

The Role of Inflammation and Immune Activation in Non-AIDS related Co-Morbidities in HIV infection: Determinants and Outcomes

Chidozie E Agu^{1*}, Ikenna K Uchendu², Chigozie N Okwuosa² and Peter U Achukwu²

¹Prime Health Response Initiative (PHRI)–Sub-recipients of Global Fund Impact HIV Project, Ilorin, Kwara State, Nigeria

²Department of Medical Laboratory Science, Health Sciences and Technology, College of Medicine, University of Nigeria Enugu Campus

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*Corresponding author: Chidozie Elochukwu Agu, Prime Health Response Initiative (PHRI) – Sub-recipients of Global Fund Impact HIV Project, Ilorin, Kwara State, Nigeria; Tel no: +2348030984682; E-mail: chidozieagu@gmail.com, cagu@phrinitiative.com

Abstract

Individuals living with HIV infection on antiretroviral therapy are at an increased risk of developing non-AIDS related diseases and experience incomplete immune restoration. The path physiology has been linked to HIV-specific mechanisms as well as non-specific generalized responses to infection which are thought to contribute to the ongoing activation of the immune system. Factors such as the early loss of gastrointestinal (GI) tract mucosal integrity, the pro-inflammatory cytokine milieu, co-infections and marked destruction of lymph node architecture all contribute to the ongoing immune activation as well as deficient immune recovery. Intensive studies on HIV are gradually aiding us understand the processes that link HIV infection to the onset of immunodeficiency. CD4+ T cells exhaustion represents the most fundamental events in HIV infection. Also, HIV-infected individuals show a strong association with individuals of old age: their immune systems are marked by a loss of regenerative capacity and an aggregation of ageing T cells. This review discusses the reason for the development of immune activation and inflammation in the early stages of HIV infection and the long-term effect of these processes to the immune system and health. The three major aspects of HIV disease pathogenesis: reduction of CD4+ T cells, immune activation and depletion of regenerative capacity shall be linked to this process

Keywords: Inflammation; Immune Activation; HIV Infection; CD4+ T Cells; Antiretroviral Therapies

Introduction

Inflammation

Inflammation is a broad term that represents the processes that takes place in the body when the immune system is stimulated to respond to a threat. Though an active immune system is important to maintain good health, in some cases persistent immune activation and inflammation due to an ongoing disease like HIV-infection can result in health-related challenges through-out the body [1]. Inflammation explains the complex cascade of events that takes place during immune recognition of

antigens and goes into action, including movement and initiation of different kinds of white blood cells (leukocytes) and release of chemical messengers referred to as cytokines [1]. The immediate immune response to infection or acute injury is often referred to as “inflammation” [2]. Macrophages and leukocytes resident in the tissues are stimulated when microorganism gain entry into the body (via a cut) through release of toxins and other signals from injured cells and blood vessels. Nuclear factor kappa-B (NF- κ B) is cellular protein is produced which turns on the genes required for immune response. Newly activated macrophages release pro-inflammatory cytokines, including interleukin 1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha). Soon, neutrophils and other immune cells move to the site and ingest the pathogens through phagocytosis or destroy them by releasing toxic substance [3].

The production of prostaglandin is stimulated by these “first-line defenders” within cells. They also stimulate an acute-phase response (APR), stimulating the liver to secrete acute-phase proteins like C-reactive protein (CRP), fibrinogen, and plasminogen. These chemical initiates physiological changes locally such as vasodilation and elevated permeability resulting to redness, swelling, heat and pain which are the classic signs of inflammation [4]. They also play a role in coagulation (blood clotting) and tissue repair [3]. “Systemically, pro-inflammatory signals act on the brain and elsewhere in the body, causing fever, loss of appetite, fatigue, and other flu-like symptoms. An extreme version of this reaction, known as a “cytokine storm,” has proven fatal in clinical trials of experimental therapies and has been proposed as an explanation for the high death rate during the 1918 influenza pandemic. First-line defenders release additional cytokines, including interferon-gamma, that promote longer-term immune activation mediated by the lymphocytes: T-cells, B-cells, and natural killer cells (NK cells). Antigen-presenting

cells such as macrophages capture pathogens and display pieces of them (antigens) on their surface [4]. Lymphocytes interact with these cells and learn to recognize and directly target those pathogens. The inflammatory response, therefore, is the result of a complex interplay of many different types of immune cells that use hundreds of chemical messengers to communicate among themselves, forming cascades and feedback loops" [5].

Under normal circumstances, the immune response is self-limiting and turns itself off when the threat is no more- for example, when a wound heals, or a bout of infection resolves. But inflammation can become chronic if the trigger persists or if suppressive control mechanisms do not work properly. Pro-inflammatory prostaglandins and acute-phase proteins are short-lived, and their effects are temporary unless there is an ongoing signal to produce more [5]. And just as some chemical messengers promote immune activation, opposing signals act to inhibit responses. Anti-inflammatory cytokines include IL-4, IL-10, and transforming growth factor-beta (TGF-beta). This fine-tuned system can go awry when the immune system is faced with a threat it cannot overcome [2]. This occurs, for example, during persistent infection. Other causes of chronic inflammation include autoimmune conditions, obesity, chronic stress, and exposure to toxins such as tobacco smoke. Numerous pathogens, including HIV, hepatitis B and C viruses, and herpes viruses, can remain in the body over the long term. Though the immune system may respond by producing antibodies and activating killer T-cells; however, this response is not always enough to clear infection. For some pathogens, such as Hepatitis CVirus, a few people can clear the infection either spontaneously or with treatment [1]. Others, like HIV, appear to always persist for life. In contrast with localized acute inflammatory responses, chronic inflammation may be systemic, affecting the entire body. The overall effect is persistent immune activation, but it is more accurately thought of as immune dysregulation, characterized by a shift in leukocyte activity [3]. During chronic inflammation, neutrophils become less active, while T-cells and other lymphocytes take on a larger role. Persistent activation of T-cells accelerates their maturation and progression through the cell cycle of growth and division. Eventually, T-cells burn out prematurely and may undergo apoptosis or lose their ability to divide. Long-term immune activation and sustained high levels of pro-inflammatory cytokines can induce damage through-out the body, and chronic inflammation is increasingly recognized as a common denominator underlying a host of progressive and age-related diseases" [4].

