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The Roles of HIV-1 Proteins and Antiretroviral Drug Therapy in HIV-1-Associated Endothelial Dysfunction

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Abstract

Since the emergence of highly active antiretroviral therapy (HAART), human immunodeficiency virus-1 (HIV-1)-infected patients have demonstrated dramatic decreases in viral burden and opportunistic infections, and an overall increase in life expectancy. Despite these positive HAART-associated outcomes, it has become increasingly clear that HIV-1 patients have an enhanced risk of developing cardiovascular disease over time. Clinical studies are instrumental in our understanding of vascular dysfunction in the context of HIV-1 infection. However, most clinical studies often do not distinguish whether HIV-1 proteins, HAART, or a combination of these 2 factors cause cardiovascular complications. This review seeks to address the roles of both HIV-1 proteins and antiretroviral drugs in the development of endothelial dysfunction because endothelial dysfunction is the hallmark initial step of many cardiovascular diseases. We analyze recent *in vitro* and *in vivo* studies examining endothelial toxicity in response to HIV-1 proteins or in response to the various classes of antiretroviral drugs. Furthermore, we discuss the multiple mechanisms by which HIV-1 proteins and HAART injure the vascular endothelium in HIV-1 patients. By understanding the molecular mechanisms of HIV-1 protein- and antiretroviral-induced cardiovascular disease, we may ultimately improve the quality of life of HIV-1 patients through better drug design and the discovery of new pharmacological targets.

Keywords

HIV; endothelium; cardiovascular disease; Tat; gp120; Nef; NRTI; NNRTI; PI; AZT

INTRODUCTION

Over 1.4 million Americans and approximately 40 million people worldwide are infected with human immuno-deficiency virus-1 (HIV-1) according to a 2006 World Health Organization report. In addition, over 4.3 million new cases are diagnosed each year with half of these infections occurring in individuals younger than 25 years. Fortunately, since the advent of highly active anti-retroviral therapy (HAART) nearly 2 decades ago, the morbidity and mortality associated with HIV-1 infection is markedly reduced. However, serious noninfectious consequences of HIV-1, such as cardiovascular disease, are being recognized with increasing frequency. Cardiovascular complications are associated with the greatest risk of death in HIV-1 patients, and are the fourth most common grade 4 event in this population.¹ Recent studies demonstrate that not only do various components of HAART contribute to endothelial cell damage and vascular dysfunction in patients, but also the viral proteins themselves increase cardiovascular risk. Human immunodeficiency virus-1-associated

cardiovascular disease progression is thus most likely a multifactorial process, resulting from a combination of distinct HIV-1 proteins as well as various components of current multidrug anti-retroviral regimens. This review seeks to summarize our current understanding of the mechanisms contributing to HIV-1-associated endothelial dysfunction by evaluating recent clinical, *in vivo*, and *in vitro* data.

THE ROLE OF HIV-1 PROTEINS IN ENDOTHELIAL DYSFUNCTION

Clinical Observations

Clinical studies examining cardiovascular disease in HIV-1-positive people before the era of HAART are relatively few, yet there is evidence of serious cardiovascular anomalies in these patients. Seminal work by Joshi et al.² discovered coronary arteriopathy in 3 of 6 children at autopsy who likely died of acquired immune deficiency syndrome (AIDS) and described vasculitis and perivasculitis with infiltration of lymphocytes and mononuclear cells in vessel walls. Other post-mortem analyses described major atherosclerotic lesions in proximal coronary arteries in 6 of 8 patients aged 23 to 32 years.³ The high frequency of abnormalities in these early studies is striking considering that cardiovascular pathologies are normally rare in these age groups. Other researchers found vasculitis in small blood vessels,^{4,5} aneurysms in medium and large arteries,⁶ and significantly lower levels of high-density lipoprotein (HDL) cholesterol⁷ in untreated HIV-1-positive individuals. These findings provided initial evidence of vascular dysfunction in HIV-1 patients, and suggested a possible role for uncontrolled HIV-1 infection or even immunodeficiency in the development of cardiovascular disease in this population.

Antiretroviral-naïve patients display markers of endothelial activation. Plasma levels of von Willebrand factor, plasminogen activator inhibitor-1 antigen, and tissue-type plasminogen activator are significantly elevated in HIV-1-positive patients.^{8,9} Furthermore, these markers of endothelial dysfunction positively correlate with anti-p24 antibody levels and disease severity.⁸ Compared with healthy controls, antiretroviral-naïve HIV-1-positive people have higher levels of soluble vascular cell adhesion molecule-1 (VCAM-1),¹⁰ intracellular adhesion molecule-1 (ICAM-1),¹¹ and E-selectin.^{9,11,12} This up-regulation of cell adhesion markers in HAART-naïve patients suggests that the virus activates and dys-regulates endothelial cells.

Indirect clinical evidence also implicates the HIV-1 virus in potentiating cardiovascular risk. An ongoing retrospective analysis by the Kaiser Permanente Medical Care Program of Northern California determined hospitalization rates for coronary heart disease and myocardial infarction in 4159 HIV-1-positive male members.¹³ The HIV-1-positive group had significantly higher hospitalization rates compared with age- and sex-matched HIV-1-negative controls after a 4-year follow-up. However, the authors discovered no correlation between antiretroviral therapy or other established risk factors (ie, smoking, hypertension, diabetes, and hyperlipidemia) and hospitalization rates in the infected group. This led the investigators to postulate that HIV-1 infection itself increases the hospitalization rate for coronary heart disease and myocardial infarction. This conclusion has been supported by other clinical studies as well. A recent 2007 study examined acute myocardial infarction in patients (3851 HIV-1-positive and 1,044,589 HIV-1-negative patients) at 2 large Massachusetts hospitals.¹⁴ The authors found a significantly increased risk for myocardial infarction in the HIV-1-positive population at all ages examined, even when adjusted for the presence of other traditional risk factors. Interestingly, the risk for myocardial infarction was roughly tripled in HIV-1-positive women compared with uninfected women, a group generally considered to be at lower risk for developing cardiovascular disease. However, there were insufficient data to adjust for the impact of HAART on this observation. Another long-term multi-institution analysis, the Strategies for Management of Antiretroviral Therapy Study Group, recently concluded that episodic cessation of antiretroviral therapy in HIV-1-positive patients increases the short-term

risk of developing cardiovascular disease.¹⁵ Because prolonged antiretroviral therapy has been associated with major metabolic and cardiovascular disorders, the authors of the study had hoped to evaluate the effectiveness of episodic antiretroviral treatment in 2720 HIV-1-positive patients based upon their CD4⁺ T-cell levels. Unfortunately, interruption of antiretroviral therapy actually increased the incidence of major cardiovascular events in HIV-1 patients.

Flow-mediated dilation (FMD) of the brachial artery is often used as an indicator of endothelial function in humans. Higher viral load inversely correlated with reduced endothelium-dependent FMD in a controlled case-study of 4 HIV-1-positive individuals, independent of HAART regimens.¹⁶ Solages et al.¹⁷ monitored FMD in 75 HIV-1-positive and 223 control subjects and found significantly impaired endothelial function in the infected population. Viral load was a significant predictor of reduced FMD. The authors did not see an association between endothelial dysfunction and the use of HAART, which may be due to the small size and relatively unrepresentative demographic characteristics of this specific population. A smaller study in patients from 3.5 to 19.5 years also showed that HIV-1-infected children had significantly reduced FMD, increased wall stiffness, and lower cross-sectional compliance and distensibility of the carotid artery than noninfected children or HAART-naïve children.¹⁸ Finally, Lorenz et al.¹⁹ recently concluded that HIV-1 infection and HAART are both independent risk factors for the development of atherosclerosis in adults. Intima media thickness (IMT) of the carotid bifurcation, a predictor of subclinical atherosclerosis, was 24.8% higher in an HIV-1-positive/HAART-naïve group compared with an uninfected control group. They also observed significantly greater IMT of the carotid bifurcation and the common carotid artery because of HAART treatment in HIV-1-positive individuals. Taken together, these clinical studies strongly demonstrate increased endothelial dysfunction and cardiovascular risk in HIV-1-infected patients independent of the effects of antiretroviral therapy.

Experimental Evidence: General Considerations for HIV-1 Proteins

Numerous mechanisms exist as to how HIV-1 proteins damage the endothelium. Multiple mechanisms play a role considering that the viral genome encodes for approximately 15 mature HIV-1 proteins,²⁰ which may interact with any number of unique targets. It is therefore likely that individual viral proteins affect the endothelium in both specific and overlapping ways. Human immunodeficiency virus-1 is a retrovirus belonging to the lentivirus (or “slow virus”) group. Its double-stranded RNA genome associates with packaging proteins in a nucleocapsid core, which is in turn surrounded by a matrix and a glycoprotein-rich envelope (for review of HIV-1 structural biology, see Refs. 20 and 21). The HIV-1 genome contains 9 main genes: *gag*, *pol*, *env*, *tat*, *rev*, *vpu*, *vpr*, *vif*, and *nef*. Proteolytic cleavage of the Gag-Pol precursor protein yields the major structural components of the viral core (ie, matrix p17, capsid p24, nucleocapsids p9 and p6, reverse transcriptase, protease, and integrase). Proteolytic cleavage of Env produces the important envelope glycoproteins gp120 and gp41. The remaining genes encode for the regulatory proteins Tat and Rev, and the accessory proteins Vpu, Vpr, Vif, and Nef. Table 1 provides a summary of the mature HIV-1 proteins, their importance in the viral replication-cycle, and their known effects on endothelial cell homeostasis. The following sections will focus on the experimental data supporting a role for the HIV-1 virus and individual viral proteins in affecting endothelial cell biology.

Infection of Endothelial Cells by HIV-1

It remains controversial as to whether or not HIV-1 actively infects vascular endothelial cells *in vivo*, and what the ramifications of this infection would be. *In vitro* infection of human umbilical vein endothelial cells (HUVECs) with HIV-1 has been demonstrated.²² Contact of HUVECs with HIV-1-infected U1 promonocytic cells significantly enhanced viral protein synthesis.²³ In addition, these endothelial cells up-regulated the production of interleukin-6 (IL-6), IL-1 β , and granulocyte colony-stimulating factor, which are cytokines that stimulate

HIV-1 synthesis.²³ Early work by Conaldi et al.²⁴ showed that HIV-1 could bind to, but not infect, nonreplicating HUVECs. On the other hand, HIV-1 could infect replicating HUVECs, which was further enhanced when cells were incubated with IL-1 β and tumor necrosis factor- α (TNF- α). The clinical importance of this observation is unclear considering that vascular endothelial cells are predominantly nonreplicative *in vivo*. The literature is also somewhat contradictory in terms of *in vitro* HIV-1 infection of other types of endothelial cells. Productive infection of brain microvascular endothelial cells (BMVECs) has been reported by some groups²⁵ and not by others.²⁶⁻²⁸ Fiala et al.³⁴ has demonstrated that although nonproductive, low levels of HIV-1 enter BMVECs by a process of macropinocytosis²⁹ and coronary artery endothelial cells through cytoplasmic vacuoles.³⁰ There is also evidence of HIV-1 DNA in BMVECs of HIV-1-positive patients,³¹⁻³³ although it is still unknown whether productive vascular endothelial infection actually occurs in the clinical setting.

Endothelial Regulation by HIV-1

Although HIV-1 is most likely not vasculotropic, the virus affects endothelial homeostasis and function in important ways. The vascular endothelium is continually exposed to a number of viral stimuli in the bloodstream. These stimuli include HIV-1-infected CD4⁺ T cells, monocytes, and macrophages; freely circulating HIV-1 viruses; HIV-1 proteins released upon host cell lysis; actively secreted viral proteins (ie, Tat and gp120); and viral-induced pro-inflammatory cytokines. Inflammatory cytokines³⁴ and exogenous substances^{35,36} increase endothelial cell permeability, and may facilitate monocyte invasion into the vessel wall. Human immunodeficiency virus-1-induced cytokines may also activate the endothelium, leading to enhanced production of reactive oxygen species (ROS), expression of cell adhesion molecules (CAMs), and the release of chemoattractants at localized areas of vascular inflammation. Although beyond the scope of the current review, HIV-1-induced cytokine expression plays an important role in altering endothelial cell homeostasis, and potentially contributes to the development of HIV-1-associated cardiovascular disease (see Refs. 37-42 for reviews.)

