Liver X Receptor α-Dependent Iron Handling in M2 Macrophages
The Missing Link Between Cholesterol and Intraplaque Hemorrhage?
Alyssa H. Hasty, Laurent Yvan-Charvet

In this issue of Circulation Research, Bories et al provide evidence of an interplay between cholesterol and iron metabolism, dominated by the nuclear receptor liver X receptor α (LXRα), in M2-like arterial macrophages. These data implicate LXRα as a primary regulator of an exquisitely controlled link between lipid and iron metabolism in macrophages and have important relevance for intraplaque hemorrhage associated with erythropagocytosis in atherosclerotic lesions.

Iron Contributes to Macrophage Diversity in Atherosclerosis
An understanding of the fate and diversity of the various monocyte/macrophage populations is needed to better comprehend the causes of atherosclerotic disease. The first evidence of macrophage diversity in human atherosclerotic plaques was reported in 2007 after identification of M1 and M2 polarized macrophages. Since then, it has become increasingly evident that macrophages display remarkable plasticity and can change their phenotype and function in response to environmental cues (Figure). Therefore, functional, rather than genotypic and phenotypic, characterization of macrophage subsets has emerged in an attempt to better understand plaque morphology and composition. In earlier studies, it was shown that M1 and M2 polarized plaque macrophages are Oil red O–positive, suggesting that both subsets can participate in lipid uptake and become foam cells in vivo. Subsequently, Staels et al identified a population of M2 macrophages in human atherosclerotic plaques that express the mannose receptor (MR). Interestingly, this subset of M2 macrophages demonstrated reduced ability to handle lipids but remained highly competent for phagocytosis. The present study by Bories et al elegantly extends these findings in vivo by showing that iron preferentially accumulates in MR-positive (MR+) M2 polarized macrophages in human atherosclerotic plaques. Interestingly, the authors also observed that these cells were abundant in areas of neovascularization and speculated they could be relevant to intraplaque hemorrhage that may result from the delivery of iron and heme from erythrocytes after microvessel rupture. Given the distinct physical separation of CD68+MR− and CD68+MR+ macrophages in human atherosclerotic plaques in the current report, it will be intriguing to determine whether these macrophages derive from circulating bone marrow–derived monocytes, or whether they are resident macrophages taking on a different phenotype and function upon exposure to the local milieu of lipids and iron.

Comparison of Iron Handling Capacity With Other Macrophage Phenotypes
One of the interesting ideas discussed in the work by Bories et al is the concept that resting, M1, and M2 macrophages all handle iron differently. A small body of literature describing macrophages of a similar phenotype as those reported by Bories et al exists. Boyle et al detected a subpopulation of MR−CD163+ alternative macrophages in areas of hemorrhage in human atherosclerotic plaques, which they refer to as Mhem (for heme-inducible macrophages). In addition, Finn et al have also described a population of M(Hb) macrophages produced by stimulation with hemoglobin–haptoglobin complexes. It is possible that Boyle, Finn, and Bories, and their coworkers are describing the same type of macrophages within the hemorrhagic plaques; however, the in vitro techniques used for mechanistic studies by the 3 groups were different, yielding nuanced differences with regard to the iron/lipid phenotype of the resultant cells. Mhem and M(Hb) macrophages are formed in vitro by uptake of heme and hemoglobin–haptoglobin complexes, respectively, via the scavenger receptor CD163 and their ferric uptake regulator (Fur). In contrast, Bories et al first polarized their macrophages to an M2 phenotype using IL–4 and then assessed iron and lipid metabolism. Thus, although the order in which iron loading versus M2 polarization was induced was different between the 3 studies, the iron handling capacity was similarly enhanced. For instance, Bories et al show that M2 macrophages were able to increase their ferroportin levels and release more iron after iron loading, consistent with earlier studies using in vitro model systems. Interestingly, in contrast to Mhem and M(Hb) cells, the newly identified Cxcl4-induced M4 macrophage subset expresses low amounts of CD163. However, it is not known whether M4 macrophages have altered iron handling or are MR+ or MR− in...
human atherosclerotic plaques. Thus, it will be of interest to further characterize whether other macrophage subpopulations preferentially accumulate iron in human atherosclerotic plaques. Furthermore, as speculated by the authors, a change in the ratio of MR<sup>−</sup>CD163<sup>+</sup> and MR<sup>+</sup>CD163<sup>−</sup> may occur during plaque progression as a result of higher or recurrent exposure to senescent erythrocytes containing iron. Clearly, the study of Bories et al<sup>1</sup> provides an important step in translational atherosclerosis research by functionally characterizing human atherosclerotic plaque M2 polarized macrophages. Furthermore, their earlier work demonstrated the possibility of promising therapeutic opportunities to diagnose and treat human atherosclerosis.