Chronic inflammation and immune activation in HIV infection

"Following the identification of HIV in the early 1980s, it was recognized that HIV-infection consists of a complex interaction between immunodeficiency, chronic inflammation

and immune activation [6]. These disturbances were found to activate essentially all cellular compartments of both the innate and adaptive immune systems, including monocytes/macrophages, NK cells, B cells, and both HIV-specific and non-HIV specific CD4+ and CD8+ T lymphocytes [7,8]. In several seminal studies, Giorgi et al. [9] demonstrated that T cell activation levels, as measured by increased expression of CD38 on CD8+ T cells, added to the predictive value of very low CD4+ T cell counts and were more prognostic of clinical progression and shorter survival than plasma viral load in people with very low CD4 T cell counts. Studies of Simian immunodeficiency virus (SIV) have provided further evidence linking inflammation and dysregulated immune activation with progressive HIV pathogenesis. Like HIV-infection, SIV- infection is characterized by high levels of viral replication and the rapid destruction of infected CD4+ T cells in sooty mangabeys and rhesus macaques. Upon infection, sooty mangabeys, which are the natural hosts of SIV and do not experience progressive immunodeficiency despite high viraemia, exhibit relatively restrained levels of immune activation in the early phases of disease in contrast to rhesus macaques, which experience high levels of T cell activation and succumb to SIV-mediated pathogenesis, much like their HIV-infected human counterparts [10]. This suggests that sustained, uncontrolled levels of inflammation and immune activation play a determinant role in distinguishing between pathogenic vs. non-pathogenic models of SIV infection. Similarly, HIV-2, which presents a milder, less pathogenic disease-course than HIV-1, is characterized by considerably lower levels of immune activation than those observed in HIV-1-infected individuals [11]. It has been hypothesized that persistently heightened levels of inflammation and immune activation manifest in the ongoing proliferation, expansion, and destruction of T cells, which leads to the exhaustion of the regenerative capacity of the immune system and ultimately immunodeficiency" [8,12]

Causes of Immune activation and inflammation in HIV-infected individuals

"During HIV-1 infection, the establishment of immune activation and inflammation involve several mechanisms that are either directly or indirectly related to viral replication [13], (Figure 1). The common cause of T cell activation during an infection is antigenic stimulation by the virus, which is the foundation of the adaptive immune response. During primary infection, HIV-1 stimulates strong T cell responses, in particular CD8+ T cells, which can persist during the chronic infection phase due to the continuous replication of the virus: around 20% of circulating CD8+ T cells can be HIV-specific in untreated chronically infected patients [14, 15]. HIV- specific CD4+ T cell responses are usually present at a lower magnitude (i.e. up to 3% of circulating CD4+ T cells), which may be related to their preferential depletion by the virus [7].

