



Thematic Review Series: Biology of Lipid Rafts

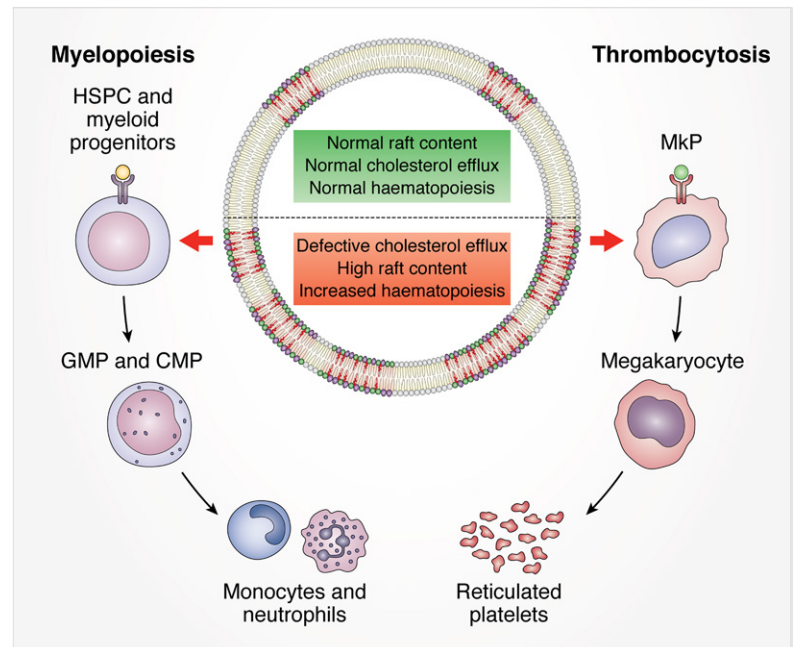
Hematopoiesis is regulated by cholesterol efflux pathways and lipid rafts: connections with cardiovascular diseases

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Abstract Lipid rafts are highly ordered regions of the plasma membrane that are enriched in cholesterol and sphingolipids and play important roles in many cells. In hematopoietic stem and progenitor cells (HSPCs), lipid rafts house receptors critical for normal hematopoiesis. Lipid rafts also can bind and sequester kinases that induce negative feedback pathways to limit proliferative cytokine receptor cycling back to the cell membrane. Modulation of lipid rafts occurs through an array of mechanisms, with optimal cholesterol efflux one of the major regulators. As such, cholesterol homeostasis also regulates hematopoiesis. Increased lipid raft content, which occurs in response to changes in cholesterol efflux in the membrane, can result in prolonged receptor occupancy in the cell membrane and enhanced signaling. In addition, certain diseases, like diabetes, may contribute to lipid raft formation and affect cholesterol retention in rafts. In this review, we explore the role of lipid raft-related mechanisms in hematopoiesis and CVD (specifically, atherosclerosis) and discuss how defective cholesterol efflux pathways in HSPCs contribute to expansion of lipid rafts, thereby promoting myelopoiesis and thrombopoiesis.

We also discuss the utility of cholesterol acceptors in contributing to lipid raft regulation and disruption, and highlight the potential to manipulate these pathways for therapeutic gain in CVD as well as other disorders with aberrant hematopoiesis.—Morgan, P. K., L. Fang, G. I. Lancaster, and A. J. Murphy. Hematopoiesis is regulated by cholesterol efflux pathways and lipid rafts: connections with cardiovascular diseases. *J. Lipid Res.* 2020. 61: 667–675.



Supplementary key words lipid rafts • apoA-I binding protein • hematopoiesis

Lipid rafts are specialized regions of organization within the plasma membrane. Lipid rafts are enriched in cholesterol and sphingolipids, and the structural rigidity of these

Abbreviations: AIBP, apoA-I binding protein; CBS, common β subunit; c-CBL, castias B-lineage lymphoma; CMP, common myeloid progenitor; CSF, colony stimulating factor; EC, endothelial cell; GM-CSF, granulocyte-macrophage-colony stimulating factor; GMP, granulocyte macrophage progenitor; HEC, hemogenic endothelial cell; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; IL, interleukin; MEP, megakaryocytic-erythroid progenitor; TGF, transforming growth factor; TLR, toll-like receptor; WTD, Western type diet.

