Research Journal of Medical Sciences 1 (5): 234-241, 2007
ISSN: 1815-9346

Total Reendothelialization of Vascular Grafts by Circulating Recipient Endothelial Stem Cells in a Transgenic Green Rat

[1]R. Torensma, [2]René H.J. Brouwer, [1,3]Karin Van Ginkel, [1]Carl G. Figdor and [2]Sandeep K. Singh
[1]Department of Tumorimmunology, Nijmegen Centre for Molecular Life Sciences, The Netherlands
[2]Department of Thoraxsurgery, Radboud University Nijmegen Medical Centre, The Netherlands
[3]Meddens Diagnostics bv, Vorden, The Netherlands

Abstract: This study was designed to unravel if arterial grafts treated with phosphate buffered saline or 10Gy irradiation to induce endothelial cell loss, still contain enough biological information to drive proper endothelial regeneration. To demonstrate that damage to donor arteries retains the biological information needed to drive proper differentiation of circulating endothelial precursor cells, arteries were either irradiated (n=10) (10Gy) or stored for 30 min in PBS (n=10) at 20°C. After treatment the arteries were grafted end to end in the aorta descendens of GFP transgenic rats. Three weeks after implantation 5 grafts were recovered and the remaining 5 after 6 weeks and were analyzed immuno histochemically using antibodies to endothelial cell lineage markers (CD31 and von Willebrand factor), Griffiona simplicifolia lectin and Green Fluorescent Protein (GFP). Arteries processed immediately after surgery served as control. Grafted arteries had an intact endothelial layer. Three weeks after graft implantation the arteries were totally denuded for both treatment protocols, while cells attach to the fibroelastic layer. Six weeks after grafting the grafts showed neointima formation and were totally reendothelialized with recipient cells. The fibroelastic layer and adventitia also contained green recipient cells. These results provide compelling evidence that mild treated arteries loose their endothelial lining but still contain the biological information to drive endothelial differentiation of recipient circulating endothelial precursor cells resulting in a intact endothelial layer six weeks after surgery. This in contrast to harsh treated grafts.

Key words: Aorta transplantation, immunohistochemistry, green fluorescent rats, reendothelialization

INTRODUCTION

There is a need for a blood vessels constructed from non-immunogenic materials that incorporate living and autologous cells (Koike *et al*., 2004; Sarraf *et al*., 2003; Poh *et al*., 005). Such materials should contain the biological information, as present *In vivo* in blood vessel tissue, to prevent the forming of unwanted scar tissue (Kasimir *et al*., 2003) and poor performance (Daly *et al*., 2004). When the cells in and on the blood vessel are of recipient origin immune suppressive treatment is unnecessary. The recent discoveries of circulating endothelial stem cells opens the possibility to use those cells to replace allogeneic endothelial cells present on the graft (Reyes *et al*., 2002; Peichev *et al*., 2000; Ashara *et al*., 1999; Warner *et al*., 2002). Those circulating cells originate from the bone marrow (Isner *et al*., 2001; Rafii, 2000). Moreover, their involvement in reendothelialization has been established (Isneret *et al*., 2001). However, the involvement of bone marrow derived circulating endothelial progenitor cells in vasculature repair is debated. In a renal transplantation study recipient endothelial cells were detected in the vessel wall of the graft suggesting they arose from circulating endothelial progenitor cells (Lagaaij *et al*., 2001; Xu *et al*., 2002). Also, patients suffering from limb ischemia showed therapeutic angiogenesis after autologous bone marrow injection in the ischemic limb (Tateisni *et al*., 2002). In contrast, no incorporation of bone marrow cells in blood vessels was observed in mouse models of hind limb ischemia (Zieglhoeffer *et al*., 2004). Moreover, no contribution of circulating vascular progenitor cells was observed during compensatory lung growth (Voswinckel *et al*., 2003). Also, in transplant arteriosclerosis no contribution of bone marrow was observed (Hillebrands *et al*., 2002). In contrast, serial transplantations clearly showed the involvement of bone marrow in endothelium repair (Grant *et al*., 2002). None of those studies denies the