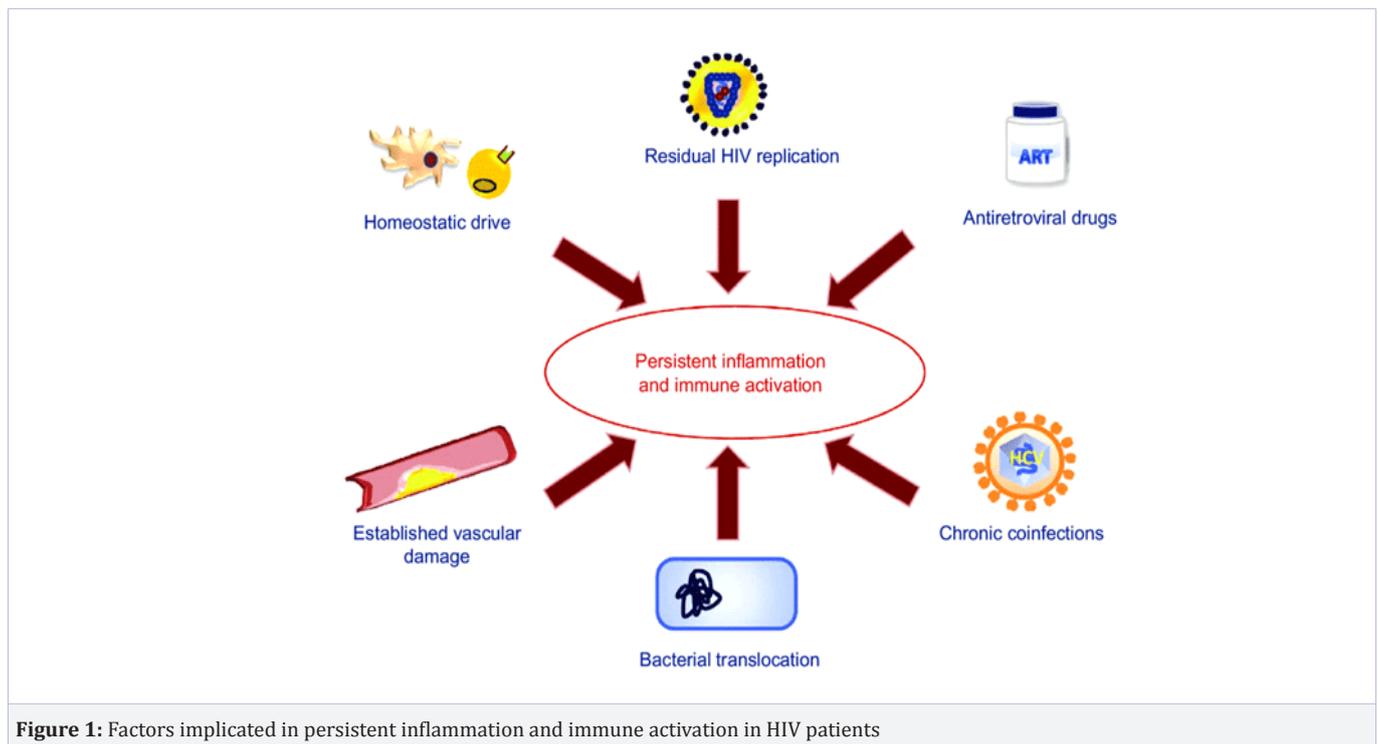


Figure 1: Factors implicated in persistent inflammation and immune activation in HIV patients

Nonetheless, the degree of activation during HIV-1 infection is such that induction with HIV antigens alone cannot account for the complete phenomenon of immune activation observed. Although the physiological impact is not yet known, *in vitro* studies suggest that HIV gene products can induce directly the activation of lymphocytes and macrophages, and the production of proinflammatory cytokines and chemokines. For instance, the envelope protein gp120 may be able to activate cells or to enhance their responsiveness to activation, even in absence of direct infection, through binding to CD4 or co-receptors [16]. The accessory protein Nef is also able to lead to lymphocyte activation either directly [17,18], or through the infection of macrophages [18]. The major drivers of immune activation in HIV-1 infection are viral replication, viral proteins, microbial translocation (from GI tract “damage”), co-infection, viral reservoirs and time of ART initiation. Of these, GI tract damage and associated stimulation of cells of the innate and acquired immune systems are possibly the most important. This is supported by the fact that the natural hosts such as SMs do not show evidence of epithelial barrier breakdown nor microbial translocation and consequently, no pathological immune activation [20]. Also, in cart treated individuals, the contribution of viral replication is minimized; therefore, ongoing immune activation is primarily due to non-viral replication factors”.

HIV-infected patients have a persistent state of inflammation and immune activation in spite of the suppression of HIV replication via ART.

Various factors might be implicated:

- 1) homeostatic drive: after reaching an immune/inflammatory set point, the immunological and inflammatory response persist in spite of eliminating the initial stimulus
- 2) Residual non-detected HIV replication
- 3) Proinflammatory effects of certain antiretroviral drugs
- 4) Translocation of bacterial products through damaged intestinal mucosa
- 5) Coexistence of chronic HCV or herpes virus infection, common in the HIV population
- 6) Established vascular lesions. Abbreviations: HIV, human immune deficiency syndrome; ART, antiretroviral therapy; HCV, hepatitis C virus. [Adopted from Luis et al. [13]]

HIV Viral proteins

“Although the physiological impact is not yet known, *in vitro* studies suggest that HIV gene products can stimulate directly the activation of lymphocytes and macrophages, and the production of pro-inflammatory cytokines and chemokines. For instance, the envelope protein gp120 may be able to activate cells or to enhance their responsiveness to activation, even in absence of direct infection, through binding to CD4 or co-receptors [16]. HIV gene products, such as Env, Tat, and Nef, have been proposed to be involved in HIV-induced immune activation. The Nef protein of HIV-1 has lost the ability to down modulate the CD3-TCR complex from the surface of infected T cells [21]. Consequently, HIV-1Nef

may directly contribute to immune activation by rendering infected CD4+ T cells highly sensitive to re-stimulation through the T-cell receptor (TCR)".

Gastrointestinal tract damage

Studies in the SIV-macaque model and in HIV-infected patients during the acute stage of infection have highlighted the massive and irreversible depletion of CD4+ memory T cells from gut mucosal tissue [22]. Early infection and rapid depletion of these cells are associated with loss of integrity of the mucosal barrier which in turn becomes a source for ongoing activation of the innate immune system [23]. Translocation of gastrointestinal (GI) tract microbes and other bacterial products such as lip polysaccharide (LPS) directly activate macrophages and dendritic cells via toll-like receptors (TLRs) to produce pro-inflammatory cytokines and reactive oxygen species (ROS), causing additional generalized activation of the immune system [23]. Importantly, the loss of memory CD4+ T cells lining the GI tract mucosa is not reversed by cART [24] and therefore, the GI "damage" is likely to remain a significant contributing factor to ongoing activation of the immune system. In addition, recent studies have demonstrated that markers of innate immune activation may be stronger predictors of death during cART than T cell activation [25]. Furthermore, plasma levels of soluble CD14, a marker of microbial translocation and monocyte activation; were shown to predict mortality in HIV infection independently" [26].