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lipids underlies lipid raft formation. As a consequence of their specific composition, certain proteins, in particular transmembrane receptors, are more likely to concentrate within the ordered environment that lipid rafts provide. Moreover, upon stimulation, lipid rafts are capable of undergoing alterations to favor protein dimerization, phosphorylation, or cross-linking of receptor modifications to trigger intracellular downstream signaling (1). Given cholesterol's critical importance in raft formation, changes in cholesterol efflux dramatically alter lipid raft abundance and the signaling pathways downstream of receptors contained within lipid rafts. Additionally, exogenous fatty acid production in settings such as obesity and diabetes can also contribute to increased lipid raft abundance to promote inflammation (2). Alterations in cholesterol efflux pathways can induce profound effects in proliferative cells, particularly those of the hematopoietic system. Hematopoiesis describes the production of mature blood cells and accumulating evidence shows that enhanced hematopoiesis occurs as a result of cellular lipid raft accumulation mediated by alterations to cholesterol efflux pathways (3, 4). This review highlights the correlation between defective cholesterol efflux pathways and lipid raft formation on disordered hematopoiesis in relation to CVDs.

OVERVIEW OF HEMATOPOIESIS WITH A FOCUS ON THE MYELOID BRANCH

Hematopoiesis is the process by which all mature blood cells are produced. Hematopoiesis occurs primarily in the bone marrow and proceeds in a hierarchical manner, with hematopoietic stem cells (HSCs) being at the apex of the hematopoietic tree. HSCs have the capability to self-renew or differentiate into all the different lineages of the immune system in addition to platelets and red blood cells. HSCs are subdivided into three major subpopulations: long-term HSCs, short-term HSCs, or multipotent progenitors (which can be further defined) (5–7). These subpopulations of HSCs differ in their self-renewal and differentiation potential/capabilities, which can be immunophenotyped (8). True HSCs predominantly exist in a nonreplicative and quiescent state; however, in the presence of growth factors or hematological stressors, HSCs enter the cell cycle and form lineage-distinct progenitor cells. These progenitor cells then mature into specific lineage cells that later populate the circulation and tissues.

Myelopoiesis is a subdivision of hematopoiesis that specifically relates to the development of myeloid cells, for example, monocytes and neutrophils. During myelopoiesis, HSCs mature to give rise first to common myeloid progenitors (CMPs) that in turn further mature into lineage-committed megakaryocytic-erythroid progenitors (MEPs) or granulocyte macrophage progenitors (GMPs) (9). While MEPs differentiate into megakaryocytes to produce platelets or erythroid progenitors to produce red blood cells, GMPs ultimately give rise to lineage-committed mature myeloid cells (9, 10). Myelopoiesis can also occur in sites other than the bone marrow in a process known as extramedullary hematopoiesis (11). The initiating steps of

extramedullary hematopoiesis involve the mobilization of hematopoietic stem and progenitor cells (HSPCs) from the bone marrow into the circulation where they populate tissues (e.g., liver and spleen) and proliferate/differentiate into lineage-specific cells, eventually establishing a primary site of hematopoiesis (11, 12). Extramedullary hematopoiesis is particularly important in CVDs, such as myocardial infarction and atherosclerosis (11, 13, 14), where it exacerbates the development of atherosclerosis.

MYELOPOIESIS IS ENHANCED IN CVDs

Atherosclerosis is a major underlying cause of CVD. Atherosclerosis is driven by the deposition of cholesterol-rich lipoproteins in the arterial wall and the consequent recruitment of myeloid cells, notably monocytes, which differentiate into macrophages once resident within the artery. Epidemiological studies have demonstrated that leukocytosis is an independent risk factor for CVD (15–18). Importantly, pioneering work over the last decade has revealed that disordered hematopoiesis, specifically enhanced myelopoiesis, is central to the increased monocyte and neutrophil numbers in CVD (3, 4, 19–25). This increased supply of circulating myeloid cells exacerbates the formation of atherosclerotic lesions. In *Apoe*^{-/-} or *Ldlr*^{-/-} atherosclerotic mice, deficiency in macrophage colony-stimulating factor, a key myeloid cell growth factor, reduced monocyte numbers and resulted in smaller atherosclerotic plaques (26–28). There is a broad consensus that hypercholesterolemia enhances atherogenesis by increasing Ly6C^{hi} monocyte entry into atherosclerotic lesions (4, 22, 29–31). Interestingly, monocytes generated in the spleen appear to preferentially migrate to the atherosclerotic plaque (11). This association was also witnessed in other inflammatory diseases that cause accelerated atherogenesis, such as diabetes and rheumatoid arthritis (19, 32). Murine models of human diseases have been pivotal in investigating the molecular processes that underlie the development and progression of CVD. Cytokines mediating intracellular and extracellular pathways have been well-described in many CVD studies, and among their various functions are myeloid cell recruitment, activation, and proliferation.

