Is the risk of cardiovascular disease altered with anti-inflammatory therapies? Insights from rheumatoid arthritis

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Cardiovascular disease (CVD) remains the leading cause of mortality worldwide. Atherosclerosis is the most common form of CVD, which is complex and multifactorial with an elevated risk observed in people with either metabolic or inflammatory diseases. Accumulating evidence now links obesity with a state of chronic low-grade inflammation and has renewed our understanding of this condition and its associated comorbidities. An emerging theme linking disease states with atherosclerosis is the increased production of myeloid cells, which can initiate and exacerbate atherogenesis. Although anti-inflammatory drug treatments exist and have been successfully used to treat inflammatory conditions such as rheumatoid arthritis (RA), a commonly observed side effect is dyslipidemia, inadvertently, a major risk factor for the development of atherosclerosis. The mechanisms leading to dyslipidemia associated with anti-inflammatory drug use and whether CVD risk is actually increased by this dyslipidemia are of great therapeutic importance and currently remain poorly understood. Here we review recent data providing links between inflammation, hematopoiesis, dyslipidemia and CVD risk in the context of anti-inflammatory drug use.

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Cardiovascular disease (CVD) is currently the leading cause of death worldwide. Atherosclerosis is the major form of CVD and is characterized by a chronic inflammatory build up, driven largely by lipid accumulation within the artery wall. Unlike acute inflammation, atherosclerosis is hallmarked by a state of unresolved low-grade chronic inflammation. Importantly, low-grade inflammation is also a feature of several diseases known to increase the risk of CVD. Obesity is a prime example of a low-grade chronic inflammatory disease that can promote insulin resistance and type 2 diabetes (T2D), and can increase the risk of CVD.1 Indeed, people with T2D have up to fourfold the risk of developing CVD compared with non-diabetic individuals. Hence, strategies targeting insulin resistance and glucose homeostasis through inflammation modulation or other mechanisms are important for the treatment of CVD. Conversely, therapies that are associated with triggering known CV risk factors including weight gain, insulin resistance and dyslipidemia are met with caution.

There has been a significant amount of research examining the immunological changes that occur within metabolically important tissues such as the adipose tissue, liver, muscle and the atherosclerotic lesion during CVD and T2D. In addition, recent advances have highlighted a significant role of the hematopoietic system in the context of metabolic diseases, which likely contributes to the elevated CVD risk due to an increase in production of white blood cells (WBCs) that feed the atherosclerotic lesion.2 In this review we will briefly outline the current understanding of the inflammatory processes linked with metabolic diseases. We will also discuss how the use of current and potential anti-inflammatory therapies in the treatment of inflammatory diseases, such as rheumatoid arthritis (RA; a pathology associated with an increased risk of CVD), alters metabolic pathways particularly in relation to cholesterol homeostasis and whether this influences CVD risk.

TARGETING INFLAMMATION FOR THE TREATMENT OF INSULIN RESISTANCE AND CVD: CURRENT THERAPIES

Since the seminal finding that tumor necrosis factor (TNF)-α causes metabolic dysfunction and the discovery of macrophages within the obese adipose tissue,3 it has become apparent that most cells of the innate and acquired immune systems are altered in obesity.1 Adipose tissue pro-inflammatory macrophages have received the greatest amount of attention, as these are the predominant leukocytes that accumulate in obese adipose tissue. These CD11c+ F4/80+ macrophages appear to be one of the main sources of the elevated cytokines TNF-α, interleukin (IL)-6 and IL-1β observed in obesity and thought to directly contribute to insulin resistance both locally and in peripheral tissues through the activation of stress-signaling pathways such as Janus N-terminal Kinase (JNK) and IkB Kinase.3 Whereas the

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activation of inflammatory pathways leading to insulin resistance and enhanced atherosclerosis has been demonstrated consistently in rodent models, it is less clear whether insulin resistance and CVD can be targeted therapeutically with anti-inflammatory drugs in humans. To date, several clinical trials have attempted to address this proposition with various anti-inflammatory regimes with varying success. We have summarized the key findings from some of these anti-inflammatory trials described below.

