Highly Active Antiretroviral Therapy–related Mechanisms of Endothelial and Platelet Function Alterations

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Highly active antiretroviral therapy (HAART) has transformed human immunodeficiency virus (HIV) infection into a chronic condition, which has allowed the infected population to age and become prone to chronic degenerative diseases common to the general population, including atherosclerotic cardiovascular disease, and coronary artery disease (CAD). Possible causative mechanisms of HIV-associated CAD are related to classic cardiovascular risk factors, such as dyslipidemia, insulin resistance, and fat redistribution, which may be due to either HIV infection or to HAART-associated toxicity. However, other mechanisms are emerging as crucial for the cardiovascular complication of HIV and HAART. This article analyzes the effects of HIV and HAART on endothelial function, endothelium-leukocyte interactions, and platelets as possible mechanisms of enhanced cardiovascular risk.

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KEY WORDS

Human immunodeficiency virus • Coronary artery disease • Highly active antiretroviral therapy • Platelet reactivity • Inflammation

Human immunodeficiency virus (HIV) remains a devastating human pathogen responsible for a worldwide epidemic of AIDS that destroys CD4+ lymphocytes, thereby impairing cell-mediated immunity and affecting multiple organs. According to estimates by the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS, 35.3 million people were living with HIV at the end of 2012. That same year, some 2.3 million people became newly infected, and 1.6 million died of AIDS-related causes.[1]

Since 1996, with the introduction of new antiretroviral therapies (highly active antiretroviral therapy [HAART]), the prognosis of HIV-infected

patients has significantly improved, with a reduction in morbidity and mortality, a striking prolongation of survival, and an improvement in patients' quality of life. Approximately 10 million people every year receive HAART.[2]

HAART has transformed HIV infection into a chronic condition, which has allowed the infected population to age and become prone to chronic degenerative diseases common to the general population, including atherosclerotic cardiovascular disease and coronary artery disease (CAD).[3] However, CAD incidence and time to appearance is increased in these individuals, even after adjusting for traditional risk factors; thus, CAD has become a major cause of morbidity and mortality in HIV-infected patients.[4,5] The WHO predicts that HIV/AIDS and ischemic heart disease will be in the top three causes for both global mortality and disability in 2030, suggesting that an intersection of these two diseases will pose a major global clinical and public health challenge in the coming decades.[6]

Possible causative mechanisms of HIV-associated CAD are dyslipidemia, insulin resistance, and fat redistribution, which may be due to either HIV infection or to HAART-associated toxicity. For the effects of HIV and HAART on classical cardiovascular risk factors, readers are referred to a recent review by Gibellini and colleagues.[5] However, other mechanisms, essentially linked to infection-elicited inflammation, are emerging as central for the cardiovascular complications of HIV. This article analyzes the effects of HIV and HAART on endothelial function, endothelium-leukocyte interactions, and platelets as possible mechanisms of enhanced cardiovascular risk.

Pathogenesis of Ischemic Cardiovascular Disease

The main risk factors for atherosclerosis and cardiovascular disease are elevated levels of low-density lipoprotein cholesterol, diabetes, smoking, hypertension, obesity, age, sex, and family history. Most, if not all, of these risk factors are also associated with inflammation, endothelial dysfunction, enhanced coagulation, and platelet reactivity, which are emerging as crucial mechanisms of atherothrombosis.

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Inflammation

Inflammation participates in atherosclerosis from its inception to its ultimate endpoint (ie, thrombotic complications). Indeed, cardiovascular risk is higher in patients with chronic inflammatory diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren syndrome, and vasculitis.[7]

Atherosclerosis begins with inflammatory changes in the endothelium, which then expresses adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1. Adhesive molecules attract monocytes, which then migrate through the endothelial layer, transform into macrophages, engulf lipids, and become foam cells. T lymphocytes also migrate into the intima, where they release proinflammatory cytokines that amplify the inflammatory response.

Inflammatory biomarkers, such as C-reactive protein (CRP), fibrinogen, and interleukin (IL)-6, are elevated in patients with atherosclerosis and may have a role in the prediction of CAD.[8] Also, platelet-activating factor (PAF), an inflammatory phospholipid with potent biologic activities, has been reported to be associated with increased cardiovascular risk.[9]

PAF is synthesized by several cell types (eg, platelets, monocytes, macrophages, foam cells, and endothelial cells) upon activation. The effects of PAF are mediated by the PAF receptor, a G protein–coupled receptor that triggers multiple intracellular signaling pathways by coupling to $G_{(i)}$ and activating phosphoinositide 3-kinase γ, which in turn promotes Akt phosphorylation.

PAF causes platelet shape change, aggregation, and histamine release, and also activates neutrophils and other circulating inflammatory cells to release growth factors, chemokines, cytokines, and IL-1β, and also to adhere to endothelial cells.[10] PAF also activates monocytes to express P-selectin glycoprotein ligand-l and to adhere to activated platelets or endothelial cells that display P-selectin. This sequence of events represents a mechanism linking inflammation to atherothrombosis.

PAF is involved in several pathophysiologic conditions related to ischemic cardiovascular disease, including the development of myocardial ischemia/reperfusion injury. PAF is released from the ischemia/reperfusion myocardium in high concentrations that negatively modulate coronary circulation, as well as electrical and contractile activities. PAF may act

either directly, via generation of secondary mediators, or indirectly through the activation of inflammatory cells, such as platelets and polymorphonuclear neutrophils, which exacerbate postischemic myocardial injury.[11]

Endothelial Dysfunction

Endothelium, the inner lining of blood vessels, is a highly specialized, metabolically active interface between blood and the underlying tissues. Given its strategic location and biologic properties, the endothelial cell layer plays an essential role in the prevention of thrombosis.[12]

Endothelial dysfunction, provoked by traditional cardiovascular risk factors and chronic inflammation, leads to the impaired production of vasodilators and platelet inhibitors, in particular, nitric oxide (NO), and to proinflammatory, proliferative, and procoagulant modifications that favor atherosclerotic progression and thrombotic events through leukocyte recruitment, platelet adhesion and aggregation, blood-clotting activation, and fibrinolysis impairment.