CYTOKINES INFLUENCE MYELOID CELLS TO PROLIFERATE AND DIFFERENTIATE

By virtue of their diverse range of functions, cytokines are involved in numerous aspects of CVD. In the early stages of atherosclerosis, interferon (IFN)- γ and transforming growth factor (TGF)- β reorganize the cytoskeleton of the extracellular matrix to change the morphology of endothelial cells (ECs), which increases plaque permeability and accumulation of immune cells (33). Moreover, colony stimulating factors (CSFs) and inflammatory cytokines can promote myelopoiesis and inflammatory activation of myeloid cells, respectively, enhancing atherosclerotic plaque progression (34). For example, macrophage-CSF deficiency reduced atherosclerotic lesion size in both

diet-induced hypercholesteremia and *Apoe*^{-/-} atherosclerotic mouse models (26, 27). In addition to atherosclerosis, following a myocardial infarction, sympathetic activity initiates extramedullary hematopoiesis via the canonical inflammatory cytokine, interleukin (IL)-1 β , which drives HSCs toward myelopoiesis (35–37). Finally, we have recently shown that rheumatoid arthritis, an auto-inflammatory condition in which numerous cytokines are chronically elevated, is associated with disordered hematopoiesis and enhanced myelopoiesis through altered cholesterol metabolism, ultimately exacerbating atherosclerosis (19).

CYTOKINE RECEPTORS LOCALIZED IN LIPID RAFTS ENHANCE MYELOPOIESIS

Lipid rafts are highly dynamic assemblies of proteins, cholesterol, and sphingolipids found within the bilayer of cellular membranes. An important function of lipid rafts is serving as a platform for cytokine and pattern recognition receptors [e.g., toll-like receptors (TLRs) and growth factor receptors]. The embedding of these receptors within lipid rafts facilitates the activation and, consequently, efficient downstream signaling. For instance, the TGF- β 1 receptor is localized in lipid rafts of HSCs. Expansion and clustering of lipid rafts following a high-fat diet was reported to enhance dysregulation of the TGF- β 1 receptor in lipid rafts (38), stimulating downregulation of TGF- β 1-induced HSC quiescence and, subsequently, promotion of hematopoiesis. Importantly, receptors for granulocyte-macrophage-CSF (GM-CSF) and IL-3, key effectors of myelopoiesis, are located within lipid rafts. GM-CSF mediates myeloid lineage specification of CMPs to monocytes and neutrophils, while IL-3 promotes proliferation and differentiation (39, 40). GM-CSF and IL-3 bind to a heterodimeric receptor complex consisting of a cytokine-specific α subunit and a common β subunit (CBS), which is shared by the receptors for GM-CSF, IL-3, and IL-5. Both α and β receptor subunits for GM-CSF and IL-3 are highly expressed on HSPCs, with the CBS in particular playing a critical role in hematopoiesis. CBS-deficient hematopoietic cells transplanted into *Apoe*^{-/-} recipient mice displayed a decrease in peripheral neutrophil and monocyte counts (41). Further investigation also revealed that depletion of CBS decreased the macrophage content of atherosclerotic lesions in *Apoe*^{-/-} mice (41), suggesting that lipid raft-located CBS is essential for hypercholesterolemia-driven myelopoiesis in both the spleen and bone marrow.

DEFECTIVE CELLULAR CHOLESTEROL EFFLUX EXPANDS LIPID RAFTS TO PROMOTE MYELOPOIESIS

It is well-established that cellular cholesterol homeostasis is an important factor in the regulation of hematopoiesis; specifically, increasing membrane cholesterol levels favors HSPC proliferation and mobilization, which increases the risk of atherosclerosis (21, 42). Specifically, we have

previously shown that either *Apoe*^{-/-} or *Ldlr*^{-/-} mice fed a Western type diet (WTD) had a significant increase in their populations of bone marrow HSPCs, CMPs, and GMPs, leading to increased blood leukocytes (4).