**LXR<sub>α</sub> Dominates Iron Recycling Phenotype in M2 Polarized Macrophages**

The major novelty in the current report is the contribution of the cholesterol-sensing nuclear receptor LXR<sub>α</sub>, which seems to play a central role in controlling Nrf2-dependent iron release in M2 polarized macrophages. Interestingly, LXR<sub>α</sub> was previously reported to dominate the M(Hb) macrophage phenotype,<sup>16</sup> whereas LXR<sub>β</sub> was required for the iron-handling capacity of Mhem macrophage subsets.<sup>15</sup> One important difference between Mhem and M2 macrophages is their level of oxidative stress. The Mhem macrophages described by Boyle et al<sup>14</sup> seemed to have reduced oxidative stress (both in vivo and in vitro), whereas the M2 macrophages loaded with iron had an increased capacity to oxidize LDL. The relevance of these findings remains to be elucidated, but one could speculate that oxidized lipids would induce an alternate newly described oxidized phospholipid macrophage phenotype, Mox. Mox cells are shown to be present in atherosclerotic lesions and relevant to regulation of redox pathways through Nrf2.<sup>20</sup> Taken together, these data strongly suggest a central role of LXR<sub>α</sub> in M2 macrophages and LXR<sub>β</sub> in Mhem macrophages to promote more flexibility with regard to iron handling compared with resting or M1 macrophages. This makes functional sense as a strategy to prevent iron-mediated lipid peroxidation in these atheroprotective macrophage subsets.

**Atheroprotective Potential of LXR<sub>α</sub> in Iron-Enriched M2 Polarized Macrophages**

Traditionally, sequestration of iron by inflammatory M1 macrophages has been reported as a mechanism of defense against bacterial proliferation.<sup>21</sup> In addition, M1 macrophages have been shown to sequester iron in the context of chronic inflammation of venous leg ulcers, leading to unrestrained inflammation and impaired wound healing.<sup>22</sup> However, the current report demonstrates that CD68<sup>−</sup>M2<sup>+</sup> M2 polarized macrophages have high iron storage capacity concomitant with a generally atheroprotective phenotype. Bories et al<sup>1</sup> show that by being more efficient in iron handling, the potential atheroprotective effects of M2 macrophages could be attributed, in part, to an increase in heme oxygenase (HO-1), previously shown to be atheroprotective in macrophages<sup>23</sup> by generation of anti-inflammatory carbon monoxide and antioxidant biliverdin from heme catabolism.<sup>24</sup> However, M2 macrophages are also known to produce IL-10 and other anti-inflammatory cytokines,<sup>4</sup> and the present study raises the question of the relevance of LXR<sub>α</sub> activation by iron loading in mediating these effects. Upregulation of LXR<sub>α</sub> by desmosterol and other oxysterols can induce several anti-inflammatory mechanisms, such as transrepression of NF-κB signaling pathways,<sup>25</sup> induction of its target genes ABCA1 and ABCG1 that have been previously shown to dampen inflammatory responses,<sup>26</sup> or promotion of alternative pathways through arginase-1 regulation.<sup>27</sup> Interestingly, Mhem and M(Hb) macrophages share similar atheroprotective potential despite induction by different signaling pathways such as ATF1/LXRβ or ROS/LXRα.<sup>15,16</sup> The different levels of oxidative stress between these macrophage subsets could reflect the degree of sterol oxidation that provides specific, and anti-inflammatory LXR activators. Comparison of the precise atheroprotective mechanisms of M2, Mhem, and M(Hb) macrophages could
ultimately lead to novel hypothesis to identify prognostic biomarkers for the residual burden of cardiovascular diseases in humans. Likewise, macrophage phenotypic switching in atherosclerotic plaque regression will be crucial in determining macrophage egress and plaque outcomes. The elegant work of Bories et al arms researchers with new perspectives for future studies.

Is LXRα a Novel Emerging Factor to Prevent Iron-Related Diseases?

The human body produces $2.4 \times 10^6$ erythrocytes per second (one-fourth of the cells in the body), with a turnover of dying cells estimated at $1 \times 10^6$ per second requiring tight regulation of this pathway. Macrophages are key players in this regulation by recycling up to 10 g per day of iron and hemoglobin through eryptosis. The idea that M2 macrophages have increased flexibility with regard to iron handling may have relevance for resident macrophages in other tissues—both in homeostatic and pathological conditions. It will be intriguing to learn more about M2 macrophage iron handling in other contexts over the coming years. The physiological role of the cholesterol-sensing nuclear receptor LXRα in M2 resident macrophages controlling the iron lifecycle at the whole body level is also of interest. Recent evidence suggests a requirement for LXRα for apoptotic cell engulfment and marginal zone macrophage differentiation, but not for red pulp macrophages, cells that are critical for phagocytosis of senescent red blood cells and iron recycling. LXR receptors have also been reported to modulate the bone marrow niche through their effect on CD169+ macrophages, cells that have recently emerged as erythroblastic island macrophages maintaining erythropoietic response to stress conditions, such as hemolytic anemia or bleeding in mice. Thus, it remains to be elucidated whether the findings of Bories et al could be extended beyond atherosclerosis, and whether LXRα could prevent iron-related diseases by acting on resident M2-like phenotype with elevated iron-handling capacity.

Clinical Perspectives

Although hypercholesterolemia, hypertension, smoking, and monocytosis are well-established independent risk factors for cardiovascular diseases, both anemia and iron overload have been reported to be associated with cardiovascular events. For instance, iron overload has been associated with increased risk of type 2 diabetes mellitus, gestational diabetes mellitus, central obesity, and metabolic syndrome. By contrast, the accelerated atherosclerosis associated with chronic kidney disease is often associated with anemia. Comparison of atherosclerotic plaques from patients with cardiovascular disease with or without iron overload disease and from patients with chronic kidney disease with or without anemia may thus offer a way to examine red blood cells and iron turnover in relation to macrophage plaque composition ex vivo by immunohistostaining or in vivo by MRI.

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Disclosures

None.

References


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