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Review Article

Disordered haematopoiesis and cardiovascular disease: a focus on myelopoiesis

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Cardiovascular (CV) diseases (CVD) are primarily caused by atherosclerotic vascular disease. Atherogenesis is mainly driven by recruitment of leucocytes to the arterial wall, where macrophages contribute to both lipid retention as well as the inflammatory milieu within the vessel wall. Consequently, diseases which present with an enhanced abundance of circulating leucocytes, particularly monocytes, have also been documented to accelerate CVD. A host of metabolic and inflammatory diseases, such as obesity, diabetes, hypercholesteraemia, and rheumatoid arthritis (RA), have been shown to alter myelopoiesis to exacerbate atherosclerosis. Genetic evidence has emerged in humans with the discovery of clonal haematopoiesis of indeterminate potential (CHIP), resulting in a disordered haematopoietic system linked to accelerated atherogenesis. CHIP, caused by somatic mutations in haematopoietic stem and progenitor cells (HSPCs), consequently provide a proliferative advantage over native HSPCs and, in the case of *Tet2* loss of function mutation, gives rise to inflammatory plaque macrophages (i.e. enhanced interleukin (IL)-1β production). Together with the recent findings of the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) trial that revealed blocking IL-1β using Canakinumab reduced CV events, these studies collectively have highlighted a pivotal role of IL-1β signalling in a population of people with atherosclerotic CVD. This review will explore how haematopoiesis is altered by risk-factors and inflammatory disorders that promote CVD. Further, we will discuss some of the recent genetic evidence of disordered haematopoiesis in relation to CVD though the association with CHIP and suggest that future studies should explore what initiates HSPC mutations, as well as how current anti-inflammatory agents affect CHIP-driven atherosclerosis.

Cardiovascular (CV) disease (CVD) is principally driven by atherosclerotic vascular disease. Atherogenesis is a complex process involving the entry and retention of both lipids and immune cells into arterial walls, and it is this entry of inflammatory leucocytes that drives disease progression and impairs plaque resolution [1-3]. Lipid-laden macrophages are vital to the highly inflammatory setting of atherosclerosis, as foam cells promote an unstable lesion and favour plaque rupture causing arterial occlusion and ultimately myocardial infarction (MI). Given the central role of monocyte-derived macrophages in lesion formation, disorders that present with higher circulating monocytes, known as monocytosis, are unsurprisingly linked to increased CVD [4]. Furthermore, co-morbidities such as obesity and diabetes, as well as autoimmune diseases like rheumatoid arthritis (RA), which are associated with increased CVD also present with disordered haematopoiesis (white blood cell production). Importantly, defects in cellular cholesterol metabolism have been connected with enhanced haematopoiesis that leads to accelerated atherosclerosis. Recently, the exciting discovery of clonal haematopoiesis of indeterminate potential (CHIP) has provided genetic evidence of how disordered haematopoiesis can elevate the risk of CVD in

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people. This review highlights the important role of enhanced myelopoiesis in accelerating atherosclerotic CVD.

Leucocytes in CVD

Monocyte-derived macrophages are essential in the progression of atherosclerosis and reduction in plaque stability, hence the contribution of circulating monocytes in the context of CVD has been widely explored [5-7]. A 7-year study looking at the association of monocyte levels with plaque formation concluded that monocyte count was an independent predictor of CVD [5]. Increasing monocyte levels were directly correlated with increased odds ratio for carotid plaque development, even after adjusting for age, sex, total cholesterol, smoking status, and systolic blood pressure. Similarly, Olivares et al. [6] have shown that people with coronary heart disease had higher levels of monocytes than those with no heart disease present, in regard to both monocyte count and percentage. In humans, monocytes can be separated into three distinct subpopulations based on their expressions of CD14 (LPS co-receptor) and CD16 (FcγIII receptor) [8]. While the classical $CD14^+CD16^-$ monocytes are the most abundant (∼85% of all monocytes), it is the intermediate $CD14^+CD16^+$ and the non-classical $CD14^-CD16^+$ monocyte subsets which are associated with vascular diseases such as stroke and MI [8-10].

Accumulating evidence has established a causal relationship between monocytosis and the development of atherosclerosis. Indeed, animal studies have confirmed this relationship quite convincingly by way of manipulating the number of circulating monocytes. For example, Ylitalo et al. [11] demonstrated that monocyte depletion reduced atherosclerosis in rabbits. In agreement with this, depletion of monocytes in mice or in models with defective monocyte development, results in blunted atherogenesis and even reduction in established plaque [12,13]. Monocytes in mice are also a heterogeneous population, separated based on Ly6-C surface expression into two subtypes; either Ly6-C^{lo} or Ly6-C^{hi} monocytes [3]. Importantly, literature suggests that both monocyte subsets can enter into atherosclerotic lesions, but the two subtypes of monocytes are thought to retain phenotypical differences in the plaque [14,15]. The so-called 'inflammatory' Ly6-C^{hi} monocytes are known to adhere to an activated endothelium, accumulate in lesions and ultimately exacerbate atherosclerosis [3,14-16]. However, Ly6-C^{lo} monocytes appear to be especially predisposed to develop into $CD11c^+$ cells once they enter in atherosclerotic lesions, which is traditionally considered a dendritic cell marker or an inflammatory macrophage [14]. Nonetheless, future work should elucidate the precise mechanisms by which Ly6-C^{lo} monocytes may influence atherosclerotic progression. Importantly, the Fisher group recently revealed the requirement of the Ly6-C^{hi} monocytes in facilitating lesion regression, suggesting the relationship between disease pathology and monocyte subsets is more complex than initially thought [17]. Macrophages within the lesion can also undergo local proliferation to expand the pool of plaque macrophages and potentially contribute to lesion progression [18]. However, it should be noted that these macrophages are mainly of the alternatively activated variety, which are enriched in scavenger receptors and as fate mapping studies suggest, this is likely a transient event and not a major source of plaque macrophages, which are usually derived from blood monocytes [18-20].

While not a major focus of this review, it should be noted that neutrophils are also important in the early stages of atherogenesis where they are known to enter smaller, developing lesions [21]. Platelets are also important drivers of atherosclerosis, where their activation or overproduction promotes plaque growth [4,22-24]. Activated platelets can bind to leucocytes to form platelet–leucocyte aggregates, which bind to the endothelium exacerbating atherogenesis. Taken together, leucocyte abundance and activation are pivotal to the development of atherosclerosis.

Myelopoiesis and extramedullary haematopoiesis

Haematopoiesis is the production of all blood cells, which occurs in the bone marrow (BM). It is a tightly regulated, ordered and efficient process that ensures the maintenance of the whole immune system. Haematopoietic stem and progenitor cells (HSPCs) are sensitive to both intrinsic and extrinsic cues such as cytokines and chemokines. Extrinsic signals dictate the role of HSPCs to either remain dormant, duplicate through symmetrical division, or to undergo asymmetrical division and to give rise to progenitor cells that can differentiate into mature lymphoid or myeloid cells.