### Aspirin/salsalate
Aspirin (acetylated salicylate) is an anti-inflammatory drug, which inhibits cyclo-oxygenase (Cox) enzymes Cox-1 and Cox-2 in the prostaglandin synthesis pathway and at higher doses inhibits the IkB Kinase-β/NF-κB pathway. Aspirin reduces CVD risk by altering platelet reactivity and preventing clot formation, but also appears to lower CVD risk through decreased levels of C-reactive protein. Unlike aspirin, salsalate is a non-acetylated salicylate and, as such, is not a cyclo-oxygenase inhibitor, nor does it induce hemostasis, but it works through inhibition of the NFκB pathway. Nonetheless, salsalate is still an effective anti-inflammatory treatment option. In the Targeting Inflammation using Salsalate in T2D (TINSAL-T2D) clinical trial, treatment of patients with T2D with salsalate consistently lowers fasting glycemia and/or Hba1c levels. It must be highlighted that, in these trials, salsalate impaired insulin clearance; thus, tissue exposure to circulating insulin was prolonged and could account for at least some of the improvements in glycemic control. However, a more recent 12-week salsalate clinical trial was found not to improve insulin sensitivity in people with glucose intolerance despite reducing fasting glycemia.

### Anti-cytokine therapies
The cytokines IL-1β and TNF-α have been therapeutically targeted in clinical trials for the treatment of insulin resistance.

Anakinra, a recombinant IL-1Ra (IL-1 receptor antagonist) molecule, has been effective in treating a wide range of inflammatory conditions. In addition, specific anti-IL-1b monoclonal antibodies such as canakunimab appear equally efficacious. Consistent with its appreciated role in preserving pancreatic β-cell function, the blockade of IL-1 signaling with anakinra reduced glucose intolerance in obese mice. Importantly, it also improved glucose metabolism in people with the metabolic syndrome or T2D by enhancing pancreatic function and not necessarily by insulin sensitivity. Furthermore, large clinical trials such as the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) have been initiated to assess whether IL-1β blockade will improve cardiovascular (CV) outcomes in patients with T2D.

TNF-α has been shown to induce insulin resistance in animal models. However, translating these findings into the clinic is yet to show any efficacy. In people with T2D or the metabolic syndrome, TNF-α neutralization failed to improve insulin sensitivity. Un fortunately, many of these studies contained major limitations such as an insufficient statistical power and short study duration, which potentially explains their lack of impact. However, a longer study in obese, insulin-resistant (non-diabetic) subjects revealed that TNF-α antagonism reduced fasting glycemia. Whether improvements in metabolism in these studies are because of a direct effect or whether they are because the underlying disease burden is reduced remains unknown. More studies are warranted to assess whether TNF-α remains a valuable target for metabolic diseases and their associated complications.

### Peroxisome Proliferator Activator Receptor gamma agonism
Thiazolidinediones (TZDs) such as rosiglitazone and pioglitazone are insulin-sensitizing Peroxisome Proliferator Activator Receptor gamma (PPARy) agonists. The complete actions of PPARy with respect to metabolism remain incompletely understood. However, TZDs are currently thought to improve insulin sensitivity by reducing lipotoxicity in tissues such as the liver and skeletal muscle, in addition to promoting lipid partitioning into adipocytes. It has become well appreciated that TZDs also elicit some anti-inflammatory effects pertaining to reductions in macrophages and/or stimulation of regulatory T cells within the adipose tissue as well as an alteration in circulating monocytes and immunomodulatory proteins. However, TZDs have also been associated with weight gain, bone fractures and heart failure because of an appearance of cardiac edema, making it a drug to prescribe with caution according to the patient history.

### CAN ANTI-INFLAMMATORY THERAPIES FOR THE TREATMENT OF METABOLIC DISEASES ALSO PROMOTE DYSLIPIDEMIA?
The number of clinical studies targeting aspects of inflammation for the treatment of metabolic diseases continues to expand. However, it is becoming a frequent observation that some anti-inflammatory treatments can cause perturbations in cholesterol homeostasis. Notwithstanding the potential benefits on fasting glycemia, salasalate treatment consistently appears to increase either low-density lipoprotein (LDL) or total cholesterol levels. Furthermore, in patients with T2D, treatment with TZDs also appears to alter circulating cholesterol concentrations and lipoprotein particle size, which may be specific to the type of TZD used. For example, rosiglitazone seems to increase total and LDL cholesterol, whereas pioglitazone does not alter total or LDL cholesterol and increases high-density lipoprotein (HDL).

