Chapter from the book *HIV Infection in the Era of Highly Active Antiretroviral Treatment and Some of Its Associated Complications*
1. Introduction

The UNAIDS global report estimated that the number of people living with HIV/AIDS by the end of 2009 was 33 million. By 2008, the global funding for HIV/AIDS had climbed to $15.6 billion (Kates et al., 2009) and by 2009, WHO estimated that 5.2 million were on ART in low and middle income countries (WHO, 2010).

Over 30 years of the AIDS epidemic and since the introduction of highly active antiretroviral therapy (ART) in 1996, the management of HIV-1 infection has gradually moved from treatment of opportunistic infections towards regular monitoring and maintenance of a suppressed viral load. As expected, this has lead to dramatic improvements in morbidity and mortality from HIV-1. Whilst the median life expectancy following diagnosis with HIV-1 prior to the advent of ART was 7 years, it has now reached 35 years in the developed world (Lohse et al., 2007). Consequently there has been a parallel growth in the complications that arise from chronic infection with HIV-1 and its treatments.

Atherosclerotic and ischemic cardiovascular disease, which once predominantly afflicted the elderly, is now increasing in prevalence in HIV-1 infected persons. In the pre-ART era, the cardiac manifestations of HIV-1 were mainly HIV cardiomyopathy and pulmonary hypertension. The first documented case reports of acute myocardial infarction in HIV-1 infected patients were described in 1998 (Bozzette et al., 2003). Supporting these findings were autopsy reports which demonstrated that HIV-1 infected patients without traditional cardiac risk factors also had unexpectedly higher rates of atherosclerosis, with endothelial lymphocytic infiltration, compared with controls (Joshi et al., 1987). The current incidence of coronary artery disease in the HIV-1 infected population is at least three-fold higher than the general population (Vittecoq et al., 2003) even in the absence of traditional risk factors, suggesting that HIV-1 is an independent risk factor for vascular disease.
The pathogenesis of endothelial dysfunction in HIV-1 infection is still being studied. However, several mechanisms have been postulated: HIV-induced endothelial cell injury, activation of endothelial cells by pro-inflammatory cytokines and mediators, and toxicity from ART which may itself have direct and indirect actions. This review will first examine endothelial dysfunction in non-HIV infected people and then explore the determinants of this process in HIV-infected patients. We will cover the most recent studies which suggest an interaction between HIV proteins and endothelium, recent developments in the link between the pro-inflammatory cascade and endothelial dysfunction and the effect of ART on both these mechanisms.

2. Background: The endothelium in non-HIV infected patients

From our knowledge of coronary artery disease in non-HIV infected subjects, we know that the earliest hallmark of vascular abnormalities is endothelial dysfunction. The endothelium is part of the barrier between the vessel wall and the circulation. It serves many purposes including regulation of muscle tone, lipid metabolism, thrombogenesis and vessel permeability (Kharbanda R, 2005). The healthy endothelium is not readily permeable, is anti-adhesive and able to relax vascular smooth muscle. This latter ability is governed by an intricate balance between vasodilatory (e.g. nitric oxide and prostacycline) and vasoconstrictive (predominantly endothelin-1, ET-1) substances that are released by the endothelial cells (Kharbanda R, 2005). Under normal conditions, the vascular endothelium is left in a predominantly dilated state; indeed, endothelial dysfunction is defined as impaired nitric oxide synthesis and vascular reactivity. However, the terminology is also used to describe the associated pro-inflammatory and pro-thrombogenic state.

2.1 Vasodilatation and vasoconstriction

In healthy endothelium, nitric oxide is produced from the precursor L-arginine via the constitutively expressed enzyme, endothelial nitric oxide synthase (e-NOS), which is activated in response to physical stimuli, such as shear stress (Kharbanda R, 2005). In addition to its vasodilatory action, the anti-thrombotic effects of nitric oxide are two-fold, inhibiting both leukocyte aggregation and platelet activation.

Factors such as smoking, dyslipidemia, diabetes, aging and sedentary lifestyle have all been shown to reduce NO synthesis and therefore impair endothelial function.

Endothelial cells also produce vasoconstrictive substances, one of the most potent being ET-1. Endothelin-1 acts via 2 receptor subtypes, Endothelin-A and Endothelin-B (ET-A and ET-B), which are expressed in varying quantities. Endogenous levels of ET-1 act via ET-A to induce coronary vasoconstriction but also serve to increase smooth muscle proliferation and induce cytokine production in vitro (Kharbanda R, 2005). Selective antagonism of ET-A receptors has been shown to improve endothelial function (Verhaar et al., 1998).

In a healthy vessel, blood flow is laminar and shear stresses of the blood flow are maximal at the vessel wall. Following shear stress, endothelial cells elongate and align to the direction of blood flow (Lowe, 2003).

