Why Don’t Macrophages Leave Atherosclerotic Lesions?

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The Neuroimmune Guidance Cue Netrin-1 Promotes Atherosclerosis by Inhibiting the Emigration of Macrophages From Plaques

Van Gils et al

A recent study proposes a novel role for inhibitory guidance cues in regulating macrophage trafficking during atherosclerosis. The study authors demonstrate that Netrin-1, a laminin-related protein with a previously established role in axon migration and tumorigenesis, contributes to atherosclerosis by preventing the emigration of macrophages from plaque.

Monocyte recruitment to areas of subendothelial lipid deposition has long been identified as a critical early step in the inflammatory pathophysiology of atherosclerosis. Less is known, however, about the signals that mediate both the retention and efflux of macrophages from atherosclerotic lesions. Evidence from prior studies has suggested that lipid accumulation promotes the retention of macrophages in atherosclerotic lesions by disrupting CCR7-dependent chemotaxis, which under normal conditions enhances macrophage efflux from sites of inflammation. Other studies have shown that inhibitory guidance cues mediated by the laminin-related protein Netrin-1 regulate leukocyte migration into infected or ischemic tissues. Netrin has an established role in neural development and axonal migration according to in vitro and in vivo studies (published elsewhere) that the editors of Circulation Research feel are of particular significance to cardiovascular medicine.

The authors found expression of Netrin-1 and the inhibitory receptor UNC5b in lesional macrophages in histological sections of human coronary arteries and also showed upregulation of Netrin-1 and UNC5b mRNA in the aortas of Ldlr<sup>−/−</sup> and Apoe<sup>−/−</sup> mice fed a high-fat diet. Peritoneal macrophage “foam cells” isolated from these mice also expressed higher levels of Netrin-1 and UNC5b, as did macrophages that had been loaded with ox-LDL (but not LDL) in vitro. Furthermore, the authors demonstrated that both recombinant Netrin-1 and Netrin-1 secreted by ox-LDL treated macrophages inhibited the in vitro chemotaxis of macrophages in response to CCL2 and the CCR7-binding chemokines CCL19 and CCL21, which are known to promote macrophage efflux from atherosclerotic plaques. These effects of Netrin were dependent on macrophage UNC5b expression and are consistent with prior findings demonstrating a broad inhibitory effect of Netrin-1 on monocyte, granulocyte, and lymphocyte chemotaxis. By contrast, the authors demonstrated that Netrin-1 promoted the migration of human coronary artery smooth muscle cells, which was consistent with the preferential expression of the activating receptor neogenin but not UNC5b by these cells in vitro and in histological sections of human coronary arteries.

To test the effect of hematopoietic deficiency of Netrin-1 on atherosclerotic disease the authors created chimeric mice by transplanting Ntn1<sup>−/−</sup> or Ntn1<sup>−/−</sup> bone-marrow into irradiated Ldlr<sup>−/−</sup> recipients (wild-type → Ldlr<sup>−/−</sup> and Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup>). Remarkably, after 12 weeks of high-fat diet, Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> mice had a 50% to 60% reduction in both aortic root and en face lesion area relative to wild-type → Ldlr<sup>−/−</sup> mice. Consistent with the in vitro findings above, immunohistochemical analysis of plaques from these animals revealed fewer macrophages and infiltrating smooth muscle cells in Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> animals, which also corresponded to reductions in the extent of lesional apoptosis and necrotic area. To demonstrate that the attenuated phenotype observed in the Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> mice was associated with an effect on macrophage efflux, the authors used a previously described technique that involves the in vivo labeling of blood monocytes with undegradable fluorescently labeled beads which can be enumerated in histological sections of atherosclerotic lesions taken at various time points after the labeling. Although peak numbers of bead-labeled monocytes in plaques were similar between the 2 groups at 3 days following injection of the fluorescent beads, by 14 days the labeled cells were retained in lesions of wild-type → Ldlr<sup>−/−</sup> mice but reduced in Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> mice. These findings suggested that the reduction in lesional macrophages observed in Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> mice resulted from an increase in macrophage efflux from the atherosclerotic plaque, presumably due to the missing inhibitory effects of Netrin-1 on macrophage chemotaxis.