In preclinical models of diabetes and atherosclerosis, rosiglitazone does appear to reduce CVD. Surprisingly, there is a lack of data in the context of diabetes regarding the effect of TNF-α blockade on cholesterol homeostasis in humans with metabolic diseases. Studies either do not report cholesterol levels or report unaltered levels or report unaltered levels or report unaltered levels or report unaltered levels or report unaltered levels or report unaltered levels. Blocking TNF-α activity in patients with RA also appears to improve indices of metabolism while concurrently increasing cholesterol levels. Recently, researchers from the Interleukin-1 Genetics Consortium analyzed genetic variants involved in IL-1Ra production to assess CV risk associated with long-term IL-1 inhibition. This consortium generated a genetic score for IL-1Ra gene variants and, surprisingly, the genetic score predicted a greater risk for coronary heart disease with increasing IL-1Ra concentration. Interestingly, the genetic score was also associated with increases in circulating LDL cholesterol, total cholesterol and triglycerides. Even though this study does not allow for the discrimination between IL-1α and IL-1β signaling, it is interesting to note that canakinumab—allowing for specific IL-1β antagonism—was recently shown to increase triglycerides (but not LDL cholesterol). Taken together, these data reveal that alterations in cholesterol homeostasis can be a side effect of treatments that target inflammation in people with metabolic diseases (Table 1).

### DYSLIPIDEMIA AND CVD RISK IN INFLAMMATORY DISEASES: THE EXAMPLE OF RA
CV risk and dyslipidemia are elevated in patients with inflammatory conditions such as chronic kidney disease, recurrent infections, myeloproliferative neoplasms, RA and systemic lupus erythematosus. However, unlike traditional metabolic diseases in which elevated CVD risk is associated with elevated cholesterol, in diseases such as RA,
cancer, sepsis and immediately post myocardial infarction, the relationship between elevated CVD risk and cholesterol is less clear. Often in these patients, increased C-reactive protein (indicative of CVD risk) is associated with reduced circulating cholesterol. This inverse relation between elevated CVD risk and cholesterol is less clear. Often in cancer, sepsis and immediately post myocardial infarction, the relationship between increased CVD risk and reduced lipids is termed the lipid paradox. Using RA as an example inflammatory disease that is associated with a two- to threefold elevated risk of CVD, many studies have found cholesterol levels to be increased with the successful treatment of RA with anti-inflammatory drugs, suggesting a link between inflammation and cholesterol homeostasis. Hence, anti-inflammatory treatments in people with RA allow for a better understanding of the cross-talk between inflammation and CVD risk and are an ideal example to discuss in the context of this review. Below we have summarized the data from some of the more recent targeted anti-inflammatories used in RA and their metabolic effects.

**Disease-modifying anti-rheumatic drugs**

Methotrexate is the frontline treatment from all existing disease-modifying anti-rheumatic drugs and has been observed to increase cholesterol and triglyceride levels in patients with RA. Importantly, CVD risk appears to be reduced with methotrexate and, although the mechanism by which this occurs is not well understood, it is likely to be through a reduction in inflammation.

**TNF-α antagonists**

TNF-α antagonism in RA patients is associated with significant alterations in circulating lipids. From meta-analyses, it is generally reported that increases in total, LDL and HDL cholesterol and triglycerides occur with TNF-α antagonism. Despite changes in circulating lipids, a meta-analysis of 3 randomized control trials and 13 observational cohort studies found that anti-TNF-α therapy in RA patients was associated with a reduction in the risk of all CV events, including myocardial infarction and stroke in the cohort studies but not the randomized clinical trials, probably because of being statistically underpowered. In patients with heart failure, however, blockade of TNF-α with infliximab adversely affected patient outcomes. More recently, data from a German biologics registry revealed reduced mortality rates in RA patients treated with anti-TNF-α compared with those treated with conventional disease-modifying anti-rheumatic drugs. However, whether the reduction in mortality was due to a reduction in CVD was not specifically assessed in this study.

### IL-6 receptor blockade

Targeting IL-6 in RA is achieved with tocilizumab, an anti-IL-6 receptor monoclonal antibody. Although tocilizumab is generally well tolerated and is efficacious in reducing disease severity, it also causes significant perturbations to lipid and cholesterol homeostasis such as increases in both LDL and total cholesterol. IL-6 biology is complex with signaling permitted through a membrane-anchored (leading to classical signaling) or soluble IL-6 receptor (termed IL-6 trans-signaling). A naturally occurring soluble glycoprotein 130 (sgp130) molecule specifically antagonizes IL-6 trans-signaling, and work over the past decade has established IL-6 trans-signaling as the major inflammatory signaling paradigm in IL-6-associated pathologies. Indeed, blockade of IL-6 trans-signaling with sgp130 was able to reduce inflammation and disease severity in experimental models of arthritis. Whether the blockade of IL-6 trans-signaling alters circulating lipids in the context of RA remains unknown. However, transgenic mice that overexpress a human sgp130Fc molecule display an IL-6 trans-signaling ‘knockout’ phenotype. These sgp130Fc transgenic mice are protected from obesity-induced white adipose tissue macrophage accumulation without having an impact on circulating lipids. Furthermore, sgp130Fc administration reduces atherosclerosis in high-fat-fed LDLr— mice without altering body mass or the blood lipid profile, suggesting that sgp130Fc may be a therapeutic target for the treatment of atherosclerosis. As bacterial infections and hyperlipidemia are major consequences of global IL-6 blockade in RA patients treated with tocilizumab, it is hypothesized that IL-6 trans-signaling antagonism with sgp130Fc may provide a more specific treatment option.