Work by Virchow, von Rotitansky and Ross (Ross et al., 1977) first generated the hypothesis that endothelial damage was characterized by a loss of the normal orientation of endothelial cells in the direction of flow resulting in low-flow and low-shear circulation of blood cells in contact with the vessel wall. As this mechanical change reduces the release of nitric oxide, vasoreactivity is impaired: this explains why the earliest stages of endothelial dysfunction are characterized by a reduced ability of vessels to vasodilate. Subsequently, there is an
accumulation of platelets, fibrin and monocytes over the injured endothelium; these then release substances (such as platelet derived growth factor, PDGF, and tissue growth factor B, TGF-B) which stimulate smooth muscle proliferation and connective tissue production. Recruited macrophages absorb circulating lipids (such as low density lipoprotein and cholesterol) and are converted to foam cells, which perpetuate a cycle of reduced laminar flow, haemostasis and inflammation (Lowe, 2003).

2.2 Haemostasis

Haemostasis, characterized by activation of the coagulation pathway and fibrin formation, is well described in atherosclerosis. The endothelium synthesizes and releases fibronectin, von Willebrand factor (vWF) and thrombospondin in response to any pro-haemorrhagic stimuli (Kharbanda R, 2005). vWF acts as a ‘glue’ linking platelets to the endothelial matrix. There is a subsequent production of fibrin, which then crosslink the mesh creating a haemostatic seal. Complete vascular occlusion by the matrix is usually prevented by endothelial synthesis and activation of specific anti-thrombotic compounds such as protein C, anti-thrombin and tPA (tissue plasminogen activator) which mediate endogenous fibrinolysis. Pro-coagulant activity is also modulated by nitric oxide which inhibits platelet aggregation and cell-cell adhesion activity.

Haemostasis and thrombosis are central to the progression of atherosclerosis and acute arterial occlusion; several studies have looked at the role of pro-coagulant factors in arterial disease and it is suggested that there may be an imbalance of haemostatic factors in the development of atherosclerosis (Signorelli et al., 2007). High plasma levels of fibrinogen have been found in patients with peripheral atherosclerosis and are prognostic predictors for the development of myocardial infarction and cardiac arrest in patients with stable intermittent claudication (Thor et al., 2002). In addition, previous studies have shown that patients with established coronary artery disease were more likely to develop ischemia, as indicated by dobutamine stress echo testing, if they had a hypercoaguable state, which comprised increased levels of fibrinogen and factor VIII (De Lorenzo et al., 2003).

2.3 The effect of inflammation in the non HIV infected-endothelium

Because atherosclerosis is typified by the cycle of haemostasis, lipid accumulation and inflammation, it is considered an inflammatory disease. Factors such as smoking and hyperlipidemia are, in effect, chronically stimulating the endothelium which in turn changes the endothelial architecture and creates a permanent state of endothelial inflammation. In recent years the importance of inflammation in the development of endothelial changes has been increasingly recognized. C-reactive protein (CRP), an acute phase protein synthesized by the liver, is a sensitive marker of inflammation. Increased levels of CRP have been demonstrated in patients with type 2 diabetes and are believed to occur in response to chronic intra-arterial inflammation (Tan et al., 2002). Several studies have shown that the CRP level is closely correlated with the extent of endothelial dysfunction and this marker has been found to be increased in patients who have developed atherosclerosis. CRP was shown to be a strong predictor of cardiovascular events in a large prospective study involving 28,000 women (Ridker et al., 2002). The CRP appears not only to be an indicator of inflammatory disease, but can also directly amplify the inflammatory response via activation of the complement cascade, tissue damage and activation of endothelial cells (Signorelli et al., 2007). Indeed systemic inflammation of any cause, including autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, may initially drive
the process of endothelial damage leading to a greater risk of cardiovascular disease (Turesson et al., 2008). It is hypothesized that HIV as a chronic inflammatory disease gives rise to atherosclerosis in a similar way.

2.4 Cytokines and endothelial dysfunction
Cytokines are polypeptide chemical messengers that play critical roles in the inflammatory cascade, following endothelial injury. Cytokines are able to act at low concentrations and over a range of time-scales; they act through paracrine, autocrine or endocrine routes (Signorelli et al., 2007). Cytokines, including Interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor alpha, Interferon-gamma (IFNγ) and monocyte-chemotactic protein-1 (MCP-1) are proinflammatory. IL-6 may be directly responsible for the production of CRP (Signorelli et al., 2007). IL-2, IFNγ and TNF-α appear to be responsible for induction of adhesion molecules and chemokines in the vascular wall (Signorelli et al., 2007) and there is mounting evidence that IL-6 works in concert with TNF-α and other cytokines to activate endothelial cells and enhance leukocyte adhesion (Mu et al., 2007).

The role of cytokines in endothelial dysfunction has mostly been studied in the obese population, those with established atherosclerosis and in diabetic patients. CRP has been shown to induce macrophage colony stimulating factor (M-CSF) release from mononuclear phagocytes, promoting a positive feedback loop with further proliferation of the macrophages which infiltrate the inflammatory plaque (Devaraj et al., 2009). Type 1 helper T cells (Th1) secrete TNF-α and IFNγ which can stimulate macrophagic internalization of modified lipoproteins leading to foam cell formation. TNF-α achieves this via up-regulation of receptors on the macrophage for uptake of modified lipoproteins (Hsu et al., 2000) and IFNγ reduces cholesterol efflux (Wang et al., 2002). The activated macrophages continue to release cytokines which increase the inflammatory response and seem to modulate smooth muscle architecture. Increased TNF levels are also believed to directly inhibit nitric oxide mediated coronary vasodilatation (Zhang et al., 2006).