The abundance and functionality of lipid rafts is influenced by changes in cholesterol levels and also by changes in sphingolipid content. For example, in macrophages, cellular cholesterol depletion via extrinsic agents such as β -cyclodextrin disrupted lipid raft formation and stability (43, 44). Alternatively, increase in lipid rafts has inflammatory consequences (45, 46). In vivo, cellular cholesterol and lipid raft content are highly modulated by cellular cholesterol efflux pathways (47), several of which exist within hematopoietic cells (3, 48, 49). In particular, the active transport of cholesterol by ABC transporters that facilitate cholesterol efflux from the cytoplasm of the cell to exogenous apolipoproteins within lipoproteins are major regulators of cellular cholesterol and lipid raft content. With the strong association between CVD and impaired cholesterol efflux pathways, ABC transporters located in lipid rafts have been shown to play a critical role in cellular cholesterol efflux pathways in HSPCs (3, 50). Genetic analysis of bone marrow HSPCs revealed high expression of *Abca1*, *Abcg1*, and *Apoe*, which was enhanced in the different progenitor cell subsets and HSPCs with the in vivo administration of LXR agonists (4). Importantly, genetic deficiencies or loss of function of ABC transporters increases the formation of lipid rafts within HSPCs, leading to a myeloproliferative phenotype **Fig. 1** (3). We have previously shown that defective cholesterol efflux via deletion of ABC transporters in HSPCs and/or their downstream myeloid cells through a number of pathways enhances inflammation in a number of tissues, while also causing HSPC proliferation, mobilization, and extramedullary hematopoiesis (3, 42, 51, 52). In *Ldlr*^{-/-} mice, bone marrow deletion of *Abcg4* in MEPs resulted in prominent platelet production and progression of atherosclerosis and thrombosis (50). Likewise, *Abca1* and *Abcg1* double knockout in macrophages or dendritic cells resulted in granulocyte-colony stimulating factor-dependent HSPC mobilization, which also likely contributes to accelerated atherogenesis (8, 42). Given the importance of ABC transporters in HSPC maintenance, the following sections highlight the role of lipid raft-resident ABC transporters on myelopoiesis and CVDs.

ABC TRANSPORTERS AND THEIR ROLE IN LIPID RAFT-MEDITATED MYELOPOIESIS

ABCA1

Early studies identified the functions of ABCA1 in cell processes such as apoptosis and inflammation (53, 54). A major advancement in the understanding of ABCA1 function was as a consequence of elucidating the basis of Tangier disease, a rare inherited disorder. In humans, mutations in the *Abca1* gene result in familial HDL deficiency in which individuals have low HDL levels and

