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Monocyte recruitment versus macrophage proliferation in atherosclerosis

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Monocytosis; Macrophages; PKCδ; atherosclerosis

Atherosclerosis, the underlying pathology in most cardiovascular disease, is a chronic inflammatory disease[1], characterized by accumulation of macrophages in the sub-endothelial space. Macrophages are protagonists of the disease, both early in the disease as well as during later stages of atherosclerotic lesion progression, with a continuous turnover of the cells[2]. Blood monocytes enter the artery wall and differentiate into macrophages, contributing to atherosclerotic lesion growth[3], but local proliferation and survival of existing artery wall macrophages also contribute to lesional macrophage plaque burden (Figure)[2].

In this issue of *Circulation Research*, Li and co-workers demonstrate an unexpected role for Protein Kinase C-δ (PKCδ) in monocytes and macrophages in the development of atherosclerosis in a mouse model[4]. PKCδ is part of a group of PKCs that are typically activated by diacylglycerol, and PKCδ is thought to play an important role in the development of insulin resistance and perhaps its complications[5], but its role atherosclerosis is unknown. Some studies[6], but not all[7], have suggested that PKCδ is involved in foam cell formation, a hallmark of atherosclerosis. Li and co-workers demonstrate that PKCδ is induced in monocytes and macrophages in response to dyslipidemia and inflammatory stimuli associated with metabolic disease and atherosclerosis, but that myeloid cell PKCδ does not appear to play any role in macrophage lipid loading *in vivo*[4].

Since PKCδ was induced in myeloid cells in response to dyslipidemia associated with atherosclerosis, one might have hypothesized that deleting this enzyme in myeloid cells would have had a protective effect. On the contrary, selective deletion of PKCδ in myeloid cells, using LysM-Cre-mediated deletion, dramatically increased atherosclerosis[4]. The increased atherosclerosis was associated with an increase in lesional macrophage accumulation, but with no evidence of increased recruitment of monocytes into the lesion. Deletion of PKCδ dramatically reduced circulating levels of more or less all blood leukocytes, including monocytes, neutrophils, B cells and T cells in response to a high-fat challenge, without altering bone marrow progenitor cells. The authors explain the reduction

Conflicts of interest

None

in all circulating cells by arguing that the observed splenomegaly is trapping circulating cells, thus resulting in generalized leukopenia. The reduction in these circulating cells, which by many would have been predictive of a reduced propensity for atherogenesis[8, 9], did not lead to reduced lesion size in the mice with myeloid cell PKCδ-deficiency. Coincidentally, also in this issue of *Circulation Research,* Williams and coworkers[10] demonstrate a reduction in atherosclerosis in mice maintained in thermoneutrality, which they attribute to a reduction in circulating monocyte numbers in response to thermoneutral ambient temperatures (30°C) [10], further highlighting the surprising and important finding by Li and colleagues. Although the animal models that were used were different in the two studies, it is becoming increasingly clear that reduced levels of blood monocytes are not always associated with an athero-protective effect.

So how does myeloid cell PKCδ-deficiency increase atherosclerosis? Robbins and coworkers demonstrated in a hallmark paper in 2013 that local proliferation dominates macrophage accumulation in more advanced atherosclerotic lesions[2]. A similar finding was reported by Lindau et al., who demonstrated that reduced circulating monocyte levels only affected early lesions but not more advanced atherosclerotic lesions[11]. In the present study, Li et al. demonstrated that myeloid cell deficiency in PKCδ indeed increase macrophage proliferation in the atherosclerotic lesion (figure)[4]. The authors could show increases in both BrdU-incorporation and Ki67-staining in PKCδ-deficient macrophages in the atherosclerotic lesion, both which are markers of proliferation. The authors also found a concomitant reduction in macrophage apoptosis. This increase in proliferation and reduction in apoptosis resulted in larger, more macrophage-rich lesions in mice with PKCδ deletion in myeloid cells. The necrotic cores were not larger in the animals with myeloid cell PKCδ-deficiency, further strengthening the authors' conclusion, as dying macrophages greatly contribute to the necrotic core formation[12]. However, one would be curious of what would happen if the lesions were allowed to progress even further, at later time points. Would having increased numbers of surviving, live macrophages aid in the clearance of necrotic debris, thereby counteracting the accelerated atherosclerosis? On a mechanistic level, Li et al. showed, using isolated macrophages, that the lack of PKCδ reduces the cell's sensitivity to apoptotic stimuli by increasing and/or maintaining phosphorylated Akt levels. The authors then went on to demonstrate the this increase in Akt phosphorylation results in a reduction in the pro-apoptotic regulator Bim, via a Foxo3a-dependet mechanism. Finally, Li and coworkers showed that myeloid cell PKCδ-deficiency results in macrophage proliferation in the spleen resulting in splenomegaly. The splenomegaly is an interesting observation. The contribution of splenomegaly to systemic leukocyte levels and atherogenesis could be further tested using for example splenectomized mice.