**JAK inhibitors**

Tofacitinib, a janus kinase inhibitor (JAK), has been developed as an orally administered anti-inflammatory. In adjuvant-induced arthritic rats, tofacitinib successfully reduced disease severity but also induced hypercholesterolemia. Recently, tofacitinib was Food And Drug Administration-approved for the treatment of RA. Similar to rodents, JAK inhibition in humans also increases circulating cholesterol.

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**Table 1 Current anti-inflammatory therapeutics and their CVD impact**

| Drug            | Disease | Target                  | Mode of action                                                                 | Metabolic impact | CVD risk | References |
|-----------------|---------|-------------------------|-------------------------------------------------------------------------------|-----------------|----------|------------|
| Salsalate       | IR/T2D  | NFκB pathway            | Inhibition of the NFκB pathway                                                | ↑ LDL ↑ TC      | Unknown  | 10,11,13,27,28 |
| Thiazolidinediones | IR/T2D  | PPARγ                   | PPARγ agonist                                                                 | ↑ Weight ↑ LDL ↑ TC ↑ HDL | ↑↑       | 29-32      |
| Anakinra        | T2D     | IL-1 receptor           | Recombinant IL-1R antagonist                                                  | ↓→ LDL          | Unknown  | 15         |
| Canakinumab     | T2D     | IL-1β                   | Anti IL-1β monoclonal antibody                                                | ↑ TG            | Unknown  | 39         |
| Methotrexate    | RA      | T cells/B cells         | Multiple mechanisms including inhibition of folate pathway/purine metabolism/T-cell activation/IL-1β/IL-1R interaction | ↑ LDL ↑ HDL ↑ TC | ↓         | 40,41      |
| Infliximab      | RA      | TNF-α signaling         | Anti TNF monoclonal antibody                                                  | ↑ TC ↓ LDL ↑ HDL ↑ TG | ↓↑ → | 42-46      |
| Tocilizumab     | RA      | IL-6 receptor           | Anti IL-6R monoclonal antibody                                                | ↑ TC ↓ LDL      | ↓        | 47-51,68   |
| Sgp130Fc        | RA      | IL-6                    | Inhibits soluble form of IL-6                                                  | Unknown         | Unknown  |            |
| Rituximab       | RA      | B cells                 | Anti-CD20 monoclonic antibody                                                 | ↑ TC ↓ LDL      | →        | 60-63      |
| Tofacitinib     | RA      | JAK-STAT signaling      | Inhibitor of the JAK1 and 3 kinases                                           | ↑ TC             | Unknown  | 58,59      |

Abbreviations: CVD, cardiovascular disease; HDL, high-density lipoprotein; IL, interleukin; JAK, Janus N-terminal Kinase; IR, insulin resistance; LDL, low-density lipoprotein; PPARγ, Peroxisome Proliferator Activator Receptor gamma; RA, rheumatoid arthritis; TC, total cholesterol; T2D, type 2 diabetes; TG, triglycerides; TNF-α, tumor necrosis factor-α.
hypercholesterolemia observed with tofacitinib is similar to that observed by IL-6 blockade with tocilizumab, which is perhaps not surprising, given that the JAK signaling pathway is activated by IL-6.

**B-cell therapies**

Rituximab is a monoclonal antibody that depletes B cells by targeting CD20, and it appears to be a promising therapeutic for the treatment of RA. Administration of rituximab can achieve sustained reductions in inflammation; however, this is at the expense of increasing total and LDL cholesterol. However, alterations in cholesterol are not always consistent with rituximab. Pooled data from clinical trials have revealed that people with RA treated with rituximab do not have an increased CVD risk.