Clinical findings support the in vitro evidence. Elevated levels of TNF-α have been reported in association with myocardial ischemia and may contribute to irreversible myocardial tissue injury (Zhang et al., 2002). Similarly, a recent meta-analysis demonstrated an odds ratio of 3.34 for myocardial infarction or coronary death per two standard deviation increase in long-term average IL-6 level (Danesh et al., 2008).

2.5 Adhesion molecules
Endothelial inflammation induces over-expression of intercellular and vascular cell adhesion molecules (ICAM-1, VCAM-1), P-selectin and E-selectin, all of which attract monocytes and neutrophils to the area (Goldberg, 2009) and hence contribute to plaque formation. ICAM-1 and VCAM-1 mediate adhesion of inflammatory cells at the vascular endothelium. Monocytes then migrate into the sub-endothelial space of the vascular wall and subsequently differentiate into macrophages (Goldberg, 2009).

In vivo, P-selectin is not expressed on normal endothelium; its expression on diseased endothelium can occur in response to a number of insults including an oxidized form of internalized low density lipoprotein (oxLDL) (Johnson-Tidey et al., 1994). In a study using rabbits fed only on an atherogenic diet, P-selectin was expressed after one week and infiltration of macrophages with lipoprotein occurred after two weeks (Sakai et al., 1997). Mice with homozygous knockout for the P-selectin gene showed a reduction in the atherosclerotic lesion size within the endothelium compared to wild-type mice (Collins et al.,
Similarly E-selectin is found in increased concentration on atherosclerotic endothelial cells and appears to be induced by TNF and IL-1 alpha (Galkina et al., 2007; Stocker et al., 2000). Combined deficiency of P-selectin and E-selectin in mice elicited an 80% protective effect in the early stages of atherosclerosis (Dong et al., 1998). Likewise, there are several reports showing increased expression of VCAM-1 on aortic endothelium in response to cholesterol accumulation within the intima (Truskey et al., 1999). Furthermore, treatment of human umbilical vein endothelial cells with TNF-α up-regulated VCAM-1 and ICAM-1 expression in vitro (Ramana et al., 2004), implying that adhesion molecule expression can be cytokine dependent.

3. The effect of HIV-1 infection on endothelial dysfunction

In recent years, reports have shown that HIV-infected patients have a greater risk of developing coronary artery disease compared to HIV-uninfected patients of the same age (Vittecoq et al., 2003). In the absence of anti-retrovirals, chronic inflammation, hypercoagulability, cell adhesion and platelet activation appear to drive the pathogenesis behind endothelial dysfunction in HIV-infected individuals (Francisci et al., 2009).

3.1 Measuring atherosclerosis and endothelial dysfunction in HIV-1
Surrogate measures of atherosclerosis include carotid artery intima-media thickness (C-IMT), which directly correlates with the extent of atherosclerosis; other techniques, such as brachial artery flow mediated dilatation, can also be used and these evaluate endothelial dysfunction.

3.1.1 Carotid intima-media thickness
Several studies have used C-IMT as a marker to assess sub-clinical atherosclerosis in HIV-infected patients. C-IMT is a non-invasive technique using high resolution B-mode ultrasonography and is a reliable predictor of myocardial infarction and stroke after adjustment for other risk factors (O’Leary et al., 1999). C-IMT can be measured over time and has therefore been used as a primary endpoint for treatment success in clinical trials with cardioprotective drugs.

C-IMT appears to be the most sensitive indicator of subclinical atherosclerosis (Hsue et al., 2010b). In a study evaluating methods for assessment of atherosclerosis in HIV-1 infection, C-IMT was compared with coronary artery calcium, measured by computerized tomography (CAC) (Hsue et al., 2010b). Older age, duration of HIV-1 infection, low nadir CD4 count and hypertension in HIV-1 infected patients were shown to be associated with significantly higher C-IMT compared to controls. In contrast, the CAC was only increased in older HIV-infected patients (Hsue et al., 2010b).

Hsue et al demonstrated that HIV-infected patients (whether or not they were on antiretroviral treatment) have a higher baseline mean C-IMT compared to age and sex-matched controls (Hsue et al., 2004). In addition, the rate of progression of C-IMT was several fold higher than in HIV-uninfected subjects. In the same study, nadir CD4 T cell count less than 200 cells/microlitre was implicated as a compounding risk factor for increased C-IMT. Similar results have been replicated elsewhere – a case control study of 77 HIV-infected men in the Netherlands showed that they had a 10.8% greater C-IMT compared with controls (van Vonderen et al., 2009). However, these findings are not universal. In an earlier study comparing well-matched cohorts (for age, sex and cardiovascular risk factors) of HIV-infected patients and non-HIV
infected controls, there was no statistically significant difference in the C-IMT as a static measure of atherosclerosis (Currier et al., 2005). One reason for the discrepancy in results may be the lack of uniform approach to C-IMT measurements. Whereas some studies measure C-IMT at the carotid bifurcation, most examine the common carotid. It is believed that the bifurcation may be more susceptible to inflammation and injury therefore could manifest early atherosclerosis (Hsue et al., 2010a).

