Cardiovascular diseases mainly caused by atherosclerosis lead death statistics in Western societies. Atherosclerosis, a chronic inflammation of the vessel wall, is most likely initiated by endothelial dysfunction followed by a constant influx of mononuclear immune cells into the intimal layer. The latter fosters growth of atherosclerotic plaques leading to vessel narrowing and occlusion. Rupture of atherosclerotic lesions can cause myocardial infarction and stroke.1 Myeloid cells, especially macrophages and their secretion products, are known to play an important role in the pathophysiology of atherosclerosis; however, some mediators exert multiple roles and might have to be revisited. In this context, Subramanian et al² revealed that the hematopoietic growth factor granulocyte macrophage colony-stimulating factor (GM-CSF; gene name Csf2), to date mostly known for its role in myeloid cell development, plays a crucial role in controlling macrophage apoptosis by induction of interleukin (IL)-23 production (Figure).

Using low-density lipoprotein receptor (Ldlr)−/−Csf2+/+ and Ldlr−/−Csf2−/− mice receiving a Western diet for 12 weeks, the authors evaluated plaque size and composition. Although overall lesion sizes and cellularity did not differ between Ldlr−/−Csf2−/− and Ldlr−/−Csf2+/+ mice, lesional T cells and CD11c MHCII (major histocompatibility complex class II)-positive dendritic cells were significantly lower in number. Similarly, necrotic core areas were smaller in Ldlr−/−Csf2−/− mice. Notably, efferocytosis was not affected, only macrophage and dendritic cell apoptosis were markedly reduced. Furthermore, lesional mRNA expression of interferon-γ, IL-2, IL-17, and IL-23 (mRNA and protein) was diminished, whereas expression of IL-10 and tumor growth factor-β was not altered in the double-knockout mice (Figure). Additional in vitro experiments showed that IL-23, but not IL-17 together with, eg, 7-ketocholesterol or oxidized low-density lipoprotein, renders macrophages apoptotic. Consequently, treatment of Ldlr−/−Csf2−/− mice with IL-23 did restore lesional apoptosis rates similar to those seen in the dendritic cells and macrophages of the control group. Neutralization of the IL-17 activity in the same mice did not alter the results. Mechanistically, the authors further reveal that IL-23 induces the expression of the phosphatase mitogen-activated protein kinase phosphatase-1, resulting in reduced B-cell lymphoma 2 (Bcl-2) phosphorylation, which leads to polyubiquitination and proteasomal degradation of Bcl-2. Reduced Bcl-2 in turn increases the apoptotic susceptibility of macrophages. In addition, IL-23-mitogen-activated protein kinase phosphatase-1 signaling enhances reactive oxygen species formation in cultured macrophages. Hence, lesional mitogen-activated protein kinase phosphatase-1 expression and reactive oxygen species formation were decreased, whereas Bcl-2 expression was increased in atherosclerotic plaques of Ldlr−/−Csf2−/− mice. Again, the latter could be reverted by the injection of IL-23.

Macrophage Apoptosis and GM-CSF/IL-23 Biology in Atherosclerotic Lesions

Inefficent clearance of apoptotic cell debris increases lesion size and fosters a proinflammatory environment. Macrophages play a key role in removing this debris hence apoptotic macrophages do (1) increase the number of cells that have to be cleaned and (2) do not function as proper phagocytes any longer.³ However, mouse studies on the role of macrophage apoptosis reveal conflicting results. Some studies suggest a beneficial role of macrophage apoptosis leading to decreased plaque development,⁴⁻⁵ whereas others show that apoptotic macrophages promote necrotic core formation and thereby lesion growth.⁶⁻⁷ In general, it is now agreed that macrophage apoptosis in early lesion development may limit plaque growth by a general dampening of the inflammation. In contrast, advanced lesions depend on adequate apoptotic cell clearance and, therefore, constant macrophage apoptosis and ineffective cell removal promote plaque growth and vulnerability.⁸⁻¹¹ Undoubtedly, macrophages take over various roles in plaque development, growth, and stability, depending on their polarization state and secretion profile.¹²⁻¹³ The secretion profile of M1 polarized macrophages comprises, eg, IL-23 and other proinflammatory mediators¹³⁻¹⁴, whereas GM-CSF does not only facilitate monocyte to macrophage differentiation but also released by lesional macrophages.²,¹⁵ Yet, mouse studies on the general role of GM-CSF in atherosclerosis have revealed conflicting results. Although Csf2 knockout mice on the apolipoprotein E (ApoE)−/− background displayed an increase in lesion size (aortic root) after 12 weeks of Western diet,¹⁶ injection of GM-CSF into Apoe−/− mice did also enhance plaque formation.¹⁶ Furthermore, Shaposhnik et al¹⁷ showed a decrease in
plaque burden (aortic root) in Ldlr−/− Csf2−/− mice after 3 months of Western diet. In addition, studies modulating the GM-CSF receptor did also reveal inconsistent outcomes.16,18,19 However, the highlighted study and the work by Shaposhnik et al17 point in the same direction depicting a moderate or no reduction in lesion size and no change in the number of lesional macrophages on the Ldlr−/− background, but a more stable plaque phenotype in case of GM-CSF deficiency. Taken together, the above-described mouse models underscore again that changes in systemic lipid profiles may significantly affect the overall immune reaction of a single protein. At that point, one could further speculate that the GM-CSF deficiency in Apoe−/− mice leads to an increase in lesion formation because reduced expression of ATP-binding cassette transporter 1 (as GM-CSF has been reported to induce its expression)15 together with the anyway impaired cholesterol efflux and increased myeloproliferation in case of Apoe knockout drive plaque growth.

