Published in final edited form as:
Circ Res. 2014 September 12; 115(7): 605–606. doi:10.1161/CIRCRESAHA.114.304925.

Do vascular smooth muscle cells differentiate to macrophages in atherosclerotic lesions?

Filip K. Swirski and **Matthias Nahrendorf**
Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA.

Keywords

vascular smooth muscle cell; macrophage; atherosclerosis

The middle layer of the healthy vessel wall, the tunica media, contains an abundant population of vascular smooth muscle cells. During atherosclerosis, lipoproteins and cells accumulate in the tunica intima, which is the innermost layer that separates the media from the lumen. It is widely accepted that the dominant cell populating the atherosclerotic intima is the macrophage, a large myeloid leukocyte known for its proficiency at scavenging just about anything from bacteria to apoptotic cells to oxidized lipoproteins. The development of atherosclerosis, the theory goes, is a story of macrophages accumulating in the vessel wall, eating lipoproteins, becoming foam cells, and wreaking inflammatory and metabolic havoc[1].

Monocytes give rise to macrophages – this statement, uttered just few years ago, would have been considered a truism. Today, somewhat dismantled, it is being reimagined. In 1968, while examining the relationship between circulating and “fixed” mononuclear phagocytes, Ralph van Furth and Zanvil Cohn posited that monocytes “migrate randomly from the peripheral blood into the tissues, where they are called macrophages”[2]. This insight has profoundly influenced our thinking; that lesional macrophages likewise derive from circulating monocytes is very much rooted in this tradition. Perhaps then, the recent observations that tissue macrophages in the steady state do not require monocytes were surprising, if not entirely unexpected[3-6]. In the context of atherosclerosis, could it be that we were wrong all along? Are lesional macrophages derived from something other than monocytes?

Enter the vascular smooth muscle cell (VSMC). Unlike other smooth muscle cells, VSMC are thought to be more plastic, capable of acquiring diverse functions in response to environmental cues[7]. In atherosclerosis, VSMC presumably migrate from the media to the intima, where they also ingest lipoproteins. The cohabitation of macrophages and VSMC in the intima has generated many questions as to whether and how these cells communicate and influence each other. Accumulation of numerous VSMC in the human intima[8] can even

Correspondence: F.K.S. (fswirski@mgh.harvard.edu) or M.N. (mnahrendorf@mgh.harvard.edu).

Disclosure Section
The authors declare no conflict of interest.

be seen as evidence that atherosclerosis is predominantly a VSMC rather than a macrophage-driven disease. Among the concepts, arguably the most provocative is the differentiation of VSMC to macrophages[9]. The idea has been difficult to test because expression of VSMC and macrophage markers on tissue sections provides a mere snapshot that is blind to lesional dynamics and cell ontogeny. Development of sophisticated lineage-tracing technologies, however, has allowed us to tackle the problem with renewed confidence.

In this issue of *Circulation Research* Feil et al.[10] suggest that vascular smooth muscle cells transdifferentiate to macrophages in atherosclerotic lesions. The authors used *Apoe*$^{-/-}$ animals expressing tamoxifen-dependent Cre in the SM22α gene locus, along with the ROSA26 Cre reporter allele which can express β-galactosidase upon Cre mediated recombination. By injecting tamoxifen, the authors permanently labeled VSMC because only SM22α^{+} cells expressed Cre recombinase, and thus β-galactosidase. Even if the cells were to lose VSMC characteristics at some later time-point, the irreversible recombination which licensed β-galactosidase activity meant that any progeny would stain blue in tissue after X-Gal administration. After labeling VSMC in young *Apoe*$^{-/-}$ animals, the authors then looked for blue cells in more advanced atherosclerosis. The identification of patches containing blue cells in the intima presumably co-staining with markers of mature macrophages led the authors to conclude that VSMC do in fact differentiate to macrophage-like cells.

Are the data convincing? The blue patches in the aorta are compelling and the co-registration of blue cells with Mac-2 and CD68 in the intima certainly argues in favor of transdifferentiation. However, the conclusions require caution. The flow cytometry data in Online Figure II show GFP^{+} cells in the aorta (for these experiments, the authors used R26R-mT/mG instead of ROSA26 LacZ Cre mice), which are presumably VSMC-derived. The important controls show no GFP^{+} cells in the blood and spleen, and no GFP^{+} monocytes and neutrophils. However, the authors neither quantify nor profile the aortic GFP^{+} cells. Without a more detailed flow cytometric analysis using established markers such CD45, F4/80, CD11b, MHCII, among others, it is difficult to ascertain whether the GFP^{+} cells are even leukocytes, let alone macrophages.