Corresponding Author: R. Torensma, Department of Tumorimmunology, Nijmegen Centre for Molecular Life Sciences, Netherlands

existence of circulating bone marrow derived endothelial progenitor cells. Apparently, not in all studies circulating endothelial progenitor cells are recruited to replace lost endothelial cells. To encompass all those apparently conflicting datasets we argued that the graft should contain 'biological cues' to drive endothelial progenitor cell attachment and differentiation. Replacement of donor endothelial cells by recipient cells has to be so subtle that the circulating endothelial progenitor cells are able to bind to the graft and differentiate into mature endothelium cells. Cells inside the matrix of the vessel wall should also be replaced for autologous cells to escape from immunological attack and maintain and remodel the structure of the matrix (Bujan *et al*., 2004; Leon and Greisler, 2003). In vitro seeding of autologous mature cells on an a cellular allograft resulted in the formation of a confluent endothelial layer and a good function (Cebotari *et al*., 2002; Numata *et al*., 2002; Lamm *et al*., 2001). However, in vitro culturing of recipient endothelial cells is a laborious and costly procedure besides that those cells have a limited life span that is overcome by retroviral infection to introduce the telomerase gene (Poh *et al*., 2005). We suggested to incorporate endothelial stem cells to replace senescent cells (Torensma, 2005).

Here, we have set out a series of experiments to find the conditions in which donor cells are replaced for recipient cells *In vivo*. Since irradiation has been described as a methodology to delay reendothelialization (Cho *et al*., 2003; Lasordo *et al*., 2003) we compared irradiation versus mild treatment by storage of the graft in phosphate buffered saline. As a first approach for blood vessel grafting we transplanted wild type aortic blood vessels in the abdomen of green fluorescent syngeneic rats. By working in a syngeneic setting the putative repopulation of the graft can be studied without immunologic involvement. Based on those data the next step is to transplant aortic vessels in a complete major histocompatibility complex mismatched recipient. Since the subtle treatment of the donor graft will leave donor endothelium on the graft, the origin of the endothelium has to be determined. By using green fluorescent transgenic animals we easily could discriminate between recipient derived endothelial cells and putative outgrowth of residual donor endothelium. Moreover, by using a large graft (≅1cm) total reendothelialization of the graft cannot totally be accomplished by recipient endothelial cells present at the site of integration (Isner *et al*., 2001). Circulating endothelial progenitor cells must have a major contribution in case total reendothelialization is observed.

MATERIALS AND METHODS

Transgenic animals: Green fluorescent protein transgenic rats (Sprague-Dawley) were raised in Japan (Okabe *et al*., 1997). After transport to the Netherlands the animals were kept in quarantine for six weeks and tested for pathogens. The animals were maintained in the University animal facilities in a special pathogen free unit that is surveyed regularly on pathogens, using sentinel animals. No pathogens were found during the experimental period. All experiments conformed to animal care protocols and were approved by the board for animal experiments of the Radboud University Nijmegen Medical Centre. For each time point and each treatment protocol five animals were used.

Experimental outline: Table 1 describes the experimental setup and the number of animals used.

In total 35 donor rats were used and 30 transgenic GFP recipient rats. Untreated aortas were collected from 5 donor animals and immediately frozen after mounting in OCT compound. Those tissue samples served as representative for the onset of the experiment. Frozen aortas were stored at -80°C. until use. Grafted aortas were collected after three and six weeks and processed in the same way.

Treatment of aortas: A 10-15 mm segment of wild type donor aorta descendens was collected after closing all side branches with suture and stored in PBS for 30 min at room temperature or stored in PBS and irradiated using a Cesium$^{(137)}$ source applying a dose of 10Gy. The latter procedure lasted also approximately 30 min. For each time point and each treatment 5 animals were used.

Implantation of aortas: Microsurgery was performed under inhalation anesthesia with methoxyflurane. Pain treatment consisted of i.p. injection of carprofen (5mg kg^{-1}) and started one day before surgery and was given once daily on three subsequent days. The wild type rat aortas were used to replace an segment of the GFP animal with end-to-end anastomoses with magnification provided by an operative microscope. Total grafting procedure lasted approximately 45 min.

Immunohistochemistry of aortas: Three and 6 weeks after grafting the aortas were collected and mounted in OCT compound (Tissue-Tek, Sakura Finetek Europe, Zoetermeer, The Netherlands) and frozen immediately thereafter and stored at -80°C. Tissue was cut into 1.5 μm sections for fluorescence labeling and 5-8 μm for immunohistochemistry and fixed with acetone (HE, vWF,CD31 and GSI and 4% paraformaldehyde (anti-GFP) prior to staining. Sections were also stained with Hematoxylin and Eosin (HE). Immunohistochemistry was performed with antibodies against GFP (Molecular Probes, Leiden The Netherlands), CD31 and von Willebrand

Table 1: the experimental setup and the number of animals used

Time point (weeks)	T = 0	T = 0	T = 0	T = 3	T = 3	T = 6	T = 6
Treatment	None control	PBS	10GY	PBS	10GY	PBS	10GY
Number of donor animals	5	5	5	5	5	5	5

factor. Antibodies were used at a concentration of 5 μg mL^{-1}. The sections were then treated with biotinylated horse anti mouse. Final development was performed with the ABC kit and AEC (Vector Burlingame, Ca.). Fluorescent immunohistochemistry was performed with endothelium specific Griffonia Simplicifolia (GS) lectin conjugated with Alexa Fluor 568 (Molecular Probes Leiden, Netherlands) and goat-anti rabbit Texas Red for anti GFP.