In recent years, evidence has accumulated that endothelial dysfunction is a major promoter of atherosclerosis and an independent predictor of future cardiovascular events.[13] Specifically, endothelial dysfunction plays a fundamental role in the pathogenesis of acute coronary syndromes, such as unstable angina and acute myocardial infarction (MI).

Another newly discovered mechanism leading to endothelial dysfunction is the generation of microparticles, a heterogeneous population of small membrane fragments shed from various cells upon activation, that interact with the endothelium disrupting NO production, modifying vascular tone, and inducing proinflammatory and proatherosclerotic changes.[14]

Platelet Activation

Platelets play a preeminent role in hemostasis and thrombosis, but also act as inflammatory cells and participate in atherogenesis. Upon activation, they release phospholipids (eg, PAF), matrix metalloproteinases, chemokines, and growth factors that participate in leukocyte recruitment and migration, the proliferation of smooth muscle cells, and the atherosclerotic remodeling of the arterial wall.

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In vivo platelet activation has been detected not only in CAD and acute coronary syndromes,[15] but also in systemic inflammatory syndromes such as rheumatoid arthritis, SLE and Sjögren syndrome. Platelet activation, in turn, may boost inflammation by the release of inflammatory mediators (eg, IL-β, thromboxane A2, CD40 ligand [CD40L], histamine, and serotonin), and may modify the phenotype of endothelial cells by the delivery of microRNAs (miRNAs): small, noncoding RNA molecules that modulate the translation of mRNAs. Platelets express an abundant array of miRNAs, with miR-223 being the most expressed. Upon activation, platelets release platelet-derived microparticles (PMPs), 45% of which contain miRNAs.[16] Coincubation of PMPs with cultured endothelial cells or leukocytes leads to the incorporation of platelet miRNAs into the latter, with consequent changes of their phenotype.[17] In particular, PMPs effectively deliver miR-223 into human umbilical vein endothelial cells (HUVECs), decreasing the expression of insulin-like growth factor 1 receptor and promoting HUVEC apoptosis.[18] Likewise, mediators derived from inflammatory cells can affect platelet function (eg, CRP has been shown to promote platelet adhesion to endothelial cells and monocytes).[19]

Clinical evidence suggests that an elevated platelet count, in vivo platelet activation, and platelet hyperreactivity contribute to adverse cardiovascular events. A mechanistic link between platelet hyperfunction and myocardial damage in patients with acute coronary syndromes is suggested by the enhanced high shear rate–induced platelet activation that represents an independent predictor of the severity of acute MI.[15] An association between platelet hyperreactivity and cardiovascular events has been shown also in patients with type 2 diabetes, myocardial ischemia, and subacute stent thrombosis.[20-22]

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Thrombus formation occurs when the mechanisms regulating the extension of platelet activation at a site of vascular damage become imbalanced. Molecules that amplify the aggregation response of platelets to activating stimuli, either released by platelets or by other cells, lead to thrombus formation.[23,24] On the other hand,

defective platelet activation control mechanisms, such as impaired NO-dependent inhibition of platelet function, are associated with increased incidence of MI.[25]

Effect of HIV Infection and HAART on Cardiovascular Risk-causative Factors

Both HIV infection and antiretroviral treatment may promote atherothrombosis by eliciting chronic inflammation and/or by altering the function of leukocytes, endothelial cells, and platelets. Chronic infection with HIV is associated with a mild, permanent inflammatory state.[26]

Endothelial dysfunction, an early marker of atherosclerosis and a predictor of cardiovascular events,[12,13,27] represents the main link between HIV infection and atherosclerosis. Indeed, an increased endothelial expression of adhesion molecules and inflammatory cytokines, molecules involved in the recruitment and adhesion of leukocytes at sites of atheroma initiation, has been observed during chronic HIV infection.[28]

HIV-infected leukocytes, lymphopenia, and lymphocyte activation are consistent features of infection. During HIV-1 infection, monocytes display an activated phenotype, and this change has an impact on the atherogenic process. HIV also infects megacaryocytes and is internalized by platelets and activates them.[28] Activated platelets may also play a role in HIV-induced atherosclerosis through the expression and release of mediators that, in turn, activate the endothelium and support the adhesion of leukocytes to the inflamed vessel wall.[29] Both of these mechanisms may have impact on initiation and progression of atherosclerotic plaques. Moreover, hyperreactive platelets may transform a normal reparative response to a mild arterial injury into an unwanted thrombotic event.[23]

Whether HAART increases the risk of cardiovascular events has been the object of extensive debate over the past decade. Some—but not all—clinical studies have suggested an association among increased cardiovascular risk, accelerated atherosclerosis, and HAART, although the reasons for this are yet to be fully clarified.

Endothelial dysfunction, an early marker of atherosclerosis and a predictor of cardiovascular events, represents the main link between HIV infection and atherosclerosis.

Findings from the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study indicated that the time of exposure to protease inhibitors, in particular indinavir, lopinavir, and ritonavir, correlates with the increased risk of MI.[30] Neither the French Hospital Database on HIV study[31] nor the D:A:D study found any significant association between the development of MI and exposure to a non-nucleoside reverse-transcriptase inhibitor (NNRTI [efavirenz or nevirapine]).

