Vascular Dendritic Cells as Gatekeepers of Lipid Accumulation Within Nascent Atherosclerotic Plaques

Gwendalyn J. Randolph, Stephane Potteaux

Recent studies, including a series of articles coauthored by Jongstra-Bilen and Cybulsky, have brought fresh and exciting insight to the earliest stages of atherosclerotic plaque formation in mouse models of the disease. Capturing outstanding images of the lesser curvature of the murine aorta, a site highly prone to atherosclerotic plaque development, the Cybulsky laboratory revealed in 2006 that cells bearing markers of dendritic cells (DCs), including CD11c (α, integrin), take up residence just beneath the endothelium in the lesser curvature of mice.1 Possible counterparts of these DCs have also been observed in lesion-prone arterial sites of rabbits and humans.2 The initial appearance of murine DCs in the lesser curvature depends on endothelium activated in response to disturbed blood flow1,3 and is independent of plasma cholesterol status, because it occurs in standard mouse strains that are not hypercholesterolemic (Figure).1 These DCs were recently visualized in the CD11c-YFP mouse and shown to possess capacity for potent antigen presentation to T cells.4 Although they sit primarily beneath the endothelium, vascular DCs occasionally extend projections into the bloodstream (Figure).4 Even in the absence of hypercholesterolemia, the density of DCs within the lesser curvature of mice varies according to strain and positively correlates with the tendency of the strains to develop fulminant atherosclerosis under conditions of hypercholesterolemia.1 Moreover, the density of this population rapidly expands in the lesser curvature of low-density lipoprotein receptor (LDLR)$^{-/-}$ mice fed a high cholesterol diet for only a few days.1,5

CD11c is not an exclusive marker of DCs but can also be found on macrophages.6,7 However, other features set at least some of the vascular DCs apart from macrophages. For instance, the intimal increase of this population in response to cholesterol feeding is substantially affected by local granulocyte/macrophage colony-stimulating factor (GM-CSF)–driven proliferation, rather than by solely building up after recruitment of blood-borne precursors (Figure). In particular, the proliferating DCs appear unrelated in origin to Ly-6C$^hi$ monocytes, thereby distinguishing them from many plaque macrophages, which arise from Ly-6C$^hi$ monocytes.8,9 Thus, the widely held view that monocytes are the earliest immune cell to contribute to and promote the development of atherosclerotic plaques may need revision. Instead, vascular DCs may act earlier than monocytes in the sequence of events that lead to plaque development. Now, in this issue of Circulation Research, Jongstra-Bilen, Cybulsky, and colleagues reveal that vascular DCs have a key role in the earliest accumulation of cholesterol in the aortic wall (Figure).10

Here, the authors continued with their work in the LDLR$^{-/-}$ mouse model, in which feeding of a cholesterol-enriched diet for only a few days leads to neutral lipid deposition in the lesser curvature. First, they show that this lipid resides within CD11c$^+$ cells. Then, to examine functional significance, they used a popular method to deplete CD11c$^+$ cells, using a strain of mice that expresses diphtheria toxin receptor (DTR) under control of the CD11c promoter (CD11c-DTR),11 here interbred with LDLR$^{-/-}$ mice. A single diphtheria toxin (DT) injection removed essentially all subintimal immune cells in these mice for about 1 week, allowing the authors to ask whether lipid would accumulate subintimally if CD11c$^+$ cells were not present. Following DT-mediated depletion, LDLR$^{-/-}$ mice were fed a high-fat diet for 5 days, and they compared lipid accumulation (reasonably assumed to be cholesterol) in the lesser curvature of these mice with that of nondepleted controls. There was a striking 55% reduction in early lipid accumulation in the aortic wall in the absence of CD11c$^+$ cells, and the lipid that did accumulate was found only in extracellular spaces, apparently ignored by circulating monocytes, at least momentarily. Thus, these data argue that vascular DCs importantly regulate the accumulation of lipid in the earliest stages of plaque formation.