Monocytes are derived from the myeloid lineage, through a sub-branch of haematopoiesis called myelopoiesis [25]. During myelopoiesis, HSPCs give rise to common myeloid progenitors (CMPs), which differentiate into granulocyte macrophage progenitors (GMPs) that in turn differentiate into myeloid-derived white blood cells such as monocytes and neutrophils [25,26]. This myeloid branch of haematopoiesis also gives rise to platelets through the megakaryocytic-erythroid progenitors (MEPs). In certain conditions, such as some infections, which require large numbers of monocytes to clear inflammatory stimuli, cells can be formed outside the medullary spaces of the BM through a process known as extramedullary haematopoiesis [25,27]. This entails mobilisation of stem cells from the

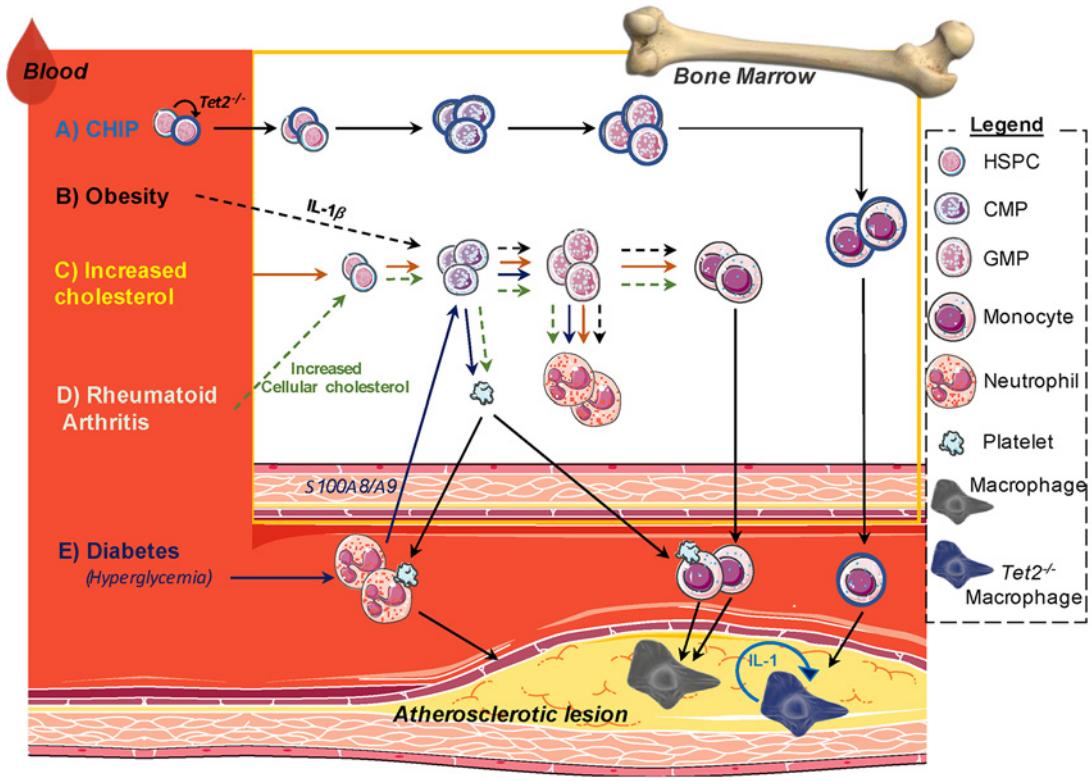

Figure 1. Disordered haematopoiesis excerabates atherosclerosis

(**A**) **CHIP:** Somatic mutations in HSPCs results in CHIP (e.g. loss of *Tet2*), providing a competitive advantage to CHIP^{+} HSPCs over normal HSPCs. This can enhance myelopoiesis by a competitive outgrowth of the clone, ultimately producing *Tet2*$^{-/-}$ lesional macrophages that produce IL-1β. (**B**) **Obesity:** Inflammed obese adipose tissue releases IL-1β, which acts on BM CMPs/GMPs to induce myelopoiesis, to promote atherogenesis. (**C**) **Hypercholesterolaemia/reduced cholesterol efflux:** Elevated circulating cholesterol levels promote HSPC proloferation, enhanced myelopoiesis, and leucocytosis. (**D**) **RA:** Systemic inflammation in RA causes cellular cholesterol defects, promoting HSPC, CMP and GMP proliferation resulting in monocytosis, neutrophilia, and thrombocytosis. Leucocyte–platelet aggregates also form to influence atherosclerosis. (**E**) **Diabetes:** Hyperglycaemia induces S100A8/A9 release from circulting neutrophils which activates BM CMPs/GMPs to stimulate myelopoiesis and platelet production (via the liver), as well as leucocyte–platelet aggregates to influence atherosclerotic lesions.

BM to the circulation where they can be home to the spleen, proliferate and contribute to the generation of mature myeloid cells [16].

Metabolic diseases display enhanced myelopoiesis

It is well-known that metabolic diseases, such as obesity and diabetes, increases the risk of CVD. While these diseases have been shown to contribute to the pathogenesis of atherosclerosis in various ways, intriguingly, they have also been linked to enhanced haematopoiesis [23,28,29]. In a murine model of diabetes, neutrophils responded to the hyperglycaemic environment by promoting the release of alarmins/damage-associated molecular patterns (DAMPs) such as S100A8/A9, which then induces myelopoiesis via stimulation of their receptors (e.g. RAGE) on myeloid progenitor cells in the BM (Figure 1) [23,29]. Hyperglycaemia-associated monocytosis and persistent entry into plaques is directly linked to impaired atherosclerotic lesion regression, independent of plasma cholesterol [28,29]. Additionally, diabetic patients also exhibit increased numbers of platelets particularly, the immature, highly reactive, and pro-atherogenic reticulated thrombocytes [23], which are correlated with heightened CVD [30]. The overproduction was also due to S100A8/A9 release from neutrophils, but in this scenario, S100A8/A9 was shown to stimulate RAGE on Kupffer cells to produce interleukin (IL)-6, which then promotes the production of the thrombopoietic hormone, thrombopoietin (TPO). Obesity also promotes monocytosis and neutrophilia [28]. Enhanced myeloid production is attributed to adipose tissue macrophage IL-1β overproduction. Subsequently, IL-1β promoted proliferation of both BM CMPs and GMPs, driving myelopoiesis and increasing circulating monocytes (Figure 1). While the mechanisms

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involved in driving the heightened myelopoiesis vary considerably, ultimately, disordered haematopoiesis is implicated in exacerbated atherosclerotic CVD in these metabolic diseases.

Hypercholesteraemia promotes leucocyte production

While there is a clearly defined relationship of increased circulating LDL, subsequent entry of cholesterol into atherosclerotic lesions and consequently increased CVD, hypercholesteraemia is also linked to increased myeloid cell production. This is highlighted in numerous studies in both swine and murine models of hypercholesteraemia [21,31-33]. Reversal of exacerbated cholesterol levels, either via diet or using statins, restores haematopoiesis. Interestingly, the level of high-density lipoprotein (HDL) cholesterol are inversely correlated to peripheral monocyte levels, particularly in the setting of hypercholesterolaemia [34]. Furthermore, the authors stipulate that the balance between LDL and HDL may be more important in hypercholesteraemia, rather than the absolute increase in cholesterol. While not a causal genetic determinant of CVD, efficient HDL function has been shown to be important in atherogenesis, as it prevents the build-up of lipid in cells by stimulating cellular cholesterol efflux [35].

Another important development with respect to the impact on hypercholesterolaemia in regulating myelopoiesis has come from the Latz group. Extending our findings that hypercholesterolaemia in atherosclerosis-prone murine models induces myelopoiesis [32,36], they found that these changes can be persistent even after the mice were switched back to a chow diet [37]. Through a series of elegant experiments, Christ et al. [37], discovered that feeding *Ldlr*$^{-/-}$ mice a Western-type diet (WTD) alters the innate immune response, such that LPS induces a larger inflammatory response. Interestingly, when mice were fed a WTD and then switched to a chow diet, monocyte levels were returned to normal levels, while neutrophils remained elevated [37]. It was found that the WTD induced epigenetic reprogramming in the GMPs that explained the persistent phenotype when the mice were switched back to the chow diet [37]. One of the main changes was enhanced IL-1β production via NLRP3, which we have shown to stimulate myelopoiesis in the context of obesity [28]. Whether epigenetic modifications are also present is the HSPCs of WTD-fed animals remain unknown but is an important question to address as it will speak to longevity of these changes in myeloid cells. However, if these are restricted to GMPs, it suggests that after the WTD exposure, GMPs will turn over and the new pool of GMPs should no longer carry these epigenetic markers, suggesting that myelopoiesis and responses to inflammatory stimuli should normalise.

