Persistent stem cell-driven inflammation in patients with prior MI and stroke

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This editorial refers to ‘Increased haematopoietic activity in patients with atherosclerosis’†, by F.M. van der Valk et al., on page 425.

Atherosclerosis is a chronic, inflammatory disease resulting in progressive atheroma formation. Progression of atherosclerosis culminates in rupture of the atheromatous plaque, causing myocardial infarction (MI). For many patients, atherosclerosis does not become clinically evident until angina, an MI, stroke, or a congestive heart failure event occurs. These sentinel events lead to re-evaluation of risk factors for atherosclerotic cardiovascular disease (ASCVD), and medical therapy is added. Beyond these steps, there is no consensus approach to determine the risk of recurrent ASCVD events. While secondary prevention strategies are often successful in the short term (1 year), it can be difficult to identify patients who have another ASCVD event. Therefore, an important goal is to develop approaches to monitor ASCVD activity, and develop new strategies for therapy that can be augmented as needed to suppress progressive atherosclerosis.

Our understanding of how the innate immune system impacts atherosclerosis has recently increased dramatically. The progression of atherosclerotic lesions requires continued monocyte proliferation and ongoing recruitment of neutrophils and monocytes from the blood stream. Elevated cholesterol levels, impaired cholesterol efflux, or impaired HDL cholesterol results in accumulation of cholesterol within the vessel wall. This leads to formation of cholesterol crystals (CCs) that activate two components of the innate immune system (see Figure 1). (i) Neutrophils respond to CCs with a specialized process known as neutrophil NETosis. In neutrophil NETosis, the chromatin of neutrophils is degraded and expelled from the cell to form extracellular chromatin nets that have antimicrobial properties, and are highly atherogenic. (ii) Exposure of neutrophil NETs to monocytes activates an inflammatory cascade known as the inflammasome, increasing interleukin-1β (IL-1β). IL-1β has several inflammatory effects in atherosclerosis, but its effects on immune cells are to activate the production of IL-17 from T_{h}17 lymphocytes, as well as act on bone marrow haematopoietic stem cells (HSCs) to promote differentiation and release of myeloid lineage cells, monocytes, and neutrophils. IL-17 also induces granulocyte colony-stimulating factor (G-CSF) from a variety of cell types including endothelium that instructs the bone marrow to release HSCs into the blood stream.

The effects of elevated cholesterol levels, or impaired ability to clear cholesterol from cells due to impaired HDL cholesterol function, also plays a role in the differentiation pattern of HSCs. Cholesterol accumulation within haematopoietic progenitor cells (HSPCs) alters the membrane of cells and the composition of structures known as lipid rafts. Lipid rafts are important structures that carry cellular signalling components including transmembrane receptor proteins, such as the interleukin-3/granulocyte-macrophage-colony stimulating factor (GM-CSF) β receptor (IL-3/CSF3R). High cholesterol levels sensitize cells to IL-3 and GM-CSF, resulting in preferential differentiation of HSPCs to monocytes and neutrophils. Activation of signalling by IL-3/CSF3R promotes preferential differentiation of HSPCs to the myeloid lineage that results in increased quantities of monocytes and neutrophils. These mechanisms sustain a pathway resulting in hypercholesterolaemia-driven inflammation and atherosclerosis that is self-perpetuating and results in sustained activation of haematopoiesis.

Recent work in animal models has shown that MI activates sustained inflammation and progressive atherosclerosis. After MI in mice with hypercholesterolaemia, mobilization of HSCs into the blood stream is increased. HSC release after MI is driven by activation of beta-3-adrenergic receptor signalling in the bone marrow. Mobilized HSCs home to the spleen where they undergo expansion and differentiation to monocytes, which contribute to progression of existing atherosclerotic lesions, and further perpetuate inflammation exacerbated by the high cholesterol environment.

Advances in cardiovascular imaging have allowed visualization of inflammatory activity in atheromatous plaques. To achieve this, [18F]deoxyglucose ([18F]FDG) positron emission tomography (PET) combined with computed tomography (CT) is used to visualize metabolic activity co-localized with the vasculature. [18F]FDG is absorbed by proliferative monocytes and macrophages, and is a surrogate for tissue inflammation. Using this approach, several groups...
have found that $^{18}$F]FDG uptake is increased in atheromatous plaques and in the spleen. The mechanism of increased avidity for glucose in inflammatory cells was recently elucidated, and is a consequence of increased activation of IL-3β receptor (IL3βR) signaling. IL3βR activation increases expression of the GLUT1 glucose transporter in HSPCs, facilitating increased glucose flux into these cells. Increased flux of glucose alters mitochondrial metabolism, enhances proliferation, and skews HSPC differentiation to the myeloid lineage. Inhibition or null mutation of GLUT1 reduces proliferation of myeloid progenitors and decreases atheroma size in animal models. These findings indicate that glucose uptake in atheromatous lesions cannot only be used as a marker of active inflammation in the vessel wall, but more importantly indicates that increased glucose uptake is mechanistically tied to production of higher levels of macrophages, monocytes, and neutrophils.

