Cardiovascular disease (CVD) is emerging as a major public health concern among HIV-positive individuals in large part because of the effectiveness of antiretroviral therapy (ART) in treating HIV infection and extending life expectancy. CVD is currently the second most frequent cause of death (after cancer) among HIV patients. Subclinical markers of atherosclerosis, such as the carotid, femoral, or iliac intima-media thickness, are consistently greater and progress earlier among the HIV-positive population than among the HIV-negative general population. HIV-positive patients have intima-media thickness measurements that are comparable with those seen among coronary artery disease patients. Development and progression of atherosclerosis, an asymmetrical focal thickening of the intima of arteries with lipid plaque, often results in myocardial infarction. HIV-positive individuals have significantly higher rates of hospitalization for coronary artery disease and risk of myocardial infarction than HIV-negative individuals.

Although atherosclerosis has been documented in early human history and the process starts in early childhood even in the general population, HIV-positive patients are especially prone to premature atherosclerosis. Thus, understanding the biological mechanisms of early atherosclerosis, specifically in the context of HIV infection, could lead to improved prevention and intervention strategies in this vulnerable group. Although the underlying mechanisms are not fully delineated, risk factors linked to atherosclerosis in HIV infection are both traditional (e.g., age, diabetes mellitus, smoking, dyslipidemia, inflammation, hypercholesterolemia, family history) and HIV specific (e.g., low CD4+ lymphocyte count, co-infection with hepatitis C virus, and certain ARTs). Infection with HIV is associated with an increase in systemic inflammation and damage to the vascular endothelium.

Herein, we review the biological network for the development of atherosclerosis in the context of HIV (Figure). We propose 3 key sequential biological processes that lead to accelerated progression of atherosclerosis: (1) inflammation leads to the recruitment of monocytes; (2) monocytes migrate to the endothelium and differentiate to macrophages and foam cells; (3) foam cells transform and undergo apoptosis because of calcium-dependent endoplasmic reticulum (ER) stress. These mechanisms are affected when HIV interacts with regulatory genes involved in any of the 3 steps in these key biological processes, including both innate and adaptive immunity. HIV kills CD4+ helper T cells and leads to persistent immune activation that modulates chronic inflammation of the arterial wall during the initial phases of atherosclerosis. Below we discuss in detail the interactions of HIV with these key sequential processes.

Inflammation
Atherosclerosis is a chronic inflammatory disease of the arterial wall, which is lined with endothelial cells (ECs) and vascular smooth-muscle cells. Macrophages are found in abundance in plaques that form arterial lesions during atherosclerosis, along with lesser numbers of dendritic cells, T cells, B cells, and NK cells. A crucial first step in atherosclerosis is activation of ECs in response to injurious factors and inflammatory mediators that activate the expression and release of cytokines and chemokines. Several studies have suggested that interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) serve as central components in the initial phase of atherosclerosis. IL-6 and its mRNA, found to be elevated among HIV-infected individuals, stimulate the production of several acute phase response markers, including C-reactive protein, serum amyloid A, and fibrinogen, although...
C-reactive protein, levels may not be strikingly elevated in HIV infection, in part because of coinfection with hepatitis C virus.\(^{17,22}\) Elevated IL-6 levels have been associated with cardiovascular mortality in patients with HIV.\(^{14}\) Furthermore, IL-6 along with IL-1 and tumor necrosis factor (TNF)-\(\alpha\) upregulate macrophage colony-stimulating factor and MCP-1 but downregulate IL-10. Also, IL-6 and macrophage colony-stimulating factor favor differentiation of monocytes into activated macrophages.\(^{19}\)

Likewise, MCP-1 has been shown to be involved in attraction, migration, and activation of monocytes.\(^{20,21}\) The HIV Tat protein can cause endothelial dysfunction in porcine coronary arteries\(^{23}\) and can promote the secretion of MCP-1.\(^{24}\) MCP-1 stimulates a cascade of upregulation of TNF-\(\alpha\) and TNF-\(\beta\) and subsequently nuclear factor-xB and IL-6.\(^{25}\) The gp120 protein of HIV has also been known to upregulate TNF-\(\alpha\).\(^{26}\) Mice rendered genetically deficient for MCP-1 or its receptor, C-C chemokine receptor (CCR)-2, are protected from vascular lesions in several atherosclerosis models. Of note, sequence variants in MCP-1, IL-6, and its receptor IL-6 receptor have been shown to be associated with atherosclerosis and coronary artery disease.\(^{3,27}\)

Several local proinflammatory cytokines (TNF-\(\alpha\), IL1, IL6, IL12, IL18, IFN-\(\alpha\))\(^{28–31}\) are also induced both by HIV replication and lipid-dependent stimuli (eg, infiltration and retention of low-density lipoprotein in the arterial intima).\(^{14,32}\)