Abacavir/lamivudine in combination and tenofovir/emtricitabine in combination are the most widely used antiretroviral drugs. Abacavir was initially considered one of the most benign antiretroviral drugs, with a better metabolic profile than other nucleoside analogues. However, since the D:A:D study[30,31] found an association between the use of abacavir and an increased risk of MI, there has been controversy around this drug.[28] Several prospective observational studies and meta-analyses have found enhanced risk of MI, whereas other meta-analyses did not confirm that exposure to abacavir was associated with an elevated cardiovascular risk.[28,32,33] A recent follow-up report reanalyzing the D:A:D data has confirmed a consistent enhanced risk of MI associated with abacavir use. Since publication of the first study findings,[32] abacavir is less frequently prescribed for patients at high CVD risk, and current use of this drug continues to be associated with an increase in MI rate (aRR 1.98 [1.72-2.29]).[34] Therefore, despite controversy, there is reason for concern. Although current guidelines continue to recommend an initial regimen of abacavir with lamivudine, caution is warranted in the use of abacavir in patients already showing a high cardiovascular risk based on traditional parameters.[33] Recent exposure to the nucleoside reverse-transcriptase inhibitor (NRTI) didasonine was also associated with an increased risk of MI.[30]

Inflammation in HIV Infection

High-sensitivity CRP (hs-CPR), a marker of inflammation and a strong predictor of acute MI,[35] is elevated in patients with HIV infection.[36] Fibrinogen, a key component of the coagulation cascade, an acute-phase reactant, and a strong contributor to cardiovascular risk, is elevated in HIV infection.[37]

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Circulating monocytes are activated in HIV-positive individuals, as shown by the enhanced expression of CD11b and CX3CR1, and independently predict carotid artery atherosclerosis.[38] Another inflammatory mediator involved in

lymphomononuclear cell recruitment and vascular permeability triggered by HIV-1 is PAF.[39] PAF seems to play a role in several AIDS manifestations, such as the AIDS dementia complex, Kaposi sarcoma, and HIV-1–associated nephropathy. Recent studies found higher PAF biosynthesis in HIV leukocytes from HIV-infected patients[40] and showed that PAF biosynthetic enzymes are inversely correlated with CD4 count and positively correlated with the viral load.[41]

Effect of HAART

HAART reduces viral load and the concentration of inflammatory markers that are likely to perpetuate cardiovascular risk (Figure 1); however, these beneficial effects may be offset by direct toxic effects on endothelium and HAART-induced metabolic syndrome.

Only limited data exist regarding the effect of specific antiretroviral drugs on inflammation, and most of them regard abacavir because of its potential link to increased cardiovascular disease. Data concerning the effects of HAART on the mechanisms favoring atherothrombosis are somewhat controversial (Table 1), but strong evidence suggests a detrimental influence of abacavir on the regulation of platelet and leukocyte interactions with the vessel wall.

Although one observational study found an association between abacavir and increased inflammatory markers,[32] two others did not.[42,43] The Simple Trial Comparing Two Strategies for Management of Anti-Retroviral Therapy (SMART) and International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) investigators showed that hs-CRP and IL-6, inflammatory biomarkers associated with increased cardiovascular risk, were higher in patients receiving abacavir.[32] However, in the Multicenter AIDS Cohort Study and the Women's Interagency HIV Study Cohort, participants who initiated abacavir showed no elevations of plasma hs-CRP, IL-6, and D-dimer.[42] Equally, data from Padilla and colleagues[43] comparing 50 patients initiating either abacavir or tenofovir revealed no differences in the levels of inflammatory biomarkers.

On the other hand, a recent study in HIV-positive, treatment-naive pregnant women randomized to either zidovudine/lamivudine/abacavir or lopinavir/ritonavir and zidovudine/lamivudine for the prevention of mother-to-child HIV-transmission showed that women treated with an abacavir-containing regimen had significantly higher expression of CD40L, IL-8, and lymphotoxin α, and significantly lower chemokine ligand 5 (CCL5) (RANTES [regulated on activation, normal T cell expressed and secreted]).[44]

A recent retrospective observational study compared changes in several markers of inflammation in HAART-naive adults starting azidothymidine/lamivudine/abacavir + NNRTI versus the same regimen without abacavir: soluble tumor necrosis factor (TNF) receptor II decreased less in the group taking abacavir, suggesting a possible pro-inflammatory effect of this drug.[45]

In HAART-experienced participants switching to either an abacavir-containing regimen or another NRTI, Kristoffersen and colleagues[46] found that plasma matrix metalloproteinase 9,

Figure 1. Reduction of viral load improves some aspects of the deleterious effects of human immunodeficiency virus (HIV) on the cardiovascular system. HIV infection, without treatment, progresses with a steady increase of the viral load. Increased viral load enhances inflammation and atherosclerotic cardiovascular disease. Biomarkers of endothelial activation and inflammation, crucial mechanisms for the cardiovascular complications, are progressively enhanced in HIV-infected persons not treated with highly active antiretroviral therapy (HAART) and have been shown to be associated with HIV replication (*red line*). For most patients, HAART initiation decreases viral load (*blue line*) to undetectable levels and in parallel it reduces HIV-related endothelial injury and dysfunction and improves (although does not completely normalize) some inflammatory and endothelial injury pathways, such as monocyte chemoattractant protein-1, soluble vascular cell adhesion molecule, von Willebrand factor, fibrinogen, asymmetric dimethylarginine, and circulating endothelial cells. Data from Neuhaus J et al,[36] Chini M et al,[41] Francisci D et al,[48] Galea P et al,[50] Blum A et al,[51] McComsey GA et al,[55] Baker JV et al,[57] and Francisci D et al.[60]