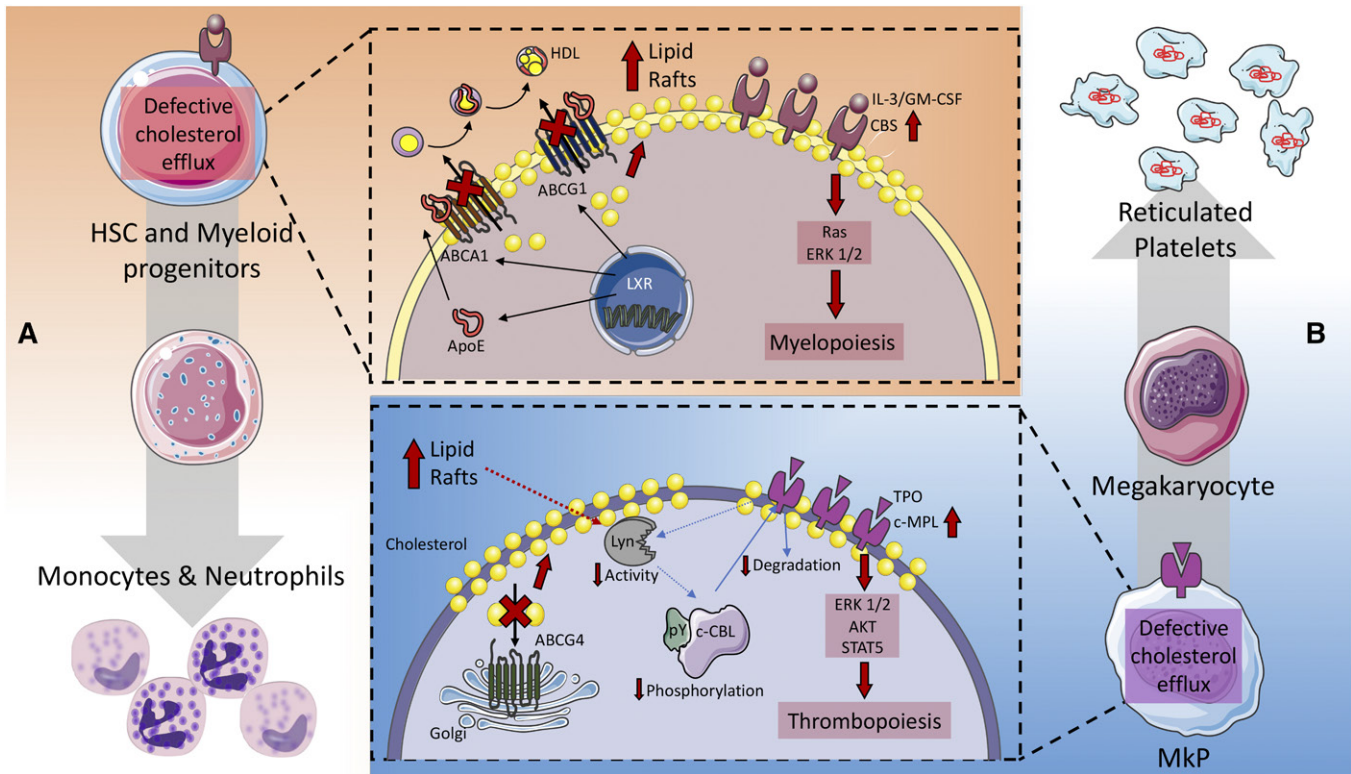


Fig. 1. Defective cholesterol efflux via the loss of ABC transporters increases lipid raft formation to trigger myelopoiesis. **A:** Co-deletion of ABCA1 and ABCG1 in HSC and myeloid progenitors results in defective cholesterol efflux and increase in lipid rafts of the plasma membrane. This increases CBS expression on lipid rafts and amplifies GM-CSF/IL-3-induced myelopoiesis and monocyte and neutrophil production. **B:** Depletion of *trans*-Golgi-localized ABCG4 in megakaryocyte progenitors (MkP) results in defective cholesterol efflux and expansion of membrane lipid rafts. This causes increased expression of the TPO receptor, c-MPL, on MkPs, thus amplifying TPO-mediated megakaryopoiesis and platelet formation. In addition, the formation of lipid rafts indirectly diminishes Lyn-kinase activity, thus downregulating the phosphorylation of c-CBL and the degradation of c-MPL, to further enhance TPO-induced myelopoiesis.

apoA-I (55–57). The discovery of the mechanism behind Tangier disease revealed the critical role played by ABCA1 in HDL synthesis via the lipidation of apoA-I (58). In addition to low HDL and apoA-I levels, patients were characterized with elevated cholesterol accumulation in macrophages and tissues, which made patients at a moderately high risk of CVD (59). A similar phenotype has been replicated in mice deficient in ABCA1 (60, 61). Conversely, the overexpression of ABCA1 increased plasma HDL and apoA-I expression in mice (62). A significant pathological finding was identified by van Eck et al. (63), where hematopoietic deletion of *Abca1* in *Ldlr*^{-/-} mice resulted in significantly advanced atherosclerotic lesions and infiltration of macrophages in major organs like the liver and spleen. Conversely, transgenic overexpression of ABCA1 specifically in macrophages led to a 65% reduction in aortic atherosclerosis compared with WT mice (64). An anti-inflammatory role of ABCA1 mediated by lipid rafts was identified in many studies (49, 65–67). Specifically, the loss of ABCA1 in macrophages impaired cholesterol efflux, resulting in the deposition of cholesterol to lipid rafts. These effects led to an increased release of inflammatory cytokines, such as TNF α , following treatment with LPS. Interestingly, another study showed that the increase in TNF α production induced ABCA1 expression through the NF- κ B signaling

pathway as a feedback mechanism to disperse lipid raft formation (68).

ABCG1

ABCG1 is a critical mediator of cholesterol efflux to mature HDL. Several human and murine studies demonstrate that, unlike ABCA1, which can efflux cholesterol to lipid-poor HDL and lipid-free apoA-I, ABCG1 promotes cholesterol efflux solely to mature HDL (69, 70). As anticipated, a high-cholesterol diet and targeted disruption of ABCG1 led to cellular lipid accumulation in tissue macrophages, while ABCG1 overexpression protected against lipid deposition (71). Paradoxically, with the exception of one study (72), transplantation of *Abcg1*-deficient bone marrow into either *ApoE*^{-/-} or *Ldlr*^{-/-} mice reduced atherosclerotic aortic lesions (73, 74). However, this decreased anti-atherogenic role was attributed to increased macrophage apoptosis due to oxidized LDL ingestion (73) or compensatory upregulation of ABCA1 and increased ApoE secretion in *Abcg1*^{-/-} macrophages (74). Gelissen et al. (75) proposed a synergistic role of ABC transporters, where ABCG1 promotes cholesterol efflux to phospholipid-rich nascent HDL particles generated by the lipidation of apoA-I via the ABCA1 transporter. Convincingly, Yvan-Charvet et al. (76) discovered that hematopoietic co-deletion of both ABCA1 and ABCG1 resulted in an exacerbated pathology