The importance of NLRP3-derived IL-1β was suggested to promote atherosclerosis, however this end point was not explored in the context of memory. Moreover, we should note that the role of NLRP3 in hypercholesterolaemia-driven atherosclerosis remains controversial as we and others failed to see an effect of NLRP3 on atherogenesis in WTD-fed *Ldlr*$^{-/-}$ or apolipoprotein (Apo)-e deficient *Apoe*$^{-/-}$ mice [38-40]. The exact reason for this discrepancy is unknown, but may be related to absence of NLRP3 inflammasome activation in WTD-fed *Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ mice. Interestingly, these studies revealed that the inflammasome did contribute to atherogenesis when it was clearly activated due to an increase in the second activation signal. This signal was either mitoROS due to Ogg-1 deficiency or increased cholesterol content due to the deficiency of the cholesterol transporters ATP-binding cassette A1 (ABCA1) and G1 (ABCG1). Intriguingly, a recent study by Tumurkhuu et al. [40,41], who previously failed to show an effect of NLRP3 inflammasome activation on atherosclerosis in *Apoe*$^{-/-}$ mice, did show that the NLRP3 inflammasome accelerates atherogenesis in the same model in their recent studies. While the authors did not comment on this discrepancy, they postulated that the secretion of IL-1β, the main product of inflammasome activation, was mediated by the cholesterol transporter ABCA1. Several pathways for IL-1β secretion have been postulated; however, these results are in clear contrast with our extensive studies showing that deficiency of *Abca1* in mice or loss-of-function mutations of *ABCA1* in humans *increase* IL-1β secretion. Moreover, we also found that humans with *ABCA1* loss-of-function mutations show increased vascular inflammation [42].

Together with the moderate reduction in CV events observed in the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) [43], it suggests that IL-1β/NLRP3 may not always be active in atherosclerotic CVD (or after an acute event). However, the findings from Christ et al. [37], in combination with various mechanisms shown to promote myelopoiesis in metabolic disorders does suggest that exposure to infection could provide a poorer prognosis driven by uncontrolled cytokine storms and requires further investigation. Another important point to consider in this context is the emerging evidence from LDL lowering clinical trials that suggest even when LDL cholesterol is very well controlled, if there is still low-grade inflammation present, then people still suffer from residual risk of CVD [44-46]. This can be clearly modelled in inflammatory disorders such as RA [47], but raises the question as to whether poor diets can be recalled by our progenitor cells to reduce the health benefits of lowering plasma cholesterol.

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Defective cellular cholesterol metabolism links haematopoiesis to CVD

Pre-clinical models have highlighted a pivotal role of disturbed cellular cholesterol efflux to amplified haematopoiesis and atherosclerosis (Figure 1) [32,48,49]. Cellular cholesterol is regulated by the cholesterol efflux genes, *ABCA1*, *ABCG1*, and *APOE*, which are under the transcriptional control of the liver X receptor (LXR) [49,50]. Indeed, the significance of LXR function in the setting of atherosclerosis was highlighted over a decade ago in a study, which revealed that $Apoe^{-/-}/Lxr\alpha^{-/-}$ mice had significantly augmented atherosclerosis compared with their $Apoe^{-/-}$ counterparts [51]. Interestingly, mice with $Lxr\alpha^{-/-}/Lxr\beta^{-/-}$ BM exhibited increased cellular cholesterol, splenomegaly, and enhanced atherosclerosis [52]. Furthermore, various BM transplant (BMT) studies have highlighted the contribution of cholesterol defects in haematopoietic cells in aggravating atherosclerosis [32,49,52-54]. Indeed, $Ldlr^{-/-}$ mice that were transplanted with $Abca1^{-/-}/Abcg1^{-/-}$ BM had increased plaque size, an unstable lesion phenotype, and increased inflammatory cell infiltration [49,54]. These atherogenic effects were independent to plasma cholesterol [53]. Together, there is now a body of evidence to highlight a pivotal role of efficient cholesterol efflux in haematopoietic cells to protect against vascular disease.

Macrophage phenotype and function play pivotal roles in altering inflammatory milieu in the plaque. Macrophages lacking *Abca1*/*Abcg1* exhibited a foam cell-like phenotype, demonstrated by impaired cholesterol efflux to HDL and apoA–I, along with excessive cellular lipid content [49,53]. Indeed, decreased ABCA1 protein has been noted in human atherosclerotic plaque [55]. Inflammatory mediators as well as minimally modified LDL (mmLDL), which are both increased in plaque, stimulate TLR4-mediated NFκB activation, in addition to suppressing LXR activity and cholesterol efflux [50]. Moreover, these lipid-laden macrophages increase inflammatory cytokine and chemokine secretion [49]. Inactivation of LXR function triggers a feed-forward loop by promoting inflammatory genes that consequently worsen the surrounding milieu, as LXR activity also instigates transrepression of NFκB genes [50,56]. Therefore, LXR inactivity simultaneously increases cellular cholesterol loading and also allows uninhibited inflammatory gene expression. Taken together, this suggests that disturbances in cellular cholesterol handling within the haematopoietic compartment contribute to exacerbated atherosclerosis.

Stem and progenitor cells require efficient cholesterol efflux

HSPCs have an efficient cholesterol efflux system, which influences their proliferation and maturation [32,48,49]. Indeed, defects in intrinsic HSPC cholesterol handling have shown to enhance haematopoiesis. Cholesterol accumulation in $Abca1^{-/-}/Abcg1^{-/-}$ HSPCs instigates an up-regulation in the granulocyte-macrophage colony stimulating factor (GM-CSF)/IL-3 common β subunit (CBS) on the cell surface. This causes a hypersensitivity to proliferative growth factors and cytokines like GM-CSF and IL-3 [32,36,57,58]. This results in excessive HSPC proliferation, myeloid skewing, extramedullary haematopoiesis, monocytosis as well as neutrophilia, which in turn promote monocytes' entry into atherosclerotic lesions [32,36,57,58]. Similarly, $Apoe^{-/-}$ mice also show enhanced HSPC proliferation and mobilisation from the BM to the spleen and consequently extramedullary haematopoiesis [32]. Collectively, these experiments highlight how impaired cholesterol efflux can contribute to leucocyte development.

Promoting cholesterol efflux improves atherosclerosis

Cellular cholesterol metabolism is central to atherogenesis, as such that promoting cholesterol efflux impedes atherosclerosis [56,59]. A prime example is when the LXRα transgenic (Tg) mouse is crossed on to an $Ldlr^{-/-}$ background. These mice have strikingly smaller atherosclerotic lesions compared with its $Ldlr^{-/-}$ counterpart [60]. Additionally, the administration of the synthetic LXR agonist GW3965 is able to reduce lesions in $Apoe^{-/-}$ mice [51]. There is likely an abundance of mechanisms through which promoting cholesterol efflux in whole body studies inhibit atherosclerosis. However, promoting cholesterol efflux has been shown to reduce the number of myeloid cells and prevent extramedullary haematopoiesis, which results in smaller atherosclerotic lesions [32,57]. The reduction in myeloid cell production by cholesterol efflux can occur in part through direct actions on the HSPCs; for example infusion of rHDL in $Apoe^{-/-}$ mice reduces lipid raft abundance in HSPCs, decreases the expression of growth factor receptors, and consequently dampens myelopoiesis [32]. The importance of promoting cholesterol efflux was also shown when $Abca1^{-/-}/Abcg1^{-/-}$ BM was transplanted into apoA–I Tg mice that have elevated levels of HDL, HSPC proliferation and myelopoiesis, which was reduced compared with apoA–I Tg mice with WT BM [36]. Moreover, short-term LXR agonist TO901317 administration increased cell surface Apoe levels and reduced HSPC abundance in the BM [32]. Additionally, LXR activation leads to increased cholesterol efflux from the macrophages within the atherosclerotic lesions, as well as macrophage egress out of lesions and in turn regression of atherosclerotic plaque

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[61]. Treatment with LXR agonists can reduce inflammatory mediators such as MCP-1, IL-6, ICAM1, E-selectin, and MMP9, through transrepression of NFκB genes [56,62,63]. Interestingly, we found that LXR agonists also inhibit atherosclerosis independent of ABCA1 and ABCG1, which we attributed to their anti-inflammatory effects [54]. Taken together, promoting LXR activity has anti-atherogenic effects by dampening inflammation via transrepression, reducing cholesterol levels in macrophages (but not plasma cholesterol) and also regulating haematopoiesis.

