Cholesterol Efflux
A Novel Regulator of Myelopoiesis and Atherogenesis

Alan R. Tall, Laurent Yvan-Charvet, Marit Westerterp, Andrew J. Murphy

Abstract—Monocytosis and neutrophilia are well-established risk factors for atherosclerosis and seem to play a causative role in lesion development. Studies in mice with defects in cholesterol efflux pathways have identified novel roles for the ATP-binding cassette transporter A1, ATP-binding cassette transporter G1, and apolipoprotein E in suppressing hematopoietic stem cell proliferation, mobilization, and the production of monocytes and neutrophils in the bone marrow. In addition, stem cell mobilization to the spleen initiates extramedullary hemopoiesis, which acts as a monocytic reservoir. Increased monocyte and neutrophil levels drive atherogenesis and its complications. Increasing high-density lipoprotein levels and cholesterol efflux can reverse excessive myelopoiesis and stem cell mobilization, suggesting a novel antiatherogenic effect of some forms of high-density lipoprotein elevation. After a myocardial infarction, splenic Ly-6C\(^{hi}\) monocyte populations are sustained by a second wave of stem cell mobilization from the bone marrow and continue to enter atheroma, accelerating atherogenesis. Because activation of cholesterol efflux pathways can inhibit stem cell proliferation, mobilization, and monocyte production, this may provide a rationale for boosting high-density lipoprotein levels after a myocardial infarction to prevent reocclusion. (Arterioscler Thromb Vasc Biol. 2012;32:2547-2552.)

Key Words: atherosclerosis ■ lipoproteins

Atherosclerosis arises in arteries as a chronic inflammatory response to hypercholesterolemia. Hypercholesterolemia leads to endothelial dysfunction in regions of disturbed arterial flow, causing adhesion of monocytes and their entry into the subendothelial space. In the subendothelium, monocytes differentiate into macrophages, take up oxidized or aggregated low-density lipoprotein that has been locally retained on proteoglycans, and give rise to macrophage foam cells. The secretion of chemokines and cytokines by foam cells and endothelium leads to further waves of monocyte entry and perpetuation of the inflammatory process. Although the macrophage foam cell has been established as having a central role in atherogenesis, there is mounting evidence that the inflammatory process driving the atherogenic response to hypercholesterolemia may be initiated in the hematopoietic organs (ie, the bone marrow [BM] and spleen). Recent findings suggest that hypercholesterolemia and defective cholesterol efflux pathways play a role in enhancing myelopoiesis.