Co-infections

Cytomegalovirus (CMV)

"Another important contributing factor to persistent activation of the immune system is the reactivation of other latent viral infections, particularly cytomegalovirus (CMV) [27]. Earlier studies had shown that patients in the sexually exposed group (as opposed to the hemophiliac or intravenous drug user groups) who were CMV antibody (Ab) positive had more rapid HIV disease progression than those who were CMVAb negative [28]. Since then, with effective cART, it has become evident that plasma CMV DNA levels are associated with progression to non-CMV AIDS-defining events [29] and that CMV-specific T cell responses persist at very high levels even during long-term cART [27]. Importantly, it has since been demonstrated that anti-CMV treatment with valganciclovir significantly reduced T cell activation levels and this effect persisted even after stopping the drug [30]. It was suggested that up to 25% of abnormal T cell activation during treated HIV disease may be due to CMV [30]. It seems reasonable to say that larger studies will be important to determine the clinical benefit of treatment of CMV".

Other co-infections

The significant contribution of co-infections to morbidity and mortality in HIV infection has been well documented [31, 32].

Many co-infections non-specifically activate the host immune system and some organisms can directly facilitate HIV replication [27]. The role played by some co-infections such as hepatitis C (HCV) remains unclear. Some have reported HCV to be a relevant predictive factor for a lack of immune recovery on cART [33]; whereas others have shown that CD4 recovery is not affected by this co-infection. Many studies have focused on the effects of treatment of various co-infections on levels of HIV viral load; with only modest reductions in plasma HIV RNA levels [33]. However, it is also relevant to investigate the impact of treatment on levels of immune activation. Co-infections have been shown to activate the cellular arm of the immune system, thereby effectively adding more activated CD4+ T cells for HIV infection and replication [31].

The HIV viral reservoir

"Despite receiving effective ART, HIV patients may have residual viral replication below the detection limits of the techniques commonly used, and/or they may have episodes of transient viral replication. There are conflicting reports in the literature regarding the relative contribution of the HIV reservoir to levels of immune activation. Residual viral replication has been associated with higher CD4+ and CD8+ T cell activation levels [34]. To address this question further, studies have considered the additional benefit of treatment intensification with the viral integrase inhibitor raltegravir. Some have demonstrated an impact on immune activation levels [34], whereas others have shown that treatment intensification has no effect on CD8+ T cell activation [35, 36]. The importance of distinguishing between effects on the CD4+ as opposed to the CD8+ T cell compartment was highlighted by the study of Massanella *et al.* [37], which demonstrated that in a long-term trial of raltegravir intensification therapy, CD38 levels on CD8+ T cells were significantly reduced; however, no effect on CD4+ T cell counts, or activation levels were detected.

Homeostatic drive and time to ART initiation

"Sustained viral replication may promote an immune/inflammatory response that cannot be reversed after a certain point. Therefore, starting ART before reaching this immune/inflammatory set point may prevent the state of persistent inflammation and immune activation. Supporting this hypothesis, the results of several studies have demonstrated that starting ART with low CD4 counts and/or a lowest CD4 nadir was associated with worse immunologic outcomes, even if patients achieved effective viral suppression [38,39]. Treating HIV infection in the acute phase significantly reduces the proportion of activated CD38+HLA-DR+CD8 T-cells when compared to non-treated patients [39,40]. In the Options Project, patients who started ART in the first 6 months after infection had a lower proportion of activated CD8 T-cells than those who initiated treatment 2 years or more after the infection [41]. In another interesting study,

Burdo et al. [42] demonstrated that patients with chronic HIV infection experienced a decrease of sCD163 levels after 3 months of ART; however, the sCD163 plasma concentration remained elevated as compared with controls. By contrast, in patients with early HIV infection (1-year post-infection), the sCD163 levels at 3 months of treatment were similar to those of controls. It seems reasonable to conclude that these studies suggest that early ART could result in the decreased activation of CD8 T-cells and monocytes-macrophages, and they support the hypothesis that the activation of these cells could be reversed to normal levels with early ART.

ART-dependent effects

Some antiretroviral drugs can induce endothelial dysfunction and oxidative stress and promote an inflammatory response. Drugs such as ritonavir, indinavir, lopinavir, zidovudine, and

abacavir have been associated with these deleterious effects. It has been suggested that other anti-retroviral drugs, such as raltegravir, may have an additional beneficial effect on these processes, independently of the suppression of viral replication. Some studies have shown that substituting a PI or non-nucleoside/nucleotide reverse transcriptase inhibitors with raltegravir reduces the levels of circulating inflammatory markers such as IL-6, hsCRP, or D-dimer.153–155

The consequences of immune activation and inflammation in HIV-1 infection

The initiation of this state of immune activation and inflammation and its long-term establishment due to persistence of the virus have extensive and detrimental effects on the immune system and human health [43], (Figure 2).