Inflammation alters cellular cholesterol metabolism to enhance haematopoiesis and atherosclerosis: observations from RA

RA is an inflammatory disease that is associated with CV events including MI, stroke, and carotid artery intimal–medial thickness (IMT), which leads to premature mortality [64-66]. Traditional CV risk factors do not account for the increased incidence of CVD, and intriguingly systemic inflammation is thought to play a central role in exacerbating atherosclerosis in RA [67-69]. Moreover, patients with active disease and high systemic inflammation were found to have a defect in cholesterol efflux capacity of HDL [70]. Interestingly, plasma from RA patients down-regulates cholesterol efflux proteins and increases lipid accumulation in a human macrophage cell line [71]. Similarly, a study in patients with RA showed an impairment of cholesterol efflux capacity of HDL via *ABCA1* and *ABCG1* [72], suggesting a defect in cellular cholesterol efflux via these transporters resulting in lipid accumulation in macrophages. In turn, this may contribute to advanced atherosclerosis in patients.

Indeed, we have recently shown that systemic inflammation in RA causes cellular cholesterol defects in HSPCs, resulting in monocytosis-driven atherosclerosis (Figure 1) [15]. This is perhaps not surprising given the magnitude of inflammatory cytokines up-regulated in RA, most of which have been linked with monocytosis previously such as IL-3, IL-6, GM-CSF, M-CSF, and IL-1β [28,57,73]. Moreover, systemic inflammation caused a reduction in the cholesterol efflux genes *Abca1*, *Abcg1*, and *Apoe* in BM HSPCs of arthritic mice, which resulted in cellular cholesterol accumulation and enhanced haematopoiesis. Consequently, daughter cells from these lipid-filled progenitor cells also carried more cholesterol, resulting in lipid-laden circulating monocytes and neutrophils. Leucocytosis in RA was associated with impaired lesion regression, where lesions also exhibited a less stable phenotype observed with increased immune cell infiltrate and lipid contact. Indeed, administration of rHDL reversed monocytosis and monocyte lesion entry in RA. In fact, controlling cholesterol efflux in another autoimmune disease with severe inflammation, systemic lupus erythematosus (SLE), where cholesterol efflux defects have also been implicated [72,74], improves atherosclerosis in the murine $Apoe^{-/-}Fas^{-/-}$ model of SLE [75]. Additionally, other methods of modulating cellular cholesterol, such as utilising statins to inhibit cholesterol synthesis, may provide additional benefits to decrease CVD in these populations, independently to modulate circulating LDL cholesterol levels. This would require formal testing and is likely only to have a partial effect on cellular cholesterol metabolism. These findings further support the notion that restoring efficient cholesterol handling in diseases with severe systemic inflammation is a potential novel approach in reducing the high CVD risk in these patients.

Disordered haematopoiesis and CVD in human: direct genetic evidence

Genetic evidence for disordered haematopoiesis and the progression of CVD has recently emerged in humans with the discovery of CHIP. CHIP increases with age and is characterised by the acquisition of somatic mutations in HSPCs which provide a competitive advantage over healthy HSCs, leading to an increase in mutated HSPCs and their mature progeny [76-80]. Consequently, this results in a progressive clonal expansion of WBCs, while the total number of WBCs remains unchanged, and ultimately an increased risk of haematological cancers, atherosclerosis, and increased mortality [77,81,82]. While these primary mutations are strongly linked, through secondary mutations, to haematological disorders such as acute myeloid leukaemia (AML)/myelodysplastic syndrome (MDS), the increased incidence of death in CHIP carriers was actually attributed to atherosclerotic CVD [77,80-82]. Interestingly, a new proof-of-concept model using basic clinical and genetic data has been shown to predict the transformation of CHIP (also described as age-related clonal haematopoiesis) to AML or pre-AML [83,84]. This is significant as the individuals not likely to develop AML should perhaps be screened for the risk of developing CV complications, as CHIP carriers were on average 1.9-times more likely to develop CVD (with some mutations associated with a 12-fold increase). Moreover, the occurrence of CHIP was five-times more prevalent in participants who have a had an MI compared with healthy controls [77]. As this is a clonal outgrowth, the investigators elegantly showed that the proportion of cells carrying the mutant allele was correlated with proportion of increased CVD risk [77].

The most commonly reported somatic mutations are the DNA (cytosine-5-)-methyltransferase 3α (*DNMT3A*) and ten-eleven translocation 2 (*TET2)* loss-of-function mutations (based on recent evidence was likely under-reported [84]), followed by *ASKL1*, *JAK2*, *TP53*, and *SF3B1* mutations [77-80]. It is already known that JAK2 mutations, famous for causing essential thrombocythemia, promote atherothrombotic vascular disease [4]. However, recent murine studies have begun to prove causality of somatic mutations in the haematopoietic compartment in exacerbating atherosclerotic CVD [76,77]. $Ldlr^{-/-}$ mice with $Tet2^{-/-}$ BM exhibited almost a doubling in atheroma size in the descending aorta, compared with control [77]. Additionally, lesions from the aortic root from these mice lacking *Tet2* in the BM, also had exacerbated atherosclerotic lesion size and complexity observed with increased immune infiltrate [76,77]. These data were comparable with $Ldlr^{-/-}$ mice transplanted with conditional myeloid deletion (Lyz2-Cre) $Tet2^{-/-}$ BM, which highlights the central role of *Tet2* deficiency macrophages in exacerbating atherogenesis in this specific example of clonal haematopoiesis (Figure 1).

Interestingly, while inflammatory diseases present with leucocytosis, which drives atherogenesis, the association of CHIP with increased monocytes is contentious. Somewhat surprisingly, while the presence of CHIP in blood cells was associated with CVD in humans, this was not associated with changes in peripheral WBC counts (with the exception of JAK-2-driven CHIP) [77]. Correspondingly, the murine experiments using the $Tet2^{-/-}$ similarly did not detect changes in circulating WBC abundance [76,77]. In contrast, an elegant study comprehensively characterising *Tet2* loss by Moran-Crusio et al. [82], have indeed shown that *Tet2* loss leads to myeloid transformation that results in enhanced haematopoiesis and leucocytosis. In HSPCs, TET2-driven DNA demethylation is thought to inhibit stem cell renewal, and hence a loss of function mutation favors excessive proliferation. Employing chimera experiments, they show that $Tet2^{-/-}$ BM outcompetes WT BM, and results in increased HSPCs, myeloid skewing through GMPs, extramedullary haematopoiesis via the spleen and ultimately monocytosis [82]. However, this was only observed in older but not in younger (4–6 weeks old) $Tet2^{-/-}$ mice. Interestingly, a recent study using CRISPR-Cas9 to delete Tet-2 or Dnmt3a in lineage negative BM cells (i.e. stem and progenitor cells), found that now the Tet-2 mutated cells outcompeted WT cells to produce more WBCs (all lineages) and deletion of both genes enhanced angiotensin II-driven cardiac fibrosis [85]. These data, together with the increased prevalence of CHIP with the aging population, suggests these mutations are progressively acquired and gradually outcompete healthy stem cells to eventually evoke leucocytosis to the detriment of the CV system.