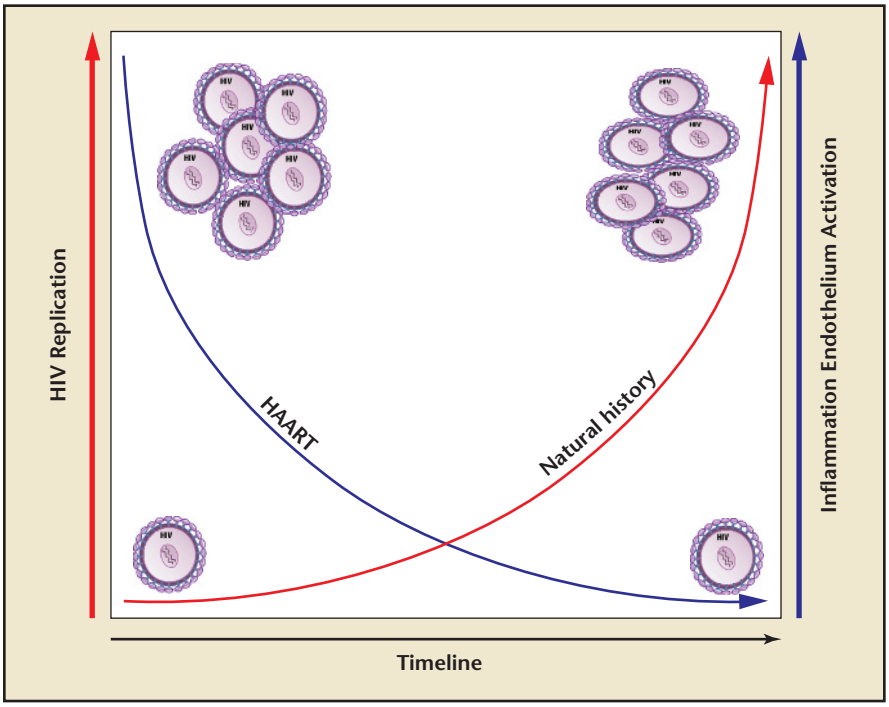


TABLE 1

Studies on the Effects of ABC on Some Causative Mechanisms Favoring Atherothrombosis

Study	Effects on	ABC Effect	Reference Drug Effect	Reference Drug
SMART/INSIGHT, D:A:D Study Groups[32]	Inflammation	↑ hs-CRP	↔ hs-CRP	TDF
SMART/INSIGHT, D:A:D Study Groups[32]		↑ IL-6	↔ IL-6	TDF
Chini M et al[41]		↑ PAF	↓ PAF	TDF
MacLeod IJ et al[44]		↑ sCD40L	↓ sCD40L	PI
Kristoffersen US et al[46]		↑ MMP-9	↔ MMP-9	ns
MacLeod IJ et al[44]		↑ IL-8	↓ IL-8	PI
MacLeod IJ et al[44]		↓ RANTES	↓ RANTES	PI
Hileman CO et al[45]		↓ TNFR	↓ TNFR	TDF
Palella FJ Jr et al[42] Padilla S et al[43]		↔ hsCRP	↔ hsCRP	TDF
Hsue PY et al[59]	Endothelium	↓ FMD	↔ FMD	ns
Francisci D et al[60]		↑ CEC	↓ CEC	TDF
McComsey GA et al[55]		↓ sVCAM	↓ sVCAM	TDF
Wang X et al[58]		↓ eNOS	↔ eNOS	nrd
Wohl DA et al[61]		↔ FMD	↔ FMD	TDF
De Pablo C et al[62] De Pablo C et al[63]		↑ leukocyte recruitment	↔ leukocyte recruitment	Lamivudine, zidovudine
Corrales-Medina VF et al[67]	Platelets	↔ PMP	↔ PMP	TDF
Falcinelli E et al[71] Satchell CS et al[74]		↑ aggregation	↔ aggregation	TDF
Falcinelli E et al[71]		↑ in vivo activation	↔ in vivo activation	TDF
Falcinelli E et al[71] Baum PD et al[73]		↓ cGMP	↔ cGMP	TDF

ABC, abacavir; CEC, circulating endothelial cells; cGMP, cyclic guanosine monophosphate; D:A:D, Data Collection on Adverse Events of Anti-HIV Drugs; eNOS, endothelial nitric oxide synthase; FMD, flow-mediated dilation; hs-CRP, high sensitivity C-reactive protein; IL, interleukin; INSIGHT, International Network for Strategic Initiatives in Global HIV Trials; MMP, matrix metalloproteinase; nrd, no reference drug; ns, not specified; PAF, platelet activating factor; PI, protease inhibitors; RANTES, regulated upon activation normal T cell expressed and secreted; sCD40L, soluble CD40 ligand; SMART, Simple Trial Comparing Two Strategies for Management of Anti-Retroviral Therapy; sVCAM-1, soluble vascular cell adhesion molecule-1; TDF, tenofovir; TNFR, tumor necrosis factor receptor.

myeloperoxidase, and hs-CRP increased in the group changed to abacavir.

In another study, several inflammatory markers, including PAF, were measured during a 12-month period after treatment initiation: although PAF biosynthesis was activated by an abacavir-containing regimen it was decreased by a tenofovir-containing regimen.[47]

Endothelium in HIV Infection

Chronic inflammation, hypercoagulability, increased cell adhesion, and platelet activation drive endothelial dysfunction in HIV-infected individuals.[28,48] HIV is able to penetrate coronary artery and brain microvascular endothelial cell membranes, and to initiate intracellular inflammatory reactions. Endothelial activation may also occur following cytokine release by mononuclear or adventitial cells activated by the virus, or by the effects of glycoprotein 120 and Tat, two secretory HIV-associated proteins that reduce endothelial NO synthase expression and upregulate several adhesion molecules in the endothelium.[49]

Endothelial dysfunction was documented in patients with HIV infection very early after the beginning of the epidemic. Increased plasma levels of TNF-α, IL-6, VCAM-1, ICAM-1, P-selectin, monocyte chemoattractant protein-1, and von Willebrand factor (vWF), all molecules released by activated endothelium and involved in the recruitment and adhesion of leukocytes at sites of atheroma initiation, have been reported during chronic HIV infection.[49,50] ICAM-1 and ICAM-2 levels correlated with the increase of β2 microglobulin and the decrease of CD4 T cells, suggesting that endothelial dysfunction worsens with the progression of HIV infection. Also, vWF plasma levels correlated inversely with the CD4+ counts.[50]