**Nonstandard Abbreviations and Acronyms**

- **ART**: antiretroviral therapy
- **CVD**: cardiovascular disease
- **ECs**: endothelial cells
- **ER**: endoplasmic reticulum
- **HDL**: high-density lipoprotein
- **IL**: interleukin
- **MCP-1**: monocyte chemoattractant protein-1
- **RYR**: ryanodine receptors
- **TLRs**: toll-like receptors
- **TNF**: tumor necrosis factor

**Figure.** Three mechanisms for atherogenesis in HIV: (I) Inflammation that occurs in the artery and blood system, (II) monocyte/macrophage recruitment that occurs on the arterial wall, and (III) calcium imbalance associated with endoplasmic reticulum (ER) stress and apoptosis of macrophages (foam cells). Green, interactions between HIV virus and human proteins and blue, interactions between proteins in humans. CAMKII indicates calmodulin-dependent protein kinase II; CRP, C-reactive protein; ICAM, intracellular adhesion molecule; IFN, interferon; IL, interleukin; IP3R, inositol 1,4,5-triphosphate receptor; MCP, monocyte chemoattractant protein; MCSF, macrophage colony-stimulating factor; MIF, migration inhibitory factor; NF-xB, nuclear factor-xB; RYR, ryanodine receptors; TLR, toll-like receptor; TNF, tumor necrosis factor; UPR, unfolded protein response; and VCAM, vascular-cell adhesion molecule.
Anti-inflammatory cytokines (TGF-β and IL10) are also released, which can decrease atherosclerotic progression. In hypercholesterolemic mice, inhibition of IL10 exacerbates coronary thrombosis and likewise, inhibition of TGF-β in T cells leads to large, unstable atherosclerotic lesions. Through cascades of cytokine signaling, several cytokines (eg, macrophage migration inhibitory factor) and metabolic inflammatory mediators (eg, reactive oxygen species) or markers (eg, C-reactive protein) related to CVD are also secreted. An abundance of chemokines (eg, C-X-C chemokine ligand [CXCL]-12, chemokine ligand [CCL]- and MCP1) have also been detected in atherosclerotic lesions and are known to be crucial in directing recruitment of monocytes and T cells. Of note, some of these chemokines are also primary ligands to the major coreceptors for HIV entry, specifically C-X-C chemokine receptor (CXCR)-4 and CCR5. Interestingly, a 32-bp deletion in the CCR5 sequence results in a nonfunctional gene, and homozygous individuals are resistant to HIV infection. The same genetic variant has been associated with reduced incidence of early myocardial infarction and lower susceptibility to severe coronary artery disease, suggesting that coreceptors and ligands associated with HIV also have a direct or indirect role in atherogenesis. A cascade of these inflammatory networks is essential in recruitment of leukocytes, especially monocytes at the sites where plaques form. Finally, inflammation induces a large number of changes in the composition of lipoproteins, including both the lipid content and the associated proteins. These changes in lipoproteins prolong the circulation of some lipoproteins, increase their penetration into the vessel wall, increase low-density lipoprotein oxidation, increase lipoprotein uptake into macrophages to form foam cells, and decrease reverse cholesterol transport, a pathway that decreases atherosclerosis. Below we describe the effects of inflammation on macrophages themselves.

**Monocyte Migration and Differentiation of Macrophage and Foam Cells**

One major consequence of the inflammatory response that contributes to atherosclerosis is the recruitment of leukocytes to the arterial sites through sequential endothelial-dependent mechanisms. Inflammatory reactions or response to hypercholesterolemia activates ECs, causing increased expression of several types of adhesion molecules, such as intracellular adhesion molecule-1 and vascular-cell adhesion molecule-1, that have been implicated in leukocyte trafficking. Several cytokines (TNF-α, IL-1, and IL-4) and chemokines (CCL3 and MCP1) also upregulate intracellular adhesion molecule-1 and vascular-cell adhesion molecule-1 in ECs. On chemo-kine-triggered activation, leukocytes, specifically monocytes and lymphocytes, express β2-integrins and α4-integrins that have a high affinity to bind with intracellular adhesion molecule-1 and vascular-cell adhesion molecule-1, respectively, and as a result monocytes adhere to the arterial walls.

Driven by inflammatory markers, in particular cytokines and chemokines, such as macrophage colony-stimulating factor, IL-6, and MCP-1, these recruited monocytes differentiate into macrophages. Lipid engulfment by macrophages then promotes further transformation to atherosclerotic foam cells. In the lipid accumulation phase, MCP-1 upregulates pattern-recognition receptors for innate immunity, specifically scavenger receptors (eg, CD163) and toll-like receptors (TLRs) that bind and facilitate the internalization of lipoproteins.