Monocytosis, Neutrophilia, and Atherogenesis
Numerous epidemiological studies have shown that the levels of blood monocytes and neutrophils are predictive of incident heart attack and stroke. In animal models, Gerrity et al first described leukocytosis and increased myeloid colony-forming units in the BM of hypercholesterolemic pigs and rabbits, leading them to suggest that augmented myeloid cell production and, in particular, monocytosis were driving the atherogenic response. Studies in mice have indicated a causal relationship between the levels of blood monocytes and neutrophils and the extent of atherosclerosis. For example, op/op mice with a mutation in macrophage colony-stimulating factor (CSF)-1 showed a gene dosage–related reduction in atherosclerosis that parallels decreases in blood monocyte counts, while pharmacological treatments that increase neutrophil production also cause an increase in early atherosclerotic lesions. Monocytosis develops in Apoe\(^{-/-}\) and, to a lesser extent, in Ldlr\(^{-/-}\) mice in response to feeding a high-fat, high-cholesterol diet. In Western diet–fed Apoe\(^{-/-}\) mice, there is increased entry of the C-C motif chemokine receptor-2\(^{+}\) Ly6-Chi subset of inflammatory monocytes into the vessel wall compared with the Ly6-C\(^{hi}\) subset of monocytes. Combining knockout of 3 major chemokine receptors on monocytes (ie, C-C motif chemokine receptor-2, fractalkine, and RANTES receptors) leads to a progressive reduction in atherosclerosis, in parallel with a stepwise reduction in blood monocyte counts. Although monocytosis in the absence of hyperlipidemia is insufficient to produce atherosclerosis, once the endothelium has become inflamed as a response to hypercholesterolemia, monocytosis and neutrophilia help to drive lesion development.
production of HDL leads to reduced atherosclerosis,25-27 The traditional view is that HDL promotes efflux of cholesterol and oxidized lipids from macrophage foam cells,28,29 reduces inflammatory responses, and interrupts the cycle of monocyte recruitment,30,31 macrophage foam cell formation, and perpetuation of the inflammatory process.32 In addition, recent studies indicate that cholesterol efflux pathways act within hematopoietic stem progenitor cells (HSPCs) to oppose the excessive production of myeloid cells.8,10,33 Several different processes may mediate cellular cholesterol efflux. The ATP-binding cassette transporter (ABC) A1 promotes cholesterol efflux to lipid-poor apolipoprotein (apo) A-I or apoE, whereas ABCG1 mediates cholesterol efflux to HDL particles.28,29,34 In addition, cholesterol exchange between cells and HDL may be mediated by scavenger receptor BI or passive diffusion. ABCA1 and ABCG1 are both targets of liver X receptor transcription factors and have complementary roles in promoting cholesterol efflux from macrophage foam cells. Accordingly, transplantation of BM deficient in Abca1 and Abcg1 into Ldlr−/− mice, followed by feeding a high-fat, high-cholesterol diet, led to accelerated atherosclerosis, compared with mice transplanted with control BM or BM singly deficient in Abca1 or Abcg1.35 Strikingly, mice transplanted with Abca1/g1 knockout BM developed dramatic monocytosis and neutrophilia, hepatosplenomegaly, lymphadenopathy, hypertrophy of intestinal Peyer patches, and infiltration of multiple organs, including the small intestine, liver, and spleen with myeloid cells and macrophage foam cells.8 Analysis of BM by flow cytometry revealed a dramatic expansion and proliferation of the HSPCs, as well as an increase in committed myeloid progenitors resulting from expression of a human APOA-I transgene into mice, this indicates a cell autonomous expansion of the myeloproliferative phenotype, in parallel with a normalization of the numbers and proliferation of HSPCs, as well as the cell surface expression of the common β-subunit.8 This suggested the existence of a backup cholesterol efflux pathway in HSPCs, possibly mediated via scavenger receptor BI, passive diffusion, or an unidentified cholesterol transporter. Overall, these studies established a key role of cholesterol efflux pathways in the control of HSPC and myeloid cell proliferation. The reversal of the myeloproliferative phenotype by the APOA-I transgene also suggested the novel concept that raising HDL might be a treatment for myeloproliferative neoplasms.

ApoE Regulates the Proliferation of Hematopoietic Stem Cells

Although HSPCs deficient in ABCA1 and ABCG1 are expanded in vivo, a subsequent investigation examined the impact of endogenous apoA-I and apoE, possible ligands of these transporters, in controlling the proliferation of HSPCs. This led to the discovery that monocytosis in Apoe−/− mice fed either a chow or Western-type diet developed in parallel to an expansion and proliferation of HSPCs.10 In contrast, chow-fed apoA-I−/− mice did not display monocytosis or proliferation of HSPCs. Notably, apoE was shown to be bound to heparan sulfate proteoglycans on the surface of HSPCs, especially on hematopoietic stem cells. Release of apoE by heparinase treatment led to increased proliferative responses of wild-type HSPCs. However, there was no further increase in the proliferation of Abca1−/−Abcg1−/− HSPCs in response to apoE release, suggesting that apoE may interact with ABCA1/G1 at the surface of HSPCs to mediate cholesterol efflux and control cell proliferation.10 Apoe−/− HSPCs demonstrated increased cell surface levels and signaling of the common β-subunit and modulation of proliferation by cholesterol efflux pathways. Consistent with these findings, competitive BM transplantation studies, carried out by mixing wild-type BM marked by CD45.1 with Apoe−/− BM marked by CD45.2, revealed a relative increase in the numbers of CD45.2/CD45.1 HSPCs and monocytes and increased accumulation of CD45.2 monocytes in atherosclerotic plaques.10 Because both CD45.2 and CD45.1 cells experience the same extracellular environment in recipient mice, this indicates a cell autonomous expansion of Apoe−/− HSPCs and monocytes in blood and lesions, exclusive of exogenous factors such as circulating apoE or levels of chemokines secreted by atherosclerotic vessels, and indicates a causal link among HSPC proliferation, monocytosis, and accumulation of monocytes in plaques, independent of circulating or vessel wall factors. Infusion of pharmacological doses of reconstituted HDL or liver X receptor activator treatment was shown to reduce HSPC proliferation and monocytosis in Apoe−/− mice.10

