Macrophage Proliferation in Atherosclerosis
An Historical Perspective

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It seems that every decade seminal observations related to the pathogenesis of atherosclerosis are revisited especially when new technology, animal models, or reagents are developed. This is the case with this study by Robbins et al from the Massachusetts General Hospital. These investigators have used a variety of powerful techniques to address the question of what roles macrophage proliferation and monocyte recruitment play in accounting for the numbers of macrophages within the intima of atherosclerotic plaques in mice at different stages of lesion development. This study makes an important new contribution because it provides quantitative evidence that monocyte recruitment followed by differentiation into macrophages predominates at early stages of lesion development in mice, whereas macrophage proliferation is predominant at later stages.

The authors started by continuously infusing the thymidine analogue, bromodeoxyuridine (BrDU) into 4-month-old apolipoprotein E-deficient (ApoE−/−) mice on a high cholesterol diet. After enzyme digestion of the aorta, they measured the BrDU incorporation into macrophages (Lin− CD11b+ CD11c−/low F4/80high cells) by flow cytometry and found that 92% of the macrophages were labeled. They corroborated these findings by also analyzing 4',6-diamidino-2-phenylindole staining for S/G2/M phase cells, H3 histone phosphorylation, and positive immunostaining for another proliferation marker Ki67 in tissue sections. Depletion of circulating monocytes had no effect on BrDU incorporation, suggesting that monocyte recruitment was not playing a role in replenishing the pool of proliferating macrophages in the established lesions. They analyzed this further using the classical technique of parabiosis. They joined the circulations of diet-fed 4-month-old CD45.1+ ApoE−/− mice into 4-month-old CD45.2+ ApoE−/− mice. By 5 months later, the CD45.1+ cells had replenished many of the macrophages within the plaques. Intriguingly, they also provided evidence that the type 1 scavenger receptor A (Msr1) seems to play a role in mediating macrophage proliferation in the mouse lesions. They transplanted irradiated 8-week-old Ldlr−/− (low-density lipoprotein receptor deficient) mice with a mixture of WT CD45.1+ and Msr1−/− CD45.2+ bone marrow cells and after BrdU infusion they compared the proliferation of Msr1+/+ and Msr1−/− macrophages at 26 weeks of age. Surprisingly, there were considerably fewer BrdU+ Msr1−/− macrophages in the lesions.

Macrophage proliferation in atherosclerotic lesions is not a new concept. In 1948, McMillan and Duff reported on mitotic activity in foam cells in atherosclerotic lesions in cholesterol-fed rabbits. In the 1960s, Spraragen et al and McMillan and Star} went on to apply the technique of 3H-thymidine autoradiography to provide more definitive data that cellular proliferation was an active process within the rabbit lesions. Shortly thereafter, thymidine incorporation was further documented in lesions from rabbits, swine, Rhesus monkeys, and humans. In the 1980s, several of us at the University of Washington applied newly developed cell type-specific monoclonal antibodies (RAM-11 and HAM-56) to definitively demonstrate that the labeled cells were both macrophages and smooth muscle cells. In studies of Watanabe Heritable Hyperlipemic (WHHL) and comparably hypercholesterolemic fat-fed rabbits, we combined the techniques of immunocytochemistry and thymidine autoradiography on single sections to identify which cell types were proliferating. We found that 30% of the labeled cells were definitively macrophages. In keeping with the observations of Robbins et al for a possible role for the type A scavenger receptor and lipid accumulation in mediating the macrophage proliferation, we also found that many of the labeled macrophages in the rabbit lesions were foam cells and that lipid accumulation regardless of whether it was from endogenous (WHHL) or exogenous (fat-fed) sources did not compromise macrophage proliferation. However, in contrast to the conclusions of Robbins et al, we observed that the early lesions in the rabbits had more macrophage proliferation (percentage of total cells) than more advanced lesions.

Concurrently, Gordon et al and Katsuda et al simultaneously applied the cell type-specific antibodies along with a monoclonal antibody to the proliferating cell nuclear antigen to demonstrate macrophage proliferation in human coronary and aortic lesions. These results were later corroborated by additional studies. Of particular note was a study by Lutgens et al on autopsy specimens of the descending aorta. These investigators used the proliferation marker Ki67 and the macrophage...
marker CD68 and in keeping with our studies of rabbits they reported that early American Heart Association type II lesions had the highest frequency of macrophage proliferation.

A major limitation of the study by Robbins et al is that it is difficult to extrapolate their observations to human atherosclerosis. Normal human muscular arteries have an intima (often referred to as diffuse intimal thickening) that contains resident macrophages.\textsuperscript{14} Normal mouse arteries do not have an intima. Thus in the mouse, monocyte recruitment leads to the formation of the intima and must predominate at early stages. In the human, it may be that resident macrophages are also induced to proliferate as part of the early inflammatory response and that macrophage proliferation contributes more to expansion of early lesions in humans than it would in mice. In fact, cells in human diffuse intimal thickenings have been shown to express proliferation markers.\textsuperscript{16} Additional unanswered questions are to what degree the macrophage proliferation actually contributes to lesion progression rather than just replenishment of the macrophage population and to what degree the scavenger receptor status regulates macrophage proliferation.

### Disclosures

None.

### References

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