The second issue concerns the blue patches presented in Figure 1 which, to be sure, are stunning. Such an abundant population of blue cells in the intima strongly argues for clonal expansion of VSMC-derived cells. Equally stunning is the observation that the patches are just that: distinct, isolated, and confined to a small region of the aorta. As panels 1E and 1F show, the vast majority of lesions are not blue. Presumably the authors selected the most instructive images, yet the root of the aorta, where abundant macrophages reside, does not contain enough blue cells to stain the plaque. While, to be fair, this could reflect the low efficiency of labeling, which the authors acknowledge, it ironically argues against the existence of VSMC-derived macrophages in the aortic root.

The authors then zoom in on the blue regions and make their most provocative observation: they identify what they believe to be VSMC-derived macrophages. There is no doubt that blue cells inhabit the same region as CD68^{+} and Mac2^{+} cells. But are these blue cells

macrophages? In fact, it appears as if CD68 and Mac2 register with regions of the intima that are largely devoid of blue, and conversely, many blue cells appear to be negative for Mac-2 and CD68. Future confocal microscopy, a technique that can better decipher whether the same cell stains for several markers in tissue, will be needed to answer whether macrophages derive from the VSMC lineage. A related problem is the choice of Mac-2 and CD68. Mac-2 (also known as galectin-3/Lgals3) is highly expressed on myeloid cells of just about every flavor. It is also expressed on different T cells, including γδT cells, as well as various stromal cells. Likewise, CD68 is expressed on the entire myeloid lineage, including neutrophils. Even if the Mac-2^+ and $CD68^+$ cells are blue, are they really macrophages? The fact that blue VSMC-derived cells do not stain for iNOS or Arg-1 again argues against the stated conclusion of the paper.

The criticisms raised above are not meant to invalidate this timely and thought-provoking study, which is important and is sure to spark intense discussion. Rather, our comments are meant to caution against over-interpretation. The authors' fate mapping tools are very elegant and powerful, even if only a fraction of VSMC are labeled. With a more precise and quantitative analysis, the authors may yet show that indeed a significant proportion of VSMC become macrophages that "look" and "act" like macrophages. Beyond the technical issues, significant conceptual roadblocks lie ahead. For example, recent macrophage fate mapping studies, which the authors cite, indicate that plaque macrophages proliferate locally and do not exclusive rely on monocyte input[11]. The authors interpret this as potential evidence in support of their claim. However, the same study also shows that the entire macrophage pool can eventually be replaced by bone marrow-derived cells, which argues that circulating cells are the ultimate source of proliferating macrophages. Many other questions remain. If VSMC give rise to macrophages, then is this fraction distinct? What is its proportion relative to other macrophages? Until we have answers to these questions, we are left with a very interesting and provocative observation that will surely challenge some of our thinking about smooth muscle cell and monocyte/macrophage biology in atherosclerosis.

Acknowledgments

Sources of Funding

This work as supported by NHLBI grants R01HL114477, R01HL117829 (to M.N.), and R01HL095612, R56AI104695 (to F.K.S.).

References

1. Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science. 2013; 339:161–166. [PubMed: 23307733]
2. van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. J Exp Med. 1968; 128:415–435. [PubMed: 5666958]
3. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. Nat Immunol. 2013; 14:986–995. [PubMed: 24048120]
4. Epelman S, Lavine KJ, Randolph GJ. Origin and Functions of Tissue Macrophages. Immunity. 2014; 41(1):21–35. [PubMed: 25035951]
5. Sieweke MH, Allen JE. Beyond stem cells: self-renewal of differentiated macrophages. Science. 2013; 342:1242974. [PubMed: 24264994]

6. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol. 2014; 14:392–404. [PubMed: 24854589]

7. Gomez D, Owens GK. Smooth muscle cell phenotypic switching in atherosclerosis. Cardiovasc Res. 2012; 95:156–164. [PubMed: 22406749]

8. Allahverdian S, Chehroudi AC, McManus BM, Abraham T, Francis GA. Contribution of intimal smooth muscle cells to cholesterol accumulation and macrophage-like cells in human atherosclerosis. Circulation. 2014; 129:1551–1559. [PubMed: 24481950]

9. Rong JX, Shapiro M, Trogan E, Fisher EA. Transdifferentiation of mouse aortic smooth muscle cells to a macrophage-like state after cholesterol loading. Proc Natl Acad Sci U S A. 2003; 100:13531–13536. [PubMed: 14581613]

10. Feil S, Fehrenbacher B, Lukowski R, Essmann F, Schulze-Osthoff, Schaller M, Feil R. Transdifferentiation of Vascular Smooth Muscle Cells to Macrophage-Like Cells druing Atherogenesis. Circ Res. 2014

11. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, Gorbatov R, Sukhova GK, Gerhardt LM, Smyth D, Zavitz CC, Shikatani EA, Parsons M, van Rooijen N, Lin HY, Husain M, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med. 2013; 19:1166–1172. [PubMed: 23933982]