Extramedullary Hematopoiesis in Spleen Contributes to Atherogenesis and Is Modulated by Cholesterol Efflux Pathways

The development of hepatosplenomegaly in some mouse models of hyperlipidemia and atherosclerosis may, in part, arise from extramedullary hematopoiesis. In this process, organs outside the BM, especially the spleen, are populated by hematopoietic stem cells, allowing expansion of hematopoiesis beyond the limited capacity of the BM. Extramedullary hematopoiesis involves the mobilization of HSPCs from the BM via the blood into the spleen and other organs.6,17 The mobilization of HSPCs occurs in response to decreased activity of local factors that normally maintain and retain stem cells in their BM niche. Extramedullary hematopoiesis is well known to occur during the progression of myeloproliferative neoplasms, such as myeloid leukemias and myelofibrosis, and contributes substantially to morbidity and mortality in these disorders.18 Recent evidence has shown that extramedullary hematopoiesis also occurs in mouse models of hyperlipidemia and atherosclerosis.8-10 Robbins et al19 discovered that the spleen is a major site of extramedullary myelopoiesis in Apoe−/− mice. This study showed that hematopoietic stem cells mobilize to the spleen and begin to produce Ly6-C6
monocytes (Figure 1) that enter atherosclerotic lesions, leading to increased cytokine release, proteolytic activity, and reactive oxygen species production.9

The infiltration of multiple organs with myeloid cells and foam cells is even more prominent in Abca1−/−Abcg1−/− mice compared with Apoe−/− mice and reflects a dramatic mobilization of stem and progenitor cell populations from the BM into the spleen, lung, and liver.8,10 Interestingly, the mechanisms responsible for stem cell mobilization are distinct from the cell-intrinsic effects that are responsible for excessive stem cell proliferation. Studies showed that defects in cholesterol efflux pathways in macrophages and dendritic cells in the sponges of Abca1−/−Abcg1−/− mice led to increased production of IL-23 and activation of a signaling axis involving IL-23/IL-17 and granulocyte-CSF39 (Figure 1). This same signaling axis had been shown by Ley and colleagues to underlie the feedback loop downregulating the production of neutrophils in the BM in response to clearance of apoptotic neutrophils by macrophages.40 Antibody neutralization experiments showed that increased granulocyte-CSF was responsible for the augmented mobilization of HSPCs from the BM of Abca1−/−Abcg1−/− mice.39 Increased abundance of granulocyte-CSF stimulated granulocyte macrophage progenitor cells in the BM to favor the production of neutrophils over monocytes/macrophages. This increased HSPC mobilization reflected decreased levels and activity of the HSPC retention receptor CXCR4 and its ligand CXCL12 (also know as stromal cell-derived factor) on mesenchymal stem cells and osteoblasts. Increasing HDL levels with...
an apoA-I transgene, or reconstituted HDL infusion, led to a reversal of these processes in 3 different models of extramedullary hematopoiesis (ie, Apoe<sup>−/−</sup> mice, Abca1<sup>−/−</sup> Abcg1<sup>−/−</sup> mice, and mice bearing an oncogenic mutation in their BM because of the expression of internal tandem duplications of fms-like tyrosine kinase receptor-3.35] Internal tandem duplications of fms-like tyrosine kinase receptor-3 is the most common mutation causing acute myeloid leukemia in humans.41,42 Increased HDL levels were shown to suppress the production of IL-23 and granulocyte-CSF and to increase levels of CXCL12 in the BM.39 These studies showed that HDL therapy has the potential to reverse extramedullary hematopoiesis relevant to both atherosclerosis and myeloproliferative neoplasms.

Stem Cell Mobilization After Myocardial Infarction Accelerates Atherosclerosis: Possible Modulation by HDL