Animal models also provide evidence of vascular derangement and endothelial dysfunction in response to HIV-1. The well-characterized LP-BM5 “murine AIDS” model of retroviral infection uses a mixture of murine leukemia viruses to cause immunosuppression and other HIV-1-like symptoms.⁴³ LP-BM5 infection time-dependently decreased maximum aortic contractile responses and impaired endothelium-dependent relaxation.⁴⁴ Isolated aortas from infected mice also expressed higher levels of ICAM-1.⁴⁴ Thus, the LP-BM5 model strongly suggests that retroviral infection, similar to that of HIV-1 in humans, causes vasculopathy and endothelial dysregulation in mice. LP-BM5 infection also increased right ventricular pressure, right ventricular hypertrophy, and medial thickness.⁴⁵ These symptoms are indices of pulmonary hypertension, which is partially characterized by endothelial dysfunction⁴⁶ and is several thousand times more common in HIV-1-positive patients than in the general population.^{47,48} Alterations in endothelial homeostasis by HIV-1 may contribute to pulmonary hypertension in humans as some clinical studies have suggested that HAART-mediated decreases in viral titer improve markers of pulmonary hypertension.^{49,50} However, it should be noted that a recent retrospective study showed no difference in the incidence of pulmonary hypertension in HIV-1 patients since the advent of HAART.⁵¹ Further insight into the potential contribution of HIV-1 to pulmonary hypertension is warranted given the extremely high incidence of this complication in HIV-1 patients compared to the general population.

The Endothelium and HIV-1-Induced Oxidative Stress

Endothelial dysfunction is an initial step in the development of cardiovascular diseases, especially atherosclerosis.⁵² Endothelial dysfunction is associated with an increase in oxidative stress, which is traditionally defined as an imbalance between pro-oxidants and antioxidants but may be more accurately defined as a disruption of proper redox signaling.

⁵³ Increases in ROS, particularly superoxide and hydrogen peroxide, arise through up-regulation of superoxide-generating sources, down-regulation of free radical scavengers (ie, antioxidants), or a combination of both events. In cells of the vasculature, the main superoxide-generating sources are the NADPH oxidases (Nox), uncoupled nitric oxide synthase (NOS), xanthine oxidase, enzymes of the mitochondrial electron transport chain, and cytochrome P450s. Reactive oxygen species play an important role in dysregulating the endothelium by oxidatively damaging cellular macromolecules, inactivating enzymatic systems, and altering transcription factor activation.^{54,55} Multiple cell types of the vascular wall including activated monocyte- derived macrophages, specific T-cell lymphocytes, vascular smooth muscle cells, and endothelial cells may produce ROS in cardiovascular disease. Thus, oxidative damage to the endothelium may arise from endothelial-specific ROS generation or by ROS derived from other vascular cell types.

In the physiological setting, oxyhemoglobin and various endogenous superoxide dismutase (SOD) isoforms are predominantly responsible for detoxifying superoxide.⁵⁶ If superoxide levels “override” the cell’s superoxide-scavenging capacity, favorable reaction rates allow superoxide to directly react with nitric oxide (NO), producing the powerful oxidant peroxynitrite.^{56,57} Thus, elevated superoxide levels deplete NO bioavailability while concurrently generating additional oxidants. Nitric oxide depletion itself contributes to cardiovascular disease because NO is the primary vasorelaxant, down-regulates CAM expression, and inhibits platelet aggregation and leukocyte adhesion to the vascular wall.^{58,59} In addition, vascular ROS production and NO depletion may cause smooth muscle cell hypertrophy and migration, and potentially endothelial cell apoptosis.^{60,61} All of these effects of NO depletion contribute to oxidative stress-induced cardiovascular disease.

Human immunodeficiency virus-1 infection is associated with increased free radical production and chronic oxidative stress,^{62,63} suggesting a role for ROS in HIV-1-induced endothelial dysfunction. Human immunodeficiency virus-1-positive patients have higher plasma levels of hydroperoxides and malondialdehyde compared with noninfected individuals, indicating enhanced ROS-mediated lipid peroxidation.⁶⁴ Immunohistochemical analysis of cardiac microvascular endothelium from HIV-1-infected/cardiovascular disease-positive people shows greater 3-nitrotyrosine staining (a marker of peroxynitrite damage) than specimens from either noninfected/cardiovascular disease-positive or HIV-1-positive/cardiovascular disease-free groups.⁴⁴ Elevated ROS levels have also been reported in peripheral blood monocytes (PBMCs) from HIV-1 patients.⁶⁵ Furthermore, HIV-1 patients have lower circulating levels of the antioxidants vitamin C, cysteine, and glutathione (GSH).^{64,66-70} Antioxidant restoration via dietary supplements improves cardiovascular health in HIV-1-positive individuals. Selenium, β -carotene, and OTC (L-2-oxothiazolidine-4-carboxylin acid; procysteine) supplementation all increased serum GSH levels.^{71,72} Treatment with the GSH precursor N-acetylcysteine decreased serum levels of the pro-inflammatory cytokine TNF- α .⁷³ Dual vitamin C and E supplementation reduced plasma lipid peroxidation and oxidative stress in HIV-1 patients,⁷⁴ and treatment with multivitamins (containing vitamins B, C, and E) significantly reduced the incidence of hypertension in HIV-1-positive pregnant women.⁷⁵ A small French study showed that, compared with placebo-fed HIV-1 patients, twice daily supplementation of either selenium or β -carotene for 1 year prevented an increase in serum markers of endothelial dysfunction.⁷⁶ Supplementation with α -tocopherol⁷⁷ or selenium⁷⁸ also decreased plasma viral load and improved T-cell numbers and viability. Direct *in vivo* measurements of endothelial cell oxidative stress and antioxidant capacity in patients are limited. Nevertheless, these clinical findings suggest that vascular endothelial cells, at the very least, are exposed to ROS in the form of lipid peroxidation products, pro-inflammatory cytokines, and activated monocytes and phagocytes of the immune system. Thus, HIV-1-induced ROS likely contribute to endothelial dysfunction through direct

effects on the endothelium and/or indirectly through monocytes and macrophages contacting the vessel wall.

Antioxidants may improve endothelial function through direct scavenging of endothelial ROS, decreased activation of circulating PBMCs and lymphocytes, or inhibition of viral replication. Many studies have shown that HIV-1 replication requires the activity of the redox-regulated nuclear transcription factor NF- κ B.⁷⁹⁻⁸⁴ Addition of the GSH precursor OTC significantly inhibits HIV-1 replication in human PBMCs and macrophages.⁸⁵⁻⁸⁸ It is possible that antioxidant supplementation decreases HIV-1 replication in circulating cells, perhaps through inhibition of redox-sensitive transcription factors such as NF- κ B. This impairment of HIV-1 replication may prevent monocyte/macrophage activation and ROS over-production, thereby ameliorating the oxidative stress environment to which endothelial cells would be exposed.

Animal experiments provide further insight into HIV-1-induced oxidative stress in endothelial cells. LP-BM5 infection selectively enhanced 3-nitrotyrosine levels in aortic endothelium compared with smooth muscle cells.⁴⁴ Expression of an HIV-1 transgene in mice also increased markers of pulmonary oxidative and nitrosative stress compared with wild-type mice.⁸⁹ Moreover, these HIV-1 effects on oxidative and nitrosative stress in the transgenic mouse occurred in the absence of changes in inflammatory cytokine levels, arguing that HIV-1 proteins themselves trigger ROS production.

Tat and Endothelial Dysfunction

Tat is the principal transactivator for HIV-1 replication and is actively secreted by infected cells.^{90,91} Tat has been detected in the sera of HIV-1 patients in concentrations of 150–550 ng/mL.⁹² Tat interacts with a number of different cell types, and as such, its interaction with endothelial cells is a major focus of research. Low concentrations of Tat (1–100 nM) significantly impair endothelium-dependent vasorelaxation in isolated pig coronary arteries without affecting smooth muscle cell function.⁹³ The authors attributed this effect to decreased mRNA and protein levels of the endothelial isoform of NOS (eNOS, NOS3). Others report that Tat induces mRNA for NOS3 and NOS2 (iNOS) in macrophages⁹⁴ and microglia⁹⁵; however, this might reflect cell type-specific effects of Tat. *In vitro* and *in vivo* studies have shown that endothelial cells of diverse origin (ie, umbilical vein, pulmonary artery, aorta, and brain) release monocyte chemoattractant protein-1^{96,97} and induce expression of endothelial CAMs in response to Tat.⁹⁸⁻¹⁰² Moreover, induction of these adhesion molecules is dependent upon activation of the redox-sensitive transcription factor NF- κ B⁹⁹⁻¹⁰¹ and facilitates adhesion of lymphoma cells,¹⁰² monocytes,^{96-98,100} and T-cells¹⁰³ to the endothelium.

Tat interacts with 3 known receptors to gain access to the endothelium: the integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ through Tat's Arg-Gly-Asp (RGD) motif,^{104,105} and the vascular endothelial growth factor receptor-2 (VEGFR-2/KDR) through Tat's basic domain.¹⁰⁶ Activation of these receptors initiates endothelial signal transduction cascades regulating diverse processes such as cytoskeletal dynamics,¹⁰⁷ endothelial permeability,^{108,109} matrix metalloproteinase activation,¹¹⁰ chemotaxis,¹¹¹ cell proliferation,^{112,113} angiogenesis,^{114,115} and apoptosis.¹¹⁶ The signaling pathways activated by Tat are incompletely defined and difficult to dissect because of considerable cross-talk. Tat-induced activation of focal adhesion kinase¹⁰⁸ and p21-activated kinase-1 (PAK-1)¹⁰⁷ is associated with increased endothelial permeability and actin cytoskeletal rearrangement, respectively. Expression of VCAM-1 is dependent upon p38 mitogen-activated protein kinase (MAPK) and activation of NF- κ B,⁹⁹ whereas expression of the pro-inflammatory cytokine IL-6 is dependent upon protein kinase C (PKC) and cAMP-dependent protein kinase A activity.¹¹⁷ Recently, Terada's group discovered that Tat activates the small GTPase Rac1 in endothelial cells, which in turn activates dual MAPK pathways.¹¹⁸ Tat activation of Rac-1 signals through RhoA, Ras, and extracellular signal-regulated kinase (ERK) to induce endothelial cell proliferation and survival. Concurrently, Tat activation

of Rac-1 signals through PAK-1 to regulate actin dynamics as well as to phosphorylate c-Jun N-terminal kinase (JNK). Much remains to be learned about the specific molecular pathways activated in response to Tat; however, it is clear that Tat causes aberrant cell signaling and leads to altered endothelial cell morphology, gene expression, and survival.

In addition to its effects on kinase activity and gene transcription, Tat signaling is a key player in HIV-1-induced oxidative stress in endothelial cells. Tat-mediated activation of PAK-1 causes phosphorylation of the Nox regulatory subunit p47^{phox}¹⁰⁷ and requires Nox2 to elicit its downstream effects.¹¹⁸ Tat activation of ERK pathways in endothelial cells also requires Nox4.¹¹⁸ Thus, superoxide production by various NADPH oxidase subunits is an upstream requirement for Tat-induced MAPK signaling. Furthermore, Tat-induced ROS activate endothelial NF- κ B and its down-stream genes,^{99,119} and decrease GSH levels.¹¹⁹ Tat also decreases expression of the mitochondrial superoxide scavenger Mn-SOD in other cell types.^{120,121} Thus, Tat activates the endothelium and increases oxidative stress through NADPH oxidase activation and through decreased antioxidant capacity.

gp120 and Endothelial Dysfunction

gp120 is a glycoprotein expressed on the surface of the HIV-1 virus and infected host cells. gp120 and gp41 interact with CD4 and the CXCR4 and CCR5 chemokine receptors to initiate viral entry into the host cell. Like Tat, circulating levels of gp120 are readily detectable in patient sera and have been reported at concentrations ranging from 0.24 ng/mL¹²² to 92 ng/mL.¹²³ Exposure to gp120 increases expression of ICAM-1, but not VCAM-1 or E-selectin, in human coronary artery, lung, brain, umbilical vein, and dermal microvascular endothelial cells.¹²⁴ Similar to Tat, gp120 significantly increases the adhesion of monocytes^{124,125} and lymphocytes^{126,127} to the endothelium. gp120 increases endothelial permeability through cytoskeletal rearrangement,³⁵ down-regulation of tight junction proteins,¹²⁸ and PKC activation¹²⁵ in gp120 transgenic mice¹²⁹ and human BMVECs. gp120 also elicits a marked release of endothelin-1 from endothelial cells,¹³⁰ suggesting that gp120 negatively affects endothelial function through the production of potent vasoconstrictors. Furthermore, gp120 induces apoptosis in human coronary endothelial cells,¹³¹ HUVECs,^{132,133} lung microvascular endothelial cells (LMVECs),¹³⁴ and BMVECs.^{135,136} Endothelial cell apoptosis in response to viral proteins is reviewed in more detail by Mu et al.⁴²

The molecular mechanism by which gp120 exerts its endothelial toxicity may involve PKC^{125,137} and p38 MAPK signaling.¹³⁵ Similar to Tat, ROS have also been implicated in gp120 toxicity of endothelial cells^{35,138} and smooth muscle cells.¹³⁹ The source of gp120-mediated free radical production is currently unknown. gp120 decreases GSH levels in BMVECs,¹³⁸ and studies in neurons show that gp120 down-regulates Mn-SOD and up-regulates NADPH oxidase activity.¹⁴⁰ It remains to be determined what role, if any, enzymatic sources of superoxide play in gp120-mediated oxidative stress in the endothelium.

