lesions in aortic allografts are primarily non-BM derived. As is illustrated in Figure 3, the host-derived VSMCs in these lesions also were predominantly, if not all, derived from a non-BM source. In addition, Hu et al found that after vein grafting in LacZ-transgenic normal and BM-chimeric mice, approximately 40% of neointimal VSMCs were recipient-derived whereas 60% remained donor derived.
No BM-derived neointimal VSMCs were found in this model, thereby excluding BM as a primary source of these cells. However, using combinations of sex-mismatched transgenic BM-chimeric mice, Sata et al. and Saiura et al. showed that in their mouse model of graft vasculopathy the BM gives rise to most (~82%) neointimal cells. However, from their data it cannot be deduced what proportion of those cells was actually SMA-positive VSMCs. Taken together, these data indicate that although the BM can contribute to the population of VSMCs found in neointimal lesions, other non-BM resources also provide precursor cells. Because most of these studies used irradiation to create BM-chimeric animals, such host-non-BM–derived VSMC precursors must be radioresistant.

Obviously, ingrowth of VSMCs into the neointimal lesions from the host side of the anastomosis cannot be excluded as a source of neointimal VSMCs. However, by analyzing longitudinal sections from the anastomosis, including both host and donor tissue, Shimizu et al. found no indications for ingrowth of adjacent host medial VSMCs. Consequently a blood-borne origin of VSMC precursors seems most likely (Figure 4). Recent data suggest that the human peripheral blood contains a population of circulating VSMC precursor cells. Simper and colleagues showed that in vitro culture of human mononuclear blood cells in the presence of platelet-derived growth factor–enriched medium resulted in the generation of smooth muscle outgrowth cells. These smooth muscle outgrowth cells displayed a variety of phenotypic characteristics of VSMCs (eg, SMA, myosin, calponin). Whether such precursors adhere to the luminal side of the vessel or migrate from the adventitia through vasa vasorum toward the NI remains unknown. Shimizu et al. argued that shear forces on the luminal side might prevent adhesion of cells. At the start of NI development, however, the first SMA-positive cells are found in a scattered pattern on the luminal side, suggesting direct entry from the lumen. Also, the observation by Sasaki et al., who studied human postmortem vein graft material from patients after coronary bypass grafting, that the process of NI formation started with the loss of ECs followed by the appearance of SMA-negative spindle-shaped cells at sites of injury suggests a blood-borne origin of these cells.

Circulating VSMC precursors might also originate from other sources. Bucala et al. 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. Also, transdifferentiation of ECs into VSMCs has been suggested. de Ruiter et al. showed that embryonic ECs can differentiate into SMA expressing cells in vivo and in vitro. Whether this is a unique property of embryonic ECs or is also shared by adult ECs remains to be proven. SMCs themselves should also be mentioned in this respect. Surprisingly, VSMCs (and fibroblasts) but not ECs were able to adhere to mechanistically de-endothelialized rat aortas. SMCs from various locations in the vessel wall display extensive heterogeneity. Noncontractile intimal (epithelioid) SMCs with morphological resemblance to ECs, lacking most VSMC-associated proteins, can transform into media-like VSMCs and generate capillary tubes in vitro consisting of ECs and VSMCs.

One should furthermore realize that VSMCs at different locations may have different embryonic origins. VSMCs in the coronary arteries, for instance, are derived from the epicardial lining and 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**

Given the growing awareness of plasticity of stem cells, it appears that neointimal VSMC precursors display similar characteristics and actually may even reside among them. We hypothesize therefore that VSMC precursors are not a single entity but can be recruited from a variety of resources depending on the severity and duration of vessel damage and the critical need for vessel repair (Table 2). In the case of limited superficial vessel damage with a remaining vascular structure, medial/intimal VSMCs themselves will probably provide sufficient repair potential. More severe vascular damage, including medial VSMC damage, over a limited period of time might signal ingrowth of VSMCs from adjacent 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. Indeed, Han et al. reported recently that BM-derived SMA-positive VSMCs in damage-induced neointimal lesions were only found after severe vascular damage, not in arteries with minimal damage. Tissue damage also proved to be the major determinant required for the transdifferentiation of primitive BM cells into cells involved in neo-angiogenesis (eg, ECs and VSMCs) in regenerating infarcted myocardium. In clinical transplantation, probably the entire spectrum of VSMC precursor derivation can be expected to occur, perhaps even in one and the same patient.

![Figure 4](image_url)
### Acknowledgments

This work was supported by a grant of the Groningen University Hospital. J.L.H. was supported by grants of the Dutch Diabetes Foundation (DFN99.028 and DFN2001.05.003) and the Ter Meulen Fund, Royal Netherlands Academy of Arts and Sciences (TMF/DA/6419).

### References


### TABLE 2: Plasticity of VSMC Replacement in TA

<table>
<thead>
<tr>
<th>Damage</th>
<th>Source of VSMC</th>
<th>TA Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little damage* over limited period</td>
<td>Medial/intimal VSMC</td>
<td>± TA</td>
</tr>
<tr>
<td>More severe damage† over limited period</td>
<td>Ingrowth of adjoining vessels (anastomosis?)</td>
<td>TA</td>
</tr>
<tr>
<td>Severe damage‡ over limited period</td>
<td>Recruitment from non-BM sources (vasculature?)</td>
<td>TA</td>
</tr>
<tr>
<td>Severe damage‡ over prolonged period</td>
<td>Recruitment from BM</td>
<td>TA</td>
</tr>
</tbody>
</table>

*Limited medial-VSMC damage. †Severe medial-VSMC damage. ‡Full medial-VSMC disruption.


Origin of Vascular Smooth Muscle Cells and the Role of Circulating Stem Cells in Transplant Arteriosclerosis
Jan-Luuk Hillebrands, Flip A. Klatter and Jan Rozing

Arterioscler Thromb Vasc Biol. 2003;23:380-387; originally published online January 30, 2003; doi: 10.1161/01.ATV.0000059337.60393.64

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/3/380

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/