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EDITORIAL

LDL-cholesterol drives reversible myelomonocytic skewing in human bone marrow

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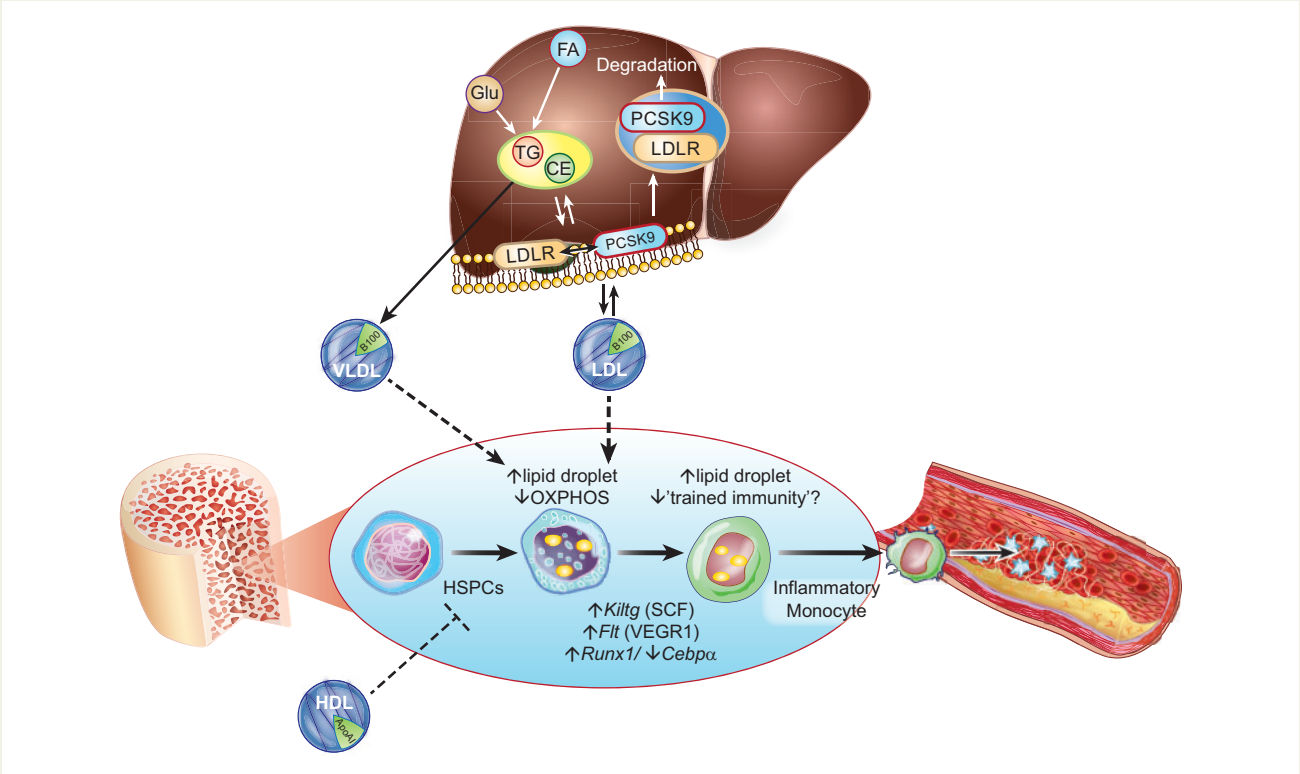
This editorial refers to 'Impact of cholesterol on proinflammatory monocyte production by the bone marrow', by L.C.A. Stiekema *et al.*, https://doi.org/10.1093/eurheartj/ehab465.



Graphical Abstract Lipoproteins regulate inflammatory monocyte production from hematopoietic stem and progenitor cells.

In this issue of the *European Heart Journal*, Stiekema *et al.*[1] show a dual proinflammatory role for plasma LDL-cholesterol (LDL-C) levels in haematopoietic stem and progenitor cells (HSPCs) from human bone marrow (BM). LDL-C promoted myelomonocytic skewing in BM HSPCs, driving monocyte production and a monocyte inflammatory signature. A maximum tolerated dose of cholesterol-lowering

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therapies (LDL-C reduction ~66%) in familial hypercholesterolaemia (FH) patients reversed myelomonocytic skewing in BM HSPCs but not the monocyte inflammatory signature. Altogether, these findings provide the first genetic evidence for the involvement of LDL-C in myelomonocytic skewing in human HSPCs and reveal how LDL-C ties chronic inflammation to cardiovascular diseases (CVD) (*Graphical Abstract*).