including foam cell infiltration of the myocardium, with larger lesion sizes in the proximal aorta. Further investigation revealed that *Abca1*^{-/-}*Abcg1*^{-/-} macrophages stimulated with oxidized LDL undergo apoptosis and express inflammatory genes (76). In addition to increased lipid raft formation, many studies have reported that *Abca1*^{-/-}*Abcg1*^{-/-} mice present with marked leukocytosis accompanied with infiltration and accumulation of inflammatory cell infiltrates in various organs (3, 52, 76–78). This inflammatory phenotype is driven via the hematopoietic compartment as bone marrow restricted deletion of *Abca1* and/or *Abcg1* resulted in accelerated hematopoiesis. (3). Combined *Abca1* and *Abcg1* deficiency led to an increase in membrane cholesterol, which increased the cellular lipid raft content of the HSPCs within the bone marrow (3). Importantly, this increase in membrane cholesterol content resulted in a secondary increase in the expression of the CBS on HSPCs, amplifying the effects of GM-CSF and IL-3, notably the activation of the signaling proteins, ERK1/2, and signal transducer and activator of transcription (STAT)5. Cumulatively, these effects lead to HSPC proliferation, enhanced myelopoiesis, and accelerated atherosclerosis (Fig. 1A). The importance of membrane cholesterol, and by extension lipid rafts, in this process is further highlighted by the fact that HSPCs from *Abca1*^{-/-}*Abcg1*^{-/-} mice that simultaneously harbor a transgene expressing apoA-I exhibit reduced CBS expression (3). As mentioned, Wang et al. (41) formally confirmed the requirement of the CBS of the IL-3/GM-CSF receptor in HSPCs through genetic studies. Additionally, Westerterp et al. (42) reported an enhancement in granulocyte-colony stimulating factor-dependent HSPC mobilization and extramedullary hematopoiesis in spleen and liver of *Abca1*^{-/-}*Abcg1*^{-/-} mice, underlining the importance of ABCA1 and ABCG1 in the regulation of cellular cholesterol levels and myelopoiesis.

ABCG4

ABCG4 is closely related to ABCG1 and was discovered by Wang et al. (69) to mediate cholesterol efflux to HDL. However, unlike ABCA1 and ABCG1, ABCG4 is not expressed in macrophage foam cells and does not regulate cholesterol efflux in macrophages (79, 80). We discovered that ABCG4 is selectively expressed in megakaryocyte progenitors and is localized in the *trans*-Golgi (50). Bone marrow *Abcg4* deficiency resulted in defective cholesterol efflux to HDL and increased cholesterol content. Importantly, the accumulation of cholesterol was accompanied by an increase in the surface expression of the thrombopoietin receptor (c-MPL) on megakaryocyte progenitors, amplifying the effects of TPO-induced proliferation of megakaryocytes and differentiation to platelets, thus accelerating atherosclerosis and thrombosis. Notably, we highlighted that the role of ABCG4 on hematopoiesis potentially acts in a Lck/Yes novel (LYN) kinase-dependent fashion with a strong association to E3 ligase castias B-lineage lymphoma (c-CBL). Loss of ABCG4 leads to elevated membrane cholesterol content due to impaired efflux, which decreases LYN kinase activity and its downstream regulatory effects on c-CBL. As a consequence, the negative feedback of

phosphorylated c-CBL on c-MPL expression is diminished and there is an increased expression of c-MPL, sensitivity to TPO, and activation of ERK1/2, AKT, and STAT5. These effects lead to enhanced megakaryocyte progenitor proliferation and platelet differentiation and increased atherosclerosis and thrombosis (Fig. 1B). Further highlighting the importance of ABCG4, a single or double dose of HDL infusion reduced megakaryocyte progenitors and platelet counts in WT mice but not in *Abcg4*^{-/-} mice, showing a strong dependence of HDL on ABCG4 for cholesterol efflux in megakaryocyte progenitors. Interestingly, this is reflected in patients with peripheral vascular disease, where a single dose of rHDL significantly decreased platelet levels as compared with placebo (50). Cumulatively, these findings emphasize a close relationship between ABCG4-regulated cholesterol content (potentially increasing lipid rafts) in megakaryocyte progenitors and disordered thrombopoiesis and atherosclerosis.