IL-23, as part of the IL-12 family, has long been recognized as a signature cytokine driving Th17 immune responses by mediating the maturation of Th17 cells and IL-17 expression. Furthermore, the Th17/IL-23 axis gains importance in various inflammatory conditions and is revealed as being a promising therapeutic target, eg, in psoriasis or Crohn disease.21,22 Although the function of IL-23 has been well described in the context of Th17 immunity,21 there is no such literature on its sole role in atherosclerosis. However, earlier work by Hauer et al23 originally addressing the role of IL-12 in atherosclerotic plaque development in Ldlr−/− mice by initiating the production of anti–IL-12 antibodies (Abs) through a specific protein vaccination technique, already point out that the Abs developing in this context also target IL-23. As a conclusion, the authors strongly assume that the increased plaque stability in their vaccinated Ldlr−/− mice partly depends on IL-23 neutralization. Hence, the presented study revealing a Th17/IL-17–independent role of IL-23 in atherosclerosis supports this finding.

Many mechanisms are discussed to initiate macrophage apoptosis in advanced lesions, eg, cholesterol overload or endoplasmatic reticulum stress inducing the unfolded protein response.3 In this context, earlier work by the authors of the study discussed here showed that Bcl-2 is an important regulator of lesional macrophage apoptosis and that macrophage-specific deletion of Bcl-2 (LysM-Cre Bcl2-flox on Apoe−/− background) resulted in a more vulnerable plaque phenotype.6 In general, Bcl-2 has been implicated as an important therapeutic target for the treatment of atherosclerosis.24 Taking this observation further, Subramanian et al2 now introduce a possible mechanism of Bcl-2 degradation by GM-CSF–induced IL-23 signaling. Simultaneously, IL-23 signaling also increases reactive oxygen species release within these apoptotic lesional macrophages.

Concluding Remarks
Understanding the mechanisms favoring macrophage apoptosis in advanced atherosclerotic lesions may reveal promising therapeutic targets for high-risk patients with cardiovascular. In support of this idea, vulnerable necrotic human plaques display increased macrophage apoptosis.25 Along this road, the discussed study highlights IL-23 as such a player. In view of the already ongoing clinical trials for IL-23–blocking antibodies in other chronic inflammatory diseases, this could be a favorable and soon available therapy for patients with
cardiovascular. However, the study by Subramanian et al leaves some open questions. It would have been interesting, for example, to not only inject IL-23 in Ldlr⁻/⁻ Csf2⁻/⁻ mice but also block IL-23 in the control group. The latter gains exceptional importance thinking of the above-mentioned translation of these findings into humans. Furthermore, one has to dissect carefully which role of different lipid profiles may play in influencing GM-CSF function or vice versa. As apparent from the different GM-CSF knockout mouse models discussed above, and despite the fact that may play in influencing GM-CSF function or vice versa. As has to dissect carefully which role of different lipid profiles to investigate whether the revealed mechanism of GM-CSF–induced and IL-23–dependent macrophage apoptosis holds true in other atherosclerotic mouse models and even more advanced lesions.

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None.

References


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Not Growth but Death: GM-CSF/IL-23 Axis Drives Atherosclerotic Plaque Vulnerability by Enhancing Macrophage and DC Apoptosis
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