Flow-mediated vasodilation (FMD) of the brachial artery, a functional marker of endothelium-derived NO, was found to be impaired in HIV-infected patients and to correlate with HIV RNA copies (Figure 1).[51,52] Arterial stiffness, a marker of early cardiovascular disease and a predictor of cardiovascular events, was also found to be enhanced in untreated HIV-infected persons when compared with control participants.[53] Finally, circulating endothelial progenitor cells, required for the endothelial damage repair, were significantly reduced in antiviral therapy-naive HIV-positive patients.[54]

Effect of HAART

There are conflicting results regarding the effects of antiretroviral therapy on endothelial dysfunction. Antiretroviral therapy may improve endothelial dysfunction reducing viral load and the infection-associated chronic inflammatory reaction. On the other hand, antiretroviral therapy may cause endothelial dysfunction in many ways: by a direct toxic effect on endothelial cells or by indirect mechanisms, either in synergy with the HIV virus or through its effects on lipids or glucose metabolism.

HIV-infected patients have increased levels of circulating markers of endothelial dysfunction and the short-term use of HAART reduces them, without differences between protease inhibitors and NNRTIs, showing that chronic infection itself, and not antiretroviral treatment, is responsible for endothelial damage (Figure 1). The effect of HAART on endothelial dysfunction was independent from lipid profile changes; on the contrary, a correlation was evident between HIV RNA copies and monocyte chemoattractant protein-1, soluble VCAM-1 (sVCAM-1), and vWF changes, further suggesting that the major role in the observed endothelium alterations is played by viral infection and that HAART, by reducing viral load and the correlated inflammatory reaction, improves endothelial dysfunction.[48]

A recent study in 244 patients initiating either abacavir/lamivudine or tenofovir/emtricitabine, confirmed the decrease of soluble TNF receptors and of the adhesion molecules sVCAM-1 and soluble ICAM-1 from baseline to week 24, without differences between the abacavir/lamivudine and tenofovir/emtricitabine arms.[55]

In agreement with these findings, in a longitudinal study, markers of endothelial activation (ICAM-1, VCAM-1, and vWF) decreased and FMD improved after 4 weeks of either protease inhibitors or NNRTI, an effect associated with the decrease of HIV RNA levels.[56] Moreover, a recent randomized comparison of immediate versus delayed initiation of HAART showed that participants randomized to immediate HAART had lower plasma levels of asymmetric dimethylarginine, an antagonist of NO biosynthesis by the endothelium, compared with those randomized to deferred HAART.[57]

The potential detrimental effects of abacavir on the endothelium have attracted attention, given the epidemiologic data suggesting enhanced incidence of MI. There is some evidence that abacavir causes endothelial NO synthase downregulation and superoxide anion production in human endothelial cells.[58] In a cohort of long-term antiretroviral-treated HIV-infected patients, those on abacavir had a greater degree of endothelial dysfunction (as shown by a lower FMD), than those on other therapies.[59]

Recent data assessing endothelial function by peripheral arterial tonometry and circulating endothelial cells confirm that chronic HIV infection impairs endothelial function, and show that treatment with tenofovir (but not with abacavir) improves it, suggesting that persistent endothelial dysfunction in abacavir-treated patients may contribute to adverse cardiovascular events.[60] On the other hand, in another randomized study, there were no significant differences in FMD or markers of inflammation and coagulation between individuals assigned to abacavir or tenofovir.[61]

Tenofovir and abacavir, instead, showed different effects on human leukocyte recruitment. In an in vitro model in which leukocytes flow over a monolayer of HUVECs, abacavir induced accumulation of leukocytes, favoring rolling and adhesion, whereas tenofovir had no effect.[62] Another study using the same in vitro model demonstrated that both abacavir and didanosine (another NRTI that has been implicated in raised risk of MI), favor the interaction between leukocytes and endothelial cells by activating

Mac-1 in neutrophils and monocytes, which in turn interacts with ICAM-1 on endothelial cells.[63]

The same authors further analyzed the effects of NRTIs (abacavir, didanosine, lamivudine, zidovudine, emtricitabine, and tenofovir) on the trafficking of leukocytes: abacavir and didanosine (didanosine, cyclic purine analogues), but not the pyrimidine analogues (lamivudine, zidovudine, and emtricitabine) or tenofovir, increased rolling, adhesion, and emigration of leukocytes through the interaction of the leukocyte's Mac-1 with its endothelial ligand ICAM-1.[64]

Platelet Function in HIV Infection

Platelets provide an additional link between HIV-mediated inflammation and cardiovascular disease, as they are activated during infection and interact with monocytes, lymphocytes, and endothelial cells. Among patients with untreated HIV infection, thrombocytopenia is a classic hematologic abnormality observed in 4% to 24% of cases, with a rate that increases with advancing HIV disease.[65]

Aside from thrombocytopenia, HIV-infected patients frequently show signs of in vivo platelet activation. Platelets have been shown to circulate in an activated state in HIV-1 infection (P-selectin, CD40L, platelet-monocyte complexes), the degree of activation correlating with the severity of disease.[66] In turn, activated platelets release proinflammatory cytokines, such as IL-1β and IL-18, which are found in enhanced amounts in the plasma of HIV-infected patients. Increased circulating levels of several markers of platelet activation (soluble P-selectin, soluble CD40L) and of platelets expressing surface activation antigens have been found in HIV-infected patients.[28,67] Moreover, an increased number of PMPs have been measured in the blood of HIV-infected patients.[67]

PMPs are enriched in bioactive molecules, including nucleic acids, and recent observations show that genetic exchange of messenger RNA and miRNA between cells can be accomplished through microparticle transfer.[68] HIV-1 replication may be restricted by certain host cellular miRNAs, in particular miRNA-223,[69] and in turn activated platelets release miRNA-223–containing microvesicles that modulate endothelial cell apoptosis.[18] On the other hand, HIV-1 is able to encode miRNAs that modify cellular defense mechanisms, thus creating an environment favorable for viral invasion and replication.[69]

Platelets provide an additional link between HIV-mediated inflammation and cardiovascular disease, as they are activated during infection and interact with monocytes, lymphocytes, and endothelial cells.

