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Blood-Borne Origin of Neointimal Smooth Muscle Cells in Transplant Arteriosclerosis

Jan-Luuk Hillebrands, Flip A. Klatter, Paul Nieuwenhuis, and Jan Rozing

Transplant arteriosclerosis (TA) is a major complication after solid organ transplantation. TA is characterized by persistent perivascular inflammation and concentric intimal thickening consisting of α -actin-positive vascular smooth muscle (VSM) cells. The current view on TA is that donor-derived medial VSM cells of affected arteries migrate and proliferate into the subendothelial space, resulting in luminal narrowing. Following this concept, the VSM cells present in the arteriosclerotic lesions are of donor origin. In this study, the authors analyzed the origin (donor vs recipient) of endothelium (EC) and neointimal α -actin-positive VSM cells in 2 different experimental transplant models. Aortic and cardiac allografting was performed in the PVG (RT-1^c) to AO (RT-1^u) rat strain combination. Aorta recipients were not immunosuppressed, whereas cardiac allograft recipients were intrathymically immune modulated to prevent acute rejection. Transplants were performed from female donor to male recipient rats. The α -actin-positive VSM cells present in arteriosclerotic lesions, in aortic as well as cardiac allografts, were of recipient, rather than donor, origin. Following aortic allografts, the ECs are completely replaced by host-derived ECs, whereas in cardiac allografts the ECs are still of donor origin.

Chronic transplant dysfunction (CTD) is the primary cause of allograft loss after the 1st perioperative year, and it is today's most important problem in clinical organ transplantation. The hallmark of CTD is development of transplant arteriosclerosis (TA), that is, progressive intimal thickening (neointima formation) consisting of α -actin-positive vascular smooth muscle (VSM) cells intermingled with inflammatory cells (T cells and macrophages).¹ The current thought on the process of TA holds that medial VSM cells of affected arteries in the graft migrate into the subendothelial space and start to proliferate in response to inflammatory cells and damaged graft endothelium, eventually resulting in luminal occlusion and deterioration of graft function.¹⁻⁴ Following this concept, neointimal VSM cells originate from the vascular media and are therefore donor (=graft) derived. This is supported

by findings of Hruban and others, who showed donor-origin of neointimal VSM cells in cardiac allografts using FISH on tissue sections.⁵ However, data have also been reported indicating recipient origin of neointimal VSM cells in experimental transplant models.⁶⁻⁸ Recently, Légaré and others demonstrated that neointima formation is dependent on, and proportional to, the extent of medial VSM cell damage.⁹ Although a clear correlation seems to exist between the disappearance of medial VSM cells, and the appearance of neointimal VSM cells, migration of medial VSM cells into the subendothelial space has not been reported so far.

Because experimental evidence proving medial origin of neointimal VSM cells is lacking, the question remains whether neointimal VSM cells are truly donor-derived. We recently hypothesized host-origin of neointimal VSM cells,¹⁰ and this hy-

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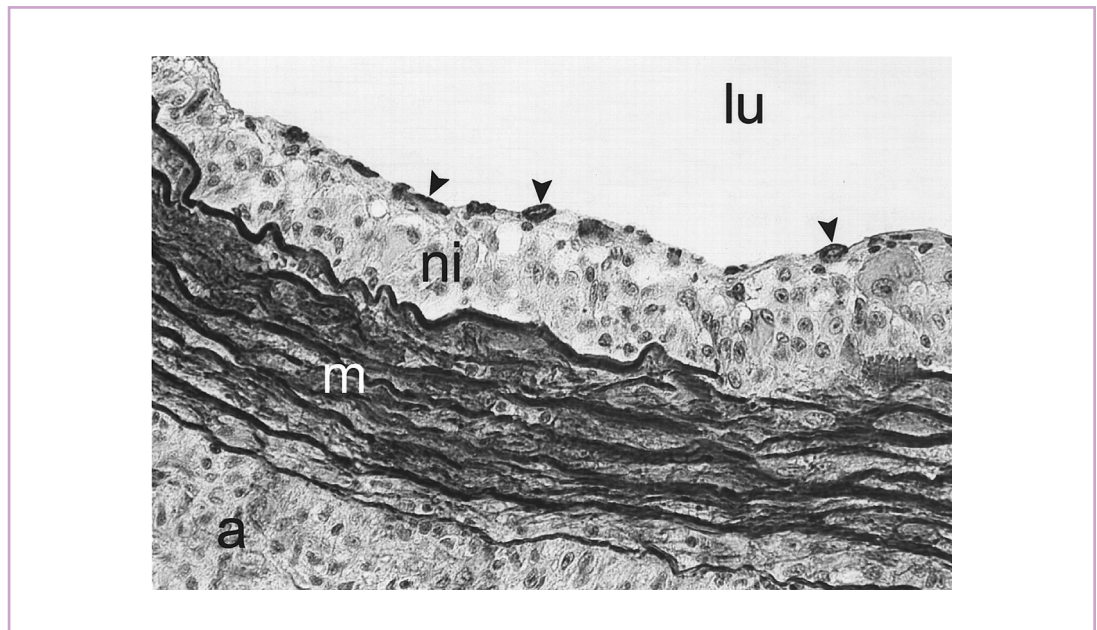


