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PERSPECTIVES

Cholesterol and Hematopoietic Stem Cells: Inflammatory Mediators of Atherosclerosis

JENNIFER K. LANG, THOMAS R. CIMATO

Clinical and Translational Research Center, Department of Medicine/Cardiology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York, USA

SUMMARY

Atherosclerosis causing heart attack and stroke is the leading cause of death in the modern world. Therapy for end-stage atherosclerotic disease using $CD34^+$ hematopoietic cells has shown promise in human clinical trials, and the in vivo function of hematopoietic and progenitor cells in atherogenesis is becoming apparent. Inflammation plays a central role in the pathogenesis of atherosclerosis. Cholesterol is a modifiable risk factor in atherosclerosis, but in many patients cholesterol levels are only mildly elevated. Those with high cholesterol levels often have elevated circulating monocyte and neutrophil counts. How cholesterol affects inflammatory cell levels was not well understood. Recent findings have provided new insight into the interaction among hematopoietic stem cells, cholesterol, and atherosclerosis. In mice, high cholesterol levels or inactivation of cholesterol efflux transporters have multiple effects on hematopoietic stem cells (HSPCs), including promoting their mobilization into the bloodstream, increasing proliferation, and differentiating HSPCs to the inflammatory monocytes and neutrophils that participate in atherosclerosis. Increased levels of interleukin-23 (IL-23) stimulate IL-17 production, resulting in granulocyte colony-stimulating factor (G-CSF) secretion, which subsequently leads to HSPC release into the bloodstream. Collectively, these findings clearly link elevated cholesterol levels to increased circulating HSPC levels and differentiation to inflammatory cells that participate in atherosclerosis. Seminal questions remain to be answered to understand how cholesterol affects HSPC-mobilizing cytokines and the role they play in atherosclerosis. Translation of findings in animal models to human subjects may include HSPCs as new targets for therapy to prevent or regress atherosclerosis in patients. STEM CELLS TRANSLATIONAL MEDICINE *2014;3:549–552*

THE IMPORTANCE OF ADVANCING THERAPIES TO PREVENT ATHEROSCLEROTIC DISEASE

Modern therapies in medicine have significantly reduced the frequency of heart attacks, strokes, and peripheral vascular disease, and have improved long-term outcomes. Advances have come from simple measures to control the major risk factors that contribute to the risk of cardiovascular disease (CVD) events. These include adequate blood pressure control, smoking cessation programs, and awareness of hypercholesterolemia as a major risk factor for atherosclerotic disease, all of which have resulted in a steady decline in deaths from CVD. Despite the progress attained, heart attack alone is still responsible for 30% of deaths worldwide and is a greater cause of mortality than cancer [1]. For those who survive a heart attack or stroke, the risk of sustaining a second heart attack, stroke, or heart failure is high, resulting in further suffering and greater health care costs. Improved application of current therapies to prevent CVD is a significant objective, and development of a greater understanding of the causes of atherosclerosis and mechanism-based therapies to prevent its progression and recurrence remain an important goal. The next advance in the prevention of atherosclerosis, and its progression, will come from a greater understanding of the chronic inflammatory component of coronary artery disease.

EVIDENCE FOR HEMATOPOIETIC STEM CELLS IN THE PATHOGENESIS OF ATHEROSCLEROSIS

Many years ago, it was recognized that patients with hypercholesterolemia had significant elevations in neutrophil and monocyte numbers in the bloodstream [1], and that higher neutrophil levels were associated with higher risk of heart disease events [2]. The significance of this finding was pursued by several subsequent investigations. Hypercholesterolemia promotes migration of monocytes into atherosclerotic lesions [3]. Diet-induced hypercholesterolemia in mice results in release of monocytes identified by expression of CD11b (mice) or $CD16^-$ $CD14^+$ monocytes (humans) [4] and directly contributes to the progression of atheroma. In animal models of hypercholesterolemia, the quantity of monocytes in the bloodstream positively correlates with atherosclerotic lesion size [5]. Neutrophils and their secreted factors play an important role in the early phases of atherosclerosis [6], and clinical studies correlating systemic neutrophil counts with severity of atherosclerosis in humans support an association of neutrophils with disease progression [7, 8]. These findings suggest a stimulatory effect of cholesterol on hematopoietic stem cells and their progenitors. Although it is known that hematopoietic stem cells circulate throughout the body to provide an immunosurveillance response to inflammatory signals [9], it remained unclear whether cholesterol had a primary effect of mobilizing

Correspondence: Thomas R. Cimato, M.D., Ph.D., State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Department of Medicine/Cardiology, Clinical and Translational Research Center, 875 Ellicott Street/Suite 7030, Buffalo, New York 14203, USA. Telephone: (716) 829-2663; E-Mail: tcimato@buffalo.edu Received November 24, 2013; accepted for publication January 6, 2014; first published online in SCTM *EXPRESS* March 19, 2014. 1066-5099/2014/$20.00/0; http://dx.doi.org/10.5966/sctm.2013-0205

hematopoietic stem cells into the circulation or to promoting localized growth and differentiation within sites of inflammation in atherosclerosis.