The atherogenic effects of clonal haematopoiesis have been linked to augmented macrophage inflammation and consequently increased monocyte recruitment into lesions. While there is limited information on inflammation and CHIP, it has been suggested that TET2 may act as a negative transcriptional regulator in response to inflammation [76]. Indeed, the loss of *Tet2* in macrophages results in an up-regulation of a whole host of inflammatory cytokines, chemokines as well as their receptors [77]. Additionally, circulating CXC chemokines CXCL1, CXCL2, and CXCL3 were elevated in the mice with $Tet2^{-/-}$ BM, which are known to play a role in monocyte adhesion to an activated endothelium [77], an important initial step in atherogenesis. Moreover, humans with a *TET2* mutation also had higher circulating levels of the CXC chemokine IL-8 levels [77]. *IL-6* mRNA expression and level also increased in $Tet2^{-/-}$ macrophages [76,77], however the atherogenic effects of murine clonal haematopoiesis were primarily attributed to increased plaque NLRP3 inflammasome activity and, ultimately, IL-1β production in lesions [76]. Subsequently, this may reveal a prominent role of CHIP in IL-1β-driven diseases that present with disordered haematopoiesis, and which carry a heightened CVD risk, which warrants further investigation.

IL-1β in CVD and haematopoiesis

Recently, the CANTOS trial revealed the benefits of the IL-1β neutralising antibody, canakinumab, in reducing CV events, in patients with a history of MI [43]. Additionally, IL-1β signalling has been reported to cause secondary MIs by promoting extramedullary haematopoiesis. While it is known that enhanced haematopoiesis drives atherosclerotic CVD, evidence also shows that stem cells mobilise into the circulation following an MI which further exacerbates CV risk [86,87]. Almost immediately following an MI, leucocytes which originate from both the circulation as well as storage organs such as the spleen, home to the infarct site. Due to the dramatic depletion of leucocytes from the circulation, this demands the induction of emergency haematopoiesis to restore leucocyte levels [87]. Recently, an elegant study using parabiosis experiments by Sager et al. [88] showed that IL-1β is released after an MI which directly homes the BM to induce haematopoiesis. Additionally, chronic IL-1β signalling was recently shown to drive myeloid differentiation in HSCs [89]. Inhibiting IL-1β signalling either with a BMT using $IL\text{-}1R1^{-/-}$ BM or utilising an IL-1β neutralising antibody, reduced extramedullary haematopoiesis after an MI [88]. Thus, it appears that IL-1β signalling is especially important in chronic inflammatory conditions that are associated with increased CVD and

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also exhibit enhanced or emergency or dysregulated haematopoiesis such as obesity, AML, RA and secondary CV events [4,28,47,58,88,90].

Concluding remarks

While it is well-known that the abundance of monocytes can drive atherosclerotic development; further studies have now highlighted additional mechanisms by which disordered haematopoiesis can exacerbate lesion development. The recent discovery of clonal haematopoiesis and CVD has emphasised the importance of inflammation in altered haematopoiesis and exacerbated atherosclerotic CVD. Indeed, over the last couple of decades the importance of inflammation-driven leucocytosis has been highlighted in many inflammatory diseases such as diabetes, obesity and RA. Collectively, studies including the CANTOS trial and TET2 mutations in clonal haematopoiesis highlight the pivotal role of IL-1β signalling in enhanced myelopoiesis and atherosclerotic CVD. Additionally, these studies suggest that inhibiting IL-1β signalling in patients with CHIP or other disease where IL-1β alters haematopoietic cells (i.e. obesity) may have the greatest CVD outcomes. Future studies should address what instigates the mutations reported in clonal haematopoiesis, and if current anti-inflammatory therapies are able to alter the CV outcome of CHIP carriers.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ABCA1, ATP-binding cassette A1; ABCG1, ATP-binding cassette G1; AML, acute myeloid leukaemia; Apo, apolipoprotein; BM, bone marrow; BMT, BM transplant; CANTOS, canakinumab anti-inflammatory thrombosis outcomes study; CHIP, clonal haematopoiesis of indeterminate potential; CMP, common myeloid progenitor; CV, cardiovascular; CVD, CV disease; DNMT3A, DNA (cytosine-5-)-methyltransferase 3α; GMP, granulocyte macrophage progenitor; GM-CSF, granulocyte-macrophage colony stimulating factor; HDL, high-density lipoprotein; HSPC, haematopoietic stem and progenitor cell; IL, interleukin; LDL, low density lipoprotein; LXR, liver X receptor; RA, rheumatoid arthritis; rHDL, reconstituted HDL; SLE, systemic lupus erythematosus; TET2, ten-eleven translocation 2; Tg, transgenic; WBC, white blood cell; WTD, western-type diet.

References

1 Bobryshev, Y.V. (2006) Monocyte recruitment and foam cell formation in atherosclerosis. *Micron* **37**, 208–222, https://doi.org/10.1016/j.micron.2005.10.007
2 Libby, P., Geng, Y.J., Aikawa, M., Schoenbeck, U., Mach, F., Clinton, S.K. et al. (1996) Macrophages and atherosclerotic plaque stability. *Curr. Opin. Lipidol.* **7**, 330–335, https://doi.org/10.1097/00041433-199610000-00012
3 Woollard, K.J. and Geissmann, F. (2010) Monocytes in atherosclerosis: subsets and functions. *Nat. Rev. Cardiol.* **7**, 77–86, https://doi.org/10.1038/nrcardio.2009.228
4 Murphy, A.J. and Tall, A.R. (2016) Disordered haematopoiesis and athero-thrombosis. *Eur. Heart J.* **37**, 1113–1121, https://doi.org/10.1093/eurheartj/ehv718
5 Johnsen, S.H., Fosse, E., Joakimsen, O., Mathiesen, E.B., Stensland-Bugge, E., Njolstad, I. et al. (2005) Monocyte count is a predictor of novel plaque formation: a 7-year follow-up study of 2610 persons without carotid plaque at baseline the Tromso Study. *Stroke* **36**, 715–719, https://doi.org/10.1161/01.STR.0000158909.07634.83
6 Olivares, R., Ducimetiere, P. and Claude, J.R. (1993) Monocyte count: a risk factor for coronary heart disease? *Am. J. Epidemiol.* **137**, 49–53, https://doi.org/10.1093/oxfordjournals.aje.a116601
7 Chapman, C.M., Beilby, J.P., McQuillan, B.M., Thompson, P.L. and Hung, J. (2004) Monocyte count, but not C-reactive protein or interleukin-6, is an independent risk marker for subclinical carotid atherosclerosis. *Stroke* **35**, 1619–1624, https://doi.org/10.1161/01.STR.0000130857.19423.ad
8 Passlick, B., Flieger, D. and Ziegler-Heitbrock, H.W. (1989) Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* **74**, 2527–2534
9 Auffray, C., Sieweke, M.H. and Geissmann, F. (2009) Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu. Rev. Immunol.* **27**, 669–692, https://doi.org/10.1146/annurev.immunol.021908.132557

