HIV infection and aging of the innate immune system

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Abstract. The increased life expectancy of HIV-infected individuals due to improved treatment has revealed an unexpected increase in non-AIDS comorbidities that are typically associated with older age including cardiovascular disease, dementia and frailty. The majority of these diseases arise as the result of dysregulated systemic inflammation, and both the aged and HIV-infected individuals exhibit elevated basal levels of inflammation. In the elderly, increased inflammation and age-related diseases are associated with a state of impaired immunity called immunosenescence, which is thought to result from a lifetime of immune stimulation. It is now apparent that HIV induces premature immunosenescence within T-cells; however, the impact of HIV on aging of cells of the innate arm of the immune system is unknown. Innate immune cells play a central role in inflammation and are thus critical for the pathogenesis of inflammatory diseases. Limited evidence suggests HIV infection mimics age-related changes to innate immune cells; however, the extent of this effect and the mechanism underlying these changes remain to be defined. This review focuses on the impact of HIV infection on the function and aging of innate immune cells and discusses potential drivers of premature immunosenescence including chronic endotoxaemia, residual viraemia, telomere attrition and altered cellular signalling.

Additional keywords: AIDS, immunosenescence, inflammation.

Introduction

Combination antiretroviral therapy (cART) has reduced the incidence of AIDS-related morbidity and mortality to such an extent that HIV is now largely considered a chronic, manageable disease within most industrialised countries, with an increasing proportion of HIV-infected individuals now entering middle and old age with a nearly normal life expectancy. This newfound longevity has uncovered an increased incidence of non-AIDS associated comorbidities including cardiovascular disease (CVD), dementia, frailty, osteopaenia and osteoporosis, liver and kidney disease, and non-AIDS-associated cancers. Although some of these conditions may be the result of mechanisms specific to HIV infection, related to cART or associated with other viral co-infections, many are thought to be due to systemic inflammation resulting from impaired regulatory mechanisms. These diseases occur prematurely in HIV-infected individuals, even in those with full virological suppression, and research has begun to unravel the complexities of immune function that remain altered despite suppressive cART. Increasing evidence suggests that HIV-associated chronic immune activation and inflammation may accelerate immune aging and senescence, leading to premature immune decline and age-related diseases.

It is well accepted that aging is associated with a progressive decline in immune function. However, increasing evidence suggests that in the elderly, defective immune responses coexist with the effects of chronic inflammation, suggesting immune dysregulation rather than immune impairment.

The aging HIV-infected population is at increased risk of non-AIDS comorbidities

By 2015, it is predicted that over half of all HIV-infected individuals living in Australia will be over 50 years of age and the situation is similar in other industrialised countries.\textsuperscript{1} Not only are these individuals living longer, placing them at risk of the age-related diseases associated with traditional risk factors (e.g. smoking, metabolic syndrome, hypertension, male sex), there is also clear evidence that HIV infection per se...
Inflammation is associated with morbidity in the elderly and in HIV-infected individuals

Chronic systemic inflammation is considered a critical process in the pathogenesis of the majority of age-related diseases. Higher basal plasma concentrations of pro-inflammatory mediators and biomarkers such as interleukin-6 (IL-6), tumour necrosis factor-α (TNFα) and highly sensitive C-reactive protein (hsCRP) are found in the elderly when compared with younger individuals, and are associated with an increased risk of atherosclerosis, muscle wasting, frailty and bone disease (reviewed in reference 9). Increased levels of inflammatory mediators predict mortality within the general population as well as in HIV-infected individuals. For example, elevated plasma concentrations of IL-6, hsCRP and d-dimer were associated with an increased risk of CVD and mortality in the Strategies for Management of Antiretroviral Therapy (SMART) study.

HIV infection induces premature immunosenescence of T lymphocytes

Immunosenescence (‘immune aging’) is defined as a dysregulation of immune function that renders individuals more susceptible to a range of infections and age-related morbidities. It is thought to result from a lifetime of exposure to infections and other immune stressors.