Taken together, these studies reveal that significant perturbations in lipid homeostasis occur in RA patients successfully treated with anti-inflammatory therapies. However, despite the increase in circulating lipids, it is generally accepted that CVD risk is either reduced or not affected (Table 1). Interestingly, it has been observed that in people who develop RA (compared with those who do not), a significant reduction in total and LDL cholesterol occurs in the 5 years preceding RA diagnosis. As such, it is now thought that the increase in cholesterol levels with anti-inflammatory treatments reflects a normalization of lipid levels to those seen in the general population, potentially explaining why CVD is not elevated in successfully treated patients. This also highlights the fact that in RA patients low levels of LDL and total cholesterol are associated with worsened CV outcomes, challenging the dogma that cholesterol levels are a reflection of the CVD risk.

**ANTI-INFLAMMATORY THERAPIES AND DYSLIPIDEMIA: DISSECTING THE MECHANISMS**

**Association between anti-inflammatory therapies and changes in lipoprotein subclasses**

A critical question that remains is whether the reduction in CVD risk is because of beneficial alterations in composition and subclasses of lipids or inflammation reduction per se. Recent evidence from the clinical trial MEASURE suggests that IL-6 blockade with tocilizumab induces beneficial alterations intrinsic to the cholesterol subfractions. In this trial, plasma lipid subfractions from RA patients treated with methotrexate alone, or with add on tocilizumab for 12 weeks, were analyzed using nuclear magnetic resonance. Despite increasing total and LDL cholesterol, the concentration of the pro-atherogenic small, dense LDL particles was increased with tocilizumab treatment. Although levels of HDL cholesterol concentration were unchanged with tocilizumab treatment, the HDL particles themselves shifted toward an anti-inflammatory phenotype, a result consistent with TNF-α antagonism. Furthermore, several proxy markers of vascular risk such as Lipoprotein(a), D-Dimer and fibrinogen were reduced in RA patients treated with tocilizumab. In a comprehensive review of the long-term safety profile of tocilizumab, data from over 4000 patients collected from five randomized, controlled tocilizumab trials were analyzed. With a mean follow-up of 4.6 years, tocilizumab improved disease severity, with no significant safety concerns over and above the ‘all-control’ population. Furthermore, tocilizumab did not increase the carotid intima-media thickness, suggesting that the hypercholesterolemia associated with IL-6 blockade does not increase CVD risk. Although these findings taken together are promising, they do not prove that CVD risk is reduced per se with tocilizumab treatment. Similar changes in HDL phenotypes were observed in the HDL particles in responders to rituximab treatment. Interestingly, the major lipoprotein of HDL, apoA-I, has potent anti-inflammatory anti-atherogenic effects, and recent evidence suggests that apoB/apoA-I ratio is a stronger predictor of CVD risk than lipid levels. These observations add credence to the notion that inflammation is the major driver of atherogenesis and CVD risk in RA. However, this hypothesis is yet to be tested formally as confounding influences such as previous drug treatments and lipid-lowering effects of other anti-inflammatory therapies can influence the contribution of CVD risk attributable to inflammation alone.

**Hematopoiesis, the missing link?**

How circulating lipids are altered by the modulation of inflammation remains incompletely understood. Several mechanisms have been proposed to explain how cholesterol levels may be reduced in states of high inflammation including enhanced hepatic LDL uptake or an impairment in cholesterol efflux from foam cells, both mechanisms being influenced by the immune system. Indeed, cells from adaptive immunity have been found to interact with cholesterol metabolism. Klingenberg et al. showed that regulatory T cells can affect lipid levels via modulation of the liver transcriptome. This leads to reduced clearance of very-low-density lipoprotein and chylomicron remnants, which ultimately increased atherosclerotic lesion formation. Moreover, recent work from the McInnes laboratory has also begun to shed some light on this topic. Using isotopically labeled cholesterol and leucine infusions, cholesterol and lipoprotein kinetics were determined in people with RA before and after 6 weeks of tofacitinib treatment and were compared with non-treated matched healthy controls. It was revealed that the reduced cholesterol levels observed in RA were because of increased cholsterol ester catabolism. Interestingly, tofacitinib administration increased cholesterol levels to those of the healthy controls. However, they did not elucidate in this study the mechanism by which cholesterol catabolism is enhanced in the context of RA. In this review, we propose that alterations in hematopoiesis could be responsible for enhanced cholesterol catabolism, which will be discussed below.