10 Kashiwagi, M., Imanishi, T., Tsujioka, H., Ikejima, H., Kuroi, A., Ozaki, Y. et al. (2010) Association of monocyte subsets with vulnerability characteristics of coronary plaques as assessed by 64-slice multidetector computed tomography in patients with stable angina pectoris. *Atherosclerosis* **212**, 171–176, https://doi.org/10.1016/j.atherosclerosis.2010.05.004
11 Ylitalo, R., Oksala, O., Yla-Herttuala, S. and Ylitalo, P. (1994) Effects of clodronate (dichloromethylene bisphosphonate) on the development of experimental atherosclerosis in rabbits. *J. Lab. Clin. Med.* **123**, 769–776
12 Stoneman, V., Braganza, D., Figg, N., Mercer, J., Lang, R., Goddard, M. et al. (2007) Monocyte/macrophage suppression in CD11b diphtheria toxin receptor transgenic mice differentially affects atherogenesis and established plaques. *Circ. Res.* **100**, 884–893, https://doi.org/10.1161/01.RES.0000260802.75766.00
13 Rajavashisth, T., Qiao, J.H., Tripathi, S., Tripathi, J., Mishra, N., Hua, M. et al. (1998) Heterozygous osteopetrotic (op) mutation reduces atherosclerosis in LDL receptor- deficient mice. *J. Clin. Invest.* **101**, 2702–2710, https://doi.org/10.1172/JCI119891
14 Tacke, F., Alvarez, D., Kaplan, T.J., Jakubzick, C., Spanbroek, R., Llodra, J. et al. (2007) Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J. Clin. Invest.* **117**, 185–194, https://doi.org/10.1172/JCI28549
15 Dragoljevic, D., Kraakman, M.J., Nagareddy, P.R., Ngo, D., Shihata, W., Kammoun, H.L. et al. (2018) Defective cholesterol metabolism in haematopoietic stem cells promotes monocyte-driven atherosclerosis in rheumatoid arthritis. *Eur. Heart J.* **39**, 2158–2167
16 Robbins, C.S., Chudnovskiy, A., Rauch, P.J., Figueiredo, J.L., Iwamoto, Y., Gorbatov, R. et al. (2012) Extramedullary hematopoiesis generates Ly-6C (high) monocytes that infiltrate atherosclerotic lesions. *Circulation* **125**, 364–374, https://doi.org/10.1161/CIRCULATIONAHA.111.061986
17 Rahman, K., Vengrenyuk, Y., Ramsey, S.A., Vila, N.R., Girgis, N.M., Liu, J. et al. (2017) Inflammatory Ly6Chi monocytes and their conversion to M2 macrophages drive atherosclerosis regression. *J. Clin. Invest.* **127**, 2904–2915, https://doi.org/10.1172/JCI75005
18 Robbins, C.S., Hilgendorf, I., Weber, G.F., Theurl, I., Iwamoto, Y., Figueiredo, J.L. et al. (2013) Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat. Med.* **19**, 1166–1172, https://doi.org/10.1038/nm.3258
19 Murphy, A.J. and Tall, A.R. (2014) Proliferating macrophages populate established atherosclerotic lesions. *Circ. Res.* **114**, 236–238, https://doi.org/10.1161/CIRCRESAHA.113.302813
20 Randolph, G.J. (2013) Proliferating macrophages prevail in atherosclerosis. *Nat. Med.* **19**, 1094–1095, https://doi.org/10.1038/nm.3316
21 Drechsler, M., Megens, R.T., van Zandvoort, M., Weber, C. and Soehnlein, O. (2010) Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation* **122**, 1837–1845, https://doi.org/10.1161/CIRCULATIONAHA.110.961714
22 Huo, Y., Schober, A., Forlow, S.B., Smith, D.F., Hyman, M.C., Jung, S. et al. (2003) Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat. Med.* **9**, 61–67, https://doi.org/10.1038/nm810
23 Kraakman, M.J., Lee, M.K.S., Al-Sharea, A., Dragoljevic, D., Barrett, T.J., Montenont, E. et al. (2017) Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. *J. Clin. Invest.*, https://doi.org/10.1172/JCI92450
24 Murphy, A.J., Bijl, N., Yvan-Charvet, L., Welch, C.B., Bhagwat, N., Reheman, A. et al. (2013) Cholesterol efflux in megakaryocyte progenitors suppresses platelet production and thrombocytosis. *Nat. Med.* **19**, 586–594, https://doi.org/10.1038/nm.3150
25 Kim, C.H. (2010) Homeostatic and pathogenic extramedullary hematopoiesis. *J. Blood Med.* **1**, 13–19, https://doi.org/10.2147/JBM.S7224
26 King, K.Y. and Goodell, M.A. (2011) Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response. *Nat. Rev. Immunol.* **11**, 685–692, https://doi.org/10.1038/nri3062
27 Murray, P.J. and Wynn, T.A. (2011) Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* **11**, 723–737, https://doi.org/10.1038/nri3073
28 Nagareddy, P.R., Kraakman, M., Masters, S.L., Stirzaker, R.A., Gorman, D.J., Grant, R.W. et al. (2014) Adipose tissue macrophages promote myelopoiesis and monocytosis in obesity. *Cell Metab.* **19**, 821–835, https://doi.org/10.1016/j.cmet.2014.03.029
29 Nagareddy, P.R., Murphy, A.J., Stirzaker, R.A., Hu, Y., Yu, S., Miller, R.G. et al. (2013) Hyperglycemia promotes myelopoiesis and impairs the resolution of atherosclerosis. *Cell Metab.* **17**, 695–708, https://doi.org/10.1016/j.cmet.2013.04.001
30 Sokunbi, D.O., Wadhwa, N.K. and Suh, H. (1994) Vascular disease outcome and thrombocytosis in diabetic and nondiabetic end-stage renal disease patients on peritoneal dialysis. *Adv. Perit. Dial.* **10**, 77–80
31 Averill, L.E., Meagher, R.C. and Gerrity, R.G. (1989) Enhanced monocyte progenitor cell proliferation in bone marrow of hyperlipemic swine. *Am. J. Pathol.* **135**, 369–377
32 Murphy, A.J., Akhtari, M., Tolani, S., Pagler, T., Bijl, N., Kuo, C.L. et al. (2011) ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. *J. Clin. Invest.* **121**, 4138–4149, https://doi.org/10.1172/JCI57559
33 Swirski, F.K., Libby, P., Aikawa, E., Alcaide, P., Luscinskas, F.W., Weissleder, R. et al. (2007) Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J. Clin. Invest.* **117**, 195–205, https://doi.org/10.1172/JCI29950
34 Tolani, S., Pagler, T.A., Murphy, A.J., Bochem, A.E., Abramowicz, S., Welch, C. et al. (2013) Hypercholesterolemia and reduced HDL-C promote hematopoietic stem cell proliferation and monocytosis: studies in mice and FH children. *Atherosclerosis* **229**, 79–85, https://doi.org/10.1016/j.atherosclerosis.2013.03.031
35 Murphy, A.J., Funt, S., Gorman, D., Tall, A.R. and Wang, N. (2013) Pegylation of high-density lipoprotein decreases plasma clearance and enhances antiatherogenic activity. *Circ. Res.* **113**, e1–e9, https://doi.org/10.1161/CIRCRESAHA.113.301112
36 Yvan-Charvet, L., Pagler, T., Gautier, E.L., Avagyan, S., Siry, R.L., Han, S. et al. (2010) ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. *Science* **328**, 1689–1693, https://doi.org/10.1126/science.1189731
37 Christ, A., Gunther, P., Lauterbach, M.A.R., Duewell, P., Biswas, D., Pelka, K. et al. (2018) Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell* **172**, 162.e14–175.e14, https://doi.org/10.1016/j.cell.2017.12.013
38 Menu, P., Pellegrin, M., Aubert, J.F., Bouzourene, K., Tardivel, A., Mazzolai, L. et al. (2011) Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome. *Cell Death Dis.* **2**, e137, https://doi.org/10.1038/cddis.2011.18