RESULTS AND DISCUSSION

Immunohistology at start of experiment: Staining for CD31, von Willebrand factor as well as GS lectin indicated that in PBS treated as well as 10Gy irradiated grafts the endothelial layer was still intact for all animals studied. Figure 1 shows the results obtained with the endothelial marker GSI. Similar results were obtained with CD31, von Willebrand factor. HE staining revealed no damage to the integrity of the vessel wall. Since the fibroelastic layer showed green auto fluorescence sections were stained with antibodies against GFP. As expected no staining was observed for the wild type aorta.

Immunohistology three weeks after grafting: A completely different view was observed for the aortas obtained three weeks after grafting. The endothelial layer was completely lost already evident from the HE staining and further demonstrated by the lack of staining with all endothelial markers (Fig. 2). Several blood cells appeared to be attached to the denuded blood vessel. In all ten animals (five irradiated and five PBS treated) similar data were observed.

Immunohistology six weeks after grafting: The endothelial layer was completely recovered six weeks after grafting as evidenced for the all endothelial markers. Also the HE staining showed an intact endothelial layer. Based on several stainings an overall coverage of 95% was estimated. For all ten animals (five irradiated and five PBS treated) similar staining patterns were observed. Fluorescence analysis also revealed GFP positive cells in the fibroelastic layer. In all animals neointima formation was observed (Fig. 3).

Isografting: To unravel the role of immunological mediated neointima formation, full syngeneic grafting was performed. Fig. 4 shows the results of a GFP SD aorta

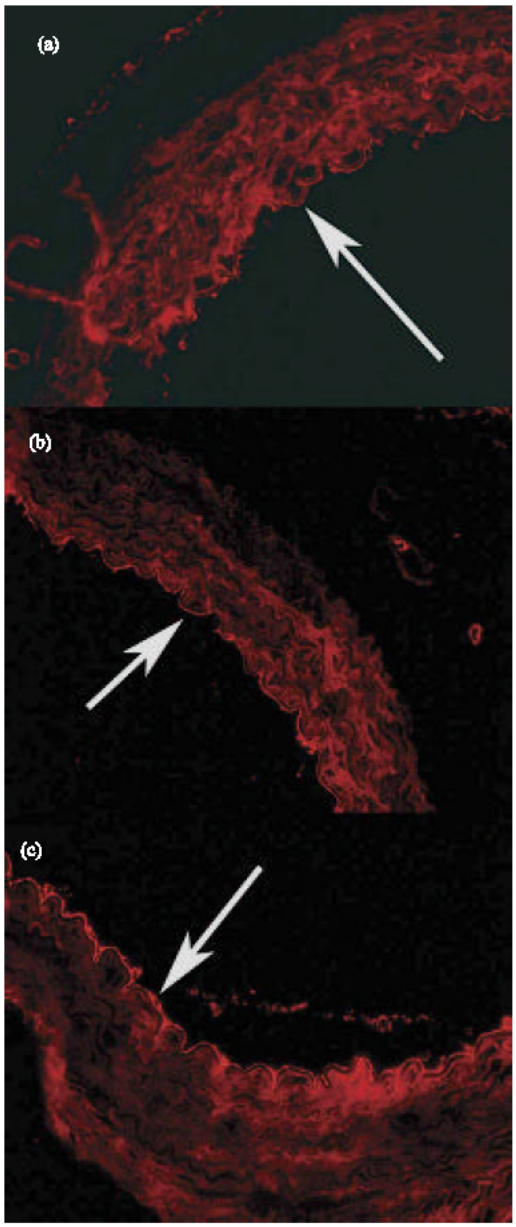

Fig. 1: a) Aortas immediately frozen after collecting and stained for endothelial integrity with Griffiona simplicifolia lectin. Arrows point at the thin endothelial lining. b) Aortas were kept for 30 min at room temperature in PBS and frozen thereafter and stained with Griffiona simplicifolia lectin. c) Aortas were irradiated 10Gy and frozen therefater and stained with Griffiona simplicifolia lectin. One out of 5 identical stainings obtained from different animals is shown

