Published in final edited form as:

Future Cardiol. 2012 May ; 8(3): 349–352. doi:10.2217/fca.12.30.

Macrophages, atherosclerosis and the potential of netrin-1 as a novel target for future therapeutic intervention

Kathryn J. Moore* and Edward A. Fisher

Marc and Ruti Bell Vascular Biology and Disease Program, Leon H. Charney Division of Cardiology, Department of Medicine, New York University School of Medicine, New York, NY, USA

Keywords

migration; macrophage; chemotaxis; atherosclerosis

Atherosclerosis is a chronic inflammatory disease that is distinguished by the persistence of cholesterol-laden macrophages in plaques of the arterial wall[1]. Based on observational studies that extend into the era of modern atherosclerosis research (which dates to the introduction in the early 90's of mouse models of the disease), the prevailing view has been that the recruitment of monocytes into atherosclerotic plaques is an unproductive exercise, in which these cells are fated to become macrophage foam cells that persist in this site, establishing chronic inflammation, until eventually dying by either necrosis or apoptosis. Unlike in other tissues, macrophages that accumulate in plaques appear to have a diminished capacity to migrate[2–5], and go from being chemotactic to chemostatic, thereby contributing to a failure to resolve the inflammatory process in arteries set in motion by the retention of atherogenic lipoproteins. Moreover, the impaired efferocytotic ability of these macrophages prevents the clearance of their dying compatriots[6], further contributing to the pro-inflammatory milieu of the plaque and ultimately resulting in the formation of a necrotic core. In such unstable plaques, inflammasome-activating cholesterol crystals[7] and pro-thrombotic tissue factor accumulate in this graveyard of dead macrophages, further adding to the mayhem.

This fatalistic scenario has been occasionally been brought into question. For example, scanning electron micrographs seemed to catch foam cells in the act of leaving porcine plaques by squeezing their way out between luminal endothelial cells[8]. However, the thesis that macrophage accumulation in plaques was reversible gained major traction from studies of atherosclerosis regression using novel mouse models. Using a transplant-based model in which plaque-bearing aortic segments are transferred from a hyperlipidemic apoE-deficient mouse into a normolipidemic wild type (WT) mouse[9], Fisher and Randolph reported that the emigration of myeloid-derived cells into draining lymph nodes and the systemic circulation ensued within days of a plaque being placed into a healthier environment, concurrent with a marked decrease in the content of macrophage foam cells[10]. In a follow-up study, they extended these observations to show that the

*Author for correspondence: Kathryn J Moore, PhD New York University Medical Center 522 First Avenue, Smilow 705 New York, NY 10016 Tel: 212-263-9259 Fax: 212-263-9115 Kathryn.moore@nyumc.org.

Financial and competing interests disclosure The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, patents received or pending or royalties.

macrophage emigration process was dependent in part on the induction of a chemokine receptor CCR7[11], implicating the CCR7-specific ligands CCL19 and CCL21 in promoting the egress of cell from the artery wall. These findings have now been reproduced in another non-surgical model of regression[12], suggesting that the increase in migratory behavior of macrophages is a common component of atherosclerosis regression. In the commentary accompanying the 2004 report[13], it was astutely observed that the results suggested that plaque macrophage content in the progression or the regression phase of the disease was determined not just by recruitment of monocytes and macrophage death, but also by a kinetic balance between monocyte recruitment and emigration of macrophages. Though this kinetic balance would be expected to be tilted towards recruitment in progression and emigration in regression, impairment in the ability to migrate would promote increased macrophage content in either phase.

The historical observations that macrophage egress from the plaque is actively inhibited in the setting of hypercholesterolemia and atherosclerosis progression, coupled with the recent discoveries that this can be reversed in models of atherosclerosis regression, argue that a better understanding of the factors that promote macrophage retention is needed from both basic science and therapeutic perspectives. Although the regulatory signals that impair this process remain largely unknown, recent studies identified one potential candidate as netrin-1, a neuronal guidance cue with unexpected immunomodulatory functions[14,15]. In a recent study in *Nature Immunology*[16], Moore and colleagues showed that netrin-1, a secreted laminin-like molecule, normally expressed during embryonic development to guide the movement of neurons, was secreted by macrophage foam cells in atherosclerotic plaques and inhibits the emigration of these cells by causing dysregulation of the actin cytoskeleton. In the developing nervous system, netrin-1 can mediate either chemorepulsion or chemoattraction of axons, and this context-dependent response is determined by the differential expression of netrin-1 receptors by the target cell[17]. For example, neurons expressing the receptors Deleted in Colon Cancer (DCC) or Neogenin interpret netrin-1 as chemoattractive, whereas cells expressing UNC5b alone, or together with DCC, sense netrin-1 as as chemorepulsive. A similar scenario was uncovered in atherosclerotic plaques, where netrin-1 would block chemokine-induced migration of macrophages expressing Unc5b, while promoting chemoattraction of smooth muscle cells expressing the Neogenin receptor[16]. The combined effect of netrin-1 on these two important cellular constituents of the plaque would be expected to promote the accumulation of these cells in the artery wall and atherosclerosis progression. Based on this suggestive evidence, a direct test of the effects of macrophage-derived netrin-1 on atherosclerosis was undertaken in LDL receptor-deficient mice using a bone marrow transplant approach with either WT or *Ntn1*^{-/-} donor marrow. The absence of netrin-1 expression in macrophages was associated with fewer of these cells in plaques, as well as a reduction in smooth muscle cells, resulting in less complex atherosclerotic lesions. Notably, in agreement with the aortic transplant-based model of atherosclerosis regression, the decreased content of macrophages in *Ntn1*^{-/-}*Ldlr*^{-/-} chimeric mice was accompanied by the emigration of macrophages from the plaques upon removal of this retention signal.

