Proliferating Macrophages Populate Established Atherosclerotic Lesions

Andrew J. Murphy, Alan R. Tall

Local proliferation dominates lesional macrophage accumulation in atherosclerosis
Robbins et al

A new study reveals that the majority of macrophages in established atherosclerotic lesions are derived from local proliferation rather than from the influx of blood-borne monocytes. Although the factors driving proliferation remain to be understood, the findings suggest that targeting macrophage proliferation could represent a new therapeutic opportunity in established atherosclerosis.

In recent years, macrophages have garnered the lion’s share of attention as the key cell populating and inciting atherosclerotic lesions. The prevalent view has been that blood monocytes are attracted to susceptible regions of arterial endothelium, migrate into the subendothelial space, take up modified lipoproteins, and become macrophage foam cells; in this model, 1 monocyte gives rise to 1 lesional macrophage. A few older studies provided hints that macrophages could proliferate in lesions1–5; however, for the most part, macrophage proliferation has not been considered to make an important contribution to macrophage burden in atherosclerotic lesions. A recent study by Swirski et al has provided compelling evidence that local proliferation is a major source of macrophages present in advanced atherosclerotic lesions in Apoe−/− and likely Ldlr−/− mice. Although the significance of these observations for human lesions is still uncertain, these novel findings are potentially paradigm-changing and have important implications for understanding the mechanisms of atherosclerotic lesion development and complications.6

In a tour de force of clever experimentation and innovative use of technology, Robbins et al6 established the role of macrophage proliferation in advanced lesions by multiple approaches. They first showed >90% bromodeoxyuridine (BrdU) labeling of macrophages in 4-month-old Apoe−/− mice fed a Western-type diet for 4 to 8 weeks, indicating a high turnover rate of macrophages in established lesions. Previous studies had shown incorporation of BrdU into macrophages in lesions but did not distinguish the point at which BrdU was incorporated because this could have occurred in bone marrow progenitors or in monocytes. Surprisingly, Robbins et al6 found that monocyte depletion by clodronate liposome treatment had no effect either on the macrophage content of advanced lesions or on BrdU incorporation into macrophages. Another interesting observation was that although the atherosclerotic lesion continued to grow, the area of the lesion made up of macrophages remained the same, suggesting that a lot of the macrophages must have died or perhaps migrated out of the lesion.

The most compelling evidence supporting a major role of macrophage proliferation in established lesions came from experiments in which pairs of Western-type diet–fed Apoe−/− mice were joined by parabiosis; in this coupling, 1 mouse was CD45.1+ whereas its partner was CD45.2+. Analysis of the CD45.1+ parabiont revealed the expected high level of chimeraism (ie, mixing of CD45.2+ with CD45.1+ cells) for blood, spleen, and aortic monocytes but, amazingly, <5% chimeraism of aortic macrophages.6 This approach was then combined with BrdU labeling and flow cytometry to show that 87% of lesional macrophages that had incorporated BrdU were derived from the CD45.1+ (host) parabiont, indicating that local proliferation of macrophages was largely responsible for accumulating macrophages in the advanced lesions. A comparable experiment in younger parabiotic mice fed a Western-type diet for 4 weeks showed that only 30% of macrophages in earlier lesions were derived from local proliferation, whereas the rest were derived by monocyte recruitment. Corroborating evidence for these conclusions was obtained by showing that CD45.1+ macrophages were proliferating locally in advanced lesions based on Ki67 antigen positivity and flow cytometry combined with single cell fluorescence imaging to show that there were increased numbers of G2M phase macrophages and increased cells undergoing mitosis. Studies of advanced human atherosclerotic lesions have shown only a low percentage of proliferating cell nuclear antigen-positive proliferating macrophages (<1% of cells).1 However, this represents a snapshot and does not provide any definite information on the quantitative importance of macrophage proliferation in human lesions.

Somewhat surprisingly, an alternative approach of bone marrow transplantation of CD45.1+ Apoe−/− bone marrow into CD45.2+ Apoe−/− mice with established lesions showed that after 3 or 5 months of further diet feeding, 74% and 97%, respectively, of lesional cells were donor-derived. These results contrast with the shorter-term parabiosis experiments and suggest that macrophage proliferation does

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not continue indefinitely and that the macrophage pool of established lesions is ultimately derived from a circulating precursor. Moreover, in Apoe \(^{-/-}\) mice net monocyte recruitment continues to increase as lesions progress throughout the aorta for \(\leq 50\) weeks, underlining the ongoing importance of monocyte recruitment in lesion formation and progression.\(^{7}\) Randolph\(^{8}\) has attempted to reconcile these seemingly paradoxical results by suggesting that most monocyte-derived macrophages may undergo rapid cell death, whereas a small pool continues to proliferate and become the dominant population in established lesions. However, this proliferating pool also seems to die after 1 or 2 months. Overall, the various studies suggest important roles for monocyte recruitment, local macrophage proliferation, and macrophage death in established lesions\(^{6,9,10}\) (Figure).