Effects of the HIV-1 Accessory Proteins Vpu, Vpr and Nef

Considerably less well-known are the effects of other HIV-1 accessory proteins on endothelial cell biology. Adenoviral expression of Vpu up-regulates the tumor necrosis factor receptor family molecule CD40, inducing VCAM-1 expression and adhesion of B-lymphoma cells to the endothelium.¹⁴¹ Treatment of BMVECs with recombinant Vpr or Nef induces TNF- α production and endothelial cell apoptosis.¹⁴² *In vitro* data suggest that Nef alters vascular homeostasis by affecting endothelial cells and macrophages through distinct pathways. For example, co-culture studies show that the presence of endothelial cells dramatically enhances HIV-1 infection of CD4⁺ T cells, and that a Nef-deficient virus attenuates viral replication.¹⁴³ This finding implies that Nef communicates with the endothelium and with HIV-1-infected T-cells to up-regulate viral replication, perhaps through direct cell-cell contact or through the

release of endothelial signals. Nef also dysregulates human monocytes and macrophages by activating MAPK¹⁴⁴ and JNK pathways,¹⁴⁵ as well as by temporally regulating the production of superoxide from macrophages.¹⁴⁶

Although enhanced Nef-mediated macrophage activation and superoxide generation may contribute to vascular dysfunction, new evidence suggests that Nef plays an additional, and no less significant, role by altering cholesterol metabolism. Nef increases cholesterol biosynthesis and binds to newly created cholesterol in order to enrich lipid rafts at the plasma membrane.¹⁴⁷ Mujawar et al.¹⁴⁸ have recently shown that HIV-1 infection of macrophages also impairs adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) cholesterol efflux in a Nef-dependent manner. Nef post-transcriptionally down-regulates ABCA1 levels, alters intracellular ABCA1 localization, and transforms macrophages into lipid-loaded foam cells. Moreover, stimulating ABCA1 expression restores cholesterol efflux and reduces HIV-1 infection. As an enveloped virus, HIV-1 requires host-cell cholesterol for structural purposes, for proper contact between viral glycoproteins and cell surface receptors during infection, and for efficient viral budding from the host cell membrane.¹⁴⁹ Thus, Nef may not only have direct effects on endothelial cell signaling, but may activate macrophages and produce foam cells. Contact of endothelial cells with these cell types would likely contribute to endothelial cell derangements and may potentially facilitate the development of atherosclerosis.

THE ROLE OF HAART IN ENDOTHELIAL DYSFUNCTION

Antiretroviral Drugs and Current HAART Regimens

There are 24 approved antiretroviral drugs targeting various viral proteins or critical points in the virus-host life cycle. These drugs are mechanistically divided into 5 classes: the nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors, and entry inhibitors (EIs). In most cases, NRTIs constitute the backbone of antiretroviral treatment strategies. The U.S. Department of Health and Human Services currently recommends initial combination therapy regimens consisting of 2 NRTIs with either 1–2 PIs or 1 NNRTI.¹⁵⁰ Although HAART has undoubtedly reduced the morbidity and mortality of HIV-1, clinical and *in vitro* evidence strongly suggests that long-term HAART results in endothelial toxicity and vascular dysfunction. Because the integrase inhibitors (ie, raltegravir) and EIs (ie, enfuvirtide and maraviroc) were only recently approved and are typically given to HAART-experienced patients demonstrating therapeutic failure, little is known about their general and endothelial-specific toxicities. Table 2 lists the currently approved antiretroviral drugs and the experimental studies that demonstrate endothelial effects. The following sections of this review will focus on the toxicities attributed to the NRTI, NNRTI, and PI classes of antiretroviral agents because these are the most commonly prescribed drugs for the initial treatment of HIV-1.

NRTIs and Endothelial Toxicity-Experimental Evidence

Nucleoside reverse transcriptase inhibitors were the first drugs approved for the treatment of HIV-1. The 8 NRTIs currently used in the U.S. are zidovudine (AZT), stavudine, didanosine, zalcitabine, abacavir, emtricitabine, lamivudine, and tenofovir (a nucleotide reverse transcriptase inhibitor). According to the Department of Health and Human Service, the 2 preferred dual NRTI backbones of current HAART regimens are either tenofovir and emtricitabine, or AZT and lamivudine.¹⁵⁰ Because AZT was the first antiretroviral drug approved for HIV-1, it is perhaps the most well-characterized NRTI and is often used as the prototype for this class of antiretrovirals.

The antiviral activity of the nucleoside analogues requires phosphorylation to their respective tri-phosphorylated moieties by nucleoside- and nucleotide-specific host cell kinases. Nucleoside reverse transcriptase inhibitor triphosphates mimic endogenous nucleotides and competitively inhibit viral reverse transcriptase. Because of the lack of a 3'-OH group on their pentose rings, incorporation of NRTI-triphosphates into viral DNA prevents subsequent phosphodiester bond formation and terminates chain elongation. Host cell toxicity to NRTIs is commonly ascribed to competitive inhibition with endogenous nucleotides for mitochondrial DNA polymerase- γ (pol- γ), the sole enzyme responsible for mitochondrial DNA (mtDNA) replication and repair. According to the DNA pol- γ hypothesis, the NRTI-triphosphate incorporates into mtDNA, inhibits mtDNA synthesis and copy number, and ultimately leads to depletion of mitochondria in affected tissues. Over time, the DNA pol- γ hypothesis has expanded to include other potential NRTI effects. Mutations in mtDNA-encoded gene products may arise through either the persistence of NRTI-triphosphate in mtDNA in the absence of chain termination or through inhibition of the exonucleolytic proofreading activity of pol- γ by excess mitochondrial NRTI-monophosphate.¹⁵¹ Because mtDNA primarily encodes for components of the electron transport chain, mutations in mtDNA and its gene products result in altered oxidative phosphorylation, aberrant cellular respiration, and electron leakage from the mitochondria leading to cell toxicity.

The diverse clinical toxicities of NRTIs are well documented and include lactic acidosis and hyperlactatemia, peripheral neuropathy, skeletal myopathy, cardiomyopathy, pancreatitis, and lipodystrophy (reviewed extensively in Refs. 152,¹⁵³ and references therein). Clinical evidence for NRTI-induced vascular/endothelial toxicity is indirect and difficult to define because NRTIs are not prescribed as monotherapy, and cardiovascular effects are often attributed to other components of HAART, namely PIs. Most experimental studies examining mitochondrial toxicity of NRTIs are conducted in liver cell lines (eg, HepG2 cells) due to their relatively large number of mitochondria, the liver's involvement in NRTI metabolism, and the observed clinical side effects implicating hepatic dysfunction. *In vitro* studies linking NRTIs to endothelial cell-specific toxicity are not as numerous; however, evidence supports a role for NRTIs in endothelial dysfunction. Aortas from mice treated with 100 mg·kg⁻¹·day⁻¹ AZT for 35 days have an approximately 34% reduction in maximum endothelium-dependent relaxation compared with vehicle-treated mice, as well as a 5-fold decrease in acetylcholine sensitivity.¹⁵⁴ AZT increases aortic endothelium superoxide levels in these mice, and treatment with the free radical scavenger tiron restores vasorelaxation, implicating ROS in NRTI-induced endothelial dysfunction. A more recent study shows that AZT (10 mg·kg⁻¹·day⁻¹ for 30 days) markedly impairs endothelium-dependent relaxation in rats without changing plasma triglycerides or total cholesterol levels, suggesting that AZT induces direct damage to the vascular endothelium.¹⁵⁵

Treatment of cultured endothelial cells with micro-molar concentrations of AZT has been shown to induce signs of cytotoxicity within 24 h¹⁵⁶ without the induction of apoptosis.^{131, 157} AZT promotes release of the vasoconstrictor and mitogen endothelin-1 and enhances proliferation of vascular smooth muscle cells and HUVECs.¹⁵⁶ In addition, AZT causes intercellular gaps and decreases transendothelial electrical resistance in human coronary artery endothelial cells.¹³¹ Mitochondria are a likely target of NRTIs in endothelial cells as AZT causes a 4-fold reduction in mitochondrial respiration, impairs ATP production, and decreases the mitochondrial membrane potential.^{156,157} The mitochondrial origin of NRTI-induced ROS is supported by studies demonstrating that over-expression of a mitochondria-targeted, but not a cytoplasmic-targeted, catalase construct prevents AZT-induced increases in ROS and endothelin-1.¹⁵⁷

Nucleoside reverse transcriptase inhibitor-induced oxidative stress, particularly mitochondrial ROS, seems to play an important role in cell culture and animal models of endothelial toxicity.

It is tempting to assume that pol- γ inhibition by NRTI-triphosphates decreases mitochondria levels or alters electron transport chain subunit activity in endothelial cells, but these parameters were not evaluated in the aforementioned studies. Furthermore, cell culture experiments showed enhanced endothelial ROS generation in response to AZT treatment within 24 h,¹⁵⁷ well short of the required 1–2 weeks necessary for mitochondrial turnover and subsequent mtDNA depletion to occur.¹⁵⁸ Thus, although mitochondrial in origin, endothelial ROS production in response to AZT (and perhaps other NRTIs) may arise from unidentified sources, and not necessarily through pol- γ inhibition. Studies using nonendothelial cell types may offer possible explanations, although one must be careful in making definitive extrapolations as NRTIs likely have cell type-specific effects. With this caveat in mind, numerous alternative targets of NRTIs have been studied. AZT decreases mitochondrial GSH in mouse cardiac cells *in vivo*¹⁵⁹ and lymphoid cells *in vitro*,¹⁶⁰ but whether decreased GSH is a cause or a consequence of mitochondrial dysfunction is unknown. Recently, Papparella et al.¹⁶¹ reported that long-term treatment of rats with AZT up-regulates the Nox subunits gp91^{phox} and p47^{phox} in heart homogenates and alters mitochondria morphology. These findings imply that NRTIs may induce expression of superoxide-generating sources. Alternatively, AZT treatment of an artificial membrane model system prevents aggregation of cardiolipin, a molecular subunit of the inner mitochondrial membrane.¹⁶² Alteration of the mitochondrial membrane by NRTIs may interfere with the membrane potential or enzymatic activities of the mitochondria and result in ROS production. Others have hypothesized that NRTI-monophosphates compete with endogenous nucleotides for host cell kinases, which would deplete mitochondrial nucleotide-triphosphate pools and impair mtDNA replication/repair.^{163,164} AZT, as the prototypical NRTI, has also been proposed to have other secondary targets in various cell types including cytochrome *c* oxidase and citrate synthase,¹⁶⁵ poly-ADP-ribose polymerase,¹⁶⁶ glycosphingolipid synthesis,¹⁶⁷ and the ADP/ATP translocator.¹⁶⁸ Recently, Lund and Wallace¹⁶⁹ showed that AZT and AZT-MP directly interact with complex I of the mitochondrial electron transport chain and prevent its cAMP-dependent phosphorylation independently of mtDNA pol- γ inhibition. Whether or not any of these targets is affected by NRTIs in endothelial cells is unknown. Future investigation into the multiple direct and indirect effects of NRTIs on mitochondrial gene expression¹⁷⁰ and ROS generation are warranted to accurately determine how this important class of drugs exerts its toxic effects on the endothelium.