The contribution of hematopoietic stem cells (HSCs) to atherosclerosis remained obscure until recently. Robbins et al. determined that the spleen represents a reservoir of hematopoietic precursors that generate monocytes that home to atherosclerotic lesions in the aorta [10]. Dutta et al. later identified the source of the hematopoietic precursors as KSL^+ HSCs [11]. Remarkably, atherosclerosis accelerates after myocardial infarction in $ApoE^{-/-}$ mice with atherosclerotic lesions providing a potent stimulus for release of HSCs from the bone marrow. HSCs then seed the spleen and provide a reservoir of new monocytes that accelerate atheroma formation. The spleen provides a source of IL-3 and granulocyte macrophage colony-stimulating factor (GM-CSF) to promote the proliferation and differentiation of monocyte precursors to monocytes. The findings provide key evidence that in the hypercholesterolemic state KSL^+ HSCs are liberated to bone marrow and reside in the spleen where they contribute to extramedullary monocyte production that contributes to atheroma formation.

Interestingly, Psaltis et al. found that the adventitial layer of the aorta is a source of rare HSCs and a more frequent population of macrophage precursor cell types [12]. In the hypercholesterolemic $ApoE^{-/-}$ mouse model, the numbers of HSCs and colonies of macrophages were significantly higher than those in normal animals, suggesting a role for cholesterol in seeding of HSCs and macrophage precursors to the aorta. Although it is unclear whether the HSCs and macrophage precursors directly participate in atheroma formation, this finding indicates that the state of high cholesterol increases the number of KSL^+ HSCs that reside in peripheral tissues and have a preferential differentiation potential to the myeloid lineage. Collectively, the evidence provided by studies from Dutta et al. [11] and Psaltis et al. [12] indicates that HSCs are mobilized to the spleen and aorta and augment monocytic differentiation. These findings suggest a role for cholesterol in the mobilization and proliferation of HSCs. The findings also indicate that extramedullary myelopoiesis is an important contributor to atherosclerosis.

Regulation of Hematopoietic Stem Cell Proliferation and Mobilization by Cholesterol

Three studies consistently illustrate the principle that cholesterol levels increase the quantity of hematopoietic stem cells in the bloodstream. In wild-type mice fed a high-cholesterol diet for a 30-day period, Gomes et al. showed that elevated cholesterol levels increased the numbers of neutrophils, lymphocytes, and hematopoietic stem and progenitor cells in the bloodstream by increasing proliferation of bone marrow cells and promoting their release from the bone marrow by increasing SDF-1 levels in peripheral blood [13]. In the low-density lipoprotein (LDL) receptor knockout ($LDLR^{-/-}$) mouse model, Feng et al. similarly found that high-fat diet consumption combined with the $LDLR^{-/-}$ background increased total cholesterol levels more than ninefold, and LDL cholesterol levels increased 40 times greater than wild-type animals [14]. The increases in total and LDL cholesterol levels in the bloodstream resulted in a twofold increase in the number of c-kit^+ Sca-1^+ Lin^- (KSL^+) hematopoietic stem cells in the bone marrow and a larger increase in KSL^+ cells in the bloodstream. The investigators further showed that LDL cholesterol promoted proliferation of KSL cells and augmented differentiation to monocytes and granulocytes, whereas high-density lipoprotein cholesterol opposed the effects of LDL cholesterol.

Additional insights into the impact of cholesterol on hematopoietic stem cell biology come from mice with defects in cholesterol efflux pathways due to inactivation of ATP-binding cassette transporters ABCA1 and ABCG1. These two proteins mediate efflux of cellular cholesterol to lipid poor ApoA-1 and high-density lipoprotein cholesterol. $ABCA1^{-/-}$ $ABCG1^{-/-}$ mice therefore represent an important model to understand how cholesterol impacts the biology of hematopoietic stem cells through a mechanism unrelated to supraphysiologic levels of cholesterol or dietary effects. Importantly, $ABCA1^{-/-}$ $ABCG1^{-/-}$ mice show accelerated atherosclerosis in hypercholesterolemic backgrounds and infiltration of various organs with macrophage foam cells [15]. $ABCA1^{-/-}$ $ABCG1^{-/-}$ mice remarkably display leukocytosis and a dramatic greater than twofold expansion of KSL^+ HSCs in the bone marrow. The effects of $ABCA1^{-/-}$ $ABCG1^{-/-}$ on proliferation are due to an increase in membrane cholesterol, resulting in increased cell-surface expression of the common β subunit of the IL-3/GM-CSF receptor leading to increased sensitivity to IL-3 and GM-CSF signals and enhanced proliferation [16]. In conclusion, the experiments from the $ABCA1^{-/-}$ $ABCG1^{-/-}$ null mouse model indicate that intracellular cholesterol accumulation results in cell intrinsic effects to promote HSC proliferation.