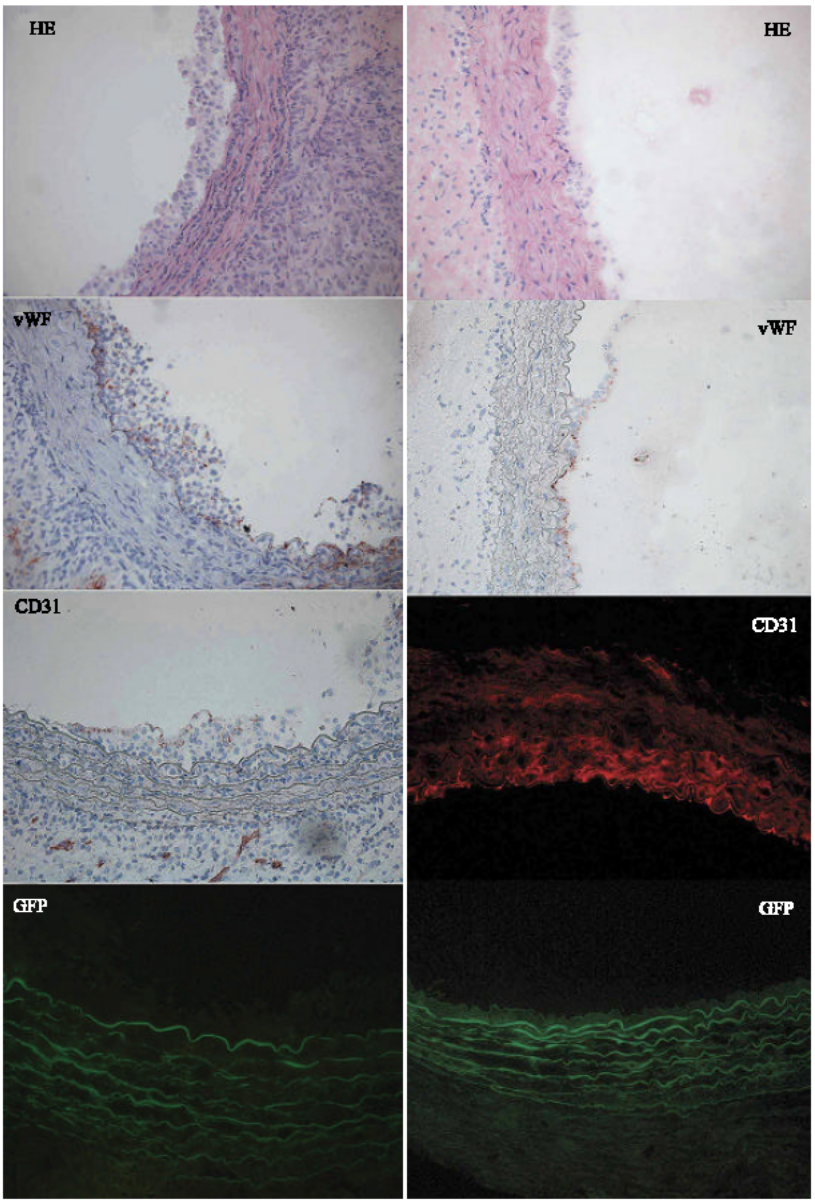

Fig. 2: Array of stained aortas obtained three weeks after grafting. The first row displays the outcome of staining with Hematoxylin Eosin (HE). The second row staining for vonWillebrand Factor (vWF). The third row staining with antibodies directed to CD31. The fourth row fluorescence for GFP One out of five identical stainings obtained from different animals is shown

grafted to GFP SD rat. Also in this situation neointima formation is observed like the previous shown results where wt aorta was grafted to GFP SD rats.

Influence of surgical procedure on neointima formation: Hardly any neointima formation was found when aortas were collected from donor animals and immediately grafted in the recipient rat. Onset of neointima formation was observed in cases where there was a delay in the grafting procedure (Fig. 5).

Long term integrity of grafted aortas: The integrity of the graft was deduced from data sets obtained 6, 8 and 15 months after grafting. After 6 , 8 as well as 15 months the endothelial layer was still intact (Fig. 6). The neointima was present and compared to the six weeks graft not significantly increased in size. This indicates that the neointima does not grow any further nor does it shrink after total reendothelialization.