A variety of TLRs are found in macrophages and are activated by pathogens, including lipopolysaccharide from Gram-negative bacteria (TLR4), lipoteichoic acid from Gram-positive bacteria (TLR2), fungi (TLR2), and viruses (TLR3). TLR signaling leads to activation of transcription factors, such as nuclear factor-xB, that promote proinflammatory activity, contributing to the development of phenotypically distinct foam cells. First, TLRs induce foam cell formation by increasing the uptake of lipoproteins and the storage of lipid (via increases in CD36, AP2, fatty acid–binding proteins, MyD88-adaptor-like, adipose differentiation-related protein, lipin 1, glycerol-3-phosphate acyltransferase 3, and diacylglycerol O-acyltransferase 2). Second, TLRs decrease the delivery of cholesterol macromolecules to high-density lipoprotein (HDL), the first step in reverse cholesterol transport (via decreases in macrophages ATP-binding cassette transporter subfamily A (ABCA)-1, ATP-binding cassette transporter subfamily G, scavenger receptor class B, member 1(CD36) antigen-like 1, and apolipoprotein E). The HIV Nef gene is also known to activate macrophages, in part, by increasing CD36, and to facilitate the transformation of macrophages to foam cells. HIV protease inhibitors also increase CD36 expression in macrophages. TLR4 and TLR2 have been shown to be involved in early stage intimal foam cell accumulation in lesion-prone aorta of mice. Increased lipopolysaccharide has been reported in the circulation of HIV-infected patients. Mutations in TLR4 in humans are associated with decreased response to lipopolysaccharide and with atherosclerosis. In addition, TLRs in foam cells produce a cascade of proatherogenic, inflammatory factors, such as chemokines (eg, CCL3, CXCL12), cytokines (eg, IL10, IL12), and matrix metalloproteinases, that promote plaque expansion and instability. Thus, active inflammation involving TLRs, complicated by HIV, adversely affects the vascular endothelium and promotes a prothrombotic environment that leads to progression of atherosclerosis.

Another way HIV Nef gene promotes transformation of macrophages to foam cells may be through decreasing cholesterol efflux from these cells. Importantly, studies have shown interdependency between cholesterol and HIV. HIV uses cholesterol-rich regions of the plasma membrane (lipid rafts) of macrophages for viral entry and budding. Also, depletion of cellular cholesterol markedly reduces HIV-1 particle production, and cholesterol sequestration drugs, such as β-cyclodextrin, render the virus incompetent for cell entry. In addition, among HIV-positive individuals who start ART to suppress HIV replication, HDL-cholesterol levels may be restored to their pre-HIV values. Apolipoprotein M, an HDL-associated apoprotein that is known to protect against atherosclerosis, is also reduced with HIV infection. Importantly, HIV-1 Nef induces several genes involved in cholesterol biosynthesis and impairs reverse cholesterol transport from macrophages. The HIV-1 Nef accessory protein also affects the normal function of ABCA1, the major regulator of cellular cholesterol. ABCA1 mediates cholesterol and phospholipid.
efflux (ie, reverse cholesterol transport) to HDL, specifically in macrophage foam cells, which is protective against atherosclerotic plaque progression. The efflux activity can also modulate macrophage expression of inflammatory cytokines and chemokines. For example, ABCA1 can modulate TLR4 signaling that can control the downstream activation of nuclear factor-κB. Inactivation of ABCA1 in macrophages has been shown to induce atherosclerosis in a mouse model, despite low plasma HDL levels.71 Thus, the effects of HIV on the inflammatory mechanisms of lipid transport could directly or indirectly affect the formation of macrophages and lipid-laden foam cells, the key intermediate process of atherosclerosis.72

**Calcium and ER Stress**

Excessive death of foam cells overloaded with cholesterol eventually forms the plaques in the arteries, induces further inflammation, and exacerbates metabolic dysregulation.73 The deleterious effects of the death of macrophages are well established, but the mechanisms are not fully known. Research suggests that ER stress (disturbance of the normal function of ER) is central to triggering apoptosis in these cells.74,75 The ER is the site of lipid and protein synthesis, modification, protein folding, maturation, and transport of assembled proteins. Disruption of ER homeostatic mechanisms, such as perturbations in intracellular calcium homeostasis, can accumulate unfolded and misfolded proteins in the ER. This results in activation of the ER stress response signaling pathway, known as unfolded protein response to reestablish normal ER function. Prolonged activation of the unfolded protein response will trigger cell apoptosis.76 In vitro studies have shown that excess stimulation of macrophages with cholesterol can cause ER stress leading to macrophage apoptosis.78