Figure 1. Micrograph showing double staining for vascular smooth muscle (VSM) cells (α -actin) and elastin (Lawson) on an aortic allograft (28 days after engraftment), transplanted in the Lewis to Brown-Norway strain combination. Arrowheads indicate α -actin-positive cells adhering to the surface of the developing neointima, suggesting blood-borne origin of these cells. Some medial α -actin-positive VSM cells are still present at this early stage after transplantation. a = adventitia; lu = lumen; m = media; ni = neointima. (Original magnification: $\times 400$)

pothesis was tested on aortic and cardiac allografts in rats using polymerase chain reaction (PCR) analysis and allowing discrimination between donor- and recipient-derived cells.

PCR analysis, based on polymorphic regions in the *Tap2* gene,¹¹ was performed on 70- μ m-thick tissue samples from aortic allografts as well as on pooled microdissected whole coronary arteries (microdissected from cardiac allografts). Although aortic, as well as cardiac, samples clearly showed the presence of recipient-derived cells, one should keep in mind that neointimal lesions can contain an appreciable number of recipient-derived infiltrating leukocytes.¹²

Since contaminating recipient-derived inflammatory cells can account for the *Tap2* PCR results, we had to use another strategy to overcome the problem of analyzing infiltrating inflammatory cells leading to false positive results. Therefore, we developed a nested PCR based on a Y-chromosome specific DNA sequence that was sufficiently sensitive to detect male-derived cells on the single-cell level (HY PCR).¹³ Since aortic and cardiac transplantation was not only performed in major histo-

compatibility complex (MHC)-incompatible strain combinations but also in a female donor to male recipient combination, we used the HY PCR to detect recipient-derived cells on the single-cell level. HY PCR analysis was performed on microdissected single nuclei from α -actin-positive neointimal VSM cells from aortic and cardiac allografts. This α -actin staining ensured positive identification of neointimal VSM cells, thereby preventing analysis of infiltrating recipient-derived inflammatory cells. In contrast to current thought, our results indicate that in both aortic and cardiac allografts, neointimal α -actin-positive VSM cells are of recipient and not of donor origin.^{13,14}

The question arises as to what the anatomical origin of these recipient-derived neointimal cells is. One possibility might be ingrowth of medial VSM cells from the recipient side of anastomosis, as suggested by Aziz and others.⁷ Another possibility might be the transdifferentiation of ECs into α -actin-positive VSM cells. Studying quail embryos, de Ruiter and others showed transdifferentiation of embryonic ECs into α -actin-expressing mesenchymal

CHRONIC TRANSPLANT DYSFUNCTION:

Progressive deterioration of graft function occurring after the first post-operative year.

PCR ANALYSIS:

Polymerase Chain Reaction analysis.

FISH:

Fluorescent In Situ Hybridization.

cells in vivo and in vitro.¹⁵ In this situation, one would also expect the neointimal ECs to be recipient derived. MHC class I haplotype-specific immunohistochemistry on aortic allografts indeed confirmed recipient origin of the neointimal ECs.¹⁴ However, ECs covering neointimal lesions in coronary arteries in cardiac allografts are still of donor type, although the neointimal VSM cells are of recipient type.¹⁴ A 3rd possibility might be the existence of VSM progenitor cells that differentiate into α -actin-positive VSM cells. Circulating VSM progenitor cells, however, have not been identified thus far. Bucala and others reported the existence of a non-bone-marrow-derived circulating cell population with fibroblast properties that specifically enters sites of tissue injury.¹⁶ In addition, Kouchi and others described the presence of α -actin-positive smooth muscle cells on Dacron grafts, which apparently originated from the blood stream.¹⁷ In our aortic transplant model, data from sequential immunohistological analysis of developing TA at an early stage after transplantation are in line with a blood-borne influx of regenerating VSM cells rather than ingrowth from the recipient side of anastomosis. As illustrated in Figure 1, the 1st α -actin-positive cells appear scattered at the luminal surface of the neointima, strongly suggesting a blood-borne origin of these cells. These data further support the notion that TA is the result of a normal vascular repair process, progressing, however, beyond the needs of functional repair.¹⁸

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