NNRTIs and Cardiovascular Health

Nonnucleoside reverse transcriptase inhibitors are non-competitive inhibitors of reverse transcriptase. In contrast to NRTIs, NNRTIs do not require phosphorylation to achieve their antiviral effects. The currently approved NNRTIs are nevirapine, efavirenz, and delavirdine. Efavirenz is the preferred NNRTI for initial treatment of HIV-1.¹⁵⁰ Hypersensitivity reactions are the predominant side effect associated with this class of drugs,¹⁷¹ and cardiovascular events appear to be relatively infrequent. In contrast to NRTIs, there are very few *in vitro* studies examining endothelial toxicity in response to NNRTI treatment. In clinical studies comparing the relative cardiovascular effects of PI- versus NNRTI-based HAART regimens, exposure to PIs, but not to NNRTIs, was associated with increased risk of premature atherosclerosis¹⁷² and myocardial infarction.¹⁷³ The use of NNRTIs in HAART regimens was also associated with a lower incidence of hypertension,¹⁷⁴ higher plasma HDL levels,^{175,176} and a more favorable lipid profile^{177,178} than either the NRTI or PI classes of antiretroviral drugs. These findings suggest that switching to an NNRTI-based HAART regimen may reduce cardiovascular side effects in atherosclerosis-prone HIV-1 patients, and highlight the role of PIs as major contributors to HAART-induced cardiovascular toxicity.

PIs and Endothelial Toxicity

PIs inhibit HIV-1 replication by preventing the cleavage of viral polypeptides into mature and functional proteins. This class of antiretrovirals includes ritonavir, atazanavir, amprenavir, indinavir, nelfinavir, darunavir, saquinavir, fosamprenavir, tipranavir, and lopinavir. Because ritonavir is a potent inhibitor of the cytochrome P450 isoenzyme 3A4, it is often given in low doses with another PI in HAART regimens (frequently atazanavir, fosamprenavir, or lopinavir) to increase its half-life.¹⁵⁰

Controversy exists regarding PI-based therapy and cardiovascular risk. Some authors have found correlations between PI exposure and higher carotid IMT^{179,180} and decreased FMD,¹⁸¹⁻¹⁸³ while others have not.^{17,184-187} Potential explanations for these clinical discrepancies include nonrepresentative population demographics, relatively short-term treatment, individual PI-specific effects, small patient sample size, and the increased incidence of compounding cardiovascular risk factors in these individuals. Finding associations between enhanced cardiovascular risk and either HIV-1 infection or antiretroviral drugs may also be complicated by the fact that patients receiving therapy have considerably longer life-spans, making the appearance of cardiovascular events more apparent in these people. Despite these contradicting reports, long-term treatment with PIs has generally been associated with a number of cardiovascular side effects including a greater incidence of diabetes mellitus, lipodystrophy, hyperlipidemia, and atherosclerosis.¹⁸⁸⁻¹⁹¹ It has been suggested that PIs increase the relative risk of myocardial infarction by up to 26% per year of exposure in HIV-1 patients,¹⁹²⁻¹⁹⁴ and that PI exposure greater than 18 months increases the risk of myocardial infarction by 2-fold in HIV-1-positive males.¹⁹⁴ Moreover, an outpatient study determined that the incidence of myocardial infarction increased in HIV-1 patients after 1996, the year that PIs were introduced as major components of HIV-1 therapy.¹⁹³

The molecular mechanisms of PI toxicity in endothelial cells has been described in great detail,¹⁹⁵ and many *in vitro* experiments implicate oxidative stress in PI-induced endothelial dysfunction. PI treatment produces serious mitochondrial disturbances as evidenced by reduced cellular respiration and ATP production,¹⁵⁶ decreased mitochondrial membrane potential, increased mitochondrial production of ROS,¹⁵⁷ and enhanced mtDNA damage.¹⁹⁶ PIs also increase endothelial cell permeability¹⁹⁷ and leukocyte adhesion¹⁹⁸ in cell culture models. *In vivo* PI administration increases superoxide and impairs endothelium-dependent relaxation of isolated rat aortic rings¹⁹⁹ and porcine coronary arteries.^{200,201} Reactive oxygen species were critical in eliciting these responses as treatment with antioxidants significantly reversed endothelial activation and dysfunction.^{197,198,201-203} The source of ROS generation has not been determined, but has been suggested to be of mitochondrial origin¹⁵⁷ or NADPH-dependent.²⁰⁰ Although ROS may impair endothelium-dependent relaxation by directly damaging the endothelium or by enhancing superoxide-mediated NO degradation, some studies suggest that various PIs reduce the mRNA and protein expression of NOS3.^{195,201-204} Thus, endothelial toxicity to PIs may result from altered mitochondrial function and integrity, direct oxidative damage, and/or ROS-mediated changes in gene expression of NO sources.

PI effects in nonendothelial cells may also contribute to cardiovascular risk. For example, PIs dysregulate fat cell homeostasis which may explain the high incidence of lipodystrophy and hyperlipidemia in HIV-1 patients. Protease inhibitors prevent the differentiation of preadipocytes by decreasing matrix metalloproteinase expression,²⁰⁵ inhibiting adiponectin secretion,²⁰⁶ and inhibiting triglyceride and very low-density lipoprotein cholesterol clearance and catabolism.^{207,208} Besides adipocytes, macrophages are also extra-endothelial targets of PI toxicity. Protease inhibitors, most notably ritonavir, cause accumulation of cholesterol in macrophages by activating the unfolded protein response²⁰⁹ and by up-regulating the scavenger receptor CD36 in a PKC- and PPAR- γ -dependent manner.²¹⁰ Interestingly,

cotreatment with the NRTIs didanosine and stavudine prevent PI-induced CD36 expression by promoting the proteasomal degradation of PKC,²¹¹ suggesting that NRTIs may counteract at least some of the pro-atherogenic properties of PIs. Ritonavir treatment of macrophages impairs cholesterol efflux that may be due in part to activation of ERK signaling and enhanced expression of Nox components.²¹² Protease inhibitors also increase expression of the pro-inflammatory cytokines TNF- α and IL-6.²¹³ Thus, PI-mediated changes in gene expression, activation of cell signaling pathways, and oxidative stress in nonendothelial cells can negatively impact cardiovascular health.

CONCLUSIONS

There is clear evidence that HIV-1 proteins and antiretroviral drugs cause endothelial dysfunction. Although differences may exist as to the relative contribution and mechanisms of toxicity, the preponderance of clinical and experimental data supports roles for both of these factors in the context of HIV-1 infection. Thus, in assessing cardiovascular risk, it is important to take into account potential contributions from both infection and therapy. Further exploration into the precise mechanisms of toxicity is warranted as endothelial dysfunction in HIV-1 patients is most likely a multifactorial process. *In vitro* experiments have demonstrated numerous overlapping, and sometimes distinct, mechanisms of toxicity for HIV-1 proteins (eg, Tat, gp120, Nef, Vpu, and Vpr) and many of the currently approved anti-retroviral drugs (eg, NRTIs and PIs), suggesting that targeting only 1 pathway may not be sufficient to reduce the incidence of cardiovascular events in HIV-1 patients. Because of its multi-factorial nature, treating cardiovascular diseases in HIV-1-positive individuals may prove challenging. For example, treatment of hyperlipidemic HIV-1 patients on PI therapy with the cholesterol-lowering drug pravastatin improved lipid profiles, yet failed to improve endothelial function as measured by FMD.²¹⁴ Thus, other factors in addition to increased pro-atherogenic lipids, likely contribute to PI-induced, and potentially other antiretroviral-induced, endothelial cell dysfunction.

To various degrees, multiple HIV-1 viral proteins and antiretroviral drugs activate cell signaling cascades, induce oxidative stress, disturb mitochondrial function, alter gene expression, and impair lipid metabolism. Moreover, these changes occur not only in endothelial cells, but also in vascular smooth muscle cells, macrophages, and adipocytes, further emphasizing the complex interactions that impact cardiovascular health in HIV-1 patients. By exploring the ways in which HIV-1 proteins and antiretroviral drugs interact with each other and with the endothelium, we may ultimately discover new ways to treat and prevent endothelial dysfunction in HIV-1-positive people.

REFERENCES

1. Reisler RB, Han C, Burman WJ, et al. Grade 4 events are as important as AIDS events in the era of HAART. *J Acquir Immune Defic Syndr* 2003;34(4):379–386. [PubMed: 14615655]
2. Joshi VV, Pawel B, Connor E, et al. Arteriopathy in children with acquired immune deficiency syndrome. *Pediatr Pathol* 1987;7(3):261–275. [PubMed: 3684808]
3. Paton P, Tabib A, Loire R, et al. Coronary artery lesions and human immunodeficiency virus infection. *Res Virol* 1993;144(3):225–231. [PubMed: 8356344]
4. Mandell BF, Calabrese LH. Infections and systemic vasculitis. *Curr Opin Rheumatol* 1998;10(1):51–57. [PubMed: 9448990]
5. Cebrian M, Miro O, Font C, et al. HIV-related vasculitis. *AIDS Patient Care STDS* 1997;11(4):245–258. [PubMed: 11361839]
6. Maniker AH, Hunt CD. Cerebral aneurysm in the HIV patient: a report of six cases. *Surg Neurol* 1996;46(1):49–54. [PubMed: 8677489]

7. Grunfeld C, Pang M, Doerrler W, et al. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J Clin Endocrinol Metab* 1992;74(5):1045–1052. [PubMed: 1373735]
8. Schved JF, Gris JC, Arnaud A, et al. von Willebrand factor antigen, tissue-type plasminogen activator antigen, and risk of death in human immunodeficiency virus 1-related clinical disease: independent prognostic relevance of tissue-type plasminogen activator. *J Lab Clin Med* 1992;120(3):411–419. [PubMed: 1517688]
9. Lafeuillade A, Alessi MC, Poizot-Martin I, et al. Endothelial cell dysfunction in HIV infection. *J Acquir Immune Defic Syndr* 1992;5(2):127–131. [PubMed: 1531074]
10. Wolf K, Tsakiris DA, Weber R, et al. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. *J Infect Dis* 2002;185(4):456–462. [PubMed: 11865397]
11. Greenwood AJ, Hughes J, Wallace G, et al. Soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) in patients with HIV/AIDS does not appear to correlate with cytomegalovirus retinitis. *Int J STD AIDS* 1998;9(11):713–714. [PubMed: 9863589]
12. Seigneur M, Constans J, Blann A, et al. Soluble adhesion molecules and endothelial cell damage in HIV infected patients. *Thromb Haemost* 1997;77(4):646–649. [PubMed: 9134636]
13. Klein D, Hurley LB, Quesenberry CP Jr, et al. Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? *J Acquir Immune Defic Syndr* 2002;30(5):471–477. [PubMed: 12154337]
14. Triant VA, Lee H, Hadigan C, et al. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* 2007;92(7):2506–2512. [PubMed: 17456578]
15. El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006;355(22):2283–2296. [PubMed: 17135583]
16. Blum A, Hadas V, Burke M, et al. Viral load of the human immunodeficiency virus could be an independent risk factor for endothelial dysfunction. *Clin Cardiol* 2005;28(3):149–153. [PubMed: 15813624]
17. Solages A, Vita JA, Thornton DJ, et al. Endothelial function in HIV-infected persons. *Clin Infect Dis* 2006;42(9):1325–1332. [PubMed: 16586393]
18. Bonnet D, Aggoun Y, Szezepanski I, et al. Arterial stiffness and endothelial dysfunction in HIV-infected children. *AIDS* 2004;18(7):1037–1041. [PubMed: 15096807]
19. Lorenz MW, Stephan C, Harmjanz A, et al. Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. *Atherosclerosis*. Jan 31;2007
20. Turner BG, Summers MF. Structural biology of HIV. *J Mol Biol* 1999;285(1):1–32. [PubMed: 9878383]
21. Krogstad P. Molecular biology of the human immunodeficiency virus: current and future targets for intervention. *Semin Pediatr Infect Dis* 2003;14(4):258–268. [PubMed: 14724791]
22. Corbeil J, Evans LA, McQueen PW, et al. Productive in vitro infection of human umbilical vein endothelial cells and three colon carcinoma cell lines with HIV-1. *Immunol Cell Biol* 1995;73(2):140–145. [PubMed: 7797233]
23. Fan ST, Hsia K, Edgington TS. Upregulation of human immunodeficiency virus-1 in chronically infected monocytic cell line by both contact with endothelial cells and cytokines. *Blood* 1994;84(5):1567–1572. [PubMed: 7915148]
24. Conaldi PG, Serra C, Dolei A, et al. Productive HIV-1 infection of human vascular endothelial cells requires cell proliferation and is stimulated by combined treatment with interleukin-1 beta plus tumor necrosis factor-alpha. *J Med Virol* 1995;47(4):355–363. [PubMed: 8636703]
25. Moses AV, Bloom FE, Pauza CD, et al. Human immunodeficiency virus infection of human brain capillary endothelial cells occurs via a CD4/galactosylceramide-independent mechanism. *Proc Natl Acad Sci U S A* 1993;90(22):10474–10478. [PubMed: 7504264]
26. Poland SD, Rice GP, Dekaban GA. HIV-1 infection of human brain-derived microvascular endothelial cells in vitro. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;8(5):437–445. [PubMed: 7697439]