**CVD RISK AND INFLAMMATION: FOCUS ON ENHANCED HEMATOPOIESIS**

**Hematopoiesis in CVD**

Hematopoiesis is the process by which blood cells are formed, and it occurs primarily within the bone marrow after birth. Hematopoiesis is a highly regulated, hierarchical and efficient process that is able to produce millions of cells daily to maintain the immune system. Hematopoietic stem and multipotent progenitor cells (HSPCs) are extremely sensitive to extrinsic cues from endocrine factors such as cytokines and chemokines as well as signals from adjacent cells within the bone marrow niche such as endothelial cells, mesenchymal stem cells, osteoblasts and macrophages. In times of stress or disease, factors such as granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor, C-X-C motif chemokine (CXCL-12), monocye chemoattractant protein-1, stem cell factor, IL-6 and thrombopoietin are critical in modulating an appropriate immune response. As such, the measurement of circulating WBCs is routinely performed to aid in the diagnosis of a wide range of pathologies and stress states from infection and inflammatory diseases to hematological malignancies.

A strong association between elevated WBCs and CVD has been identified in numerous epidemiological studies. Preclinical studies of atherosclerosis have revealed that circulating monocytes,
neutrophils and platelets have an important role in disease initiation and progression.40,81 Leukocyte abundance is also known to influence heart failure after a myocardial infarction92 and may also contribute to secondary CV events.83 Of the circulating leukocytes, cells of the myeloid compartment, namely monocytes and neutrophils, are the most highly associated ones with cardiac events.84–87 However, an important relationship between adaptive immune cells and CVD has emerged over the past decade (see reviews Hedrick88, Wigren et al.89 and Ammirati et al.90). The balance of different leukocyte subsets has a critical role in CVD. Indeed, in the context of atherosclerosis, hematopoiesis and, in particular, WBC production outside the bone marrow (extramedullary hematopoiesis) is skewed toward the production of pro-inflammatory monocyte subsets (Ly6-C/ in mice and CD14+/CD16−/ in humans93), which are known to be strongly pro-atherogenic. Alongside the Ly6-C/Ly6-C/ monocyte balance, many different T-cell subsets have been shown to participate in atherosclerotic lesion development. Most of these T-cell subpopulations prove to be inflammatory, accelerating lesion development and stability.90,92 Regulatory T cells are the main cells from adaptive immunity that have been shown to have an atheroprotective effect in CVD mostly through the secretion of anti-inflammatory cytokines and the inhibition of some inflammatory T-cell subsets.72,93 Over the past decade, a number of discoveries have allowed a better understanding of the inflammatory pathways involved in promoting enhanced hematopoiesis and its impact on WBC homeostasis in the context of CVD.94 This knowledge will be useful in targeting these pathways when developing novel or prescribing existing anti-inflammatory therapies to treat CVD.

Cholesterol homeostasis and hematopoiesis

A direct correlation between hyperlipidemia and leukocyte number is routinely reported in humans.95,96 Furthermore, lowering LDL and increasing HDL with pravastatin treatment in people with coronary artery disease was associated with reductions in monocytes and neutrophils.97 These effects with statins have also been modeled in hypercholesterolemic Apo−/− mice where statin use reduced inflammatory Ly6-C/ monocytes.98 Thus, a close relationship exists between cholesterol homeostasis and circulating leukocytes. Recent work has begun to unravel an important role for intrinsic cellular cholesterol-handling in bone marrow HSPCs in modulating leukocyte production (reviewed recently99). Cellulord cholesterol levels are determined by the balance between de novo synthesis and cellular flux (that is, uptake versus efflux). Critical regulators of cellular cholesterol levels are the ATP-binding Cassette Transporters (ABC)A1 and ABCG1, which transport cholesterol from membranes to HDL particles. In a hallmark study, Yvan-Charvet et al.100 revealed that mice deficient of ABCA1 and ABCG1 develop prominent leukocytosis and accelerated atherosclerosis. It was found that leukocytosis was due to altered proliferation of HSPCs in the bone marrow and in extramedulIary locations.101 In these mice the hyperplofferation of the HSPCs was linked with increased plasma membrane lipid rafts and levels of the common β-subunit of the receptor for IL-3 and GM-CSF. Thus, these data reveal that impaired cholesterol efflux and the subsequent cholesterol accumulation in HSPCs result in enhanced proliferation because of enhanced sensitivity to growth factor signaling. A similar mechanism was also discovered in western-type-diet-fed Apo−/− mice.102 The proliferative defects in both the Abca1−/−Achg1−/− and Apoe−/− mice were cell-intrinsic, meaning that it was not simply because of changes in systemic inflammation. Importantly, restoring the cholesterol efflux in the Abca1−/−Achg1−/− and Apoe−/− mice restored this proliferative defect.100,102 It is also of interest that the cholesterol transporter ABCG4 is highly expressed in bone marrow megakaryocyte progenitors and has been found to regulate thrombopoiesis.103 Thus, this newly discovered link between intrinsic cellular cholesterol metabolism in regulating hematopoiesis and atherosclerosis highlights the potential importance of maintaining efflux potential in HSPCs.