In the plaque, Netrin-1 inhibits chemokine-directed migration of macrophages by disrupting the Rac1 signaling cascade, re-organization of the actin cytoskeleton and cell polarization. However, these effects on macrophage actin dynamics may have implications in atherosclerosis beyond inhibiting macrophage movement out of the plaque. Efferocytosis, the clearance of apoptotic cells by surrounding macrophages, becomes impaired in advanced lesions and ultimately leads to the formation of the necrotic core. Given the significant overlap between the cellular mechanisms involved in efferocytosis and those involved in cell migration (eg., changes in the actin cytoskeleton, formation of lamellipodia, activation of Rho family GTPases and PI-3 Kinases) it is plausible that netrin-1 may also disrupt

macrophage efferocytosis, making the targeted inhibition of these negative guidance cues in the plaque even more appealing.

Based on the results from these mouse models, there appear to be factors that either promote (e.g., CCR7) or retard (e.g., netrin-1) macrophage emigration from plaques, much like the accelerator or brake of an automobile, respectively. Thus, the net movement of macrophages in atherosclerosis will likely be determined by the integration of these opposing forces. Undoubtedly, there are other members of both classes of factors to be discovered, but the general concept is strongly supported by the pre-clinical data. As instructional roles outside of the nervous system are emerging for other neuronal guidance cues, including members of the semaphorin, ephrin and slit family, it is becoming appreciated that these factors may regulate cell migration in a broader context. In fact, several of these molecules have already been shown to guide the migration of cells of the immune system, making them interesting candidates for regulating inflammation and the accumulation of myeloid-derived cells in atherosclerotic plaques. Notably, recent studies by our groups identified semaphorin 3E as a gene differentially expressed in macrophages from progressing versus regressing plaques using microarray analysis (EA Fisher, manuscript submitted). Like netrin-1, we find that semaphorin 3E can inhibit macrophage responses to chemokines implicated in the migration of these inflammatory cells to draining lymph nodes (EA Fisher & KJ Moore, manuscript in preparation). These exciting discoveries suggest that netrin-1 and semaphorin 3E may be just two of a suite of negative guidance factors that promote macrophage retention, and atherosclerosis progression. It is tempting to speculate that certain co-existing morbidities that increase the risk of cardiovascular disease, and remain incompletely responsive to risk factor modification (such as diabetes and chronic renal disease), press more lightly on the accelerator or harder on the brake.

Although there are good pre-clinical data to support the role of netrin-1 in the retention of macrophages in atherosclerosis progression, and to hypothesize a similar effect in regression, there are relatively scant data in people, except for the observation that it is expressed in macrophages in human plaques[16]. On the other hand, the effect of netrin-1 in vitro on macrophage migration was not confined to murine cells, but in fact, it was equally potent in cells of human origin [14,16]. Nonetheless, most of the advances in the therapeutic potential for manipulating netrin-1 expression in atherosclerosis will likely continue to come from the study of pre-clinical models. We have based our scenario on the role of macrophage emigration from plaques on the particular mouse models of atherosclerosis used. Recent data from another mouse model of atherosclerosis regression has shown, however, that the relative roles of recruitment, retention, and cell death may vary somewhat from model to model[18]. Nonetheless, taken together, the studies to date indicate that achieving the optimal balance between monocyte entry and macrophage exit may help to limit the chronic inflammation in the plaque, allowing the resolution phase to begin. Additional studies will be needed to get a more complete view of the general impact of each process on atherosclerosis and to determine how concurrent diseases known to increase cardiovascular risk may in turn alter these processes.