In the current issue of the journal, van der Valk and co-workers determined if persistent inflammation could be observed in human subjects with MI or stroke 1 year previously. To achieve this aim, they relied on PET imaging using $^{18}$F]FDG uptake within vessels as a surrogate for metabolic activity of monocytes and macrophages in the vessel wall. They found significantly elevated $^{18}$F]FDG uptake within vessels in subjects with history of prior ASCVD event, indicating ongoing metabolic activity within the vessel wall. Seminal work by Ye and co-workers has shown that $^{18}$F]FDG uptake predominantly occurs in macrophages within atherosclerotic plaques, indicating the specificity of this imaging approach to assess atherosclerotic activity non-invasively.

Increased production of myeloid cells, monocytes, and neutrophils is critical to the progression of atherosclerosis. They arise from HSCs released from bone marrow and are directed to differentiate to the myeloid lineage (macrophages, monocytes, and neutrophils) by inflammatory stimuli including IL-1β, IL-6, and GM-CSF. Elevated lipids contribute to myeloid skewed haematopoiesis by providing higher levels of lipid rafts harbouring growth factor receptors on HSPCs, making them more sensitive to the effects of inflammatory cytokines. van der Valk and co-workers found that subjects with prior ASCVD had no significant differences in the levels of total CD34$^+$ cells in their blood stream, but that the CD34$^+$ cells from subjects with prior ASCVD events had significantly greater differentiation to granulocytes and macrophages using an in vitro assays. This finding indicates that stem cells from subjects with prior ASCVD have a preferred differentiation pattern to the myeloid lineage. How this occurs remains to be determined and may involve effects of chronic IL-1β stimulation on the bone marrow, or epigenetic effects on HSCs to skew their differentiation pattern towards myeloid. Further insights may be achieved by a more in-depth analysis of the components of the total CD34$^+$ cells using multicolour flow cytometry approaches to determine if the circulating CD34$^+$ cell pool contains higher levels of early myeloid committed cells such as common myeloid or granulocyte–macrophage progenitors.

While the work of van der Valk and co-workers represents early evidence of chronic vascular inflammation after MI in human subjects, there are a few limitations of their study. van der Valk and co-workers made use of CD34$^+$ HSPCs obtained from subjects with cancer mobilized by treatment with the bone marrow-mobilizing agent G-CSF. Recent studies in subjects with cancer showed that cancer itself causes an increase in the mobilization of HSCs and HSPCs into the blood stream. Cancer also increases
the circulating quantity of granulocyte–macrophage progenitors in the blood stream that directly contribute to immunosuppressive myeloid suppressor cells, assisting the tumour in evading immune detection. The effect of cancer on myeloid differentiation remains a potential confounding factor to be verified in subjects without cancer. A second potential limitation of van der Valk’s study is the use of oxidized LDL (oxLDL) cholesterol to promote myeloid differentiation of HSPCs. The definition, composition, and preparation of oxLDL cholesterol is variable, and its relationship to naturally occurring oxLDL in human subjects is not well defined. The role of oxLDL as a stimulus for myeloid differentiation of HSPCs remains an open question.

Further study is needed to determine if vascular [18F]FDG uptake, or HSPC levels are useful in predicting future ASCVD events, and whether serial measures of these parameters could be used to guide treatment after an ASCVD event. Serial studies of vascular inflammatory activity using PET imaging or combined PET/CT is not widely available and would require serially exposing patients to ionizing radiation. Given the chronic nature of atherosclerosis, it would be most useful to determine if a blood biomarker such as the levels of haematopoietic precursors of monocytes and neutrophils (common myeloid or granulocyte–macrophage progenitors), or the differentiation potential of CD34+ cells to myeloid cells, can be linked to vascular [18F]FDG uptake in vessels. Therapeutically, new approaches are needed to suppress ongoing vascular inflammation after an ASCVD event, and normalize the myeloid differentiation potential of HSPCs. Candidate pathways that may result in reduced plaque inflammation and reduced HSPC numbers are shown in Figure 1 including neutrophil NETosis, IL-1

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References