In summary, Li and colleagues[4] have highlighted the complexity of macrophage biology in atherosclerosis. Not only did deletion of PKCδ not have the anticipated athero-protective effect, but deletion of this kinase in myeloid cells also demonstrated the interplay between recruitment of blood monocytes and local macrophage proliferation and reduced apoptosis. Monocyte recruitment may be a less significant contributor to lesional macrophage accumulation during the later stages of disease, where local macrophage proliferation dominates. However, it is not inconceivable that certain situations are changing the dynamics of monocyte recruitment versus lesional macrophage proliferation and survival, such as

diabetes[13] or myocardial infarction[14], both thought to accelerate atherosclerosis via monocytosis. In both diabetes and myocardial infarction, monocytosis does not appear to be driven by dyslipidemia, but rather by hyperglycemia[13] and activation of the sympathetic nervous system[14], respectively. So, is the relative contribution of these mechanisms different in different animal models? Furthermore, the relevance and contribution of these pathways in human disease remains to be elucidated. Finally, all monocytes and macrophages are not created equally, and will not behave the same way. If we can find ways to reduce their inflammatory potential and stimulate them to take on a more reparative phenotype, the number of monocytes and macrophages might become less relevant.

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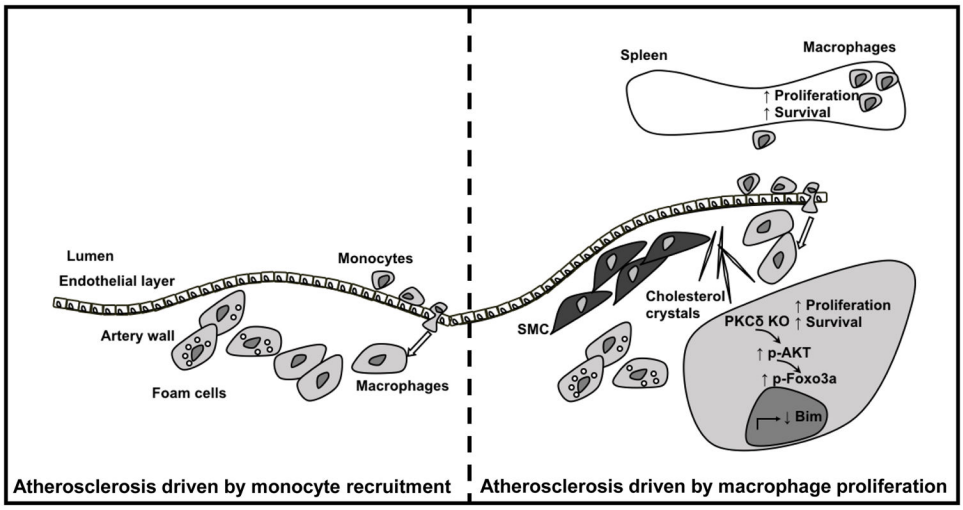

Figure 1.

Macrophages play an important role in all phases of atherosclerosis. Monocyte recruitment is critical during early atherosclerotic disease (and perhaps during certain other situations; left part of figure) and thus at this stage of the disease, blood monocyte levels are an important determining factor. Local macrophage proliferation dominates macrophage accumulation in later stages of atherosclerosis (right part of figure). Macrophage PKCδ is induced by modified lipids that can be found in the plaque. Myeloid cell PKCδ primarily impairs macrophage survival and proliferation, via regulation of phosphorylation of Akt and Foxo3a and ultimately the apoptotic regulator Bim. Deletion of PKCδ results in increased proliferation and survival, both in the artery wall and in the spleen resulting in more atherosclerosis and enlargement of the spleen. SMC-smooth muscle cells.