27. Ades EW, Comans TW, Nicholson JK, et al. Lack of evidence that human immunodeficiency virus can infect human endothelial cells in vitro. *J Acquir Immune Defic Syndr* 1993;6(1):104. [PubMed: 8417169]
28. Ades EW, Nicholson JK, Browning SW, et al. Inability of human immunodeficiency virus to infect human microvascular endothelial cells. *Pathobiology* 1992;60(6):330–331. [PubMed: 1290591]
29. Liu NQ, Lossinsky AS, Popik W, et al. Human immunodeficiency virus type 1 enters brain microvascular endothelia by macropinocytosis dependent on lipid rafts and the mitogen-activated protein kinase signaling pathway. *J Virol* 2002;76(13):6689–6700. [PubMed: 12050382]
30. Gujuluva C, Burns AR, Pushkarsky T, et al. HIV-1 penetrates coronary artery endothelial cells by transcytosis. *Mol Med* 2001;7(3):169–176. [PubMed: 11471553]
31. An SF, Groves M, Giometto B, et al. Detection and localisation of HIV-1 DNA and RNA in fixed adult AIDS brain by polymerase chain reaction/in situ hybridisation technique. *Acta Neuropathol (Berl)* 1999;98(5):481–487. [PubMed: 10541871]
32. An SF, Groves M, Gray F, et al. Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. *J Neuropathol Exp Neurol* 1999;58(11):1156–1162. [PubMed: 10560658]
33. Bagasra O, Lavi E, Bobroski L, et al. Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: identification by the combination of in situ polymerase chain reaction and immunohistochemistry. *AIDS* 1996;10(6):573–585. [PubMed: 8780811]
34. Fiala M, Looney DJ, Stins M, et al. TNF-alpha opens a paracellular route for HIV-1 invasion across the blood-brain barrier. *Mol Med* 1997;3(8):553–564. [PubMed: 9307983]
35. Shiu C, Barbier E, Di Cello F, et al. HIV-1 gp120 as well as alcohol affect blood-brain barrier permeability and stress fiber formation: involvement of reactive oxygen species. *Alcohol Clin Exp Res* 2007;31(1):130–137. [PubMed: 17207111]
36. Fiala M, Eshleman AJ, Cashman J, et al. Cocaine increases human immunodeficiency virus type 1 neuroinvasion through remodeling brain microvascular endothelial cells. *J Neurovirol* 2005;11(3):281–291. [PubMed: 16036808]
37. Chi D, Henry J, Kelley J, et al. The effects of HIV infection on endothelial function. *Endothelium* 2000;7(4):223–242. [PubMed: 11201521]
38. Maslin CL, Kedzierska K, Webster NL, et al. Transendothelial migration of monocytes: the underlying molecular mechanisms and consequences of HIV-1 infection. *Curr HIV Res* 2005;3(4):303–317. [PubMed: 16250878]
39. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. *Clin Sci (Lond)* 2005;108(3):205–213. [PubMed: 15540988]
40. Sudano I, Spieker LE, Noll G, et al. Cardiovascular disease in HIV infection. *Am Heart J* 2006;151(6):1147–1155. [PubMed: 16781213]
41. Copeland KF. Modulation of HIV-1 transcription by cytokines and chemokines. *Mini Rev Med Chem* 2005;5(12):1093–1101. [PubMed: 16375755]
42. Mu H, Chai H, Lin PH, et al. Current Update on HIV-associated Vascular Disease and Endothelial Dysfunction. *World J Surg* 2007;31(4):632–643. [PubMed: 17372667]
43. Clark S, Duggan J, Chakraborty J. Tsl and LP-BM5: a comparison of two murine retrovirus models for HIV. *Viral Immunol* 2001;14(2):95–109. [PubMed: 11398815]
44. Baliga RS, Chaves AA, Jing L, et al. AIDS-related vasculopathy: evidence for oxidative and inflammatory pathways in murine and human AIDS. *Am J Physiol Heart Circ Physiol* 2005;289(4):H1373–H1380. [PubMed: 15923317]
45. Gillespie MN, Hartsfield CL, O'Connor WN, et al. Pulmonary hypertension in a murine model of the acquired immunodeficiency syndrome. *Am J Respir Crit Care Med* 1994;150(1):194–199. [PubMed: 8025749]
46. Naeije R, Rondelet B. Pathobiology of pulmonary arterial hypertension. *Bull Mem Acad R Med Belg* 2004;159(Pt 2):219–226. [PubMed: 15615096]
47. Seoane L, Shellito J, Welsh D, et al. Pulmonary hypertension associated with HIV infection. *South Med J* 2001;94(6):635–639. [PubMed: 11440333]
48. Speich R, Jenni R, Opravil M, et al. Primary pulmonary hypertension in HIV infection. *Chest* 1991;100(5):1268–1271. [PubMed: 1935280]

49. Pugliese A, Isnardi D, Saini A, et al. Impact of highly active antiretroviral therapy in HIV-positive patients with cardiac involvement. *J Infect* 2000;40(3):282–284. [PubMed: 10908024]
50. Zuber JP, Calmy A, Evison JM, et al. Pulmonary arterial hypertension related to HIV infection: improved hemo-dynamics and survival associated with antiretroviral therapy. *Clin Infect Dis* 2004;38(8):1178–1185. [PubMed: 15095226]
51. Sitbon O, Lascoux-Combe C, Delfraissy JF, et al. Prevalence of HIV-related pulmonary arterial hypertension in the current antiretroviral therapy era. *Am J Respir Crit Care Med* 2008;177(1):108–113. [PubMed: 17932378]
52. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340(2):115–126. [PubMed: 9887164]
53. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal* 2006;8(9–10):1865–1879. [PubMed: 16987039]
54. Castro L, Rodriguez M, Radi R. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. *J Biol Chem* 1994;269(47):29409–29415. [PubMed: 7961920]
55. Hausladen A, Fridovich I. Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. *J Biol Chem* 1994;269(47):29405–29408. [PubMed: 7961919]
56. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* Nov;1996 271(5 Pt 1):C1424–C1437. [PubMed: 8944624]
57. Wink DA, Mitchell JB. Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radic Biol Med* 1998;25(4–5):434–456. [PubMed: 9741580]
58. Gewaltig MT, Kojda G. Vasoprotection by nitric oxide: mechanisms and therapeutic potential. *Cardiovasc Res* 2002;55(2):250–260. [PubMed: 12123764]
59. Fenster BE, Tsao PS, Rockson SG. Endothelial dysfunction: clinical strategies for treating oxidant stress. *Am Heart J* 2003;146(2):218–226. [PubMed: 12891188]
60. Zafari AM, Ushio-Fukai M, Akers M, et al. Role of NADH/NADPH oxidase-derived H₂O₂ in angiotensin II-induced vascular hypertrophy. *Hypertension* 1998;32(3):488–495. [PubMed: 9740615]
61. Shankar SS, Dube MP. Clinical aspects of endothelial dysfunction associated with human immunodeficiency virus infection and antiretroviral agents. *Cardiovasc Toxicol* 2004;4(3):261–269. [PubMed: 15470273]
62. Pace GW, Leaf CD. The role of oxidative stress in HIV disease. *Free Radic Biol Med* 1995;19(4):523–528. [PubMed: 7590404]
63. Baruchel S, Wainberg MA. The role of oxidative stress in disease progression in individuals infected by the human immunodeficiency virus. *J Leukoc Biol* 1992;52(1):111–114. [PubMed: 1640166]
64. Favier A, Sappey C, Leclerc P, et al. Antioxidant status and lipid peroxidation in patients infected with HIV. *Chem Biol Interact* 1994;91(2–3):165–180. [PubMed: 8194133]
65. Elbim C, Pillet S, Prevost MH, et al. Redox and activation status of monocytes from human immunodeficiency virus-infected patients: relationship with viral load. *J Virol* 1999;73(6):4561–4566. [PubMed: 10233914]
66. Peterhans E. Reactive oxygen species and nitric oxide in viral diseases. *Biol Trace Elem Res* 1997;56(1):107–116. [PubMed: 9152514]
67. Buhl R, Jaffe HA, Holroyd KJ, et al. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet* 1989;2(8675):1294–1298. [PubMed: 2574255]
68. Dröge, W.; Eck, HP.; Mihm, S. Oxidant-antioxidant status in human immunodeficiency virus infection. In: Packer, L., editor. *Oxygen Radicals in Biological Systems*. Academic Press; San Diego, CA: 1994. p. 594–601.
69. Droge W, Eck HP, Mihm S. HIV-induced cysteine deficiency and T-cell dysfunction—a rationale for treatment with N-acetylcysteine. *Immunol Today* 1992;13(6):211–214. [PubMed: 1378279]
70. Stephensen CB, Marquis GS, Jacob RA, et al. Vitamins C and E in adolescents and young adults with HIV infection. *Am J Clin Nutr* 2006;83(4):870–879. [PubMed: 16600941]
71. Delmas-Beauvieux MC, Peuchant E, Couchouron A, et al. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of

- supplementation with selenium or beta-carotene. *Am J Clin Nutr* 1996;64(1):101–107. [PubMed: 8669404]
72. Barditch-Crovo P, Noe D, Skowron G, et al. A phase I/II evaluation of oral L-2-oxothiazolidine-4-carboxylic acid in asymptomatic patients infected with human immunodeficiency virus. *J Clin Pharmacol* 1998;38(4):357–363. [PubMed: 9590463]
 73. Akerlund B, Jarstrand C, Lindeke B, et al. Effect of N-acetylcysteine(NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial. *Eur J Clin Pharmacol* 1996;50(6):457–461. [PubMed: 8858271]
 74. Allard JP, Aghdassi E, Chau J, et al. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. *Aids* 1998;12(13):1653–1659. [PubMed: 9764785]
 75. Merchant AT, Msamanga G, Villamor E, et al. Multi-vitamin supplementation of HIV-positive women during pregnancy reduces hypertension. *J Nutr* 2005;135(7):1776–1781. [PubMed: 15987864]
 76. Constans J, Seigneur M, Blann AD, et al. Effect of the antioxidants selenium and beta-carotene on HIV-related endothelium dysfunction. *Thromb Haemost* 1998;80(6):1015–1017. [PubMed: 9869176]
 77. de Souza Junior O, Treitinger A, Baggio GL, et al. alpha-Tocopherol as an antiretroviral therapy supplement for HIV-1-infected patients for increased lymphocyte viability. *Clin Chem Lab Med* 2005;43(4):376–382. [PubMed: 15899652]
 78. Hurwitz BE, Klaus JR, Llabre MM, et al. Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation: a randomized controlled trial. *Arch Intern Med* 2007;167(2):148–154. [PubMed: 17242315]
 79. Palmieri C, Trimboli F, Puca A, et al. Inhibition of HIV-1 replication in primary human monocytes by the IkappaB-alphaS32/36A repressor of NF-kappaB. *Retrovirology* 2004;1:45. [PubMed: 15613239]
 80. Victoriano AF, Asamitsu K, Hibi Y, et al. Inhibition of human immunodeficiency virus type 1 replication in latently infected cells by a novel IkappaB kinase inhibitor. *Antimicrob Agents Chemother* 2006;50(2):547–555. [PubMed: 16436709]
 81. Williams SA, Kwon H, Chen LF, et al. Sustained induction of NF-kappa B is required for efficient expression of latent human immunodeficiency virus type 1. *J Virol* 2007;81(11):6043–6056. [PubMed: 17376917]
 82. Alcamí J, Lain de Lera T, Folgueira L, et al. Absolute dependence on kappa B responsive elements for initiation and Tat-mediated amplification of HIV transcription in blood CD4 T lymphocytes. *Embo J* 1995;14(7):1552–1560. [PubMed: 7729429]
 83. Beauparlant P, Kwon H, Clarke M, et al. Transdominant mutants of I kappa B alpha block Tat-tumor necrosis factor synergistic activation of human immunodeficiency virus type 1 gene expression and virus multiplication. *J Virol* 1996;70(9):5777–5785. [PubMed: 8709193]
 84. Harhaj E, Blaney J, Millhouse S, et al. Differential effects of I kappa B molecules on Tat-mediated transactivation of HIV-1 LTR. *Virology* 1996;216(1):284–287. [PubMed: 8615004]
 85. Ho WZ, Starr SE, Sison A, et al. L-2-oxothiazolidine-4-carboxylic acid inhibits human immunodeficiency virus type 1 replication in mononuclear phagocytes and lymphocytes. *Clin Diagn Lab Immunol* 1997;4(3):352–357. [PubMed: 9144376]
 86. Oiry J, Puy JY, Mialocq P, et al. Synthesis and in vitro anti-HIV activity in human monocyte-derived macrophages of 2-oxothiazolidine-4(R)-carboxylic acid derivatives. *J Med Chem* 1999;42(23):4733–4740. [PubMed: 10579837]
 87. Lederman MM, Georges D, Dando S, et al. L-2-oxothiazolidine-4-carboxylic acid (procysteine) inhibits expression of the human immunodeficiency virus and expression of the interleukin-2 receptor alpha chain. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;8(2):107–115. [PubMed: 7834394]
 88. Raju PA, Herzenberg LA, Herzenberg LA, et al. Glutathione precursor and antioxidant activities of N-acetylcysteine and oxothiazolidine carboxylate compared in in vitro studies of HIV replication. *AIDS Res Hum Retroviruses* 1994;10(8):961–967. [PubMed: 7811547]
 89. Jacob BA, Porter KM, Elms SC, et al. HIV-1-induced pulmonary oxidative and nitrosative stress: exacerbated response to endotoxin administration in HIV-1 transgenic mouse model. *Am J Physiol Lung Cell Mol Physiol* 2006;291(4):L811–L819. [PubMed: 16728526]