Hematopoiesis and CVD risk: lessons learned from RA

Reductions in neutrophil numbers are consistently reported in RA patients treated with tocilizumab, which coincides with increases in cholesterol.48,50,51 Whether the increase in cholesterol by IL-6 blockade is caused by a suppression of hematopoiesis remains to be tested experimentally. However, IL-6 has been shown to stimulate granulopoiesis in the absence of G-CSF and GM-CSF in times of increased neutrophil need,104,105 an effect most likely regulated by IL-6 trans-signaling.106 Furthermore, two studies in the New England Journal of Medicine revealed that treatment of RA with the JAK inhibitor tofacitinib caused reductions in neutrophils, which mirrored increases in LDL cholesterol.107,108 Whereas these data reveal that inhibitors of JAK and IL-6 can reduce neutrophil levels, they do not show that WBC production is enhanced in RA. However, it is well recognized that patients with RA generally present with leukocytosis due primarily to monocytosis and neutrophilia.109 There remains, however, a relative paucity of data, directly assessing hematopoiesis and cholesterol homeostasis in RA. Looking at another pathology, people with myeloproliferative diseases also exhibit hypcholesterolemia which, importantly, is reversed with successful disease treatment.110 Furthermore, the administration of the hematopoietic growth factors macrophage colony-stimulating factor or GM-CSF into non-human primates and rabbits or humans, respectively, causes a reduction in cholesterol levels.111,112 Conversely, immunosuppressants required for successful hematopoietic stem cell transplantation and solid organ transplantation cause dyslipidemia.113,114 Collectively, these studies reveal that altered hematopoiesis (by either pathological or therapeutically induced means) can have a direct impact on cholesterol homeostasis and provide a potential mechanism by which cholesterol levels are reduced in states of high inflammatory burden. To date, very few studies have directly investigated whether anti-inflammatory treatments alter RA-induced leukocytosis. In one particular study, a significant increase in circulating neutrophils, monocytes and platelets was found utilizing a rodent model of adjuvant-induced arthritis. Importantly, the treatment of these arthritic rodents with tofacitinib caused a normalization of platelet, reticulocyte and neutrophil levels in the blood, which was associated with an increase in cholesterol levels.115 Taken together, these data reveal an important link between arthritis-induced hematopoiesis and perturbations in circulating cholesterol levels.

Metabolic dysfunctions and hematopoiesis: consequences on atherosclerosis

Circulating myeloid cells are also elevated in states of metabolic dysfunction such as obesity, insulin resistance and diabetes,115–118 and are thought to contribute directly to the enhanced atherosclerosis observed in patients with these conditions. Importantly, even when lipids are controlled with statins, significant residual CVD risk remains in people with diabetes.119 Indeed, it was found in streptozotocin-treated diabetic mice that hyperglycemia per se promoted monocytosis by inducing bone marrow myeloid progenitor cell expansion and proliferation. In addition, it was shown that diabetic animals have an impaired potential for atherosclerotic lesion regression.120 Importantly, it seems that in diabetic animals, monocytosis is mostly linked
to hyperglycemia as monocyte levels were not affected by changes in circulating cholesterol and insulin levels and was resolved following glucose normalization.

Interestingly, a recent clinical trial of over 7000 patients with T2D (EMPA-REG OUTCOME) showed that a glucose-lowering treatment (using empagliflozin, a sodium glucose co-transporter 2 inhibitor (SGLT2i) that blocks renal re-absorption of glucose) was associated to lower rates of death from CV causes and from any cause compared with placebo,121 making this the first antidiabetic drug to lower CV events to date. However, this was primarily because of a reduction in heart failure through an unknown mechanism, and no readouts on inflammatory profiles were reported. A recent meta-analysis has suggested that there is also a reduction in myocardial infarction in SGLT2i-treated diabetic patients.122 Hence, further longitudinal studies are still required to investigate the anti-atherogenic effects of glucose-lowering with SGLT2is in people with diabetes.

Interestingly, prominent monocytosis and neutrophilia due to altered hematopoiesis are also observed in mouse models of obesity.123 Unlike the streptozotocin model, hyperglycemia was moderate and glucose-lowering had no effect on monocytosis, signifying an alternative mechanism of action.123 Together, these data provide mechanistic evidence by which diabetes and obesity can promote atherosclerosis by modulating hematopoiesis and monocytosis.