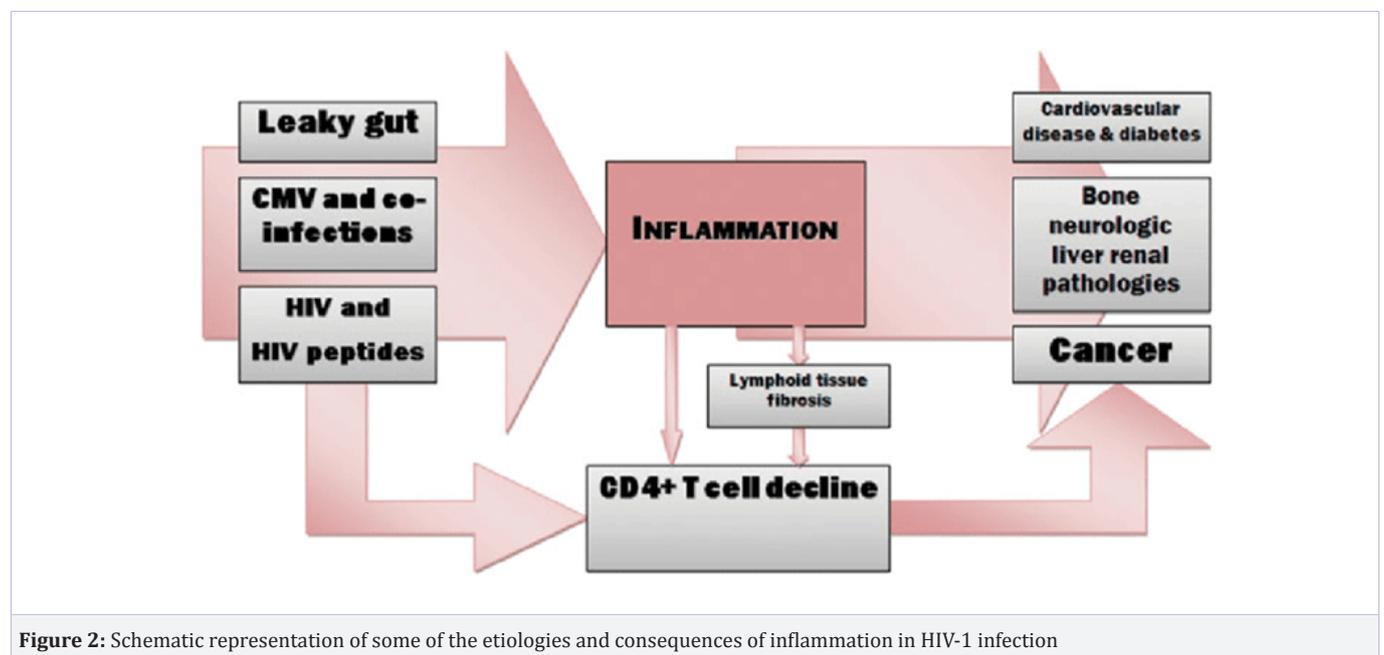


Figure 2: Schematic representation of some of the etiologies and consequences of inflammation in HIV-1 infection

CD4+ T cell depletion

The hallmark depletion of CD4+ T cells is central to the pathogenesis of HIV-infection and AIDS. Direct and indirect mechanisms are delineated involving both the virus itself and non-specific responses to infection and immune activation [44]. Early studies showed that CD4+ T lymphocytes from HIV-infected persons had an enhanced propensity to apoptosis. Furthermore, this was directly related to the degree of lymphocyte activation and correlated with disease progression [44, 45]. To limit the potentially harmful effects of ongoing cell proliferation, well-coordinated death signals are unregulated coincident upon repetitive T cell receptor (TCR) engagement by antigen [46]. Therefore, persistent exposure to antigen, whether in the form of HIV peptides or other foreign antigens, will elicit the signal

for T cells to undergo the physiological form of death, apoptosis. This process is termed activation-induced cell death (AICD) and is mediated in CD4+ T cells predominantly via the engagement of the death receptor Fas (CD95) by its ligand (FasL) [46]. This is relevant in the context of HIV infection in that levels of both Fas and FasL have been shown to be increased and further, that CD4+ T cells have an enhanced propensity to AICD [47]. In addition to this, HIV peptides such as Nef and Tat, without direct infection of CD4+ T cells, have been shown to induce the up-regulated expression of Fas and FasL [48]. The cytokine environment and particularly the T cell growth factor, interleukin-2 (IL-2), can also modulate Fas-mediated apoptosis [49]. Thus, it is pertinent to note that the very process inducing CD4+ T cells to proliferate is simultaneously predisposing them to death.

Lymphoid tissue pathology

“Chronic antigen stimulation and inflammation result in lymphoid tissue hyperplasia and ultimately in the diffuse effacement of lymph node architecture [50]. Even with effective ART, HIV structural proteins have been shown to persist in the germinal centers (GC) of lymph nodes, providing a source for ongoing immune activation. In addition, there is an accumulation of T follicular helper cells (TFH) within the GC [51] which promote the skewing of B cell subsets and efficiently support ongoing viral replication even during negative plasma viremia [52]. However, recently it was shown that immune activation (as measured by sCD14) rather than direct infection with SIV or HIV was associated with *in vivo* accumulation of TFH cells within GC [51]. The pro-inflammatory cytokine IL-6 drives the accumulation of T cells in the GC with concomitant up-regulation of bcl-6 (a transcription factor required for TFH formation and B cell help), and this was associated with hyper secretion of IgG1 [53]. Ongoing inflammation promotes the deposition of collagen which disrupts the fibro-reticular network within lymphoid tissues [54]. This impairs homeostatic mechanisms required for T cell homing and survival by limiting the direct contact of naive T lymphocytes with the important T cell growth cytokine, interleukin-7 (IL-7), resulting in decreased naive T cell proliferation [54]. Thus, chronic inflammation leads not only to an increased demand for CD4+ T cells but also to the fibrotic “scarring” of lymphoid tissue which impairs the supply of CD4+ T cells during ART [55]. The possibility that this process may be partially reversible if treated early bodes well for the use of anti-fibrotic agents such as Pirfenidone”.

HIV-related lymphomas

Such activity and pathology in the lymph nodes as described above would predispose HIV-infected individuals to significantly higher incidences of B-cell non-Hodgkin lymphomas (NHL). All sub-types of NHL show a 60--200-fold increase in HIV-infected persons. Importantly, HIV itself is not classified as a direct carcinogen [56]; but rather facilitates the development of lymphomas via indirect mechanisms; including chronic inflammation and immunodeficiency. In addition, the reactivation or acquisition of other directly oncogenic viruses such as Epstein-Barr virus (EBV) and human herpes virus 8 (HHV-8) provide the “second hit” required for the development of lymphomas [57]. The introduction of ART saw a significant decrease in incidence in HIV-related lymphomas in first world countries; however, this does not appear to have been the case in resource-limited countries [58]. It is possible that in these settings, late presentation of the lymphomas with higher levels of general antigenic exposure and inflammation may preclude the beneficial effects seen from ART in resource- rich countries.