90. Goldstein G. HIV-1 Tat protein as a potential AIDS vaccine. *Nat Med* 1996;2(9):960–964. [PubMed: 8782444]
91. Chang HC, Samaniego F, Nair BC, et al. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. *Aids* 1997;11(12):1421–1431. [PubMed: 9342064]
92. Poggi A, Carosio R, Fenoglio D, et al. Migration of V delta 1 and V delta 2 T cells in response to CXCR3 and CXCR4 ligands in healthy donors and HIV-1-infected patients: competition by HIV-1 Tat. *Blood* 2004;103(6):2205–2213. [PubMed: 14630801]
93. Paladugu R, Fu W, Conklin BS, et al. Hiv Tat protein causes endothelial dysfunction in porcine coronary arteries. *J Vasc Surg* 2003;38(3):549–555. [PubMed: 12947275]discussion 555–546
94. Chen F, Lu Y, Castranova V, et al. Nitric oxide inhibits HIV tat-induced NF-kappaB activation. *Am J Pathol* 1999;155(1):275–284. [PubMed: 10393859]
95. Liu X, Jana M, Dasgupta S, et al. Human immunodeficiency virus type 1 (HIV-1) tat induces nitric-oxide synthase in human astroglia. *J Biol Chem* 2002;277(42):39312–39319. [PubMed: 12167619]
96. Park IW, Wang JF, Groopman JE. HIV-1 Tat promotes monocyte chemoattractant protein-1 secretion followed by transmigration of monocytes. *Blood* 2001;97(2):352–358. [PubMed: 11154208]
97. Pu H, Tian J, Flora G, et al. HIV-1 Tat protein upregulates inflammatory mediators and induces monocyte invasion into the brain. *Mol Cell Neurosci* 2003;24(1):224–237. [PubMed: 14550782]
98. Dhawan S, Puri RK, Kumar A, et al. Human immunodeficiency virus-1-tat protein induces the cell surface expression of endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 in human endothelial cells. *Blood* 1997;90(4):1535–1544. [PubMed: 9269771]
99. Liu K, Chi DS, Li C, et al. HIV-1 Tat protein-induced VCAM-1 expression in human pulmonary artery endothelial cells and its signaling. *Am J Physiol Lung Cell Mol Physiol* 2005;289(2):L252–L260. [PubMed: 15805138]
100. Pieper GM, Olds CL, Bub JD, et al. Transfection of human endothelial cells with HIV-1 tat gene activates NF-kappa B and enhances monocyte adhesion. *Am J Physiol Heart Circ Physiol* 2002;283(6):H2315–H2321. [PubMed: 12427593]
101. Cota-Gomez A, Flores NC, Cruz C, et al. The human immunodeficiency virus-1 Tat protein activates human umbilical vein endothelial cell E-selectin expression via an NF-kappa B-dependent mechanism. *J Biol Chem* 2002;277(17):14390–14399. [PubMed: 11827962]
102. Chirivi RG, Taraboletti G, Bani MR, et al. Human immunodeficiency virus-1 (HIV-1)-Tat protein promotes migration of acquired immunodeficiency syndrome-related lymphoma cells and enhances their adhesion to endothelial cells. *Blood* 1999;94(5):1747–1754. [PubMed: 10477700]
103. Matzen KDA, oude Egbrink MG, Speth C, et al. HIV-1 Tat increases the adhesion of monocytes and T-cells to the endothelium in vitro and in vivo: implications for AIDS-associated vasculopathy. *Virus Res* 2004;104(2):145–155. [PubMed: 15246652]
104. Barillari G, Sgadari C, Fiorelli V, et al. The Tat protein of human immunodeficiency virus type-1 promotes vascular cell growth and locomotion by engaging the alpha5beta1 and alphavbeta3 integrins and by mobilizing sequestered basic fibroblast growth factor. *Blood* 1999;94(2):663–672. [PubMed: 10397733]
105. Mitola S, Soldi R, Zanon I, et al. Identification of specific molecular structures of human immunodeficiency virus type 1 Tat relevant for its biological effects on vascular endothelial cells. *J Virol* 2000;74(1):344–353. [PubMed: 10590123]
106. Albin A, Soldi R, Giunciuglio D, et al. The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells. *Nat Med* 1996;2(12):1371–1375. [PubMed: 8946838]
107. Wu RF, Gu Y, Xu YC, et al. Human immunodeficiency virus type 1 Tat regulates endothelial cell actin cytoskeletal dynamics through PAK1 activation and oxidant production. *J Virol* 2004;78(2):779–789. [PubMed: 14694110]
108. Avraham HK, Jiang S, Lee TH, et al. HIV-1 Tat-mediated effects on focal adhesion assembly and permeability in brain microvascular endothelial cells. *J Immunol* 2004;173(10):6228–6233. [PubMed: 15528360]

109. Andras IE, Pu H, Deli MA, et al. HIV-1 Tat protein alters tight junction protein expression and distribution in cultured brain endothelial cells. *J Neurosci Res* 2003;74(2):255–265. [PubMed: 14515355]
110. Toschi E, Barillari G, Sgadari C, et al. Activation of matrix-metalloproteinase-2 and membrane-type-1-matrix-metalloproteinase in endothelial cells and induction of vascular permeability in vivo by human immunodeficiency virus-1 Tat protein and basic fibroblast growth factor. *Mol Biol Cell* 2001;12(10):2934–2946. [PubMed: 11598182]
111. Arese M, Ferrandi C, Primo L, et al. HIV-1 Tat protein stimulates in vivo vascular permeability and lymphomononuclear cell recruitment. *J Immunol* 2001;166(2):1380–1388. [PubMed: 11145723]
112. Margheri F, D'Alessio S, Serrati S, et al. The urokinase-type plasminogen activator, its receptor and u-PA inhibitor type-1 affect in vitro growth and invasion of Kaposi's sarcoma and capillary endothelial cells: role of HIV-Tat protein. *Int J Oncol* 2005;27(1):223–235. [PubMed: 15942664]
113. Fiorelli V, Barillari G, Toschi E, et al. IFN-gamma induces endothelial cells to proliferate and to invade the extracellular matrix in response to the HIV-1 Tat protein: implications for AIDS-Kaposi's sarcoma pathogenesis. *J Immunol* 1999;162(2):1165–1170. [PubMed: 9916748]
114. Barillari G, Sgadari C, Palladino C, et al. Inflammatory cytokines synergize with the HIV-1 Tat protein to promote angiogenesis and Kaposi's sarcoma via induction of basic fibroblast growth factor and the alpha v beta 3 integrin. *J Immunol* 1999;163(4):1929–1935. [PubMed: 10438928]
115. Urbinati C, Mitola S, Tanghetti E, et al. Integrin alphavbeta3 as a target for blocking HIV-1 Tat-induced endothelial cell activation in vitro and angiogenesis in vivo. *Arterioscler Thromb Vasc Biol* 2005;25(11):2315–2320. [PubMed: 16166568]
116. Park IW, Ullrich CK, Schoenberger E, et al. HIV-1 Tat induces microvascular endothelial apoptosis through caspase activation. *J Immunol* 2001;167(5):2766–2771. [PubMed: 11509621]
117. Zidovetzki R, Wang JL, Chen P, et al. Human immunodeficiency virus Tat protein induces interleukin 6 mRNA expression in human brain endothelial cells via protein kinase C- and cAMP-dependent protein kinase pathways. *AIDS Res Hum Retroviruses* 1998;14(10):825–833. [PubMed: 9671211]
118. Wu RF, Ma Z, Myers DP, et al. HIV1 Tat activates dual Nox pathways leading to independent activation of ERK and JNK MAP kinases. *J Biol Chem*. Oct 16;2007
119. Toborek M, Lee YW, Pu H, et al. HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. *J Neurochem* 2003;84(1):169–179. [PubMed: 12485413]
120. Flores SC, Marecki JC, Harper KP, et al. Tat protein of human immunodeficiency virus type 1 represses expression of manganese superoxide dismutase in HeLa cells. *Proc Natl Acad Sci U S A* 1993;90(16):7632–7636. [PubMed: 8395050]
121. Westendorp MO, Shatrov VA, Schulze-Osthoff K, et al. HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. *Embo J* 1995;14(3):546–554. [PubMed: 7859743]
122. Gilbert M, Kirihaara J, Mills J. Enzyme-linked immunoassay for human immunodeficiency virus type 1 envelope glycoprotein 120. *J Clin Microbiol* 1991;29(1):142–147. [PubMed: 1993748]
123. Oh SK, Cruikshank WW, Raina J, et al. Identification of HIV-1 envelope glycoprotein in the serum of AIDS and ARC patients. *J Acquir Immune Defic Syndr* 1992;5(3):251–256. [PubMed: 1740750]
124. Ren Z, Yao Q, Chen C. HIV-1 envelope glycoprotein 120 increases intercellular adhesion molecule-1 expression by human endothelial cells. *Lab Invest* 2002;82(3):245–255. [PubMed: 11896203]
125. Kanmogne GD, Schall K, Leibhart J, et al. HIV-1 gp120 compromises blood-brain barrier integrity and enhances monocyte migration across blood-brain barrier: implication for viral neuropathogenesis. *J Cereb Blood Flow Metab* 2007;27(1):123–134. [PubMed: 16685256]
126. Takano Y, Shimokado K, Hata Y, et al. HIV envelope protein gp120-triggered CD4+ T-cell adhesion to vascular endothelium is regulated via CD4 and CXCR4 receptors. *Biochim Biophys Acta* 2007;1772(5):549–555. [PubMed: 17346946]
127. Bragardo M, Buonfiglio D, Feito MJ, et al. Modulation of lymphocyte interaction with endothelium and homing by HIV-1 gp120. *J Immunol* 1997;159(4):1619–1627. [PubMed: 9257821]
128. Kanmogne GD, Primeaux C, Grammas P. HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. *J Neuropathol Exp Neurol* 2005;64(6):498–505. [PubMed: 15977641]