Only a few studies so far have assessed ex vivo platelet function in HIV-infected patients. One study reported both hyper- and hyporeactivity depending on the agonist tested. Platelets from HIV patients were more reactive to epinephrine, whereas less platelet aggregation was observed in response to submaximal concentrations of other agonists (thrombin receptor-activated peptide [TRAP]-6 and collagen), suggesting the existence of multiple underlying defects in platelet function in HIV infection.[70] In another study, markers of in vivo platelet activation and ex vivo platelet reactivity were consistently and significantly higher in HIV-infected patients than in healthy control participants.[71]

Effect of HAART

Few studies have analyzed the impact of HAART, and of abacavir in particular, on platelet activation. Recent data have shown persistently elevated in vivo platelet activation in HIV-infected patients despite effective HAART; however, no distinction was made between different antiretroviral regimens.[72] A case-control study assessing ex vivo platelet function by light transmission aggregometry showed both hyper- and hyporeactivity, depending on the platelet agonist tested, suggesting multiple underlying alterations of platelet function. Here too, no investigation on the influence of different antiretroviral treatments was made.[70]

Increased plasma levels of several platelet-derived inflammatory mediators (eg, RANTES, soluble CD40L, P-selectin, and LIGHT [lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells]), with raised levels persisting or even increasing (eg, neutrophil-activating protein-2) during successful HAART, were reported.[72]

Few studies have assessed the effects of abacavir on platelet function in the search for a mechanistic explanation of the suggested enhanced incidence of MI associated with this treatment (Table 1). Given that the reported enhanced risk of MI associated with abacavir use is precocious (within 6 mo) and rapidly reversible upon drug discontinuation, it seems likely that the cause may be the facilitation of platelet-dependent intracoronary thrombus formation rather than accelerated atherosclerosis.

In vitro incubation of human whole blood with abacavir, a guanosine analogue, increased adenosine diphosphate (ADP)-induced platelet

activation as assessed by P-selectin expression. Of the two other guanosine analogues in clinical use, ribavirin also demonstrated a moderate enhancing effect on platelet activation whereas entecavir did not.[73] Carbovir triphosphate, the active metabolite of abacavir, mimics the structure of guanosine triphosphate. However, it lacks a hydroxyl group and, therefore, it cannot be transformed into cyclic nucleotide, thus competitively inhibiting soluble guanylyl cyclase (sGC) and preventing the intracellular formation of cyclic guanosine monophosphate (cGMP; an essential negative regulator of platelet function), thus increasing platelet reactivity.[25,73]

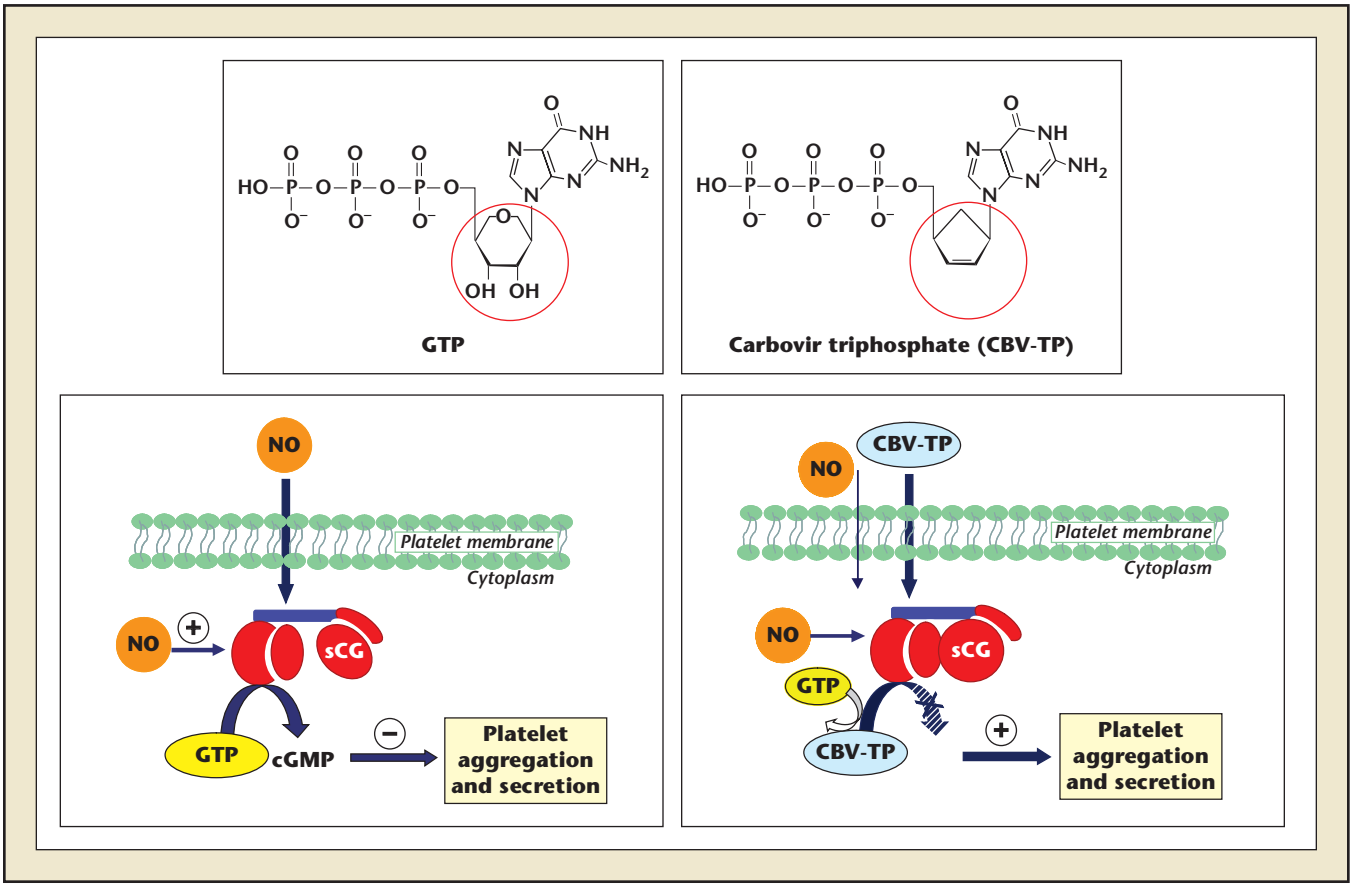
Circulating PMPs are significantly increased among HIV-infected patients; no differences between patients receiving protease inhibitor-based therapy and those receiving abacavir have been shown.[67]