39 Westerterp, M., Fotakis, P., Ouimet, M., Bochem, A.E., Zhang, H., Molusky, M.M. et al. (2018) Cholesterol efflux pathways suppress inflammasome activation, NETosis and atherogenesis. *Circulation*, https://doi.org/10.1161/CIRCULATIONAHA.117.032636
40 Tumurkhuu, G., Shimada, K., Dagvadorj, J., Crother, T.R., Zhang, W., Luthringer, D. et al. (2016) Ogg1-dependent DNA repair regulates NLRP3 itherosclerosis. *Circ. Res.* **119**, e76–e90, https://doi.org/10.1161/CIRCRESAHA.116.308362
41 Tumurkhuu, G., Dagvadorj, J., Porritt, R.A., Crother, T.R., Shimada, K., Tarling, E.J. et al. (2018) *Chlamydia pneumoniae* hijacks a host autoregulatory IL-1beta loop to drive foam cell formation and accelerate atherosclerosis. *Cell Metab.*, https://doi.org/10.1016/j.cmet.2018.05.027
42 Bochem, A.E., van der Valk, F.M., Tolani, S., Stroes, E.S., Westerterp, M. and Tall, A.R. (2015) Increased systemic and plaque inflammation in ABCA1 mutation carriers with attenuation by statins. *Arterioscler. Thromb. Vasc. Biol.* **35**, 1663–1669, https://doi.org/10.1161/ATVBAHA.114.304959
43 Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C. et al. (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.* **377**, 1119–1131, https://doi.org/10.1056/NEJMoa1707914
44 Bohula, E.A., Giugliano, R.P., Leiter, L.A., Verma, S., Park, J.G., Sever, P.S. et al. (2018) Inflammatory and cholesterol risk in the FOURIER trial. *Circulation* **138**, 131–140, https://doi.org/10.1161/CIRCULATIONAHA.118.034032
45 Koenig, W. (2018) Low-grade inflammation modifies cardiovascular risk even at very low LDL-C levels: are we aiming for a dual target concept? *Circulation* **138**, 150–153, https://doi.org/10.1161/CIRCULATIONAHA.118.035107
46 Pradhan, A.D., Aday, A.W., Rose, L.M. and Ridker, P.M. (2018) Residual inflammatory risk on treatment with PCSK9 inhibition and statin therapy. *Circulation* **138**, 141–149, https://doi.org/10.1161/CIRCULATIONAHA.118.034645
47 Dragoljevic, D., Kraakman, M.J., Nagareddy, P.R., Ngo, D., Shihata, W., Kammoun, H.L. et al. (2018) Defective cholesterol metabolism in haematopoietic stem cells promotes monocyte-driven atherosclerosis in rheumatoid arthritis. *Eur. Heart J.* **39**, 2158–2167, https://doi.org/10.1093/eurheartj/ehy119
48 Tall, A.R. and Yvan-Charvet, L. (2015) Cholesterol, inflammation and innate immunity. *Nat. Rev. Immunol.* **15**, 104–116, https://doi.org/10.1038/nri3793
49 Yvan-Charvet, L., Ranalletta, M., Wang, N., Han, S., Terasaka, N., Li, R. et al. (2007) Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J. Clin. Invest.* **117**, 3900–3908
50 Westerterp, M., Bochem, A.E., Yvan-Charvet, L., Murphy, A.J., Wang, N. and Tall, A.R. (2014) ATP-binding cassette transporters, atherosclerosis, and inflammation. *Circ. Res.* **114**, 157–170, https://doi.org/10.1161/CIRCRESAHA.114.300738
51 Bradley, M.N., Hong, C., Chen, M., Joseph, S.B., Wilpitz, D.C., Wang, X. et al. (2007) Ligand activation of LXR beta reverses atherosclerosis and cellular cholesterol overload in mice lacking LXR alpha and apoE. *J. Clin. Invest.* **117**, 2337–2346, https://doi.org/10.1172/JCI31909
52 Tangirala, R.K., Bischoff, E.D., Joseph, S.B., Wagner, B.L., Walczak, R., Laffitte, B.A. et al. (2002) Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 11896–11901, https://doi.org/10.1073/pnas.182199799
53 Out, R., Hoekstra, M., Habets, K., Meurs, I., de Waard, V., Hildebrand, R.B. et al. (2008) Combined deletion of macrophage ABCA1 and ABCG1 leads to massive lipid accumulation in tissue macrophages and distinct atherosclerosis at relatively low plasma cholesterol levels. *Arterioscler. Thromb. Vasc. Biol.* **28**, 258–264, https://doi.org/10.1161/ATVBAHA.107.156935
54 Kappus, M.S., Murphy, A.J., Abramowicz, S., Ntonga, V., Welch, C.L., Tall, A.R. et al. (2014) Activation of liver X receptor decreases atherosclerosis in Ldlr (-)/(-) mice in the absence of ATP-binding cassette transporters A1 and G1 in myeloid cells. *Arterioscler. Thromb. Vasc. Biol.* **34**, 279–284, https://doi.org/10.1161/ATVBAHA.113.302781
55 Forcheron, F., Legedz, L., Chinetti, G., Feugier, P., Letexier, D., Bricca, G. et al. (2005) Genes of cholesterol metabolism in human atheroma: overexpression of perilipin and genes promoting cholesterol storage and repression of ABCA1 expression. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1711–1717, https://doi.org/10.1161/01.ATV.0000174123.19103.52
56 Joseph, S.B., McKilligin, E., Pei, L., Watson, M.A., Collins, A.R., Laffitte, B.A. et al. (2002) Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 7604–7609, https://doi.org/10.1073/pnas.112059299
57 Westerterp, M., Gourion-Arsiquaud, S., Murphy, A.J., Shih, A., Cremers, S., Levine, R.L. et al. (2012) Regulation of hematopoietic stem and progenitor cell mobilization by cholesterol efflux pathways. *Cell Stem Cell* **11**, 195–206, https://doi.org/10.1016/j.stem.2012.04.024
58 Murphy, A.J., Dragoljevic, D. and Tall, A.R. (2014) Cholesterol efflux pathways regulate myelopoiesis: a potential link to altered macrophage function in atherosclerosis. *Front. Immunol.* **5**, 490, https://doi.org/10.3389/fimmu.2014.00490
59 Terasaka, N., Hiroshima, A., Koieyama, T., Ubukata, N., Morikawa, Y., Nakai, D. et al. (2003) T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. *FEBS Lett.* **536**, 6–11, https://doi.org/10.1016/S0014-5793(02)03578-0
60 Teupser, D., Kretzschmar, D., Tennert, C., Burkhardt, R., Wilfert, W., Fengler, D. et al. (2008) Effect of macrophage overexpression of murine liver X receptor-alpha (LXR-alpha) on atherosclerosis in LDL-receptor deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **28**, 2009–2015, https://doi.org/10.1161/ATVBAHA.108.175257
61 Feig, J.E., Pineda-Torra, I., Sanson, M., Bradley, M.N., Vengrenyuk, Y., Bogunovic, D. et al. (2010) LXR promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression. *J. Clin. Invest.* **120**, 4415–4424, https://doi.org/10.1172/JCI38911
62 Castrillo, A., Joseph, S.B., Marathe, C., Mangelsdorf, D.J. and Tontonoz, P. (2003) Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J. Biol. Chem.* **278**, 10443–10449, https://doi.org/10.1074/jbc.M213071200
63 Yu, M., Amengual, J., Menon, A., Kamaly, N., Zhou, F., Xu, X. et al. (2017) Targeted nanotherapeutics encapsulating liver X receptor agonist GW3965 enhance antiatherogenic effects without adverse effects on hepatic lipid metabolism in Ldlr−/− mice. *Adv. Healthcare Mater.* **6**, https://doi.org/10.1002/adhm.201700313
64 van Halm, V.P., Peters, M.J., Voskuyl, A.E., Boers, M., Lems, W.F., Visser, M. et al. (2009) Rheumatoid arthritis versus diabetes as a risk factor for cardiovascular disease: a cross-sectional study, the CARRE Investigation. *Ann. Rheum. Dis.* **68**, 1395–1400, https://doi.org/10.1136/ard.2008.094151