Context of the study

It is well established that monocytosis, the expansion of blood monocytes, is associated with CVD.[2,3] However, the underlying mechanisms of monocyte expansion remain poorly understood. Several factors driving monocytosis have been identified in pre-clinical models, including inflammation *per se*, but also obesity, diabetes, and dyslipidaemia, which are all associated with the metabolic syndrome and drive CVD.[2,3] Seminal studies have shown a link between cellular cholesterol metabolism and monocytosis. Cholesterol accumulation due to defective cholesterol efflux, mediated by the cholesterol transporters ATP-binding cassette A1 and G1 (ABCA1 and ABCG1), drives proliferation, expansion, and skewing of HSPCs towards myeloid cells.[4] These effects were attributed to increased membrane cholesterol accumulation, which enhances the surface expression of the common β subunit of the interleukin-3/granulocyte–macrophage-colony-stimulating factor (IL-3/GM-CSF) receptor that stimulates HSPC proliferation and a bias towards the myeloid lineage.[4] Ultimately, this leads to enhanced monocyte recruitment into the vessel wall and accelerated atherosclerosis,[4] the major cause of CVD. Follow-up studies have shown that apolipoprotein (apo)E, which is present on several classes of lipoprotein particles including HDL, LDL, and very-low-density lipoprotein (VLDL), binds proteoglycans on the surface of HSPCs to mediate cholesterol removal and subsequently limits myelopoiesis.[5] Later studies revealed that LDL-C uptake by HSPCs also enhances HSPC expansion and proliferation,[6,7] with reversal by HDL, presumably mediated by cholesterol efflux.[8] More recent studies have shown that HSPC cholesterol accumulation and myeloid skewing can also occur during 'trained immunity'.[9] 'Trained immunity' is the long-term functional reprogramming of innate immune cells, especially monocytes and macrophages, mediated by epigenetic modifications, evoked by an exogenous or endogenous insult.[9] Together, these studies introduced the concept that cholesterol accumulation in HSPCs is one of the major culprits promoting myeloid cell fate and monocytosis in pre-clinical models of hypercholesterolaemia-driven atherosclerosis.

High plasma LDL-C levels at the origin of enhanced haematopoietic activity in CVD patients

Previous studies in humans revealed that enhanced bone marrow and splenic metabolic activity predicts risk of future CVD events as shown by [^{18}F]fluorodeoxyglucose positron emission tomography (PET) imaging.[10] The Stroes Laboratory confirmed increased haematopoietic activity in patients with atherosclerosis.[11] Moreover, by performing PET/computed tomography (CT) imaging using [^{18}F]DPA714, a radioligand of translocator protein (TSPO), they showed persistent BM and splenic haematopoietic activity up to 3 months after a first major cardiovascular event.[12] The authors showed that HSPCs from CVD patients had increased myeloid potential, which they attributed to oxidized LDL (oxLDL) uptake.[11] Although previous epidemiological studies in the UK Biobank and National Health and Nutrition Examination Survey (NHANES) have suggested that plasma triglyceride levels rather than LDL-C correlated positively with monocyte counts,[13,14] a recent study identified a positive correlation of plasma LDL-C with blood HSPCs in ~70 healthy volunteers, highlighting a link between LDL-C and HSPC expansion.[6] Using an inherited genetic defect leading to an extreme condition of elevated plasma LDL-C, the study by Stiekema *et al.* now provides the first genetic evidence of a role for LDL-C in HSPC expansion and myelomonocytic skewing in FH patients, with reversal by cholesterol-lowering therapies, including statins.[1] From previous studies, we have learned that reducing LDL-C by statin therapy decreases plasma levels of high-sensitivity C-reactive protein (hs-CRP), a standard marker of systemic inflammation, but residual inflammatory risk (hs-CRP >2 mg/L) persists.[15] However, blood monocyte counts and hs-CRP differently predict future CVD.[16] In the EPIC-Norfolk Prospective Population Study (comprising 12 304 individuals), the positive correlation between plasma LDL-C and blood monocytes was adjusted for plasma hs-CRP levels, age, gender, smoking, and body mass index,[1] suggesting that LDL-C may enhance inflammatory and CVD risk via monocytosis independently of its effects on hs-CRP, and downstream of its role in HSPC expansion.

Mechanistic studies on the causal role of LDL-C in promoting myelomonocytic skewing