AIBP: A NEW PLAYER IN CHOLESTEROL-MEDIATED HEMATOPOIESIS AND INFLAMMATION

Several current therapeutic interventions aim at promoting cholesterol efflux via HDL mimetics or by increasing the expression of ABC transporters via LXR agonists (81–83). These approaches are designed to improve cholesterol homeostasis, consequently reducing lipid raft content by promoting cholesterol efflux from target cells (82, 83). However, the feasibility of these interventions to date has been limited in clinical studies, as these methods ultimately require cell-specific and organ-selective cholesterol efflux to avoid side effects (for example, LXR agonists that do not cause *de novo* lipogenesis). Recent studies suggest that the non-cell autonomous acting protein, apoA-I binding protein (AIBP), is important in the regulation of cholesterol efflux in hematopoiesis and has a potential therapeutic function (84). AIBP is a secreted protein that is physically associated with apoA-I (85). AIBP binds to both apoA-I and HDL, thereby augmenting cholesterol efflux and disrupting lipid rafts in ECs and macrophages (86–90). *Apoa1bp*^{-/-} mice on a WTD exhibited a greater abundance of inflammatory macrophages with exacerbated hyperlipidemia and atherosclerosis. Furthermore, in what appears to be a negative feedback pathway, AIBP binds to activated TLR4 dimers localized within lipid rafts. This promotes the recruitment of HDL/apoA-I and removal of cholesterol from lipid rafts leading to the disassociation of the active TLR4 dimer and, hence, an inhibition of TLR4 signaling and reduced inflammation (89). Recently, AIBP has been shown to play a role in regulating hematopoiesis via two mechanisms: *i*) regulation of the hemogenic endothelium; and *ii*) regulation of the HSPCs themselves (84). *Aibp2* (the zebrafish functional paralog of AIBP) transgenic overexpressing zebrafish had increased expression of *Srebf2*, the gene encoding Srebp2. Srebp2 is the master regulator of cholesterol synthesis in cells, and the increased expression of *Srebf2* would indicate that AIBP is a regulator of Srebp2 activity (likely by cholesterol removal), promoting

cholesterol synthesis. Because AIBP augments cholesterol efflux via HDL, cotreatment with HDL and AIBP in ECs revealed a dose-dependent increase of Srebp2 activity (84). The HDL-AIBP induction of *Srebp2* is likely a response to depleted cellular cholesterol, in keeping with Srebp2's well-defined role (91). In cholesterol-rich environments, Srebp2 is downregulated, preventing cholesterol synthesis (e.g., *Hmgcr* expression) and uptake (e.g., *Ldlr* expression) to avoid lipotoxicity.

Interestingly, while classical Srebp2 target binding motifs (i.e., *Srebf2*, *Hmgcr*, *Ldlr*) were confirmed in hemogenic ECs (HECs), *Notch* was also shown to be occupied by Srebp2. A comparative RNA-seq analysis of ECs, HECs, HSCs, and progenitors with lymphoid potential from E10.5 mouse embryos also revealed that *Notch* and some *Notch* target genes (e.g., *Hey1* and *Hey2*) were enriched in HECs as they transitioned to HSCs (92). Interestingly, aside from Scap, Srebp2 cholesterol metabolism target genes were not strongly represented in this data set. Considering that there is remarkable enrichment of the Notch signaling pathway in Srebp2-regulated genes, it is possible that the role of Srebp2 is diverted to Notch signaling during endothelial to hematopoietic transition, a process that perhaps does not rely on cholesterol synthesis.

The role of Srebp2 in hypercholesterolemia has been explored in *Ldlr*^{-/-} mice fed a WTD. Consistent with our previous finding (4), WTD-fed *Ldlr*^{-/-} mice had an expansion of bone marrow HSPCs, and blocking Srebp2 prevented HSPC expansion (84). While these studies provide a clue that Srebp2 transactivation of Notch is associated with HSPC expansion, HSPC-specific inhibition of Srebp2 in *Ldlr*^{-/-} mice is required to confirm this. Importantly, this effect of Srebp2 may also involve its classical cholesterol synthesis mechanism, as we have previously shown that HSPCs use the LDLR to uptake cholesterol in order to proliferate (23). In the hypercholesterolemic milieu, it is likely that in response to the inflammatory environment in WTD-fed *Ldlr*^{-/-} mice, intrinsic cholesterol synthesis is dysregulated in the *Ldlr*-deficient HSPCs. In humans with elevated LDL cholesterol, circulating HSPCs have increased protein levels of Srebp2 and Notch. While LDL levels are correlated with circulating HSPCs, no correlation was observed with the white blood cell population. However, subjects in this study were either healthy or had only mild hypercholesterolemia, which may not necessarily lead to the typically observed increase in mature myeloid cells that occurs in