Inflammatory-associated aging

Diseases traditionally associated with aging include liver and kidney disease; cardiovascular, bone loss with associated fractures, cognitive impairment and cancers. As ART has improved the survival of patients with HIV, they are living to older age and the shift has moved from typical HIV-associated diseases and opportunistic infections to disorders associated with aging. However, these are occurring at a younger age in these persons [59]. Inflammation and immune dysfunction may contribute to the increased prevalence of age-related disorders in this population. Higher levels of inflammatory markers such as IL-6 and C-reactive protein (CRP), as well as the coagulation marker, D-dimer; have been associated with increased mortality [60]. The adaptive immune system has an important role in normal aging. It becomes less competent resulting to a state of chronic inflammation [61]. As described above, HIV is also associated with a chronic inflammatory state. HIV-infected subjects older than 50 years show a slower response to treatment. Although ART generally improves immune function, its effects may also contribute to accelerated aging in HIV-infected persons. Certain ART’s may induce mitochondrial dysfunction and oxidative stress and telomere shortening [62]. Nucleoside reverse transcriptase inhibitor (NRTI) use may lead to mitochondrial toxicity which results in increased reactive oxygen species and slowing down of cell division [62]. Tenofovir has been shown to inhibit telomerase activity *in vitro* leading to shortening of telomere length in peripheral blood mononuclear cells [63]. Protease inhibitors can induce senescence markers, oxidative stress and inflammation in human coronary artery endothelial cells *in vitro* [64]. The implications hereof with regards to patient management remain to be determined”.

HIV and neuro cognitive disorders

“The neurocognitive disorders associated with HIV range from mild neuropsychological impairment to HIV-associated dementia [65]. Although the prevalence of severe dementia associated with HIV has decreased owing to the introduction of ART, neurological effects persist. These are thought to be a result of the increased incidence of age-associated disorders in HIV as described above, the ongoing inflammation, increased use of drugs of abuse in HIV-infected individuals and the effects of ART [65]. The inflammation that has been described to occur with HIV-infection also occurs locally within the central nervous system resulting in macrophage activation and the severity of neurological disorders in HIV correlates with the amount of microglia activation [66]. Some ART’s may have neurotoxic side-effects and exacerbate central nervous system disorders in HIV-infected individuals [66]. As described above, HIV-infection is associated with the same immune system disorders as aging which may be particularly relevant for the development of dementia. In addition, HIV is associated with an increased prevalence of atherosclerosis,

coagulopathy, cardiovascular disease, hypertension, diabetes and the metabolic syndrome. These are all risk factors associated with the development of cerebrovascular accidents (CVAs) which have a higher incidence in HIV infection” [66].

Cardiovascular disease (CVD)

The risk of cardiovascular disease (CVD) is significantly increased in HIV infection and is likely to be the combined result of traditional risk factors, ART-induced cardio-toxicity and HIV-related immune dysfunction. In addition, an increased incidence of traditional CVD risk factors such as smoking, dyslipidemia, diabetes, hypertension and central obesity has been found in HIV-infected individuals [67]. Atherosclerosis is increased in HIV infection. Immune activation induces coronary artery endothelial cells to produce chemokines and adhesion molecules which enhance the development of atherosclerosis [68]. The HIV virus itself via its tat protein can directly activate endothelial cells leading to the up-regulated expression of adhesion molecules such as E-selectin [69]. The HIV envelope glycoprotein gp120 can also increase T cell adherence. In addition, both these proteins have been shown to induce endothelial cell apoptosis. Levels of soluble immune activation markers such as IL-6, adhesion molecules and D-dimer correlate with endothelial dysfunction in HIV infection [61]. It is postulated that the persistent immune activation in HIV infection may result in atherosclerosis and thickened carotid intimal thickness [70]. The risk of acute myocardial infarction (AMI) has also been found to be higher in HIV-infected individuals with a recent study showing a 50% increased risk [71].

Another important contributing factor to the risk of CVD is CMV co-infection. CMV has been shown to infect endothelial cells resulting in endothelial damage and accelerated atherosclerosis. Microbial translocation with increased levels of LPS may also perpetuate chronic inflammation and endothelial dysfunction, thereby facilitating accelerated atherosclerosis. The important causal link between microbial translocation and the development of both atherosclerosis and thrombotic disease came from the study of Pandrea *et al.* [72]; their data demonstrated that pigtail macaques developed both atherosclerosis and thrombotic diseases during chronic pathogenic SIV-infection, mimicking the vascular pathology observed in HIV-infected individuals. In addition, vascular pathology in the pigtail macaque SIV model was associated with increases in systemic monocyte/ macrophage activation and coagulation markers” [72].