3.1.2 Brachial artery flow-mediated dilatation
The hallmark of endothelial dysfunction is impaired endothelial dependent vasodilation. This can be non-invasively measured using a technique called brachial artery flow mediated vasodilation (FMD). The technique provokes the release of nitric oxide resulting in vasodilation following transient forearm ischemia and can be quantified as a measure of vasomotor function. FMD measures endothelial dysfunction in response to shear stress whereas C-IMT measures structural defects and reflects more long term exposure to atherogenic factors (Ho et al., 2009).

Studies have previously shown that HIV-1 infected patients have impaired endothelial function as assessed by FMD when compared to non-infected controls (Solages et al., 2006). The severity of impairment may be related to the level of viral replication.

3.2 Pathogenesis
The molecular mechanisms by which HIV-1 induces endothelial dysfunction have yet to be fully elucidated but several theories have been proposed and are currently being researched (Monsuez et al., 2009):
1. Direct endothelial injury from the HIV-1 virus and the component proteins of HIV-1
2. HIV-induced chronic inflammation
3. HIV-induced dyslipidaemia and metabolic syndrome
4. Direct endothelial injury from antiretroviral therapy
5. ART-induced dyslipidaemia and metabolic syndrome

It is likely to be the combination of viraemia, elevated inflammatory markers and adhesion molecules, a pro-atherogenic lipid profile and the effects of ART, which heighten the risk of cardiovascular disease in HIV-infected persons.

3.2.1 The effects of HIV viral load
It is likely that the increased viral load provides a permanent “on-switch” which constantly activates the endothelium: this may be via direct toxic insult, the concomitant inflammatory response in HIV infection, or both. One study demonstrated a 4-fold greater cardiovascular mortality in patients with higher viral loads (defined by at least 5 Log_{10} copies/ml) which was independent of CD4 count – this study suggested that the viral load was a surrogate marker for endothelial activation and IL-6 release (Marin et al., 2009). A study conducted in Argentina showed that patients with detectable HIV-1 viraemia had significantly higher levels of von Willebrand Factor (vWF) which implies endothelial activation and therefore may predict future cardiovascular risk (de Larranaga et al., 2003). Although some studies have shown no relationship between peak viral load and cardiovascular risk (Friis-Moller et al., 2007), there is now a general consensus on the association between viral load, chronic inflammatory activity and endothelial dysfunction. Moreover, recent results confirm that HIV viraemia is a significant predictor of acute myocardial infarction irrespective of CD4 cell count (Triant et al., 2010).
The strategies for the management of antiretroviral therapy (SMART) longitudinal study demonstrated that patients who were initially assigned to intermittent ART therapy had increased cardiovascular events compared to the constant treatment arm which is believed to be due to ‘rebound viraemia’ after stopping treatment (El-Sadr et al., 2006). Similarly, fluctuations in viral load during ART correlate with adverse changes in flow mediated dilatation (Torriani et al., 2008).

3.2.2 The effect of the component proteins within the HIV virion

One of the genes within the HIV virion, “env”, encodes a single protein called Gp160. When Gp160 is synthesized, carbohydrate molecules are attached to it and the complex is turned into a glycoprotein (Wilson et al., 2008). The glycoprotein migrates to the cell surface envelope where it is cleaved into a trimeric complex comprised of a transmembrane protein (Gp41) and a surface glycoprotein (Gp120) which is embedded in the lipid bilayer. The Gp120 facilitates viral entry through interaction with the CD4 receptor and co-receptors on the receiving cell, which are either CXCR4 or CCR5.

Studies have shown that during this interaction there may be some damage to the endothelium, which itself expresses CD4 receptors and co-receptors (Ullrich et al., 2000). Contact between Gp120/Gp160 and the CXCR4 co-receptor initiates the apoptotic cascade in umbilical vein endothelium (Huang et al., 2001). Another study showed that Gp120 significantly increased the expression of human endothelial intercellular adhesion molecules (ICAM-1) at both m-RNA and protein levels, although it did not alter expression of VCAM-1 and E-selectin (Ren et al., 2002). Furthermore, Gp120 has been shown to significantly reduce eNOS expression and endothelium dependent vasorelaxation in porcine and coronary arteries pre-treated with TNF-α; the authors also demonstrated that the combination of Gp120 and TNF-α substantially up-regulated ICAM-1 expression in these arteries (Jiang et al., 2010). In a different study the same authors showed that the HIV viral proteins Tat and Nef could also inhibit eNOS expression in endothelial cells. Tat additionally appears to induce expression of several adhesion molecules on endothelium. These results suggest that several viral proteins potentially contribute to the vascular complications seen in HIV-infected patients (Duffy et al., 2009).