These studies add to a growing body of literature indicating that macrophages can proliferate locally in tissues; for example, after interleukin-4 treatment or helminth exposure, high numbers of M2 macrophages in tissues seem to be derived by local proliferation.\(^{11}\) The underlying mechanisms responsible for macrophage proliferation in advanced lesions are not well understood. Robbins et al\(^{5}\) ruled out a role for granulocyte macrophage colony-stimulating factor, a cytokine that would have been a leading candidate as a proliferative stimulus, based on previous studies showing it to be responsible for dendritic cell proliferation in the hypercholesterolemic aortic intima.\(^{12}\) They also showed using a parabiosis approach that a wild-type mouse joined to a Western-type diet–fed Apoe \(^{-/-}\) mice did not experience local macrophage proliferation in aorta of the wild-type mouse, suggesting a role of the microenvironment of the lesion. However, in this experiment, apolipoprotein E expression in the wild-type mouse could have lowered plasma cholesterol,\(^{13,15}\) so an alternative interpretation would be that ongoing hypercholesterolemia was required as a stimulant to macrophage proliferation. This is also plausible in view of mounting evidence that cellular cholesterol accumulation enhances hematopoietic cell proliferation in response to a variety of growth factors.\(^{16-18}\) A mixed chimera model using CD45.2\(^{-/-}\)Msr1\(^{-/-}\) and CD45.1\(^{+}\) bone marrow cells transplanted into irradiated Ldlr\(^{-/-}\) mice showed a role of the scavenger receptor A in lesional macrophage proliferation. Earlier work showed a key role of the scavenger receptor A in macrophage proliferation stimulated by oxidized low-density lipoprotein.\(^{19}\) Thus, the continued entry of atherogenic lipoproteins into lesions with uptake of modified particles via scavenger receptor A may provide an ongoing stimulus for macrophage proliferation, perhaps involving growth factors such as macrophage colony-stimulating factor (Figure).

These studies further illustrate the complex life cycle of monocyte/macrophages and their progenitors in mouse models of atherosclerosis. Recent work has demonstrated that hypercholesterolemia and defective cholesterol efflux pathways result in excessive proliferation of hematopoietic stem and progenitor cells in the bone marrow, hematopoietic stem cell mobilization to the spleen, myeloid progenitor, and monocyte proliferation in the spleen, all of which fuel monocytosis and increased entry of inflammatory monocytes into atherosclerotic lesions.\(^{17,18,20,21}\) Macrophage proliferation may be viewed as the final amplification step in this overall process of multistage inflammatory cell expansion.\(^{6,20}\) Robbins et al show that there may be new therapeutic opportunities aimed specifically at reducing macrophage proliferation in advanced plaques, as shown for fluorouracil or potentially other cell-cycle regulators. Improvements in cholesterol homeostasis, for example, via low-density lipoprotein–lowering, liver X receptor activation, or reconstituted high density lipoprotein infusion, could

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**Figure.** Monocyte production, recruitment, and local macrophage proliferation in atherosclerosis. A, Defective cholesterol homeostasis results in bone marrow (BM) hematopoietic stem and multipotential progenitor cells (HSPCs), common myeloid progenitors (CMPs), and granulocyte-macrophage progenitors (GMPs) expansion, and enhanced monocyte production. B, BM HSPCs also mobilize to the spleen, initiating extramedullary hematopoiesis and further increasing monocyte production. C, In atherogenesis, BM and splenic-derived monocytes are recruited to the plaque and differentiate into macrophages. D, As the lesion develops macrophages via scavenger receptor A (SRA) sense–modified low-density lipoprotein (LDL; eg, oxLDL) and this, along with local cytokines, induces macrophage proliferation, which sustains the majority of the population of lesional macrophage population. E, Although there is still recruitment of monocytes to the developed lesion, some of these cells can undergo early apoptosis. F, Macrophages can also undergo late apoptosis or senescence after an unknown number of divisions. (Illustration Credit: Derek Ng.)
also be antiproliferative.\(^1^8\) One potential downside to directly targeting lesional macrophage proliferation may be the yin and yang between cell proliferation and cell death, with potential adverse effects on plaque stability. Overall, this elegant study provides a surprising new view of the multifaceted lesional macrophage and suggests potential novel approaches for the treatment of established atherosclerosis.

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**Disclosures**

None.

**References**

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