The finding that selective deletion of netrin-1 in bone marrow derived cells markedly reduces atherosclerosis and is associated with macrophage emigration from plaques not only suggests, as noted above, that inhibition of such molecules may have therapeutic value for the treatment of atherosclerosis. However, plaguing almost all therapeutic strategies that would promote either decreased recruitment of monocytes or the increased emigration of macrophages is the potential for interfering with normal immune function in sites other than the plaque. Local delivery systems to block the actions of netrin-1 or its receptor Unc5B in plaques, such as atherosclerosis-directed nanoparticles containing novel small molecules, siRNA[1], anti-sense oligonucleotides, and microRNA mimics or antagomirs[19,20] may

overcome this problem, while limiting plaque progression, and potentially favoring plaque regression. The potential to use such directed therapies to target negative regulators of leukocyte migration to promote the resolution of inflammation offers promise not only for the treatment of atherosclerosis, but potentially other chronic inflammatory disorders in which these guidance cues may become dysregulated. Currently available therapies for coronary artery disease are woefully inadequate, preventing less than half of the heart attacks in intervention trials. In addition to guiding neurons and macrophages, perhaps netrin-1 will also lead investigators to the new directions so clearly needed.

Acknowledgments

This work was supported by the National Institutes of Health (HL100815; HL084312; HL098055).

Bibliography

1. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011; 145(3):341–355. [PubMed: 21529710]
2. Nagao T, Qin C, Grosheva I, Maxfield FR, Pierini LM. Elevated cholesterol levels in the plasma membranes of macrophages inhibit migration by disrupting RhoA regulation. *Arterioscler Thromb Vasc Biol*. 2007; 27(7):1596–1602. [PubMed: 17495238]
3. Park YM, Febbraio M, Silverstein RL. CD36 modulates migration of mouse and human macrophages in response to oxidized LDL and may contribute to macrophage trapping in the arterial intima. *J Clin Invest*. 2009; 119(1):136–145. [PubMed: 19065049]
4. Qin C, Nagao T, Grosheva I, Maxfield FR, Pierini LM. Elevated plasma membrane cholesterol content alters macrophage signaling and function. *Arterioscler Thromb Vasc Biol*. 2006; 26(2):372–378. [PubMed: 16306428]
5. Randolph GJ. Emigration of monocyte-derived cells to lymph nodes during resolution of inflammation and its failure in atherosclerosis. *Curr Opin Lipidol*. 2008; 19(5):462–468. [PubMed: 18769227]
6. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nature reviews. Immunology*. 2010; 10(1):36–46.
7. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010; 464(7293):1357–1361. [PubMed: 20428172]
8. Gerrity RG. The role of the monocyte in atherogenesis: II. Migration of foam cells from atherosclerotic lesions. *Am J Pathol*. 1981; 103(2):191–200. [PubMed: 7234962]
9. Reis ED, Li J, Fayad ZA, et al. Dramatic remodeling of advanced atherosclerotic plaques of the apolipoprotein E-deficient mouse in a novel transplantation model. *Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter*. 2001; 34(3):541–547.
10. Llodra J, Angeli V, Liu J, Trogan E, Fisher EA, Randolph GJ. Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. *Proc Natl Acad Sci U S A*. 2004; 101(32):11779–11784. [PubMed: 15280540]
11. Trogan E, Feig JE, Dogan S, et al. Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc Natl Acad Sci U S A*. 2006; 103(10):3781–3786. [PubMed: 16537455]
12. Feig JE, Parathath S, Rong JX, et al. Reversal of hyperlipidemia with a genetic switch favorably affects the content and inflammatory state of macrophages in atherosclerotic plaques. *Circulation*. 2011; 123(9):989–998. [PubMed: 21339485]
13. Ludewig B, Laman JD. The in and out of monocytes in atherosclerotic plaques: Balancing inflammation through migration. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(32):11529–11530. [PubMed: 15292506]
14. Ly NP, Komatsuzaki K, Fraser IP, et al. Netrin-1 inhibits leukocyte migration in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2005; 102(41):14729–14734. [PubMed: 16203981]

15. Rosenberger P, Schwab JM, Mirakaj V, et al. Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. *Nat Immunol.* 2009; 10(2):195–202. [PubMed: 19122655]
16. van Gils JM, Derby MC, Fernandes LR, et al. The neuroimmune guidance cue netrin-1 promotes atherosclerosis by inhibiting the emigration of macrophages from plaques. *Nature immunology.* 2012; 13(2):136–143. [PubMed: 22231519]
17. Cirulli V, Yebra M. Netrins: beyond the brain. *Nat Rev Mol Cell Biol.* 2007; 8(4):296–306. [PubMed: 17356579]
18. Potteaux S, Gautier EL, Hutchison SB, et al. Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe^{−/−} mice during disease regression. *The Journal of clinical investigation.* 2011; 121(5):2025–2036. [PubMed: 21505265]
19. Rayner KJ, Sheedy FJ, Esau CC, et al. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *The Journal of clinical investigation.* 2011; 121(7):2921–2931. [PubMed: 21646721]
20. Rayner KJ, Esau CC, Hussain FN, et al. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature.* 2011; 478(7369):404–407. [PubMed: 22012398]