Using 10 untreated FH adult patients, Stiekema *et al.* uniquely show a 1.4-fold expansion of CD34$^+$ HSPCs obtained from sternal BM aspiration in FH patients compared with age- and-gender-matched normocholesterolaemic controls.[1] Despite BM and blood monocyte counts being similar in FH patients and normocholesterolaemic controls, the authors identified a gene signature in BM CD34$^+$ HSPCs from FH patients promoting myelomonocytic skewing along with a promigratory profile, while the expression of genes involved in cell cycling was decreased, and there were differences in expression of genes that regulate metabolic flux. Based on this transcriptomic analysis, the authors proposed that perturbed oxidative phosphorylation (OXPHOS) could be due to a switch from glucose to fatty acid utilization (based on higher *Pdk4* expression in HSPCs from FH patients compared with controls).[1] Metabolic flux measurement indeed suggested a trend towards reduced basal respiration in HSPCs from FH patients. Consequently, this could drive their expansion and myelomonocytic skewing [based on higher expression of *Kiltg* and *Flt1*, genes that encode the stem cell survival protein stem cell factor (SCF) and the vascular endothelial growth factor (VEGF), respectively]. Although the down-regulation of gene sets related to the cell

cycle makes intuitive sense when HSPCs enter differentiation, the down-regulation of gene sets related to Runx1-regulated HSPC differentiation is intriguing, because *Runx1*-deficient mice display lack of foetal liver haematopoiesis. However, in adult haematopoiesis, RUNX1 may dictate HSPC fate depending on the growth hormone environment.[17] In an attempt to address this important point, the authors assessed the potential of GM-CSF and erythropoetin (EPO) in a colony-forming unit (CFU) assay to induce myelomonocytic and erythroid lineage commitment, respectively. While myelomonocytic skewing was enhanced in HSPCs from FH patients, as shown by the GM-CFU assay, in the EPO-CFU assay HSPC fate between FH and normocholesterolaemic controls was not different.[1] Future studies will be required to address alternative HSPC fates in FH patients. In particular, it will be of interest to evaluate the reason for the increased monocyte but not neutrophil bias in HSPCs in view of the transcriptomic signature that the authors identified.

LDL lowering in FH patients to dissect mechanisms linking hypercholesterolaemia to myelomonocytic skewing

Stiekema *et al.* then continued, using an approach previously used by the Stroes Laboratory aimed at combining an LDL-lowering intervention in FH patients.[18,19] They used treatment with maximally tolerated proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies and statins, or a combination thereof (with or without ezetimibe), which reduced plasma LDL-C levels by ~66%.[1] Doing so, the authors reversed the transcriptomic signature in HSPCs from FH patients almost completely, including OXPHOS-, HSPC cycle-, and differentiation-related genes, as such proving that LDL-C was the driving force for these specific transcriptomic changes.[1] They validated restoration of OXPHOS by metabolic flux measurements and reduced myelomonocytic fate using CFU assays with GM-CSF. The latter could reflect enhanced cell surface expression of the IL-3/GM-CSF receptor previously found to be regulated by increased membrane cholesterol accumulation in pre-clinical models as mentioned above.[4] Consistent with this hypothesis, the authors show an enhanced lipid droplet content in BM $CD34^+$ HSPCs from FH patients that was reversed by LDL lowering. Similarly, a negative correlation of plasma HDL levels with blood monocytes was found in a cohort of FH children (n = 49),[8] presumably downstream of HDL-induced cholesterol efflux. Together with the findings of Stiekema *et al.*,[1] these data suggest that the balance between cholesterol loading and cholesterol efflux in the HSPCs of these patients is very sensitive in dictating myelomonocytic fate. The authors also identified an up-regulation of two key transcription factor genes, *Gata1* and *Cebpa*, in HSPCs after cholesterol-lowering therapy.[1] These transcription factors can be regulated by receptor tyrosine kinases and could divert HSPCs from myelomocytic fate. In sum, the findings of Stiekema *et al.*[1] provide novel downstream signalling targets that will need further investigation and functional validation in terms of their role in myelomonocytic fate.

Does the lack of reversible monocyte inflammatory signature by LDL lowering in FH patients reflect sustained residual inflammatory risk in CVD patients?

Previous studies found an increased lipid accumulation in circulating monocytes in FH patients independent of significant changes in monocyte counts but with higher migratory capacity, suggesting that hypercholesterolaemia directly contributes to monocyte activation, amplifying their effector function.[18,19] This was attributed to epigenetic reprogramming due to cholesterol loading that mimicked 'trained immunity'. However, modulation of myeloid progenitor cholesterol metabolism has also been previously suggested to be an integral component of 'trained immunity'.[9] Stiekema *et al.* now extend these observations to BM HSPCs of FH patients, suggesting that 'trained immunity' acts at an early stage of the monocyte life span.[1] Because of the potential epigenetic reprogramming associated with these effects, it may not be surprising that a persistent proinflammatory monocyte signature still occurs in BM HSPCs after cholesterol-lowering therapy, similar to what was previously observed for blood monocytes.[18,19] Identifying the mechanisms to bypass this epigenetic reprogramming may offer novel therapeutic perspectives.

In sum, this study provides new insights into how defects in hepatic lipoprotein metabolism as observed in FH patients impact myelomonocytic fate. This study also provides new perspectives that may open up further investigation into the liver–BM communication related to low grade inflammation in cardiometabolic diseases.

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