3.2.3 HIV induced inflammatory cascade and adhesion markers

Another mechanism by which HIV-1 may contribute to endothelial dysfunction is via systemic inflammation. We know from non-HIV infected patients that inflammation plays an important role in endothelial dysfunction and atherosclerosis. As we have mentioned previously, raised CRP has been implicated in the pathogenesis of atherosclerosis in HIV uninfected individuals. Similarly, higher levels of CRP have been found in HIV-infected patients compared to controls and this has been shown to predict cardiovascular mortality and morbidity even after accounting for viral load and CD4 count (Hsue et al., 2004). Levels of CRP do appear to reduce following ART initiation, but not back to normal levels – data from the AIDS clinical trial group (ACTG 5095) showed that CRP levels did not normalize after 96 weeks of treatment (Shikuma et al., 2011).

The CRP is not the only marker of inflammation in HIV-1 infection; HIV-1 appears to be associated with a generalized inflammatory activation of the vascular wall. Proinflammatory markers and adhesion molecules that are implicated in the pathogenesis of cardiovascular disease in non-HIV individuals are similarly studied in the context of HIV-1. TNF-α, for example, is expressed in large quantities by macrophages in HIV-infected
individuals (Herbein et al., 1994). Studies by the Tanga Aids Working Group in Tanzania showed a significant increase in many proinflammatory cytokines in HIV-1 infected people and these displayed a positive correlation with HIV-1 RNA levels, suggesting that HIV-1 replication itself may cause a pathological cytokine response (Haissman et al., 2009). The plasma levels of IL-6 are also higher in HIV-infected patients and are directly associated with the HIV-1 viral load (de Larrañaga et al., 2003).

Of note, a study which examined the cardiovascular characteristics of a group of HIV-1 positive “elite controllers” (Deeks et al., 2007) (so called as they can maintain undetectable viral loads in the absence of ART), demonstrated raised CRP levels even in these patients (Hsue et al., 2009a). Elite controllers are likely to exhibit a state of viral replication which is not detected by current assays; this low level of replication may be sufficient to increase T-cell specific responses with subsequent IL-6 and CRP release. Likewise, patients who are clinically well on long term ART may still have a low level of replication which is not detectable but which may be driving an atherogenic response. Not only does the increase in pro-inflammatory cytokines correlate with HIV-1 plasma viral load, but also with pro-thrombotic molecules such as vWF. Platelet activation is increased in HIV-1, resulting in increased thrombogenesis (Aukrust et al., 2000). Several studies have shown increased circulating levels of the endothelial adhesion markers VCAM-1 and ICAM-1 as well as selectins in HIV-infected patients and this may also correlate with disease progression (Galea et al., 1997). Moreover, raised levels of vWF, ICAM-1 and VCAM-1 have been associated with raised D-dimer levels, which are fibrin-degradation products produced when fibrinolysis occurs following coagulation (Wolf et al., 2002). The significance of this association may be that endothelial activation correlates with activation of the coagulation cascade and therefore increased thrombogenic potential. Consistently, these biomarkers closely correlate with HIV-1 plasma viraemia corroborating the interplay between inflammatory biomarkers, HIV-1 viral load and endothelial dysfunction (figure 1).

In keeping with these results, another study which looked specifically at risk factors for increased cardiovascular mortality demonstrated that levels of D-dimer and VCAM-1 in HIV-infected patients positively correlated with cardiovascular risk; the D-dimer was identified as an independent risk factor for cardiovascular disease in addition to the traditional risk factors of hypercholesterolaemia and smoking (Ford et al., 2010). These findings may suggest a role for biomarkers in future risk stratification in HIV-infected patients.

### 3.2.4 Antiretroviral Therapy (ART) and endothelial dysfunction

There are currently 6 classes of antiretrovirals that have been approved for use. These are the nucleoside reverse transcriptase inhibitors (NRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, CCR5-antagonists and integrase inhibitors. Recommended initial regimens usually include combinations of two NRTIs and one NNRTI, or two NRTIs and one PI.

Antiretroviral therapy appears to be somewhat of a ‘double edged sword’ in terms of cardiovascular effects. Treatment with ART reduces viral load and the concentration of inflammatory markers that are likely to perpetuate cardiovascular risk. However, this may be offset by direct toxic effects of ART on endothelium and ART-induced metabolic syndrome. It seems likely that the effects of ART on endothelial dysfunction may depend on nadir CD4 count and peak viral load prior to ART, the type of antiretroviral given and other contributing ‘classical’ cardiovascular risk factors.
Endothelial Dysfunction in HIV

Fig. 1. The key processes involved in plaque formation in anti-retroviral naïve, HIV-1 infected patients. The HIV-1 virus leads to increased levels of adhesion molecules, ICAM-1 and VCAM-1 and increased levels of vWF. Heightened plasma levels of IL-6 contribute to C-reactive protein synthesis from the liver. The adhesion molecules and CRP promote monocyte recruitment to the area. Monocytes migrate to the sub-endothelial tissue and mature into macrophages. Macrophages contribute to foam cell formation and release TNF-α. TNF-α and gp120 inhibit eNOS expression and endothelium dependent vasorelaxtion. The net result is a proliferation of macrophages, foam cell formation and atheromatous plaques with associated haemostasis exacerbated by increased vWF levels.