Satchell and collagues[74] explored platelet function in HIV-infected patients receiving antiretroviral therapy compared with treatment-naive HIV-infected patients, and showed that platelets of abacavir-treated patients are more reactive to TRAP-6, ADP, epinephrine, and collagen.[74] Other results showed an increased thromboxane A_2 receptor-mediated platelet aggregation in abacavir-treated patients compared with tenofovir-treated patients.[75]

We have recently evaluated in vivo platelet activation in HIV-infected patients treated with either abacavir or tenofovir, before and after 6 to 12 months and 28 to 34 months of therapy. Treatment with abacavir for 6 to 12 months, but not with tenofovir, enhanced in vivo platelet activation as shown by enhanced levels of platelet activation markers (soluble P-selectin, soluble CD40L, soluble phospholipase A2, and soluble glycoprotein V). Moreover, abacavir- but not tenofovir-treated patients showed enhanced ex vivo platelet aggregation by TRAP, U466619, collagen, and ADP, a shorter PFA-100® (Dade-Behring, Marburg, Germany) /ADP closure time and enhanced platelet-surface expression of P-selectin, CD40L, and lysosomal integral membrane protein-1, confirming treatment-induced platelet hyperresponsiveness.[71]

In vitro studies showed that incubation with carbovir triphosphate

Figure 2. Schematic representation of the effect of ABC treatment on platelet sGC. NO is a powerful platelet inhibitor that suppresses platelet aggregation induced by all platelet agonists and also high-shear stress-induced platelet activation, by directly stimulating sGC and thus increasing platelet cGMP. Structurally, CBV-TP, the active metabolite of ABC, mimics the natural GC substrate, GTP, but lacking a 3' hydroxyl group cannot give rise to cyclic nucleotide formation, thus generating a competitive inhibition of the enzyme. This inhibition leads to an impaired platelet cGMP formation and thus causes platelet hyperreactivity. ABC, abacavir; CBV-TP, carbovir triphosphate; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; NO, nitric oxide; sGC, soluble guanylyl cyclase.



not only blunted the increase of platelet cGMP induced by exogenous NO, but it concomitantly enhanced platelet aggregation by ADP. Moreover, 2 hours after oral abacavir administration, at peak plasma drug concentrations, NO-induced increase of platelet cGMP was blunted and, consensually, platelet aggregation was enhanced in an abacavir concentration-dependent way.[71]

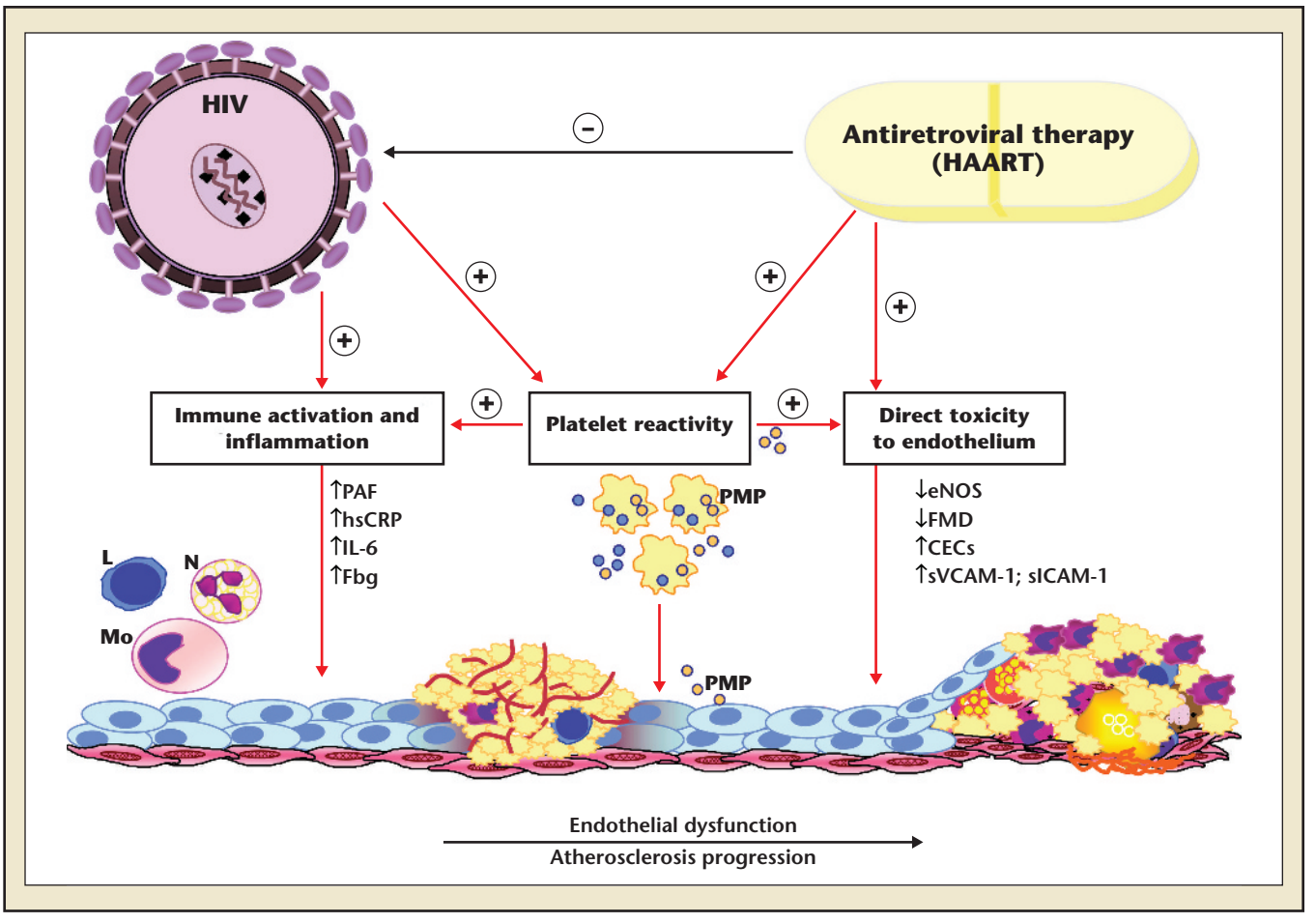
Therefore, abacavir treatment enhances platelet activation in vivo and magnifies the platelet hyperreactivity typical of HIV-infected patients in part by competing with the inhibitory activity of NO on platelets, a possible mechanism of the increased ischemic cardiovascular risk associated with abacavir (Figure 2).