129. Cioni C, Annunziata P. Circulating gp120 alters the blood-brain barrier permeability in HIV-1 gp120 transgenic mice. *Neurosci Lett* 2002;330(3):299–301. [PubMed: 12270651]
130. Didier N, Banks WA, Creminon C, et al. HIV-1-induced production of endothelin-1 in an in vitro model of the human blood-brain barrier. *Neuroreport* 2002;13(9):1179–1183. [PubMed: 12151765]
131. Fiala M, Murphy T, MacDougall J, et al. HAART drugs induce mitochondrial damage and intercellular gaps and gp120 causes apoptosis. *Cardiovasc Toxicol* 2004;4(4):327–337. [PubMed: 15531776]
132. Ullrich CK, Groopman JE, Ganju RK. HIV-1 gp120- and gp160-induced apoptosis in cultured endothelial cells is mediated by caspases. *Blood* 2000;96(4):1438–1442. [PubMed: 10942389]
133. Huang MB, Khan M, Garcia-Barrio M, et al. Apoptotic effects in primary human umbilical vein endothelial cell cultures caused by exposure to virion-associated and cell membrane-associated HIV-1 gp120. *J Acquir Immune Defic Syndr* 2001;27(3):213–221. [PubMed: 11464139]
134. Kanmogne GD, Kennedy RC, Grammas P. Analysis of human lung endothelial cells for susceptibility to HIV type 1 infection, coreceptor expression, and cytotoxicity of gp120 protein. *AIDS Res Hum Retroviruses* 2001;17(1):45–53. [PubMed: 11177382]
135. Khan NA, Di Cello F, Stins M, et al. Gp120-mediated cytotoxicity of human brain microvascular endothelial cells is dependent on p38 mitogen-activated protein kinase activation. *J Neurovirol* 2007;13(3):242–251. [PubMed: 17613714]
136. Kanmogne GD, Kennedy RC, Grammas P. HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: possible pathway for HIV entry into the brain and HIV-associated dementia. *J Neuropathol Exp Neurol* 2002;61(11):992–1000. [PubMed: 12430716]
137. Huang MB, Bond VC. Involvement of protein kinase C in HIV-1 gp120-induced apoptosis in primary endothelium. *J Acquir Immune Defic Syndr* 2000;25(5):375–389. [PubMed: 11141237]
138. Price TO, Uras F, Banks WA, et al. A novel antioxidant N-acetylcysteine amide prevents gp120- and Tat-induced oxidative stress in brain endothelial cells. *Exp Neurol* 2006;201(1):193–202. [PubMed: 16750528]
139. Schecter AD, Berman AB, Yi L, et al. HIV envelope gp120 activates human arterial smooth muscle cells. *Proc Natl Acad Sci U S A* 2001;98(18):10142–10147. [PubMed: 11504923]
140. Jana A, Pahan K. Human immunodeficiency virus type 1 gp120 induces apoptosis in human primary neurons through redox-regulated activation of neutral sphingomyelinase. *J Neurosci* 2004;24(43):9531–9540. [PubMed: 15509740]
141. Henderson WW, Ruhl R, Lewis P, et al. Human immunodeficiency virus (HIV) type 1 Vpu induces the expression of CD40 in endothelial cells and regulates HIV-induced adhesion of B-lymphoma cells. *J Virol* 2004;78(9):4408–4420. [PubMed: 15078922]
142. Acheampong E, Mukhtar M, Parveen Z, et al. Ethanol strongly potentiates apoptosis induced by HIV-1 proteins in primary human brain microvascular endothelial cells. *Virology* 2002;304(2):222–234. [PubMed: 12504564]
143. Choi J, Walker J, Boichuk S, et al. Human endothelial cells enhance human immunodeficiency virus type 1 replication in CD4+ T cells in a Nef-dependent manner in vitro and in vivo. *J Virol* 2005;79(1):264–276. [PubMed: 15596822]
144. Biggs TE, Cooke SJ, Barton CH, et al. Induction of activator protein 1 (AP-1) in macrophages by human immunodeficiency virus type-1 NEF is a cell-type-specific response that requires both hck and MAPK signaling events. *J Mol Biol* 1999;290(1):21–35. [PubMed: 10388555]
145. Varin A, Manna SK, Quivy V, et al. Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *J Biol Chem* 2003;278(4):2219–2227. [PubMed: 12419805]
146. Olivetta E, Pietraforte D, Schiavoni I, et al. HIV-1 Nef regulates the release of superoxide anions from human macrophages. *Biochem J* 2005;390(Pt 2):591–602. [PubMed: 15847608]
147. Zheng YH, Plemenitas A, Fielding CJ, et al. Nef increases the synthesis of and transports cholesterol to lipid rafts and HIV-1 progeny virions. *Proc Natl Acad Sci U S A* 2003;100(14):8460–8465. [PubMed: 12824470]
148. Mujawar Z, Rose H, Morrow MP, et al. Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. *PLoS Biol* 2006;4(11):e365. [PubMed: 17076584]

149. Bukrinsky M, Sviridov D. Human immunodeficiency virus infection and macrophage cholesterol metabolism. *J Leukoc Biol* 2006;80(5):1044–1051. [PubMed: 17056763]
150. OARAC. DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Dec 1. 2007 available at: <http://aidsinfo.nih.gov>
151. Lewis W, Day BJ, Copeland WC. Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. *Nat Rev Drug Discov* 2003;2(10):812–822. [PubMed: 14526384]
152. Dagan T, Sable C, Bray J, et al. Mitochondrial dysfunction and antiretroviral nucleoside analog toxicities: what is the evidence? *Mitochondrion* 2002;1(5):397–412. [PubMed: 16120293]
153. Pinti M, Salomoni P, Cossarizza A. Anti-HIV drugs and the mitochondria. *Biochim Biophys Acta* 2006;1757(5–6):700–707. [PubMed: 16782042]
154. Sutliff RL, Dikalov S, Weiss D, et al. Nucleoside reverse transcriptase inhibitors impair endothelium-dependent relaxation by increasing superoxide. *Am J Physiol Heart Circ Physiol* 2002;283(6):H2363–H2370. [PubMed: 12388299]
155. Jiang B, Hebert VY, Zavec JH, et al. Antiretrovirals induce direct endothelial dysfunction in vivo. *J Acquir Immune Defic Syndr* 2006;42(4):391–395. [PubMed: 16810107]
156. Hebert VY, Crenshaw BL, Romanoff RL, et al. Effects of HIV drug combinations on endothelin-1 and vascular cell proliferation. *Cardiovasc Toxicol* 2004;4(2):117–131. [PubMed: 15371629]
157. Jiang B, Hebert VY, Li Y, et al. HIV antiretroviral drug combination induces endothelial mitochondrial dysfunction and reactive oxygen species production, but not apoptosis. *Toxicol Appl Pharmacol* 2007;224(1):60–71. [PubMed: 17669453]
158. Gross NJ, Getz GS, Rabinowitz M. Apparent turnover of mitochondrial deoxyribonucleic acid and mitochondrial phospholipids in the tissues of the rat. *J Biol Chem* 1969;244(6):1552–1562. [PubMed: 5773057]
159. de la Asuncion JG, Del Olmo ML, Gomez-Cambronero LG, et al. AZT induces oxidative damage to cardiac mitochondria: protective effect of vitamins C and E. *Life Sci* 2004;76(1):47–56. [PubMed: 15501479]
160. Yamaguchi T, Katoh I, Kurata S. Azidothymidine causes functional and structural destruction of mitochondria, glutathione deficiency and HIV-1 promoter sensitization. *Eur J Biochem* 2002;269(11):2782–2788. [PubMed: 12047388]
161. Papparella I, Ceolotto G, Berto L, et al. Vitamin C prevents zidovudine-induced NAD(P)H oxidase activation and hypertension in the rat. *Cardiovasc Res* 2007;73(2):432–438. [PubMed: 17123493]
162. Cazzalini O, Lazze MC, Iamele L, et al. Early effects of AZT on mitochondrial functions in the absence of mitochondrial DNA depletion in rat myotubes. *Biochem Pharmacol* 2001;62(7):893–902. [PubMed: 11543724]
163. McKee EE, Bentley AT, Hatch M, et al. Phosphorylation of thymidine and AZT in heart mitochondria: elucidation of a novel mechanism of AZT cardiotoxicity. *Cardiovasc Toxicol* 2004;4(2):155–167. [PubMed: 15371631]
164. Song S, Wheeler LJ, Mathews CK. Deoxyribonucleotide pool imbalance stimulates deletions in HeLa cell mitochondrial DNA. *J Biol Chem* 2003;278(45):43893–43896. [PubMed: 13679382]
165. Pan-Zhou XR, Cui L, Zhou XJ, et al. Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells. *Antimicrob Agents Chemother* 2000;44(3):496–503. [PubMed: 10681309]
166. Szabados E, Fischer GM, Toth K, et al. Role of reactive oxygen species and poly-ADP-ribose polymerase in the development of AZT-induced cardiomyopathy in rat. *Free Radic Biol Med* 1999;26(3–4):309–317. [PubMed: 9895221]
167. Yan JP, Ilsley DD, Frohlick C, et al. 3'-Azidothymidine (zidovudine) inhibits glycosylation and dramatically alters glycosphingolipid synthesis in whole cells at clinically relevant concentrations. *J Biol Chem* 1995;270(39):22836–22841. [PubMed: 7559416]
168. Valenti D, Barile M, Passarella S. AZT inhibition of the ADP/ATP antiport in isolated rat heart mitochondria. *Int J Mol Med* 2000;6(1):93–96. [PubMed: 10851273]
169. Lund KC, Wallace KB. Adenosine 3',5'-cyclic monophosphate (cAMP)-dependent phosphoregulation of mitochondrial complex I is inhibited by nucleoside reverse transcriptase inhibitors. *Toxicol Appl Pharmacol* 2008;226(1):94–106. [PubMed: 17904600]

170. Cote HC. Possible ways nucleoside analogues can affect mitochondrial DNA content and gene expression during HIV therapy. *Antivir Ther* 2005;10(Suppl 2):M3–M11. [PubMed: 16152702]
171. Kontorinis N, Dieterich DT. Toxicity of non-nucleoside analogue reverse transcriptase inhibitors. *Semin Liver Dis* 2003;23(2):173–182. [PubMed: 12800070]
172. de Saint Martin L, Vandhuick O, Guillo P, et al. Premature atherosclerosis in HIV positive patients and cumulated time of exposure to antiretroviral therapy (SHIVA study). *Atherosclerosis* 2006;185(2):361–367. [PubMed: 16137695]
173. Friis-Moller N, Reiss P, Sabin CA, et al. Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med* 2007;356(17):1723–1735. [PubMed: 17460226]
174. Thiebaut R, El-Sadr WM, Friis-Moller N, et al. Predictors of hypertension and changes of blood pressure in HIV-infected patients. *Antivir Ther* 2005;10(7):811–823. [PubMed: 16315369]
175. Tashima KT, Bausserman L, Alt EN, et al. Lipid changes in patients initiating efavirenz- and indinavir-based antiretroviral regimens. *HIV Clin Trials* 2003;4(1):29–36. [PubMed: 12577194]
176. van der Valk M, Kastelein JJ, Murphy RL, et al. Nevirapine-containing antiretroviral therapy in HIV-1 infected patients results in an anti-atherogenic lipid profile. *Aids* 2001;15(18):2407–2414. [PubMed: 11740191]
177. Murphy RL, Smith WJ. Switch studies: a review. *HIV Med* 2002;3(2):146–155. [PubMed: 12010362]
178. Young J, Weber R, Rickenbach M, et al. Lipid profiles for antiretroviral-naïve patients starting PI- and NNRTI-based therapy in the Swiss HIV cohort study. *Antivir Ther* 2005;10(5):585–591. [PubMed: 16152752]
179. van Wijk JP, de Koning EJ, Cabezas MC, et al. Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients. *J Am Coll Cardiol* 2006;47(6):1117–1123. [PubMed: 16545639]
180. Johnsen S, Dolan SE, Fitch KV, et al. Carotid intimal medial thickness in human immunodeficiency virus-infected women: effects of protease inhibitor use, cardiac risk factors, and the metabolic syndrome. *J Clin Endocrinol Metab* 2006;91(12):4916–4924. [PubMed: 17003092]
181. Stein JH, Klein MA, Bellehumeur JL, et al. Use of human immunodeficiency virus-1 protease inhibitors is associated with atherogenic lipoprotein changes and endothelial dysfunction. *Circulation* 2001;104(3):257–262. [PubMed: 11457741]
182. Shankar SS, Dube MP, Gorski JC, et al. Indinavir impairs endothelial function in healthy HIV-negative men. *Am Heart J* 2005;150(5):933. [PubMed: 16290967]
183. Blanco JJ, Garcia IS, Cerezo JG, et al. Endothelial function in HIV-infected patients with low or mild cardiovascular risk. *J Antimicrob Chemother* 2006;58(1):133–139. [PubMed: 16702174]
184. Currier JS, Kendall MA, Henry WK, et al. Progression of carotid artery intima-media thickening in HIV-infected and uninfected adults. *Aids* 2007;21(9):1137–1145. [PubMed: 17502724]
185. Currier JS, Kendall MA, Zackin R, et al. Carotid artery intima-media thickness and HIV infection: traditional risk factors overshadow impact of protease inhibitor exposure. *Aids* 2005;19(9):927–933. [PubMed: 15905673]
186. Grubb JR, Dejam A, Voell J, et al. Lopinavir-ritonavir: effects on endothelial cell function in healthy subjects. *J Infect Dis* 2006;193(11):1516–1519. [PubMed: 16652279]
187. Nolan D, Watts GF, Herrmann SE, et al. Endothelial function in HIV-infected patients receiving protease inhibitor therapy: does immune competence affect cardiovascular risk? *Qjm* 2003;96(11):825–832. [PubMed: 14566037]
188. Brown TT, Cole SR, Li X, et al. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Arch Intern Med* 2005;165(10):1179–1184. [PubMed: 15911733]
189. Riddler SA, Li X, Chu H, et al. Longitudinal changes in serum lipids among HIV-infected men on highly active antiretroviral therapy. *HIV Med* 2007;8(5):280–287. [PubMed: 17561873]
190. Fisher SD, Miller TL, Lipshultz SE. Impact of HIV and highly active antiretroviral therapy on leukocyte adhesion molecules, arterial inflammation, dyslipidemia, and atherosclerosis. *Atherosclerosis* 2006;185(1):1–11. [PubMed: 16297390]