3.2.4.1 The effects of ART on viral load and cardiovascular disease

It is well established that HIV-1 viraemia and proinflammatory markers are intrinsically linked. Therefore one would presume that by reducing the viral load, ART should also arrest inflammatory processes. The SMART (Lundgren et al., 2008) study showed that interrupted ART was associated with a higher risk of cardiovascular events implying that viral replication and inflammation following treatment cessation is linked with cardiovascular disease. A similar interruption study demonstrated a significant increase in the pro-inflammatory markers IL-6 and D-dimer and an associated increase in cardiovascular mortality in the ART sparing arm compared to those who continued therapy (Kuller, 2008).

3.2.4.2 The effect of ART on inflammatory markers and endothelial dysfunction

Various biological markers of endothelial dysfunction have been shown to increase in HIV-1 infected patients. A longitudinal study comparing biomarkers in HIV-infected patients at ART initiation to two months and then 14 months into treatment demonstrated a normalization or significant reduction in levels of E-selectin, ICAM-1, VCAM-1 and CRP at the two month interval. These changes persisted up to 14 months except for E-selectin which
did not change (Kristoffersen et al., 2009). Similar findings have been reported in other cohort studies, showing significant reductions in both vWF and VCAM-1 levels after six months following initiation of ART, with no demonstrable difference between PI and NNRTI containing regimes (Francisci et al., 2009). In another study the levels of VCAM-1 and vWF correlated positively with viral load in the ART-naïve group; following five months of treatment with a regimen which included either a PI or an NNRTI, there was a significant reduction in levels of VCAM-1 and vWF suggesting a marked reduction in endothelial activation following ART (Wolf et al., 2002).

In recent years, the NRTI, abacavir, has been linked with increased cardiovascular risk in certain observational studies (Sabin et al., 2008). The SMART study has also suggested that the use of abacavir is independently associated with a significant increase in plasma levels of CRP and IL-6 (Lundgren et al., 2008), implying that abacavir itself has pro-inflammatory effects compared to other NRTIs. However, these results have not been uniformly replicated in other randomized trials. Indeed the HEAT study, which compared efficacy between abacavir/lamivudine/kaletra and tenofovir/emtricitabine/kaletra regimens, retrospectively showed that there was a decline of CRP, IL-6 and VCAM-1 in both regimens along with viral load reduction. Neither regime was specifically associated with increased cardiovascular events (Smith et al., 2009).

3.2.4.3 The effects of ART on flow mediated dilatation

It seems logical that by reducing systemic inflammation in HIV-1 infected patients, there would be a concurrent improvement in brachial flow mediated dilatation. This concept was demonstrated in a pilot trial which examined the effects of the anti-inflammatory nuclear factor kappaB-inhibitor, salsalate, on HIV-1 infected patients who were not on ART: a significant improvement in FMD was witnessed after 8 weeks of salsalate therapy (Gupta et al., 2008). Since ART also reduces systemic inflammation it might be expected to improve flow mediated dilatation (FMD).

Indeed, a study which examined the effects of ART (two NRTIs and one NNRTI, two NRTIs and one PI or one PI and one NNRTI) on FMD, viral load and lipid profile showed that after 24 weeks of treatment there was an increase in brachial artery FMD in all 3 ART regimens. This occurred despite an associated increase in total cholesterol and low density lipoprotein levels in all participants, suggesting that decreased inflammation with ART may have a protective effect on the endothelium (Torriani et al., 2008).

However, in another study vascular dilatation in HIV-1 infected patients on ART was significantly impaired compared with ART naïve patients (with no demonstrated difference between PI and NRTI containing regimes) (Andrade et al., 2008). Another study comparing 37 HIV-1 infected patients receiving ART against age and diabetes matched non-HIV-1 infected patients showed that the FMD was equally reduced between the HIV-1 infected patients virally controlled on ART and HIV-1 negative diabetic patients (van Wijk et al., 2006). Again, certain antiretrovirals may be particularly implicated: in HIV-1 infected patients treated with abacavir and achieving virological suppression, a significant reduction in FMD was observed compared to those receiving abacavir-sparing regimes (Hsue et al., 2009b).

3.2.4.4 The effects of ART on vasomotor activity and endothelial cells

Impairment of FMD demonstrates the macro-structural alterations elicited by ART. However, ART also leads to direct microvascular changes. ART can provide a direct insult and subsequent cell death through mitochondrial DNA damage and necrotic pathways, a theory that was demonstrated on human endothelial cells treated with ritonavir in vitro.
(Zhong et al., 2002). In vitro cytotoxic effects have also been exhibited by zidovudine (AZT) and indinavir, both damaging intercellular gaps between adjacent endothelial cells (Fiala et al., 2004) thus providing a platform for the inflammatory cascade.

There may be a causal relationship between the toxic effects of ART and impaired vasomotor reactivity. Ritonavir has been shown to reduce endothelial NO synthase (eNOS) mRNA and protein levels in cultured human coronary endothelial cells (Fu et al., 2005). Similarly, administration of combination antiretrovirals, which included zidovudine and indinavir in the regimen treatment rats have been shown to increase levels of endothelin-1, a marker of endothelial injury and inducer of vasoconstriction (Jiang et al., 2006).