The above observations may take on extra significance in the light of an exciting series of recent studies showing that in Apoe<sup>−/−</sup> mice the spleen acts as a hematopoietic reservoir, producing Ly6-C<sup>+</sup> inflammatory monocytes.43 After a myocardial infarction (MI), there is rapid recruitment of Ly6-C<sup>+</sup> monocytes from the spleen to the site of injury.43,44 In the setting of hypercholesterolemia, the increased production of Ly6-C<sup>+</sup> monocytes in the spleen also disturbs the resolution of inflammation, hampering the transition toward an anti-inflammatory healing microenvironment and impairs left ventricular function.45,46 After an MI caused by ligation of the coronary arteries in Apoe<sup>−/−</sup> mice, a second wave of HSPC mobilization to the spleen occurs, which in turn drives myelopoiesis and accelerates atherosclerosis47 (Figure 2). The underlying mechanism was shown to be excessive sympathetic nervous system activation, which was blocked by administration of a β<sub>3</sub>-adrenoreceptor blocker. It had been previously shown that sympathetic nerve endings are in intimate contact with Nestin<sup>+</sup> mesenchymal stem cells within the BM niche48 and that sympathetic activation results in decreased expression of stem cell maintenance factors, such as CXCL12, stem cell factor (also known as Kit-ligand), and angiopoietin in mesenchymal stem cells, leading to the mobilization of hematopoietic stem cells in response to stress or diurnal variation.49,50 Reduced expression of stem cell maintenance factors, including Cxcl12, Scl, and Vcam1, was also shown in the setting of acute MI.47 This novel set of observations is likely to be in play in humans because there was an increase in c-kit<sup>+</sup> progenitors cells in the spleens of humans after an MI47 and CD14<sup>+</sup>CD16<sup>+</sup> monocytes are significantly elevated.51,52 Together, these results may partly explain why recurrent atherothrombotic events are so common after an initial heart attack or stroke in humans.

Interestingly, in patients being treated for an acute coronary syndrome (ACS), baseline HDL levels are strongly predictive of recurrent events and death in the ensuing year.53 A small number of infusions of reconstituted HDL particles consisting of apoA-I<sup>Flavo</sup> or apoA-I<sup>WT</sup> combined with phospholipids caused a reduction in atherosclerotic plaque burden when administered to patients after an ACS event.26,27 Furthermore, in a post hoc analysis of the DEFINE study, coronary revascularization procedures were much less frequent in patients treated with the HDL-raising cholesteryl ester transfer protein inhibitor anacetrapib compared with control treatments with background statin therapy alone.54 Although the premature termination of the AIM-HIGH<sup>55</sup> and Dal-OCTIMES<sup>56</sup> study for futility would seem to speak against a beneficial effect of HDL raising after an ACS event, it is possible that more dramatic elevations of endogenous HDL levels or infusions of large amounts of cholesterol-poor reconstituted HDL are needed to produce clinically meaningful results. Although requiring further evaluation in clinical trials, these observations suggest that raising HDL in patients who have ACS may have a particular beneficial role, in part by suppressing myelopoiesis and stem cell mobilization/extramedullary hematopoiesis. HDL also has anti-inflammatory effects on monocytes and can inhibit platelet activation,26,57 supporting the rationale for increasing HDL levels in the setting of ACS.

Summary

To summarize, the elevation of HDL has 2 independent beneficial effects in hematopoiesis. First, activation of cholesterol efflux pathways acts directly within HSPCs to control their proliferation and monocyte and neutrophil production. Second, by suppression of inflammatory responses in macrophages and dendritic cells, cholesterol efflux pathways suppress the mobilization of HSPCs and the ensuing contribution of extramedullary hematopoiesis to plaque growth and development (Figure 2). This may be especially important in the setting of hypercholesterolemia or metabolic syndrome, where excessive production of myeloid cells may lead to monocytosis and accelerated plaque growth, or after an acute ischemic event, where activation of the sympathetic nervous system leads to mobilization of HSPCs. Overall, these observations led us to propose that in addition to having beneficial effects on macrophage foam cells and dysfunctional endothelium in atherosclerotic plaques, intensive therapeutic elevations of HDL may have important beneficial effects on myelopoiesis and accelerated atherosclerotic plaque growth (Figure 2).

Sources of Funding

Drs Murphy and Yvan-Charvet are supported by grants from the American Heart Association (12POST11890019 and SDG2160053). Dr Westerterp is supported by the Netherlands Organization of Scientific Research (VENI 916.11.072).

Disclosures

Dr Tall is a consultant for Merck, Pfizer, Roche, Amgen, CSL, and Arisaph and receives research support from CSL. The other authors have no conflicts to report.

References


Cholesterol Efflux: A Novel Regulator of Myelopoiesis and Atherogenesis
Alan R. Tall, Laurent Yvan-Charvet, Marit Westerterp and Andrew J. Murphy

doi: 10.1161/ATVBAHA.112.300134
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/32/11/2547

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/