In a study of mice that were fed a Western diet, oxidized low-density lipoprotein induced a dose-dependent rise in intracellular calcium in aortic ECs and increased MCP-1 production. Chemically inhibiting intracellular calcium alleviated the effect of oxidized low-density lipoprotein on MCP-1 production that also indicated a role of calcium signaling in the inflammatory processes of atherogenesis.77

The ER serves as the storage site of free calcium ion (Ca2+) in humans and has the ability to induce rapid efflux of Ca2+ in response to a variety of cellular signals. Recently, calcium homeostasis has been implicated in macrophage apoptosis during advanced atherosclerosis. The key distal unfolded protein response effector responsible for cholesterol-induced macrophage death is the molecule C/EBP-homologous protein (CHOP) (GADD153). CHOP enables apoptosis by promoting calcium release from the ER.79 This apoptosis mechanism is initiated by the downstream activation of calcium/calmodulin-dependent protein kinase II.80 Other calcium-binding proteins in the cytosol (CALM1, CALM2, and CALM 3) are also involved in Ca2+ homeostasis in the ER.80

Ca2+ is released from the ER to cytosol via several channels, in particular inositol 1,4,5-trisphosphate (IP3) receptors and ryanodine receptors (RYR). HIV Nef gene directly interacts with inositol triphosphate receptor and activates Ca2+ influx but not Ca2+ release, thus causing an accumulation and imbalance of Ca2+ that can lead to apoptosis.82,84 RYRs are the largest known calcium ion channels and exist as 3 mammalian isoforms, RYR1 to RYR3, encoded by 3 different genes.82 Of particular interest is that variants in RYR3 and RYR2 genes have been associated with carotid intima-media thickness, a subclinical atherosclerosis marker, in HIV patients from 2 independent cohorts.85,86 Supporting the Ca2+-mediated apoptotic hypothesis. HIV-1 Tat protein causes endothelial dysfunction23 and can induce rapid loss of ER Ca2+ through the mediation of RYRs.87 RYRs are also upregulated in the aorta of atherosclerosis-prone mice when compared with atherosclerosis-resistant mice.77

Various other risk factors of atherosclerosis and CVD can also mediate ER stress and apoptosis of cells. Diabetes mellitus lowers Ca2+ in the ER of smooth-muscle cells, macrophages, and platelets.88 Homocysteine depletes ER Ca2+ in aortic smooth muscle, induces ER stress, and also accelerates atherosclerosis through this mechanism.89

It is possible that the HIV virus, which activates inflammation and lipid-markers, also triggers ER stress through its interaction with host genes that result in an imbalance of Ca2+. Although the HIV virus is found in low concentrations in treated HIV-positive patients, it could still drive atherogenesis in untreated patients or those who are intermittently viremic because of suboptimal treatment or nonadherence. For instance, in The Strategies for Management of Antiretroviral Therapy study, individuals on intermittent ART had a significantly higher rate of CVD events than those on continuous ART88 and when they were reinitiated on continuous therapy, the CVD risk substantially decreased.89,90 These results suggest that HIV or related immunologic factors play a role in CVD risk and potentially interact with host genes when they are not immunologically suppressed.

The role of ART with CVD or atherosclerosis is not clear and remains controversial. Some ART, alone or in combination, is associated with severe lipoatrophy and metabolic abnormalities.90-92 For example, nucleoside reverse transcriptase inhibitors, especially stavudine (d4T), are associated with peripheral fat wasting (lipoatrophy).93 Lipoatrophy is associated with high triglycerides and low HDL.94,95 Specific drugs (eg, ritonavir and efavirenz) have been linked to dyslipidemia, including elevated triglycerides and non–HDL-cholesterol.96,97 HIV itself is associated with increased triglycerides and decreased HDL.98 Other studies also show that ART improves vascular function and CVD in HIV-positive patients.99,100 Further research is needed to elucidate how HIV itself or in combination with treatment influences vascular function.

**Summary**

The process of atherosclerosis can be viewed in relation to a 3-phase pathway: (1) inflammation of the ECs lining the arterial wall; (2) recruitment, migration, and transformation of monocytes/lipid-laden macrophages; and (3) ER stress and apoptosis of foam cells. The HIV virus itself, or together with treatment, affects this progression by increasing inflammation, promoting transformation of monocytes, and increasing apoptosis through ER stress and an imbalance of Ca2+. Understanding the variants in the genes and their expressions and functions in this network could help elucidate mechanisms of atherosclerosis in the context of HIV. The information could
then be used to develop innovative interventions to reduce the burden of atherosclerosis in this high-risk population.

Disclosures

None.

References


HIV, Inflammation, and Calcium in Atherosclerosis
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