Recent data using next-generation sequencing identified two heterozygous mutations in two functionally related genes, *GUCY1A3*, which encodes the α1 subunit of sGC (α1-sGC), and *CCT7*, encoding a protein that stabilizes sGC, in a large family with several individuals suffering from early MI. Platelets from digenic mutation carriers contained less sGC protein and displayed reduced NO-induced cGMP formation; moreover, mice deficient in the α1 isoform of sGC showed enhanced thrombus formation, confirming the crucial role that an impaired response to NO has in ischemic cardiovascular risk.[25]

Conclusions

Ischemic cardiovascular events have become the first non–infection-related cause of morbidity and mortality in HIV-infected patients. HIV-infection enhances cardiovascular risk by inducing a chronic inflammatory condition by provoking endothelial dysfunction,

Figure 3. Interplay between HIV, HAART, platelet activation, and endothelial dysfunction. The figure displays the pathogenesis of HIV-related platelet and endothelial dysfunction with a schematic representation of the possible interplay with HAART. Direct toxicity on endothelium has been reported only for some antiretroviral drugs. Untreated HIV infection amplifies several proatherogenic mechanisms, such as immune activation, inflammation, and platelet activation that lead to endothelial dysfunction and to progression of atherosclerosis and consequent development of cardiovascular disease. Despite effective HAART, which suppresses HIV replication, most HIV-infected persons have evidence of persistent inflammation, platelet dysfunction, and endothelium alterations contributing to enhanced cardiovascular risk by enhancing thrombus formation and/or atheroma progression. CECs, soluble circulating endothelial cells; eNOS, endothelial nitric oxide synthase; Fbg, fibrinogen; FMD, flow-mediated vasodilation; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; L, lymphocyte; Mo, monocyte; N, neutrophil; PAF, platelet activating factor; PMP, platelet microparticle; sICAM-1, soluble intercellular adhesion molecules-1; sVCAM-1, soluble vascular adhesion molecule-1.



and by generating a condition of in vivo platelet hyperreactivity. HAART may reduce the deleterious effects of HIV on the cardiovascular system by decreasing viral load (Figure 1), but some antiretroviral drugs may enhance cardiovascular risk due to direct adverse effects on platelets or the endothelium (Figure 3). In particular, abacavir may enhance the risk of MI by impairing the platelet-inhibitory and vasodilatory effects of NO, a powerful endogenous natural antithrombotic agent (Figure 2).

Given that the AIDS epidemic is destined to continue, and that the use of HAART, including abacavir, is essential for the survival and well-being of HIV-infected patients, studies prospectively assessing platelet-inhibitory and endothelium-protective treatments in HIV-infected, abacavir-treated patients are highly warranted. ■

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MAIN POINTS

- Highly active antiretroviral therapy (HAART) has transformed human immunodeficiency virus (HIV) infection into a chronic condition, which has allowed the infected population to age and become prone to chronic degenerative diseases common to the general population, including atherosclerotic cardiovascular disease and coronary artery disease (CAD).
- Possible causative mechanisms of HIV-associated CAD are dyslipidemia, insulin resistance, and fat redistribution, which may be due to either HIV infection or to HAART-associated toxicity.
- Both HIV infection and antiretroviral treatment may promote atherothrombosis by eliciting chronic inflammation and/or by altering the function of leukocytes, endothelial cells, and platelets; chronic infection with HIV is associated with a mild, permanent inflammatory state.
- The use of HAART, including abacavir, is essential for the survival and well-being of HIV-infected patients. Although HAART may reduce the deleterious effects of HIV on the cardiovascular system by decreasing viral load, some antiretroviral drugs may enhance cardiovascular risk due to direct adverse effects on platelets or the endothelium. In particular, abacavir may enhance the risk of myocardial infarction by impairing the platelet-inhibitory and vasodilatory effects of nitric oxide, a powerful endogenous natural antithrombotic agent.

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