The paper by van Gils et al provides convincing evidence that hematopoietic Netrin-1 expression plays an important role in atherogenesis, potentially by preventing the efflux of macrophage foam cells from atherosclerotic lesions. Although the plausibility of this mechanism for Netrin-1 action during atherosclerosis is certainly supported by the in vitro studies.
studies described above, the relative importance of macrophage efflux versus recruitment in the overall pathophysiology of atherosclerosis remains an open question. Initial evidence that reduced macrophage efflux contributed to atherosclerosis came from studies in which investigators surgically transplanted aortas from Apoe<sup>−/−</sup> mice into Apoe<sup>−/−</sup> or Apoe<sup>+/−</sup> recipients. They then compared the dynamics of leukocyte egress from plaque under normal versus hyperlipidemic conditions, respectively. In those studies, monocyte efflux from atherosclerotic lesions was found to be a feature of regressive plaques in normolipidemic recipients (Apoe<sup>+/−</sup>), but not progressive plaques in hyperlipidemic recipients (Apoe<sup>−/−</sup>). Furthermore, the lymph-node homing chemokine receptor CCR7 and its associated chemokines CCL19 and CCL21 were found to be characteristically upregulated in foam cells in regressive plaques and blockade of CCR7 signaling prevented plaque regression, presumably by inhibiting the egress of these cells from the lesion. However, other studies using a similar methodology to track monocyte influx and egress have suggested that the contribution of increased recruitment predominates in determining the extent of atherosclerotic disease. In one recent paper, Apoe<sup>−/−</sup> mice on high-fat diet were treated with apoE-encoding or control adenoviral vectors to model normolipidemic/regressive versus hyperlipidemic/progressive atherosclerotic lesions. In that study, the authors found that a decrease in lesional monocyte-derived cells was attributable to marked reductions in monocyte recruitment but could not be explained by increased egress or CCR7-dependent mechanisms.

Another interesting question highlighted by the work of van Gils et al relates to the recruitment and egress of different monocyte and macrophage subsets during atherosclerosis. Although the study authors show a decrease in labeled monocyte-derived cells in plaques of Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> mice, it remains uncertain if reduced macrophage efflux fully accounts for the striking phenotype observed in these mice, compared to controls. Apart from the fact that only 3 mice per group were included in the reported monocyte bead-labeling experiment, and that very few beads were detected in the lesions, the labeling technique used may not necessarily reflect the behavior of all monocyte subsets that are recruited into the plaque. For example, the bead-technique the authors used selectively labels only Ly6C<sup>lo</sup> monocytes and the initial labeling-efficiency of this approach was not reported. It is unknown therefore what effect Netrin-1 deficiency would have on the trafficking of Ly6C<sup>hi</sup> monocytes, which have been shown to be preferentially increased in the blood in atherosclerotic mice and possess distinct migration characteristics that allow them to adhere to endothelium and infiltrate atherosclerotic plaque in a more efficient manner. The hypothesis that Netrin-1 promotes atherosclerosis via the retention of plaque macrophages could be further supported by future studies on the effects of expression and/or blockade of Unc5b and Netrin-1 on Ly6C<sup>hi</sup> chemokinesis, and by a demonstration that Ly6C<sup>hi</sup> cells (which can be selectively labeled by beads<sup>14,18</sup>) are reduced in atherosclerotic lesions in Ntn1<sup>−/−</sup> → Ldlr<sup>−/−</sup> bone-marrow chimeras.

In contrast to the proinflammatory function that hematopoietic Netrin-1 appears to exhibit in the setting of atherosclerosis, other evidence suggests a clear antiinflammatory function for Netrin-1 in certain disease contexts. As the authors point out, Netrin-1 is also expressed by endothelial and epithelial cells and can have a negative regulatory role in determining the extent of leukocyte influx during inflammation. This has been particularly apparent in several recent studies of acute kidney injury, wherein Netrin-1 appears to play a protective role in limiting leukocyte influx and apoptosis following ischemia-reperfusion. Consequently, one might expect the net effect of host (nonhematopoietic) deletion of Netrin-1 expression to be more, not less, initial leukocyte recruitment in the setting of chronic atherosclerosis. Unfortunately, global deletion of Ntn1 is lethal due to severe defects in neuronal development and so further interrogation of the contribution of nonhematopoietic Netrin-1 expression would have to be performed in conditional or inducible knock-out models, or in heterozygous mutants.

The context-dependent role of Netrin-1 during inflammation has particular relevance to the translational and therapeutic implications of Netrin-1 signaling in human atherosclerosis. Although the findings of the present study point to pharmacological blockade of Netrin-1 or its receptor Unc5b as a possible therapeutic strategy to promote macrophage egress from plaque and limit atherosclerotic progression, systemic administration of such blockers would likely have pleiotropic and possibly conflicting effects depending on the target cell in question. In light of the above evidence that Netrin-1 plays an important negative regulatory role in leukocyte recruitment in certain models of inflammation, one can imagine a scenario whereby Netrin-1 blockade exacerbates rather than ameliorates inflammatory responses, particularly in the acute setting. Given the clear reduction in atherosclerosis with hematopoietic Netrin-1 deficiency, however, this question certainly warrants further study to determine the net effect of Netrin-1 expression by different cellular sources in the chronic inflammatory milieu of atherosclerotic vascular disease.

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**References**


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