We are left pondering the heterogeneity and origin of these vascular DCs. How different are these cells from monocyte-derived macrophage foam cells in development and function? Although at least a portion of vascular DCs do not arise from Ly-6C$^hi$ monocytes,12–14 their overall relationship to the monocyte lineage, widely accepted as precursors of macrophages, is uncertain. One of the important DC markers that the authors identified on the cells was 33D1.5 33D1 is not expressed on all monocytes,15–18 it may indeed be the case that these cells are not within the monocyte lineage. On the other hand, there is evidence that vascular DCs are not the phenotypic equivalents of spleen DCs.4

Even more importantly, are all vascular CD11c$^+$ cells involved in retaining lipid in the artery wall, or is this role...
held only by a subset of the DCs described? In their previous work, the authors reveal heterogeneity among vascular DCs, although this is scarcely discussed in the present study. Thus, 33D1 is a marker of many, but not all, vascular DCs. Another fraction of CD11c+ DCs in nascent plaques are CD11b+ cells that likely arise from Ly-6Chi monocytes. These monocyte-derived cells accumulate in nascent plaques simultaneously with the proliferation of 33D1+ vascular DCs. Apparently, DT treatment affected both of these populations in the CD11c-DTR mouse. In contrast to CD11c, neither the 33D1 nor CD11b marker was demonstrated to colocalize with lipid staining, so it remains unclear whether the possibly more DC-related 33D1+ cells accumulated lipid or whether it was in the CD11b+ cells, or both. The LysCre reporter mouse provides a useful approach for confirming which DC populations are not of monocyte origin and would be useful to apply here. Furthermore, application of CD11b-DTR mice would potentially allow for the removal of CD11b+ DCs, but not 33D1+ DCs, to determine whether these different DC populations have distinct roles with regard to the accumulation of cholesterol in the artery wall. In addition, use of other DTR models besides the CD11c-DTR mouse model, especially DTR models in which no changes in lipid deposition are found, would help to ensure that any spike in TGFβ1 released from DTR-induced apoptosis did not account for the changes in lipid deposition observed.

In summary, the appearance of vascular DCs in lesion-prone areas of arteries before the onset of hypercholesterolemia had already positioned these cells as suspect players in disease initiation. Now, the findings of the present study, together with images of vascular DCs extending projections into the lumen of the aorta, raise the tantalizing question of whether vascular DCs actually capture LDL that may interact

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Figure. Role of dendritic cells in atherosclerotic plaque development and progression. CD11c+ DCs accumulate in the subintima of plaque-prone regions in the arterial tree, even in the absence of hypercholesterolemia in a manner that is partially dependent on vascular cell adhesion molecule 1, setting up a “preplaque sentinel post.” These CD11c+ DCs are comprised of at least 2 populations that can be distinguished based on differential expression of 33D1 and CD11b. 33D1+ DCs proliferate locally in response to GM-CSF. Cholesterol feeding leads to lipid accumulation in vascular DCs that, as the feature article shows, function as a major reservoir for retaining cholesterol in nascent atherosclerotic plaques. This cholesterol accumulation triggers plaque progression. Over time, progressive plaques recruit large numbers of monocytes; some of these are CD11c+ in the mouse, and others are not. How and whether DCs, such as the proliferative 33D1+ DCs, participate in more progressed plaques remains to be explored. Intracellular lipid droplets here depicted as white circles in the cytoplasm of schematized cell types.
with the activated endothelium as it flows past in the circulation, thereby collecting much of the first cholesterol that accumulates in the arterial intima. Because at least some DCs can retain intact protein for long periods after ingesting it,19–21 in contrast to macrophages,20 the idea that DCs are the earliest depot of cholesterol deposition in plaques may not be inconsistent with earlier studies that cholesterol accumulation in nascent plaques of rabbits involves selective retention of “intact” LDL within plaque-prone sites,22,23 even though those studies argued against the idea that the earliest plaque cholesterol was localized within phagocytes. To potentially reconcile the present study with the aforementioned earlier work in rabbits and add to our understanding of the sequence of early events in plaque formation, it will be interesting in future studies to determine whether vascular DCs indeed retain LDL-associated proteins in intact form for rather long periods. It will also be important to understand how this mechanism interfaces with lipoprotein retention mediated by proteoglycans in the artery wall24,25 and whether DC- versus proteoglycan-mediated retention operate cooperatively, possibly even through a positive feedback, to bring on rapid plaque growth or whether they represent independent mechanisms for cholesterol retention in the artery wall. In either case, vascular DCs, likely activated in response to lipid loading, would be in a position to produce a cascade of factors that orchestrate the recruitment of large numbers of monocytes and increased amounts of cholesterol that follow, thereby fueling plaque progression in a way that proteoglycan-mediated cholesterol retention alone may not. We eagerly wait for the next chapter in this series of studies to unfold.

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**Disclosures**

None.

**References**


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