65 Meune, C., Touze, E., Trinquart, L. and Allanore, Y. (2010) High risk of clinical cardiovascular events in rheumatoid arthritis: Levels of associations of myocardial infarction and stroke through a systematic review and meta-analysis. *Arch. Cardiovasc. Dis.* **103**, 253–261, https://doi.org/10.1016/j.acvd.2010.03.007
66 Kumeda, Y., Inaba, M., Goto, H., Nagata, M., Henmi, Y., Furumitsu, Y. et al. (2002) Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis. *Arthritis Rheum.* **46**, 1489–1497, https://doi.org/10.1002/art.10269
67 Ogdie, A., Yu, Y., Haynes, K., Love, T.J., Maliha, S., Jiang, Y. et al. (2015) Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. *Ann. Rheum. Dis.* **74**, 326–332, https://doi.org/10.1136/annrheumdis-2014-205675
68 Chung, C.P., Giles, J.T., Petri, M., Szklo, M., Post, W., Blumenthal, R.S. et al. (2012) Prevalence of traditional modifiable cardiovascular risk factors in patients with rheumatoid arthritis: comparison with control subjects from the multi-ethnic study of atherosclerosis. *Semin. Arthritis Rheum.* **41**, 535–544, https://doi.org/10.1016/j.semarthrit.2011.07.004
69 del Rincon, I.D., Williams, K., Stern, M.P., Freeman, G.L. and Escalante, A. (2001) High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum.* **44**, 2737–2745, https://doi.org/10.1002/1529-0131(200112)44:12%3c2737::AID-ART460%3e3.0.CO;2-
70 Charles-Schoeman, C., Lee, Y.Y., Grijalva, V., Amjadi, S., FitzGerald, J., Ranganath, V.K. et al. (2012) Cholesterol efflux by high density lipoproteins is impaired in patients with active rheumatoid arthritis. *Ann. Rheum. Dis.* **71**, 1157–1162, https://doi.org/10.1136/annrheumdis-2011-200493
71 Voloshyna, I., Modayil, S., Littlefield, M.J., Belilos, E., Belostocki, K., Bonetti, L. et al. (2013) Plasma from rheumatoid arthritis patients promotes pro-atherogenic cholesterol transport gene expression in THP-1 human macrophages. *Exp. Biol. Med. (Maywood, NJ)* **238**, 1192–1197, https://doi.org/10.1177/1535370213503262
72 Ronda, N., Favari, E., Borghi, M.O., Ingegnoli, F., Gerosa, M., Chighizola, C. et al. (2014) Impaired serum cholesterol efflux capacity in rheumatoid arthritis and systemic lupus erythematosus. *Ann. Rheum. Dis.* **73**, 609–615, https://doi.org/10.1136/annrheumdis-2012-202914
73 Schuettpelz, L.G. and Link, D.C. (2013) Regulation of hematopoietic stem cell activity by inflammation. *Front. Immunol.* **4**, 204, https://doi.org/10.3389/fimmu.2013.00204
74 Westerterp, M., Gautier, E.L., Ganda, A., Molusky, M.M., Wang, W., Fotakis, P. et al. (2017) Cholesterol accumulation in dendritic cells links the inflammasome to acquired immunity. *Cell Metab.* **25**, 1294.e6–1304.e6, https://doi.org/10.1016/j.cmet.2017.04.005
75 Voloshyna, I., Teboul, I., Littlefield, M.J., Siegart, N.M., Turi, G.K., Fazzari, M.J. et al. (2016) Resveratrol counters systemic lupus erythematosus-associated atherogenicity by normalizing cholesterol efflux. *Exp. Biol. Med. (Maywood, NJ)* **241**, 1611–1619, https://doi.org/10.1177/1535370216647181
76 Fuster, J.J., MacLauchlan, S., Zuriaga, M.A., Polackal, M.N., Ostriker, A.C., Chakraborty, R. et al. (2017) Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* **355**, 842–847, https://doi.org/10.1126/science.aag1381
77 Jaiswal, S., Natarajan, P., Silver, A.J., Gibson, C.J., Bick, A.G., Shvartz, E. et al. (2017) Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N. Engl. J. Med.* **377**, 111–121, https://doi.org/10.1056/NEJMoa1701719
78 Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F. et al. (2014) Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* **371**, 2477–2487, https://doi.org/10.1056/NEJMoa1409405
79 Zink, F., Stacey, S.N., Norddahl, G.L., Frigge, M.L., Magnusson, O.T., Jonsdottir, I. et al. (2017) Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* **130**, 742–752, https://doi.org/10.1182/blood-2017-02-769869
80 Jaiswal, S., Fontanillas, P., Flannick, J., Manning, A., Grauman, P.V., Mar, B.G. et al. (2014) Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* **371**, 2488–2498, https://doi.org/10.1056/NEJMoa1408617
81 Xie, M., Lu, C., Wang, J., McLellan, M.D., Johnson, K.J., Wendl, M.C. et al. (2014) Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med.* **20**, 1472–1478, https://doi.org/10.1038/nm.3733
82 Moran-Crusio, K., Reavie, L., Shih, A., Abdel-Wahab, O., Ndiaye-Lobry, D., Lobry, C. et al. (2011) Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **20**, 11–24, https://doi.org/10.1016/j.ccr.2011.06.001
83 Abelson, S., Collord, G., Ng, S.W.K., Weissbrod, O., Mendelson Cohen, N., Niemeyer, E. et al. (2018) Prediction of acute myeloid leukaemia risk in healthy individuals. *Nature*, https://doi.org/10.1038/s41586-018-0317-6
84 Desai, P., Mencia-Trinchant, N., Savenkov, O., Simon, M.S., Cheang, G., Lee, S. et al. (2018) Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nat. Med.* **24**, 1015–1023, https://doi.org/10.1038/s41591-018-0081-z
85 Sano, S., Ohshima, K., Wang, Y., Katanasaka, Y., Sano, M. and Walsh, K. (2018) CRISPR-mediated gene editing to assess the roles of Tet2 and Dnmt3a in clonal hematopoiesis and cardiovascular disease. *Circ. Res.*, https://doi.org/10.1161/CIRCRESAHA.118.313225
86 Massa, M., Rosti, V., Ferrario, M., Campanelli, R., Ramajoli, I., Rosso, R. et al. (2005) Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood* **105**, 199–206, https://doi.org/10.1182/blood-2004-05-1831
87 Dutta, P., Courties, G., Wei, Y., Leuschner, F., Gorbatov, R., Robbins, C.S. et al. (2012) Myocardial infarction accelerates atherosclerosis. *Nature* **487**, 325–329, https://doi.org/10.1038/nature11260
88 Sager, H.B., Heidt, T., Hulsmans, M., Dutta, P., Courties, G., Sebas, M. et al. (2015) Targeting interleukin-1β reduces leukocyte production after acute myocardial infarction. *Circulation*, https://doi.org/10.1161/CIRCULATIONAHA.115.016160
89 Pietras, E.M., Mirantes-Barbeito, C., Fong, S., Loeffler, D., Kovtonyuk, L.V., Zhang, S. et al. (2016) Chronic interleukin-1 drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nat. Cell Biol.* **18**, 607–618, https://doi.org/10.1038/ncb3346
90 Carey, A., Edwards, D.K., Eide, C.A., Newell, L., Traer, E., Medeiros, B.C. et al. (2017) Identification of interleukin-1 by functional screening as a key mediator of cellular expansion and disease progression in acute myeloid leukemia. *Cell Rep.* **18**, 3204–3218, https://doi.org/10.1016/j.celrep.2017.03.018