**Should we tolerate dyslipidemia as an acceptable side effect when suppressing inflammation to treat CVD?**

Having discussed the importance of hematopoiesis in metabolic disease and its impact on CVD, it appears that, despite promoting dyslipidemia, some of the current anti-inflammatory treatments for metabolic diseases are able to correct the hematopoietic dysfunctions. In T2D patients treated with salalate, increased total and LDL cholesterol was associated with significant reductions in neutrophils and lymphocytes.10,11 Recently, IL-1β was identified in preclinical studies as a contributor to obesity-induced monocytosis by directly modulating bone marrow myeloid progenitor cell expansion and proliferation via the IL-1 receptor.123 When obese mice were treated with the IL-1R antagonist anakinra, circulating monocytes and neutrophils were reduced. Similarly, anakinra reduces blood cell counts in humans with T2D, neonatal-onset multisystem inflammatory disease and following a stroke.15,124,125 Similarly, an anti-IL-1β antibody LY2189102 reduces neutrophils and WBCs in patients with T2D.126 In the EMPA-REG OUTCOME trial, despite a reduction in HbA1c with empagliflozin treatment, an increase in circulating LDL and HDL cholesterol was observed. Similar increases in cholesterol have been observed with canagliflozin, another SGLT2i.127 Unfortunately, in the study by Zinman et al.121 WBCs were not reported; however, based on the study by Nagareddy et al.,128 it could be expected that leukocytosis would be reduced by SGLT2i in the context of hyperglycemia-induced inflammation, but this pathway would likely be over-ridden if obesity was also present.123

**WHAT IS THE FUTURE FOR CURRENT THERAPIES?**

One of the staple treatments in people with CVD is statins. This class of drugs effectively lowers plasma cholesterol level by blocking endogenous cholesterol synthesis, primarily in the liver, and by causing the upregulation of the LDL receptor. By targeting endogenous cholesterol pathways and the formation of synthesis intermediaries, statins also show some anti-inflammatory properties; however, this is probably a mild effect compared with targeted anti-inflammatory therapies. Thus, now that CVD is defined as a chronic inflammatory disease, the question is how important is plasma cholesterol levels versus inflammation in the pathogenesis of CVD (Figure 1). The link between elevated cholesterol levels and CVD risk is well appreciated and should not be lightly dismissed. Considerable epidemiological and experimental evidence has reinforced the mechanisms by which elevated cholesterol (particularly, LDL cholesterol) drives atherogenesis and its associated CV complications. As such, statins remain the frontline drug treatment for the treatment of hypercholesterolemia, and their impact on the reduction of CVD risk and mortality cannot be understated with randomized clinical trials reporting a reduction of up to 30% in coronary events associated with statin use.129 Epidemiological studies also show that increased plasma cholesterol is associated with monocytosis,95 and this can be modeled in mice when manipulating genes involved in cholesterol metabolism (that is, Apoe, Abc transporters, Ldlr and Apoai).100,102,130 As previously discussed, mouse models advocate that, even with an adverse lipid profile, reduced monocytes clearly equate to smaller atherosclerotic lesions.131 Whereas statins or other lipid-modulating therapies will always remain staple drugs in the treatment of CVD, these genetic models do highlight the importance of also targeting inflammation in the etiology of CVD.

**CONCLUSION**

Inflammation clearly has a role in elevating CVD risk in disease populations irrespective of whether cholesterol levels are elevated (obesity, insulin resistance and diabetes) or reduced (RA, HIV, infection, sepsis, systemic lupus erythematosus and several cancers). An emerging trend is that several anti-inflammatory treatments appear to increase cholesterol levels. Current evidence suggests that these increases in cholesterol are not associated with any obvious adverse CV effects in the context of sustained inflammation suppression. Whether this is because of a beneficial alteration in cholesterol subclasses, the reduction in inflammation or a combination of both remains unknown. In addition, understanding how anti-inflammatories promote increased cholesterol levels and whether this
is due to the suppression of hematopoiesis and stem cell proliferation is a critical question with potential wide-reaching implications, and certainly warrants further investigation.

The long-term CV safety of anti-inflammatory therapies will become more apparent with data to emerge from current trials in future years and will allow us to decipher whether the contribution of inflammation to CVD is equally or more important than the metabolic abnormalities. However, until we better understand this relationship, a close screening of all patients receiving anti-inflammatory drugs should be mandated in order to closely monitor CV risk in case of subsequent dyslipidemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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