Circulating endothelial progenitors cells originating from the bone marrow are considered to play a role in

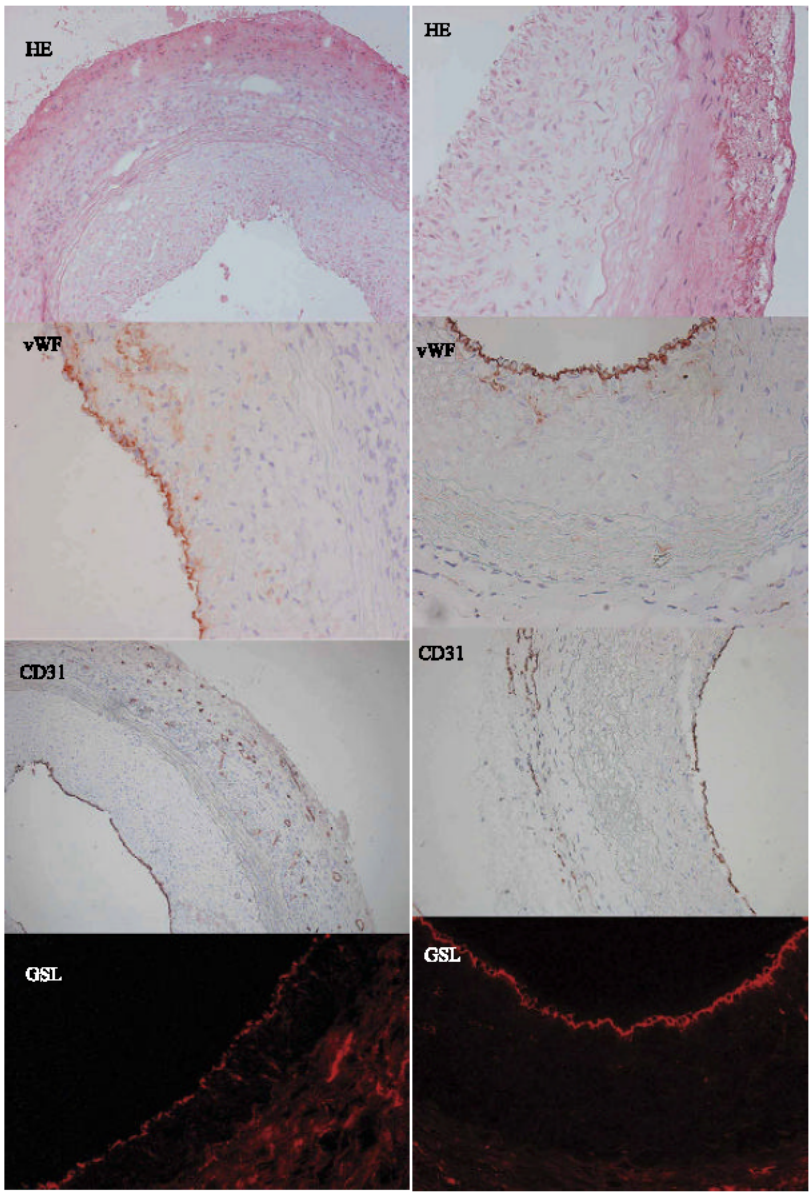

Fig. 3: Array of stained aortas obtained six weeks after grafting. The first row displays the outcome of staining with Hematoxylin Eosin (HE). The second row staining for vonWillebrand Factor (vWF). The third row shows nstaining with antibodies directed to CD31. The 4th row displays the staining with Griffiona Simplicifolia (GS)A. The results with aortas that were kept for 30 min at room temperature in PBS. B. The results obtained with aortas that were irradiated. One out of five identical stainings obtained from different animals is shown

postnatal angiogenesis (Reyes *et al*., 2002; Asahara *et al*., 1999; Isner *et al*., 2001; Gill *et al*., 2001; Cogle *et al*., 2004). Vascular damage will recruit endothelial progenitors cells from the bone marrow via elevation of the SDF-α. (Schober *et al*., 2003) In general, mechanical and decellularized and cross linked animal grafts are unable to recruit endothelial cells. However, reendothelialization appeared to depend on the decellularization procedure (Kasimir *et al*., 2003; Numata *et al*., 2002; Booth *et al*., 2002). Therefore, the biological information that is retained in the vessel wall will drive differentiation into the appropriate cells. Here, we studied the putative repair of wild type ischemic (mild treatment) and 10 Gy irradiated (less mild treatment) abdominal aortas after transplantation into transgenic green fluorescent rats. Three and six weeks after grafting the aorta's were

Fig. 4: Neointima formation observed in aortas obtained from GFP rats grafted to GFP rats