Cholesterol Metabolism Intersects the IL-23 IL-17 G-CSF Axis in Hematopoietic Stem Cell Mobilization

The $ABCA1^{-/-}$ $ABCG1^{-/-}$ mouse model provides additional insights into the mobilization of KSL^+ HSCs into the bloodstream. Westerterp et al. found that $ABCA1^{-/-}$ $ABCG1^{-/-}$ knockout animals had threefold higher levels of KSL^+ HSCs in the bloodstream and in the spleen, indicating increased mobilization of HSCs from the bone marrow [17]. Mechanistically, $ABCA1^{-/-}$ $ABCG1^{-/-}$ knockout mice had greater than twofold increased granulocyte colony-stimulating factor (G-CSF) levels in the bloodstream, mediating the mobilization of HSCs from bone marrow. Precisely how G-CSF secretion is regulated by disordered cholesterol metabolism is unclear. G-CSF secretion is induced by IL-17 produced by T cells, and indeed IL-17-neutralizing antibodies decreased G-CSF levels in the blood of $ABCA1^{-/-}$ $ABCG1^{-/-}$ mice, suppressing HSC mobilization. A previously characterized homeostatic mechanism regulating granulopoiesis was described in which macrophages and dendritic cells secrete IL-23 to augment neutrophil production when phagocytic activity of apoptotic neutrophils is reduced because of low neutrophil counts in tissues [18]. IL-23 then acts on T cells to induce production of IL-17, which induces G-CSF secretion and mobilization of HSCs from bone marrow stores. Infusion with recombinant HDL cholesterol reduced serum IL-17 and G-CSF levels, leading to lower levels of HSCs in the bloodstream, and reversed the effects of $ABCA1^{-/-}$ $ABCG1^{-/-}$ knockout. These findings highlight the role of the IL-23 IL-17 G-CSF axis in mobilization of HSCs, and suggest a role for cholesterol in regulation of HSC mobilization. The cholesterol-sensitive component of the cell mobilization pathway may represent a novel approach to therapy in atherosclerosis by reducing cholesterol-induced HSC mobilization.

Links Between Cholesterol Levels and Circulating HSPCs in Humans

Variation in CD34 Levels by Cholesterol, Myocardial Infarction, and Stroke

Although the findings in the mouse models described above are a useful starting point to develop new approaches to prevent and regress atherosclerosis, it is important to note that the degree of hypercholesterolemia found in murine models of atherosclerosis is substantially higher than that found in humans. For example, Feng et al. found that LDLR$^{-/-}$ mice fed a normal diet had total cholesterol levels of 100 $\pm$ 9.6 mg/dl and LDL cholesterol levels 44 $\pm$ 8.4 mg/dl [14]. Upon feeding the mice a high-fat diet, total cholesterol levels increased roughly 5-fold and LDL cholesterol levels 10-fold. In contrast, most human subjects who sustain a myocardial infarction have total cholesterol levels ranging between 200 and 250 mg/dl. Consequently, the degree of cholesterol elevation in the murine model is well above the levels normally found in humans. Therefore, it is important that verifying studies confirm the findings of HSC mobilization and quantity in the bloodstream, and determine whether there is any correlation between cholesterol and HSC levels.

The quantity of CD34$^+$ cells in the bloodstream of humans has been the subject of numerous studies under varying contexts. CD34 expression by itself in humans identifies several cell types including hematopoietic stem and progenitor cells [19], angiogenic cells [20], and endothelial colony-forming cells [21]. The quantity of CD34$^+$ cells in the bloodstream has been used as an assessment of vascular health, as well as an important source of cell therapy for patients with ischemic heart and limb diseases. The largest study to measure the levels of CD34-expressing cells in the bloodstream of humans and determine the clinical and genetic parameters that regulate their numbers was performed by Cohen et al. as part of the Framingham Offspring Study [22]. In that study, 1,786 subjects underwent clinical assessment and determination of CD34 cell levels in the bloodstream by flow cytometry. Correlations between CD34$^+$ cell numbers in the bloodstream and clinical parameters were determined. Positive effects on CD34 numbers after multivariate adjustment included weight, total cholesterol levels, and HMG-CoA reductase inhibitor use, and age, sex, and cigarette smoking correlated with reduced CD34$^+$ cell levels in the bloodstream. Remarkably, the clinical parameters assessed accounted for only 6.3% of the variability in CD34$^+$ cell numbers. CD34$^+$ cell quantity was found to be highly heritable with a high degree of correlation in CD34$^+$ cell numbers between siblings but not between spouses. The study is the only large-scale study of its kind to assess CD34$^+$ cell numbers in the bloodstream, but the study cohort had relatively low cholesterol levels compared with those of subjects with myocardial infarcts and only measured CD34$^+$ cells with no costaining markers.