Inflammation and thrombosis

“An important paradigm is the link between inflammation and thrombosis: inflammation promotes thrombosis, and thrombosis can amplify inflammation [73]. HIV-infection is associated with an increased risk of thrombosis which may be worsened by certain ART regimens [73]. Inflammation induces an imbalance between endothelial pro-coagulant and anti-coagulant properties and this

is facilitated by the interaction of leukocytes, endothelial cells and platelets [74]. The marker of coagulation, the D-dimer, has been shown consistently to be a valuable marker of risk for adverse events. A study in untreated late stage HIV-infection found that D-dimer levels were elevated and were strongly associated with mortality after initiation of ART. It was suggested that increased D-dimer levels may be useful to identify those who may need aggressive clinical monitoring after the initiation of ART [74]. Another study highlighted monocyte as an important cell that provides the link between inflammation and thrombosis [75]. It was demonstrated that monocytes from HIV-infected patients showed significantly up-regulated expression of the pro-coagulant tissue factor and that this correlated with markers of immune activation and soluble levels of CD14, the receptor for LPS [75]. The up-regulated expression of this tissue factor promotes the synthesis of thrombin which directly activates platelets, further predisposing patients to thrombus formation”.

Diabetes mellitus

“HIV infection is associated with lip dystrophy which involves mitochondrial dysfunction, adipose tissue redistribution, altered differentiation of adipocytes, increased adipocyte lipolysis and apoptosis [76]. This leads to altered adipokine secretion and the release of pro-inflammatory cytokines and free fatty acids which exacerbate chronic inflammation, dyslipidemia and insulin resistance (76). Lip dystrophy may be worsened by ART as mitochondrial toxicity, described with thymidine-based NRTIs, may combine with a direct role of HIV-1 infection via Vpr and tat proteins [77]. The atherogenic lipid profile found in HIV- infected individuals is also referred to as the “diabetic dyslipidemia” and is strongly associated with diabetes [77]. HIV inflammation may lead to insulin resistance due to pro-inflammatory cytokines such as IFN- α and the disturbed secretion of adipokines [77]. Study such as the Swiss HIV cohort study is along-term study which showed that HIV-infected individuals receiving ART had a higher prevalence of diabetes [78]. An important consideration is that diabetes, especially when poorly controlled, may lead to an increased risk of infection and reduced immunity which has been found to increase the prevalence of tuberculosis (TB) infection in some cohorts”[79].

Bone disease

“Bone disease in HIV includes osteoporosis, osteonecrosis and osteomalacia [80]. This is likely due to the combination of traditional risk factors, direct effects of HIV-1 infection (peptides and inflammation) and effects of ART (80). Various studies have shown that the prevalence of osteopenia in HIV is 22–71% and the prevalence of osteoporosis in HIV is 3–33% [78]. HIV-infected individuals tend to have a higher incidence of traditional risk factors such as smoking and alcohol use, they tend to weigh less, use medication that may affect bone mineral density such as

selective serotonin re-uptake inhibitor (SSRI) anti-depressants and steroids, and have more diarrhea and malabsorption [81]. High viral load has been associated with a decreased bone mineral density and low CD4+ T cell count is an independent risk factor. Both these factors have been associated with increased frailty [80]. Certain viral proteins such as Vpr and gp120 may stimulate osteoblast activity leading to increased bone resorption [82]. Gp120 can also stimulate apoptosis of osteoblasts and shift the differentiation of mesenchymal cells from osteoblasts to adipocytes. P55-gag suppresses osteoblast activity and leads to increased osteoblast apoptosis [82].

Osteoblasts are derived from precursors of monocyte-macrophage lineage and have a membrane surface receptor known as receptor activator NF- κ B (RANK). Its ligand is known as RANKL and has important immunological functions including the regulating of T-cell growth and dendritic cell function [83]. In addition, RANK-RANKL interaction stimulates the formation of osteoclasts. The inflammatory cytokines associated with HIV infection can activate RANKL, stimulate osteoclast formation and induce the apoptosis of osteoblasts [84]. LPS from microbial translocation can also directly stimulate osteoclast synthesis by producing inflammatory cytokines and RANKL [83]. The effects of ART are also important in the development of bone pathology. Most bone loss occurs early after initiation of ART and stabilizes in a year or two [84]. Some ARTs lead to mitochondrial toxicity, which elevates lactic acid levels and the bone is resorbed to act as a buffer in this situation. Other ARTs can inactivate vitamin D or cause its catabolism by stimulating cytochrome p450 enzymes [84]. Tenofovir acts directly on the proximal renal tubules and can induce renal phosphate wasting with increased parathyroid hormone levels thereby facilitating bone resorption [79].

Renal and liver complications

Kidney disease in HIV-infected individuals may be due to direct effects of HIV, the chronic inflammation associated with HIV-infection, traditional risk factors of kidney disease and the toxic effects of certain ART [85]. After the introduction of ART in the 1990s, the prevalence of certain diseases traditionally associated with HIV-infection decreased; however others including kidney and liver disease, increased [86]. A study in France has found that the following are risk factors for the development of renal complications in HIV-infection: female gender, older age, diabetes, hyperlipidaemia, low CD4 count and the use of tenofovir [87]. A study conducted in the US found similar risk factors but added African-American race and higher viral loads [88]. HIV-associated nephropathy (HIVAN), the typical kidney disorder associated with HIV-infection and presenting with severe proteinuria and progressive kidney damage, is found to be more prevalent in Blacks [87]. This disorder is caused by HIV-1 infection of the kidney itself [89], however, may also have an underlying genetic component [87]. Kidney disease may affect

vitamin D metabolism leading to decreased activation of vitamin D and this may worsen the bone diseases associated with HIV-infection described above. HCV co-infection is also associated with kidney disease as reported in a study [89].