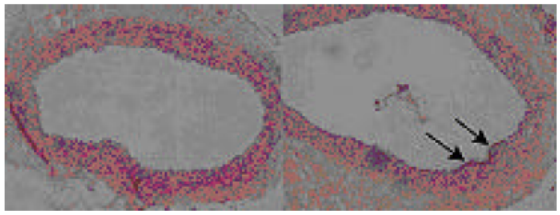

Fig. 5: Lack of neointima formation observed after immediate grafting of aorta with a fast procedure (A). Onset of neointima when the surgical procedure lasted longer (B). Arrows show onset of neointima formation

recovered. After three weeks the endothelial lining was to a great extend lost in all tested animals. After six weeks new endothelial cells were present on the graft forming a continuous lining in all animals. Those cells were from the green recipient rat excluding outgrowth of residual wild type non-green endothelial cells from the graft donor. Irradiation showed damage to the extracellular matrix of the aorta while PBS treatment of the grafted aorta left the structure intact (data not shown). Remarkably, no difference in denuding the graft, as seen after three weeks, as well as total reendothelialization after six weeks for the two treatment protocols was observed. The damage to the matrix was minimal since after six weeks the elastic layers of the aorta were intact and covered with endothelium. The fibroelastic layer became populated with recipient cells indicating that all biological information to drive proper differentiation was retained irrespective of the treatment protocol. The rapid removal of donor cells from the graft and the subsequent rapid recovery with recipient cells opens more clinical feasible grafting with allografts and treat recipients for the first few weeks with immune suppression to circumvent

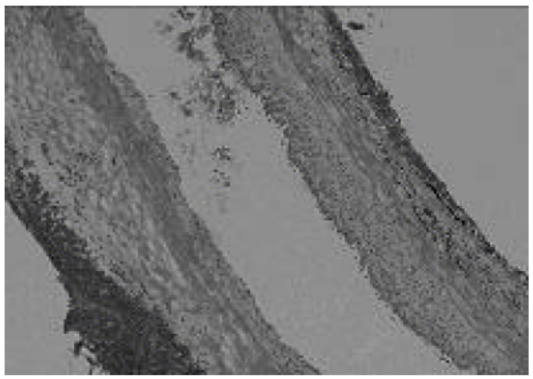

Fig. 6: Stable neointima 8 months after grafting aortas from SD rats to SD recipients (syngeneic grafting)

immunological destruction of the vessel wall with concomitant loss of biological information.

Besides total endothelial coverage of the vessel wall neointima formation occurred. Neointima formation can have different causes as evidenced from several treatments that prevented neointima formation. Long-term inhibition of Rho kinases (Matsumoto *et al*., 2003), statin therapy (Walter *et al*., 2002), triggering of the bradykinin receptor, (Agata *et al*., 2000;) prostacyclin synthase gene transfer, (Numagueri *et al*., 1999) immediate early response gene induction, (Schulz *et al*., 2003) macrophage depletion, (Donenberg *et al*., 2003) C-type natriuretic gene delivery, (Ohno *et al*., 2002) immunosuppressive drugs, (Matsumoto *et al*., 2003) or treatment with carbon monoxide (Otterbin *et al*., 2003) all reduced neointima formation. We observed neointima formation even when isografts were transplanted excluding an immunological cause of the observed neointima formation in our case. In an attempt to unravel the cause of the neointima we grafted aortas immediately after collecting them from the donor animal. Remarkably when the grafting proceeded smoothly no intima formation was observed while the beginning of neointima was observed when the surgical operation took somewhat longer. This points to a mechanism that the more the graft is exposed to ischemia the more damage is inflicted causing rapid loss of the endothelial lining. Three weeks after grafting the graft is totally denuded and cells attach to fibroelastic layer. Subsequently this cell layer is covered by recipient endothelial lining. This demonstrates that reendothelialization is slower than the attachment of cells to the denuded fibroelastic layer. Faster replacement of the endothelial layer can prevent neointima formation. Recent data suggest that this could be achieved by treatment mobilizing endothelial stem cells with GM-CSF (Cho *et al*., 2003).

Based on these results, we propose that minimal damage to endothelial lining but with retaining the biological information, recruits circulating stem to the damaged tissue and differentiates them into endothelium that replace the damaged cells completely.

ACKNOWLEDGEMENT

The expert surgical assistance of D. Smits of the Central Animal Facility is acknowledged.

Dr Masaru Okabe is acknowledged for supplying the green rats.

This research was supported by a 'Vrije Beleidsruimte' grant (2001-F2) from the Radboud University Nijmegen Medical Centre.

REFERENCES

Agata, J., R.Q. Miao, K. Yayama, L. Chao, J. Chao and B. Bradykinin, 2000. Receptor mediates inhibition of neointima formation in rat artery after balloon angioplasty. Hypertension, 36: 364-370.

Asahara, T., H. Masuda, T. Takahashi, C. Kalka, C. Pastore and M. Silver *et al.*, 1999. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovasculrization. Circ. Res., 85: 221-228.