As with other chronic insults, ART-induced endothelial dysfunction may progress to established vascular disease over time. A study by Jiang et al showed that short term treatment (five days) of mice with AZT resulted in a reduction in endothelium-dependent vessel relaxation; however after two weeks of treatment the authors showed a significant increase in injury-induced vascular smooth muscle proliferation and neo-intimal hyperplasia (Jiang et al., 2010). In the same study the authors demonstrated that this increase in neo-intimal hyperplasia correlated with an increase in vascular cell adhesion molecule staining, providing a link between ART and induction of cell adhesion molecules.

4. The metabolic profile of chronic HIV infection and ART and its impact on cardiovascular risk

Both HIV and antiretrovirals may also induce endothelial damage via modification of 'classical' vascular insults, especially blood lipids and glucose. HIV-1 is a known risk factor for hypertriglyceridaemia, elevated low density lipoprotein cholesterol, depressed levels of high density lipoprotein cholesterol and insulin resistance (Oh et al., 2007). In treatment naïve patients, higher HIV-1 RNA levels independently associate with very low density lipoprotein (VLDL) and triglyceride levels. In patients with low CD4 cell counts there is also a higher risk of insulin resistance (El-Sadr et al., 2005). Thus, the metabolic changes that are often attributed to ART may be difficult to interpret because of established abnormalities already present due to infection alone. Nevertheless, it is generally accepted that the PIs and NRTIs are associated with metabolic side effects such as lipodystrophy, and shift the lipid profile to a proatherogenic pattern.

The ongoing Data Collection on Adverse Events of Anti-HIV drugs (D:A:D) study showed that the relative risk of a myocardial infarction (MI) associated with cumulative PI use was 1.16 per year of exposure (whereas NNRTIs did not appreciably increase the risk of an MI) (Friis-Moller et al., 2007). However, there appears to be a metabolic difference between various types of PIs. In the CASTLE study, patients treated with lopinavir/ritonavir had significantly raised fasting total cholesterol and triglyceride levels compared to patients given atazanavir/ritonavir (Molina et al., 2010). Other studies have now demonstrated that boosted lopinavir appears to elicit a worse lipid profile compared to other PI-containing regimens (Molina et al., 2010; Mills et al., 2009).

The data supporting the role of NRTIs in generating metabolic abnormalities is mainly found in studies which used them in combination with a PI. Again, certain drugs are particularly implicated. The ACTG 5052 study compared efficacy in HIV-infected patients between abacavir/lamivudine and tenofovir/emtricitabine given with efavirenz or ritonavir-boosted atazanavir for 96 weeks. At week 48, fasting lipid levels had significantly increased in the arm receiving abacavir/lamivudine.
The use of NNRTIs may also be associated with adverse lipid effects – recent data from the ACTG 5095 study showed that a regime containing efavirenz significantly increased lipid levels above the baseline values and above those seen in ‘NRTI only’ combinations (Shikuma et al., 2007) 96 weeks after treatment initiation.

5. Assessment and management of patients with increased cardiovascular risk

Given that HIV-1 itself is an independent risk factor for cardiovascular disease, there is an increasing need to apply a cardiovascular risk stratification score in HIV-1 infected patients. A cross-sectional study of HIV-1 infected patients in a Spanish outpatient setting demonstrated that the traditionally used Framingham risk calculation score identified a higher proportion of HIV-1 infected men with a moderate cardiovascular risk compared to other available risk stratification tools (Knobel et al., 2007). However, this tool may not be equally applicable to all populations – for example, in a study which examined the predicted cardiovascular risk in an HIV-1 infected Thai population, the Framingham calculation overestimated the risk of cardiovascular disease compared to other cardiovascular risk equations (Edwards-Jackson et al., 2011).

When managing cardiovascular risk in the HIV-1 infected patient, one must advise in the same way as HIV-1 uninfected individuals; for example, addressing lifestyle factors as well as measuring lipid levels, blood pressure and signs of glucose intolerance. However, trials of non-drug therapies and dietary advice alone may not be sufficient to control HIV and ART associated dyslipidaemia.

After addressing lifestyle measures it may then be prudent to review the current antiretroviral therapy. Firstly, it may be possible to switch within the class – for example, changing from nelfinavir to atazanavir can reduce the total cholesterol and triglyceride level sufficiently (Calza et al., 2005; Oh et al., 2007). Another strategy, if the patient is on a PI, is to switch them to another class providing there is established viral suppression and a compatible viral resistance profile (Dube et al., 2003).

In terms of lipid-lowering pharmacotherapy, the Adult ACTG (Dube et al., 2003) have provided some guidance in approaching HIV-patients with dyslipidaemia and raised cardiovascular risk. There are few changes in management compared to the general population and the use of statins (hydroxyl-methyl-glutaryl coenzyme A reductase inhibitors) as a therapy is widely advocated in patients with established isolated hypercholesterolemia (elevated total and LDL-cholesterol and triglyceride level less than 5mmol/l).