Several different populations of CD34$^+$ cells in the bloodstream are of clinical relevance in heart disease and tissue repair. Massa et al. determined the quantity of more specific subpopulations of CD34 cell types including CD34$^+$ CD33$^+$ and CD34$^+$ CD38$^+$ hematopoietic progenitors and CD34$^+$ CD133$^+$ VEGFR2$^+$ endothelial progenitor cells in the setting of acute myocardial infarction (AMI) [23]. Of note, the CD133$^+$ VEGFR2$^+$ cells have been identified as a population of angiogenic monocytes [24]. The investigators found no change in the levels of CD34$^+$ CD38$^-$ hematopoietic progenitors after AMI. More differentiated hematopoietic progenitor cell types that were CD34$^+$ and CD33$^+$, CD38$^+$ and CD117$^+$, as well as CD34$^+$ CD133$^+$ VEGFR2$^+$ endothelial progenitor cells (EPCs) increased in quantity 5.8-fold after the onset of symptoms in AMI. Similar increases in blood colony-forming units were noted in the AMI group compared with unaffected control subjects. Interestingly, although the investigators did not report specific cholesterol levels in the cohort, they did report that only 20% of the AMI population had dyslipidemia. Similar increases in CD34$^+$ cell numbers in the bloodstream have been reported in patients with an acute stroke; however, subfractionation of specific CD34$^+$ cell types was not performed to distinguish hematopoietic stem cells, hematopoietic progenitor cells, and EPCs.

A recent study by our group aimed to examine the effects of cholesterol levels on the quantity of CD34$^+$ Lineage$^-$ CD45^{dim} HSPCs in humans using a unique strategy [25]. We modulated the levels of cholesterol in human subjects with no known history of atherosclerosis using three statins of differing potencies for a 2-week treatment duration. The quantity of CD34$^+$ HSPCs was determined after treatment. All three statin treatments reduced the quantity of CD34$^+$ HSPCs, and CD34$^+$ HSPC levels positively correlated with serum LDL cholesterol levels. Mechanistically, LDL cholesterol increases the number of CD34$^+$ HSPCs in the bloodstream by increasing proliferation, and by increasing the levels of mobilizing cytokines IL-17 and G-CSF. The findings provided confirmatory evidence for the mechanism defined by studies in ABCA1$^{-/-}$ ABCG1$^{-/-}$ knockout mice, in which increased IL-17 and G-CSF levels mobilized KSL$^+$ HSCs in mice. How cholesterol levels modulate IL-17 levels remains to be determined but may represent an additional target to reduce activation of inflammatory cells in hypercholesterolemia.

IL-17 is produced primarily by T cells, which are known to contribute to atherosclerosis [26]. Although a mechanistic connection between cholesterol levels and IL-17 remains to be determined, IL-17–producing T_H17 cells and counter-regulatory T_{reg} cells appear to become imbalanced in acute coronary syndromes. A recent study by Ma et al. identified a significant increase in the numbers of IL-17-producing T_H17 cells with corresponding reductions in T_{reg} cell types through an increase in IL-6 levels in patients with acute coronary syndromes [27]. As noted in the studies described above, HSPCs are mobilized in animal models of myocardial infarction and contribute to the progression of atherosclerosis after a heart attack. The IL-17 G-CSF axis appears to stimulate the mobilization of HSPCs in both mice and humans, and IL-17-producing T-cell subsets are augmented in myocardial infarction, representing a potential but unexplored target to block the inflammatory component of atherosclerosis.

Conclusion

The interaction of cholesterol, hematopoietic stem cells, and the innate immune system has remained somewhat unclear until recently. Translation of the connection between cholesterol and HSCs identified in animal models to human subjects represents an important avenue to determine whether the biology of humans is the same and whether antagonism of HSC mobilization and differentiation in response to cholesterol is of benefit for prevention and regression of atherosclerosis.

Author Contributions

J.K.L.: conception and design, manuscript writing, final approval of manuscript; T.R.C.: conception and design, financial support, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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