Booth, C., S.A. Korossis, H.E. Wilcox, K.G. Watterson, J.N. Kearney and J. Fisher *et al.*, 2002. Tissue engineering of cardiac valve prostheses I: development and histological characterization of an acellular porcine scaffold. J. Heart Valve. Dis., 11: 457-462.

Bujan, J., N. Garcia-Honduvilla and J.M. Bellon, 2004. Engineering conduits to resemble natural vascular tissue. Biotechnol. Applied Biochem., 39: 17-27.

Cebotari, S., H. Mertsching, K. Kallenbach, S. Kostin, O. Repin and A. Batrinac *et al.*, 2002. Construction of autologous human heart valves based on an acellular allograft matrix. Circulation, 106: I63-I68.

Cho, H.J., H.S. Kim, M.M. Lee, D.H. Kim, H.J. Yang and J. Hur *et al.*, 2003. Mobilized endothelial progenitor cells by granulocyte-macrophage colony-stimulating factor accelerate reendothelialization and reduce vascular inflammation after intravascular radiation. Circulation, 108: 2918-2925.

Cogle, C.R., D.A. Wainman, M.L. Jorgensen, S.M. Guthrie, R.N. Mames and E.W. Scott, 2004. Adult human hematopoietic cells provide functional hemangioblast activity. Blood, 103: 133-135.

Daly, C.D., G.R. Campbell, P.J. Walker and J.H. Campbell, 2004. *In vivo* engineering of blood vessels. Front Biosci., 9: 1915-24.

Danenberg, H.D., I. Fishbein, H. Epstein, J. Waltenberger, E. Moerman and J. Monkkonen *et al.* 2003. Systemic depletion of macrophages by liposomal bisphosphonates reduces neointimal formation following balloon-injury in the rat carotid artery. J. Cardiovasc. Pharmacol., 42: 671-679.

Gill, M., S. Dias, K. Hattori, M.L. Rivera, D. Hicklin and L. Witte *et al.*, 2001. Vascular trauma induces rapid but transient mobilization of VEGFR2 (+)AC133(+) endothelial precursor cells. Circ. Res., 88: 167-174.

Grant, M.B., W.S. May, S. Caballero, G.A. Brown, S.M. Guthrie and R.N. Mames *et al.*, 2002. Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. Nat. Med., 8: 607-612.

Hillebrands, J.L., F.A. Klatter, W.D. Van Dijk and J. Rozing, 2002. Bone marrow does not contribute substantially to endothelial-cell replacement in transplant arteriosclerosis. Nat. Med., 8: 194-195.

Isner, J.M., C. Kalka, A. Kawamoto and T. Asahara, 2001. Bone marrow as a source of endothelial cells for natural and iatrogenic vascular repair. Ann. N.Y. Acad. Sci., 953: 75-84.

Kasimir, M.T., E. Rieder, G. Seebacher, G. Silberhumer, E. Wolner and G. Weigel *et al.*, 2003. Comparison of different decellularization procedures of porcine heart valves. Int. J. Artif. Organs., 26: 421-427.

Koike, N., D. Fukumura, O. Gralla, P. Au, J.S. Schechner and R.K. Jain, 2004. Tissue engineering: Creation of long-lasting blood vessels. Nature, 428: 138-139.

Lagaaij, E.L., G.F. Cramer-Knijnenburg, F.J. van Kemenade, L.A. van Es, J.A. Bruijn and J.H. van Krieken, 2001. Endothelial cell chimerism after renal transplantation and vascular rejection. Lancet, 357: 33-337.

Lamm, P., G. Juchem, S. Milz, M .Schuffenhauer and B. Reichart, 2001. Autologous endothelialized vein allograft: A solution in the search for small-caliber grafts in coronary artery bypass graft operations. Circulation, 104: 108-114.

Laufs, U., N. Werner, A. Link, M. Endres, S. Wassmann and K. Jurgens *et al.*, 2004. Physical training increases endothelial progenitor cells, inhibits neointima formation and enhances angiogenesis. Circulation, 109: 220-226.

Leon, L. and H.P. Greisler, 2003. Vascular grafts. Expert Rev. Cardiovasc. Ther., 1: 581-594.

Losordo, D.W., J.M. Isner and L.J. Diaz-Sandoval, 203. Endothelial recovery: The next target in restenosis prevention. Circulation, 107: 2635-2637.

Matsumoto, Y., A. Hof, Y. Baumlin and R.P. Hof, 2003. Differential effect of cyclosporine A and SDZ RAD on neointima formation of carotid allografts in apolipoprotein E-deficient mice. Transplantation, 76: 1166-70.