familial hypercholesterolemia patients, who have 2- to 3-fold higher LDL-C levels (23, 93). It appears paradoxical that high LDL-C content activates Srebp2 in circulating HSPCs. However, oxidative stress and oxidized phospholipids, both of which are likely abundant in 4 month WTD-fed *Ldlr*^{-/-} mice, may contribute to Srebp2 activation, as has been reported previously (94, 95). Another possibility is that endoplasmic reticulum stress can trigger noncanonical caspase2-dependent Srebp2 activation (96). All of these speculations warrant further investigation to explore the underlying mechanism of hypercholesterolemia regulation of Srebp2 activity. In the context of lipid raft disruption, it would also be important to determine whether modulation of lipid rafts by alternative methods also alters Notch. Nonetheless, these studies define a novel mechanism for hematopoiesis in which disruption of lipid rafts by AIBP causes SCAP-mediated Srebp2 activation, which regulates Notch signaling **Fig. 2**. This pathway was characterized in detail during developmental HSPC emergence largely in zebrafish, but requires further understanding in hypercholesterolemia-induced HSPC expansion, where cell cholesterol accumulation and increased lipid raft content have been found to contribute to hyperproliferation and enhanced myelopoiesis (3, 4, 97).

CONCLUSIONS

Defective cholesterol efflux promotes the accumulation of membrane cholesterol and an increase in lipid rafts. The lipid composition of lipid rafts enables the preferential localization of cytokine receptors to these microdomains on the plasma membrane. The deficiency of ABC transporters and the consequent reduced ability to efflux cholesterol leads to an increase in lipid rafts. As a consequence, the expression of lipid raft-localized cytokine receptors (e.g., CBS and c-MPL) that favors myeloid skewing is increased, leading to the progression of atherosclerosis. Current treatments to lower plasma LDL-C using statins largely achieve this via shutting down cholesterol synthesis in the hepatocytes causing the induction of the LDLR to clear plasma lipoproteins. This balance of cellular cholesterol metabolism occurs in most cells and, thus, statins likely promote cholesterol uptake in HSPCs in this way. Therefore, direct modulators of lipid rafts in combination with statins may be more effective in restoring normal

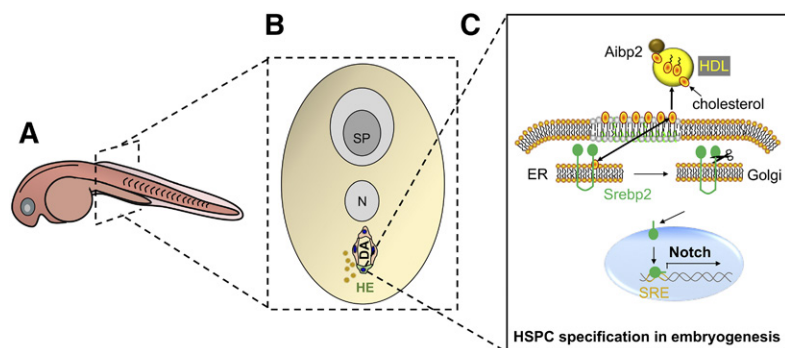



Fig. 2. AIBP regulation of hematopoiesis in zebrafish. A: Transverse view of the zebrafish trunk. B: AIBP (brown) is secreted from the sclerotome and acts on ECs in a paracrine fashion. C: AIBP accelerates cholesterol efflux from the hemogenic endothelium (floor of dorsal aorta), reducing cellular cholesterol levels. This promotes the activation of Srebp2, which in turn transactivates Notch contribution to HSPC emergence. SP, spinal cord; N, notochord; DA, dorsal aorta; SRE, sterol responsive element.

hematopoiesis driven by hypercholesterolemia and the associated inflammation. AIBP is capable of disrupting lipid raft integrity. Studies in macrophages and microglial cells indicate that AIBP binds TLR4 and disrupts the lipid rafts where TLR4 resides (86, 89, 98). It remains to be determined whether the same mechanism acts in HSPCs. Further studies should address the hematopoietic- and niche-specific deletion of AIBP and its role on Srebp2 and Notch signaling in HSPCs. Nonetheless, cholesterol metabolism is critically important in hematopoiesis, and manipulating this in a targeted manner will likely provide beneficial effects in the setting of CVD and other disorders with aberrant hematopoiesis. 

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