The advantages of using a statin are two-fold; firstly, statins reduce the levels of cholesterol, which is implicated in endothelial dysfunction and atheroma formation. Secondly, there is increasing evidence that statins also exhibit anti-inflammatory effects (Jain et al., 2005). Recently a double-blinded cross-over trial (8 weeks of high dose 80mg atorvastatin versus placebo in HIV-1 infected ART naïve patients) showed a significant reduction in immune activation with statin therapy, as measured by a fall in activated CD8+ T cells, without any effect on HIV-1 RNA viral load (Ganesan et al., 2011). This further supports the use of statins in HIV-1 infected patients even without established lipid abnormalities. Lowering of oxidized LDL-cholesterol and total LDL cholesterol with 40mg pravastatin has also been shown to improve endothelial dysfunction, as measured by FMD, in patients on a PI-containing ART regime (Hurlimann et al., 2006).

However, some caution must be exercised when using statins as there may be significant interactions with PIs. The concentration of pravastatin has been shown to markedly increase
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when used with boosted darunavir, although its levels are decreased with all other PIs. Therefore, whilst there is a potential increase in the side effect profile of all statins, pravastatin is usually considered the safest to use with PIs other than darunavir (Seker, 2007).

Statins are not the only lipid-lowering drug available; the combination of a statin and a fibrate should be considered (albeit with close monitoring due to the exaggerated side effect profile) when the triglyceride level is above 5mmol/l and may be the best approach to achieve lipid targets in these patients. Ezetimibe is a newer agent that acts by reducing intestinal cholesterol absorption and has been shown to be better tolerated but equally efficacious compared to statins in HIV-infected patients with hypercholesterolaemia (Negredo et al., 2006). Ezetimibe may be used when statins are not tolerated or as an adjunct to other anti-lipid agents in severe lipid disturbance.

In non-HIV-1 infected patients, the beneficial effects of aspirin have largely been attributed to its action on thromboxane synthesis and platelet aggregation; however, there is also evidence suggesting that aspirin improves endothelial dysfunction through endothelium dependent vasodilatation (Husain et al., 1998). Current guidelines, as outlined by the U.S Preventative Services Task Force, recommend the use of aspirin in male patients between 45-79 years old when the benefits of a reduction in risk of myocardial infarction, taking into account overall cardiovascular risk, outweighs the potential risks associated with aspirin therapy (Calonge N, 2009). Spanish researchers applied these standards to their HIV-infected cohort and found that aspirin would be indicated in 30.8% of their male patients (Tornero et al., 2010). Moreover, salsalate, a compound which exists within the same class as aspirin, has been shown to significantly improve flow mediated dilatation in HIV-infected patients after 8 weeks (as discussed above), perhaps suggesting a role for these agents in reducing endothelial dysfunction (Gupta et al., 2008). Whether or not aspirin should be considered as primary prevention in HIV-infected patients is still debatable and certainly it should not be seen as a replacement for timely ART.

6. Conclusions

As the HIV-1 infected population grows, management of patients is increasingly focused on chronic care issues such as cardiovascular comorbidity and metabolic disturbances including lipodystrophy and glucose intolerance. In recent years, there has been increasing recognition that endothelial dysfunction plays a pivotal role in atherosclerosis in HIV-1 infected patients, and that HIV-1 may be as important as other more “traditional” risk factors for accelerated coronary artery disease. The pathology is complex and multifactorial; the HIV-1 virus and its component proteins are likely to perpetuate a cycle of chronic inflammation, haemostasis and endothelial activation. The role of ART is even less well understood, with the benefits of viral suppression being offset by the toxic and metabolic effects of ART itself (figure 2).

However, what is certain is that early detection and appropriate management of HIV-1 and its complications is imperative in attempting to reduce the devastating global impact that HIV-1 has had within the last 30 years. Effective viral suppression, establishing coronary risk and modifying behavioral risk factors may provide the best initial approach to endothelial dysfunction. Following this, the option to switch antiretroviral drugs and treat the patient with pharmacotherapeutic agents aiming to optimize lipids, glucose and blood pressure may then be effective.
Fig. 2. Interplay between HIV, ART and endothelial dysfunction. Schematic diagram explaining how endothelial dysfunction is potentially caused through the effects of HIV itself and the effects of ART. Treatment naïve individuals can develop endothelial dysfunction as a consequence of direct toxicity of the virus, HIV-induced metabolic disturbances and associated chronic inflammation. ART acts to reduce these phenomena but can itself be toxic to endothelium and induce dyslipidaemia and glucose intolerance. ART = antiretroviral therapy.

7. References


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HIV Infection in the Era of Highly Active Antiretroviral Treatment and Some of Its Associated Complications


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Human immunodeficiency virus (HIV) infection is a complex illness affecting the immune system. Acquired immunodeficiency syndrome (AIDS) is an advanced form of HIV infection in which the patient has developed opportunistic infections or certain types of cancer and/or the CD4+ T cell count has dropped below 200/µL. More than 40 million persons around the world are infected with HIV, with approximately 14,000 new infections every day. The disease causes 3 million deaths worldwide each year, 95% of them in developing countries.

Optimal management of human immunodeficiency virus requires strict adherence to highly active antiretroviral treatment (HAART) regimens, but the complexity of these regimens (e.g., pill burden, food requirements, drug interactions, and severe adverse effects) limits effective treatment. However, more patients with HIV are surviving longer today because of these drugs. This allows further study of commonly associated adverse effects. These may affect all body systems and range from serious toxicities to uncomfortable but manageable events. This book reviews some of HAART-related metabolic and neurological complications.

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