Matsumoto, Y., T. Uwatoku, K. Oi, K. Abe, T. Hattori, K. Morishige *et al*., 2003. Long-Term Inhibition of Rho-kinase Suppresses Neointimal Formation After Stent Implantation in Porcine Coronary Arteries--Involvement of Multiple Mechanisms. Arterioscler Thromb. Vasc. Biol., 2003.

Numaguchi, Y., K. Naruse, M. Harada, H. Osanai, S. Mokuno and K. Murase *et al*., 1999. Prostacyclin synthase gene transfer accelerates reendothelialization and inhibits neointimal formation in rat carotid arteries after balloon injury. Arterioscler. Thromb. Vasc. Biol., 19: 727-733.

Numata, S., K. Niwaya, T. Fujisato, S. Funamoto, T. Nakatani and T. Yagihara *et al*., 2002. Decellularized allograft valve for tissue engineering: Experimental study of heart valves using decellularized cryopreserved allografts. Heart Surg Forum, 6: 2.

Ohno, N., H. Itoh, T. Ikeda, K. Ueyama, K. Yamahara and K. Doi *et al*., 2002. Accelerated reendothelialization with suppressed thrombogenic property and neointimal hyperplasia of rabbit jugular vein grafts by adenovirus-mediated gene transfer of C-type natriuretic peptide. Circulation, 105: 1623-1626.

Okabe, M., M. Ikawa, K. Kominami, T. Nakanishi and Y. Nishimune, 1997. 'Green mice' as a source of ubiquitous green cells. FEBS. Lett., 407: 313-319.

Otterbein, L.E., B.S. Zuckerbraun, M. Haga, F. Liu, R. Song and A. Usheva *et al*., 2003. Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury. Nat. Med., 9: 183-190.

Peichev, M., A.J. Naiyer, D. Pereira, Z. Zhu, W.J. Lane and M. Williams *et al*., 2000. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood, 95: 952-958.

Poh, M., M. Boyer, A. Solan, S.L. Dahl, D. Pedrotty and S.S. Banik *et al*., 2005. Blood vessels engineered from human cells. Lancet, 365: 2122-2124.

Rafii, S., 2000. Circulating endothelial precursors: Mystery, reality and promise. J. Clin. Invest., 105: 17-19.

Reyes, M., A. Dudek, B. Jahagirdar, L. Koodie, P.H. Marker and C.M. Verfaillie, 2002. Origin of endothelial progenitors in human postnatal bone marrow. J. Clin. Invest. 109: 337-346.

Sarraf, C.E., A.B. Harris, A.D. McCulloch and M. Eastwood, 2003. Heart valve and arterial tissue engineering. Cell. Prolif, 36: 241-54.

Schober, A., S. Knarren, M. Lietz, E.A. Lin and C. Weber, 2003. Crucial role of stromal cell-derived factor-1alpha in neointima formation after vascular injury in apolipoprotein E-deficient mice. Circulation, 108: 2491-2497.

Schulze, P.C., G.W. de Keulenaer, K.A. Kassik, T. Takahashi, Z. Chen and D.I. Simon *et al*., 2003. Biomechanically induced gene iex-1 inhibits vascular smooth muscle cell proliferation and neointima formation. Circ. Res., 93: 1210-1217.

Tateishi-Yuyama, E., H. Matsubara, T. Murohara, U. Ikeda, S. Shintani and H. Masaki *et al*., 2002. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: A pilot study and a randomised controlled trial. Lancet, 360: 427-35.

Torensma, R., 2005. Blood vessels engineered from human cells. Lancet, 366: 892.

Voswinckel, R., T. Ziegelhoeffer, M. Heil, S. Kostin, G. Breier and T. Mehling *et al*., 2003. Circulating vascular progenitor cells do not contribute to compensatory lung growth. Circ. Res., 93: 372-379.

Walter, D.H., K. Rittig, F.H. Bahlmann, R. Kirchmair, M. Silver and T. Murayama *et al*., 2002. Statin therapy accelerates reendothelialization: A novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation, 105: 3017-3024.

Werner, N., J. Priller, U. Laufs, M. Endres, M. Bohm and U. Dirnagl *et al*., 2002. Bone marrow-derived progenitor cells modulate vascular reendothelialization and neointimal formation: Effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition. Arterioscler Thromb. Vasc. Biol., 22: 1567-1572.

Xu, W., H.J. Baelde, E.L. Lagaaij, E. De Heer, L.C. Paul and J.A. Bruijn, 2002. Endothelial cell chimerism after renal transplantation in a rat model. Transplantation, 74: 1316-20.

Ziegelhoeffer, T., B. Fernandez, S. Kostin, M. Heil, R. Voswinckel and A. Helisch, *et al*., 2004. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ. Res., 94: 230-238.