A Central Role of Inducible NO Synthase for Progenitor Cell Recruitment and a New Antiinflammatory Mechanisms of Statins

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Coronary artery bypass grafting (CABG) is performed using autologous vein and arterial grafts. Patency rates of arterial grafts are significantly higher than those of vein grafts. Early vein graft occlusion occurs in up to 12%, potentially attributable to graft thrombosis. The subsequent occlusion rate is 2% to 4% per year, and this problem has been attributed to accelerated vein graft arteriosclerosis, a process preceded by intimal hyperplasia that occurs in the first year after implantation. The neointima that forms consists largely of cells expressing smooth muscle markers which are derived in part from circulating progenitor cells (CPC).

In animal vein graft models, massive endothelial cell apoptosis has been observed during the first weeks after implantation and endothelial cell loss is thought to promote neointimal hyperplasia. Reendothelialization is usually achieved within 4 weeks after implantation, and elegant studies in the mouse indicate that this neo-endothelium consists of former circulating progenitor cells that attach to the arterial lumen and then differentiate into endothelial cells. Despite this hallmark insight, numerous questions remain unsolved. For example; what are the mechanisms guiding CPCs to the vein graft? From which population of cells do the engrafting CPCs derive? What determines whether an adhering CPC acquires a muscle smooth or endothelial phenotype? And finally, what is the significance of CPC endothelial engraftment for the development of vein graft neointima?

In the present issue of Circulation Research, Mayr et al report a central role for the inducible NO synthase (iNOS) in vein graft reendothelialization by CPC in mice. By using a surgically challenging transplant model of vena cava segments implanted into the common carotid artery, the authors observed that deletion of the iNOS gene resulted in enhanced neointima formation, attenuated reendothelialization by CPC, and attenuated production of the vascular endothelial growth factor (VEGF). Inhibition of NO synthases or of the VEGF receptor 2 also resulted in enhanced neointima formation and impaired endothelial repair. Conversely, the application of VEGF in iNOS−/− mice promoted reendothelialization and prevented neointima formation. This demonstrates that the induction of VEGF in the vein graft occurs via a pathway involving NO and that VEGF plays a central role for reendothelialization by CPC.

It is important to point out that, at least in the mouse, it seems that the smooth muscle cells that form the neointima are also differentiated CPC. Thus, it is possible that neointimal smooth muscle cells and endothelial cells derive from the same pool of circulating cells. Therefore the question arises which factor may determine the fate of adhering CPC. Differentiation of CPC into endothelial cells is known to involve VEGF, whereas TGF-β1 and PDGF promote the expression of smooth muscle markers. Certainly of relevance to the work by Mayr et al is the fact that interaction between iNOS and the TGF-β1 and PDGF cascades have been demonstrated. Although both growth factors prevent the induction of iNOS in smooth muscle cells, NO inhibits the synthesis and the actions of the two growth factors on smooth muscle cells. Therefore, it is attractive to speculate that NO, generated from iNOS in the vein graft model is a central fate-determining switch for vasculogenic CPC. Consequently, it might be that the enhanced neointima formation observed in iNOS−/− mice is a consequence of a fate change of the CPC. The observation of Mayr et al that exogenous VEGF prevented the effect of iNOS gene deletion on neointima formation, however, argues that VEGF, and not inhibitory effects of NO on the smooth muscle differentiating system, is the predominant fate-determining factor. Most importantly, the data obtained with VEGF suggest that the inhibitory effect of NO on neointima formation is not a consequence of a direct effect of NO on CPC or smooth muscle cell function (Figure). VEGF, however, has effects above and beyond those on CPCs and endothelial cells; such as the recruitment of “inflammatory cells” to the vascular wall. The contribution of the latter cells in angiogenesis, for example, is so central, that a role for inflammatory cells should also be considered in the vein graft model.

If NO acts primarily to promote reendothelialization, it seems obvious to ask about the role of reendothelialization in the prevention of neointima formation. Given that the neointima largely comprises differentiated CPC, it could be speculated that the restoration of endothelial integrity prevents CPCs homing to tissues. Currently there are more open than solved questions regarding the mechanisms, factors, and conditions that control CPC homing. However, integrins are thought to be central to this process, and this class of adhesion molecules mediates the attachment of CPC to the matrix.
Putative mechanisms leading to the reendothelialization of vein grafts by circulating progenitor cells. EC indicates endothelial cell; CPC, circulating progenitor cell; SMC, smooth muscle cells; iNOS, inducible nitric oxide synthase; NO, nitric oxide; VEGF, vascular endothelial growth factor.

Consequently, reendothelialization would prevent CPC-integrin interactions and should therefore block the process of CPC recruitment to the neointima.

What can be done to accelerate the reendothelialization process by CPC? HMG-CoA–reductase inhibitors (statins) mobilize CPC from the bone marrow,13,14 increase the number of PCPs in blood,15 promote CPC differentiation,16 and accelerate reendothelialization.17 Indeed, pravastatin (albeit at high concentrations) suppresses intima hyperplasia in a rabbit vein graft model.18 HMG-CoA–reductase inhibitors also improve vein graft patency rates in humans, although it is unclear whether this effect is a consequence of the lipid lowering action of the statins, of their pleiotropic effects on CPC, or of the antiinflammatory actions of this class of compounds.19

It is only recently that the antiinflammatory effects of statins have gained more attention.20 Most of these pleiotropic effects are attributed to the inhibition of protein isoprenylation. Consequently, statins affect a very broad range of signaling molecules, in particular small GTPases. In this issue of Circulation Research, Paumelle et al report that in mouse macrophages and neutrophils the antiinflammatory effects of statins are mediated by the peroxisome proliferator-activated receptor-α (PPAR-α).21 Using an in vivo model of carrageenan footpad edema as well as lipopolysaccharide (LPS) and isolated neutrophils the authors determined the effect of PPAR-α on the expression of inflammatory proteins such as iNOS and interleukin-6. Interestingly, by comparing PPAR-α wild-type and knock-out mice the authors discovered that PPAR-α was required to mediate the antiinflammatory effects of statins. Previously, the group had observed that PPAR-α inhibits nuclear factor-κB (NF-κB) by transrepression and that the phosphorylation of PPAR-α prevents this effect.22,23 In the present article, Paumelle et al report that PPAR-α phosphorylation, which is mediated by PKC-α isoforms, is prevented by statins. Thus, statins, by preventing the inhibition of PPAR-α, block signaling via NF-κB.21

The studies by Mayr et al27 and Paumelle et al21 both use mouse model systems. The data clearly advance our understanding of the mechanisms underlying neointima formation and the antiinflammatory action of statins. The next challenge will be to determine whether or not the mechanisms described are operative in man, and are of clinical and pathophysiological importance.

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References


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