191. Friis-Moller N, Weber R, Reiss P, et al. Cardiovascular disease risk factors in HIV patients—association with antiretroviral therapy. Results from the DAD study. *Aids* 2003;17(8):1179–1193. [PubMed: 12819520]
192. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003;349(21):1993–2003. [PubMed: 14627784]
193. Holmberg SD, Moorman AC, Williamson JM, et al. Protease inhibitors and cardiovascular outcomes in patients with HIV-1. *Lancet* 2002;360(9347):1747–1748. [PubMed: 12480430]
194. Mary-Krause M, Cotte L, Simon A, et al. Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men. *Aids* 2003;17(17):2479–2486. [PubMed: 14600519]
195. Wang X, Chai H, Yao Q, et al. Molecular mechanisms of HIV protease inhibitor-induced endothelial dysfunction. *J Acquir Immune Defic Syndr* 2007;44(5):493–499. [PubMed: 17245228]
196. Zhong DS, Lu XH, Conklin BS, et al. HIV protease inhibitor ritonavir induces cytotoxicity of human endothelial cells. *Arterioscler Thromb Vasc Biol* 2002;22(10):1560–1566. [PubMed: 12377730]
197. Chen C, Lu XH, Yan S, et al. HIV protease inhibitor ritonavir increases endothelial monolayer permeability. *Biochem Biophys Res Commun* 2005;335(3):874–882. [PubMed: 16105660]
198. Mondal D, Pradhan L, Ali M, et al. HAART drugs induce oxidative stress in human endothelial cells and increase endothelial recruitment of mononuclear cells: exacerbation by inflammatory cytokines and amelioration by antioxidants. *Cardiovasc Toxicol* 2004;4(3):287–302. [PubMed: 15470276]
199. Baliga RS, Liu C, Hoyt DG, et al. Vascular endothelial toxicity induced by HIV protease inhibitor: evidence of oxidant-related dysfunction and apoptosis. *Cardiovasc Toxicol* 2004;4(2):199–206. [PubMed: 15371635]
200. Conklin BS, Fu W, Lin PH, et al. HIV protease inhibitor ritonavir decreases endothelium-dependent vasorelaxation and increases superoxide in porcine arteries. *Cardiovasc Res* 2004;63(1):168–175. [PubMed: 15194474]
201. Chai H, Yang H, Yan S, et al. Effects of 5 HIV protease inhibitors on vasomotor function and superoxide anion production in porcine coronary arteries. *J Acquir Immune Defic Syndr* 2005;40(1):12–19. [PubMed: 16123675]
202. Chai H, Yan S, Lin P, et al. Curcumin blocks HIV protease inhibitor ritonavir-induced vascular dysfunction in porcine coronary arteries. *J Am Coll Surg* 2005;200(6):820–830. [PubMed: 15922191]
203. Chai H, Zhou W, Lin P, et al. Ginsenosides block HIV protease inhibitor ritonavir-induced vascular dysfunction of porcine coronary arteries. *Am J Physiol Heart Circ Physiol* 2005;288(6):H2965–H2971. [PubMed: 15681703]
204. Fu W, Chai H, Yao Q, et al. Effects of HIV protease inhibitor ritonavir on vasomotor function and endothelial nitric oxide synthase expression. *J Acquir Immune Defic Syndr* 2005;39(2):152–158. [PubMed: 15905730]
205. Bourlier V, Zakaroff-Girard A, De Barros S, et al. Protease inhibitor treatments reveal specific involvement of matrix metalloproteinase-9 in human adipocyte differentiation. *J Pharmacol Exp Ther* 2005;312(3):1272–1279. [PubMed: 15537822]
206. Xu A, Yin S, Wong L, et al. Adiponectin ameliorates dyslipidemia induced by the human immunodeficiency virus protease inhibitor ritonavir in mice. *Endocrinology* 2004;145(2):487–494. [PubMed: 14592951]
207. Shahmanesh M, Das S, Stolinski M, et al. Antiretroviral treatment reduces very-low-density lipoprotein and intermediate-density lipoprotein apolipoprotein B fractional catabolic rate in human immunodeficiency virus-infected patients with mild dyslipidemia. *J Clin Endocrinol Metab* 2005;90(2):755–760. [PubMed: 15522931]
208. Lee GA, Seneviratne T, Noor MA, et al. The metabolic effects of lopinavir/ritonavir in HIV-negative men. *Aids* 2004;18(4):641–649. [PubMed: 15090769]
209. Zhou H, Pandak WM Jr, Lyall V, et al. HIV protease inhibitors activate the unfolded protein response in macrophages: implication for atherosclerosis and cardiovascular disease. *Mol Pharmacol* 2005;68(3):690–700. [PubMed: 15976036]

210. Dressman J, Kincer J, Matveev SV, et al. HIV protease inhibitors promote atherosclerotic lesion formation independent of dyslipidemia by increasing CD36-dependent cholesteryl ester accumulation in macrophages. *J Clin Invest* 2003;111(3):389–397. [PubMed: 12569165]
211. Bradshaw EL, Li XA, Guerin T, et al. Nucleoside reverse transcriptase inhibitors prevent HIV protease inhibitor-induced atherosclerosis by ubiquitination and degradation of protein kinase C. *Am J Physiol Cell Physiol* 2006;291(6):C1271–C1278. [PubMed: 16822947]
212. Wang X, Mu H, Chai H, et al. Human immunodeficiency virus protease inhibitor ritonavir inhibits cholesterol efflux from human macrophage-derived foam cells. *Am J Pathol* 2007;171(1):304–314. [PubMed: 17591975]
213. Zhou H, Jarujaron S, Gurley EC, et al. HIV protease inhibitors increase TNF-alpha and IL-6 expression in macrophages: involvement of the RNA-binding protein HuR. *Atherosclerosis* 2007;195(1):e134–e143. [PubMed: 17531241]
214. Stein JH, Merwood MA, Bellehumeur JL, et al. Effects of pravastatin on lipoproteins and endothelial function in patients receiving human immunodeficiency virus protease inhibitors. *Am Heart J* 2004;147(4):E18. [PubMed: 15077088]

HIV-1 Proteins and Their Effects on Viral Function and Endothelial Cell Biology

TABLE 1

Gene	Protein	Category	Viral Function	Endothelial Cell Effects	Reference
<i>gag</i>	p9	structural (core)	packaging signal; RNA binding	—	n/a
<i>gag</i>	p6	structural (core)	virion building; vpr interaction	—	n/a
<i>gag</i>	p24	structural (core)	predominant core component	—	n/a
<i>gag</i>	p17	structural (core)	virion stabilization; viral DNA transport	—	n/a
<i>pol</i>	protease	enzyme	cleavage of viral proteins	—	n/a
<i>pol</i>	reverse transcriptase	enzyme	viral DNA synthesis	—	n/a
<i>pol</i>	integrase	enzyme	incorporation of viral DNA into host genome	—	n/a
<i>vif</i>	Vif	accessory	increase infectivity	—	n/a
<i>vpr</i>	Vpr	accessory	block cell division; nuclear import	↑ apoptosis	142
<i>rev</i>	Rev	regulatory	nuclear export of unspliced RNA	↑ apoptosis, ↓ relaxation,	n/a, 116, 142
<i>tat</i>	Tat	regulatory	transcriptional transactivator of RNA	↑ CAMs,	93
				↑ monocyte chemoattractant protein-1,	98-102
				↑ adhesion,	96-97
				regulate cytoskeleton,	96-103
				↑ permeability,	107
				↑ MMP,	108, 109
				↑ chemotaxis,	110
				↑ proliferation,	111
				↑ angiogenesis,	112, 113
				↑ ROS	114, 115
					107-119
<i>vpu</i>	Vpu	accessory	virus release; ↓ CD4	↑ CAMs	141
<i>env</i>	gp120	structural (envelope)	receptor binding	↑ apoptosis, ↑ CAMs,	125, 131-134, 136, 142
				↑ adhesion,	124
				regulate cytoskeleton,	124-127
				↑ permeability,	39
				↑ vasoconstrictors,	39, 125, 129
				↑ ROS	130
					39, 138
<i>env</i>	gp41	structural (envelope)	fusion of viral and host membranes	—	n/a
<i>nef</i>	Nef	accessory	↓ CD4 and Class I MHC; ↑ viral infectivity	↑ apoptosis	142

MHC indicates major histocompatibility factor; n/a, not applicable; ↑, increase; ↓, decrease; CAM, cell adhesion molecule; MCP, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; ROS, reactive oxygen species.

TABLE 2

Antiretroviral Drugs Currently Approved by the Food and Drug Administration for the Treatment of HIV-1 and Their Known Effects on Endothelial Cell Homeostasis

<i>Generic Name</i>	<i>Abbreviation</i>	<i>Class</i>	<i>Endothelial Cell Effects</i>	<i>Reference</i>
Zidovudine	AZT, ZDV	NRTIs	↓ vasorelaxation	154,155
Stavudine	d4T		↑ ROS	154,157,214
Didanosine	ddI		↑ vasoconstrictor release	156
Zalcitabine	ddC		↑ proliferation	156
Abacavir	ABC		↑ permeability	131
Emtricitabine	FTC		↓ mitochondrial function	156,157
Lamivudine	3TC		↓ GSH	214
Tenofovir	TDF			
Nevirapine	NVP	NNRTIs	—	n/a
Efavirenz	EFV			
Delavirdine	DLV			
Ritonavir	RTV	PIs	↑ carotid IMT	179,180
Atazanavir	ATV		↓ FMD	181-183
Amprenavir	APV		↔ IMT	184,185
Indinavir	IDV		↔ FMD	21,186,187
Nelfinavir	NFV		↓ mitochondrial function	156
Darunavir	DRV		↑ ROS	157,195,199-202
Saquinavir	SQV		↑ mtDNA damage	196
Fosamprenavir	FPV		↑ permeability	197
Tipranavir	TPV		↓ vasorelaxation	198-200
Lopinavir	LPV		↓ eNOS	200,203
Raltegravir	RAL	Integrase Inhibitor	—	n/a
Enfuvirtide	ENF	EIs	—	n/a
Maraviroc	MVC			

GSH indicates reduced glutathione; HDLc, high-density lipoprotein cholesterol; n/a, not applicable; ↑, increase; ↓, decrease; ↔, no change; ROS, reactive oxygen species; IMT, intima media thickness; FMD, flow-mediated dilation; eNOS, endothelial nitric oxide synthase.