In addition, kidney disease is a CVD risk factor and may worsen the CVD risk in HIV-infected individuals described above. Liver disease is associated with the increased co-infection with hepatitis viruses in HIV-infected individuals [90]. Hepatitis C virus HCV is transmitted parenterally; therefore, co-infection with HIV is common, especially in intravenous drug users. Although there is now a vaccine available for hepatitis B virus (HBV), co-infection with HIV is common, especially amongst intravenous drug users. The SMART study found that HIV immunodeficiency was exacerbated by HBV infection [91]. Other viral hepatitis, such as hepatitis D and E may also lead to liver disease in HIV-infection [91]. Certain ART may cause mitochondrial dysfunction promoting the development of liver diseases and others are associated with lipid disturbances like those found in the metabolic syndrome, which may result in non-alcoholic fatty liver disorder [92]. Importantly, all these factors described heighten the risk of developing hepatocellular carcinoma in HIV infection [92].

Non-HIV-related malignancies

“In the early pre-ART era of HIV infection, AIDS-defining cancers (ADC) such as Kaposi’s sarcoma, non-Hodgkin’s lymphoma (NHL) and cervical cancer were highly documented [93]. However, since the early introduction of ART in 1996 and the introduction of the regimes presently used in 2002, there has been a decline in ADC’s and an increased reporting on “non-AIDS-defining cancers” (NADC’s) such as melanomas, Hodgkin’s lymphoma, anal, prostate, hepatocellular, lung and colorectal cancers [93,94]. The potential causes of NADC’s include direct and indirect oncogenic effects of other viruses and HIV, immunosuppressant, chronic inflammation and immune activation, ART and the traditional risk factors [94]. As successful ART improves survival, age may also play an important role in the increased prevalence of NADC’s. The immunosuppressant associated with HIV-1 infection has been directly associated with an increased risk of ADC [93]. CD4+ T cell counts are inversely associated with NADC risk, whereas the association is not as strong as for ADC [95]. Additionally, the HIV tat protein can block tumor suppressor genes, inhibit cell apoptosis and affect the cell cycle. HIV-infected individuals have also been shown to have impaired DNA repair ability [96].

Chronic inflammation and immune activation promote increased cell proliferation and the generation of potentially damaging reactive oxygen species [95]. Pro-carcinogenic cytokines and growth factors may also be stimulated [95]. The immune dysfunction associated with HIV infection may also

result in impaired immune surveillance with the impaired ability to detect early tumor cells [97]. An increased incidence of traditional risk factors such as smoking, and alcohol consumption has been found in HIV-infected individuals and this may increase the risk of certain NACD's. Earlier initiation of ART has not been proven to decrease the incidence of cancer [98] and in fact some studies have found a higher incidence of cancer in those on ART [94]. As HIV-infected individuals are followed up regularly and may have more regular medical examinations than the public, this may facilitate earlier cancer detection and a perceived higher prevalence than in the general population. However, HIV-infected individuals tend to present at a younger age with more advanced aggressive disease; which is associated with a worse prognosis and more metastases" [94]

Approaches to "switching off" inflammation and immune activation in HIV infection

"Several approaches have attempted to "dampen" the activated state associated with HIV-infection with varying degrees of success. Early work using general immunosuppressant's such as hydroxyurea, prednisone and mycophenolic acid showed limited benefit or even harm in some cases, illustrating that blanket immunosuppressant is counter-productive [99]. A randomized trial that added cyclosporine A to the ART regimen in the chronic stage of the disease showed only minimal and transient increases in CD4+ T cell counts [100]. Selective anti-inflammatory approaches are more likely to be successful. Targeting LPS-induced monocyte/macrophage expression of the enzyme cyclooxygenase 2 with cox-2 inhibitors such as Celecoxib has shown promising results. A small randomized placebo-controlled trial (RPCT) in patients with stable viral loads on long-term ART significantly improved markers of immune activation [101]. Subsequently, for patients not yet on ART, the use of Celecoxib for 12 weeks was shown to reduce chronic immune activation with the additional evidence of improved T cell function in vivo [102]. In a similar approach targeting cells of the innate immune system; a study utilizing the anti-malarial agent hydroxychloroquine, which is known to inhibit endosomal TLR, was shown to decrease immune activation levels significantly in HIV-infected, ART-treated, immunological non-responders" [103].

"The lipid-lowering agents, the statins, have well-described anti-inflammatory properties [104]. Results of a double-blind RPCT using Atorvastatin showed modest but significant reductions in the proportions of activated T lymphocytes. In addition, statins have been shown to reduce levels of CRP and improve patients' outcome as shown in the JUPITER study [105]. Future studies will be of value to determine the impact of these agents on both pro-inflammatory and T cell activation markers. In addition, it will be important to establish the degree to which these markers should be decreased for clinical benefit to be attained [106]. The value of aspirin in the context of HIV

management remains contentious. Early studies did not appear to show any benefit; however, this was prior to the advent of ART. In view of the heightened risk for CVD in HIV-infection, the possibility of revisiting aspirin for these individuals has been explored with some promising results. However, another study showed that aspirin was poorly tolerated and failed to improve endothelial function in virologically suppressed HIV-infected adults" [107]

Conclusion

"Persistent immune activation and inflammation are key driving forces in the loss of CD4+ T cells, progression to AIDS and other complications, as shown in Figure 1. The ongoing priming of the immune system results in the release of pro-inflammatory cytokines and recruitment of immune cells to sites of inflammation or infection. An inability to switch into "anti-inflammatory mode" results in the constant erosion of immune protection. Immunotherapy's that limit ongoing immune activation or selectively "switch off" the attendant inflammation will be important avenues for future research. The field of inflammation in HIV-1 infection is extensive and further investigation is required to determine which of the causes impact to a greater or lesser extent on the various complications".

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References

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