Immunosenescence of the adaptive immune system is characterised by involution of the thymus, with reduced numbers of naïve T lymphocytes and T lymphocyte proliferation, and a concomitant expansion of mature memory and effector CD8+ T-cells, which have shortened telomeres and a senescent CD28- phenotype. Replicative senescence of the T-cells due to shortened telomeres is associated with age-related diseases including CVD. HIV infection is also associated with expansion of CD8+ CD28- T-cells and this expansion is seen even in individuals on cART with full virological suppression (HIV RNA levels below the limit of clinical detection). CD8+ CD28- T-cells may also express CD57, a marker of immunosenescence that can be detected in the elderly and in the pathogenesis of age-related diseases. The herpes virus cytomegalovirus (CMV) is ubiquitous throughout the world and although healthy adults show asymptomatic infection, intermittent viremia is common. CMV seropositivity is significantly associated with immunosenescence and mortality risk in the elderly, and drives many of the functional and phenotypic T-cell changes described above. CMV infection results in oligoclonal expansion of senescent CD8+ CD28- CMV-specific T-cells which are functionally defective. Approximately 10% of T-cell responses are directed towards CMV in seropositive hosts and this is amplified in the elderly, where over 20% of all CD8+ T-cells can be specific for CMV. This large immunological burden is thought to impair immunity to other antigens. Indeed, CMV infection is associated with a reduced response to Epstein–Barr virus in the elderly, impaired response to the influenza vaccination and HIV disease progression. CMV infection accelerates telomere shortening in both CD4+ and CD8+ T lymphocytes, which may explain the increased risk of age-related diseases such as CVD in CMV-seropositive individuals (reviewed in reference 33). Chronic hepatitis C infection also accelerates telomere shortening, and CD4+ T-cell telomere length is a predictor of clinical endpoints and antiviral success. Thus, immunosenescence and age-related diseases are driven by persistent antigen stimulation by several chronic viral pathogens.

HIV infection is associated with accelerated age-related changes to innate immune function

In contrast to the adaptive immune system, much less is known about how aging affects the innate immune system. This is surprising, given the central role played by innate immune effector cells in inflammation, in defence against infection and in the pathogenesis of age-related diseases. The following sections discuss specific changes induced by HIV infection and aging in key cells of the innate immune system; similarities between the two groups are summarised in Table 1. Although there is no universal definition of what ages constitute ‘young’ and ‘elderly’, the majority of gerontological studies utilise participants aged 18–35 and...
over 60–65 years for analysis of young and aged individuals respectively.

**Monocytes and macrophages**

In addition to functioning as phagocytes and antigen-presenting cells, peripheral blood monocytes and tissue macrophages play central roles in responding to and controlling inflammation, and are central to the development of age-related inflammatory diseases.

Macrophage activation is critical for the function of these cells. Phenotypic characterisation of monocytes from elderly humans generally indicates reduced activation as measured by decreased expression of the major histocompatibility complex Class II HLA-DR activation molecules. There is expansion of the minor CD14+ CD16– monocyte subset in the elderly that has a mature phenotype. The proportion of these pro-inflammatory CD14+ CD16– monocytes is also increased in HIV-infected individuals not receiving suppressive cART, but is not significantly altered in individuals on cART.

Data regarding the impact of age on monocyte and macrophage phagocytic function are difficult to assess and conclusions often conflicting, probably due to variations in experimental design such as the assay used, the cell type studied (primary versus cell line), the activation state, murine rather than human studies, a lack of strict criteria for subject recruitment and a definition of ‘elderly’. In the setting of HIV infection, there is a consensus that monocyte phagocytic function is impaired, where binding of targets to Fc and complement receptors on the surface of the cell has been shown to trigger defective intracellular signals and is associated with the development of HIV-associated opportunistic infections such as Candida albicans, Toxoplasma gondii and Mycobacterium avium, which normally are ingested and controlled by these pathways.

Investigation of the effects of cART on monocyte function has shown that some nucleoside analogue reverse transcriptase inhibitor (NRTI) drugs may induce changes in complement-mediated phagocytosis. Not all aspects of monocyte function are reduced in the elderly. Elevated basal plasma levels of the monocyte- and macrophage-derived pro-inflammatory cytokine IL-6 have been reported, as well as increased levels of both the high affinity p55-soluble TNF-α receptor (sTNFR-I) and p75 sTNFR-II, supporting a pro-inflammatory response in the elderly. These findings are of clinical relevance, as heightened production of IL-6 is considered a robust marker for subsequent risk of atherosclerosis. The pro-inflammatory cytokine profile in HIV infection is remarkably similar to that observed in the elderly, with plasma IL-6 and IL-6 mRNA being markedly elevated in HIV-infected patients compared with healthy donors. Elevated basal plasma levels of the monocyte- and macrophage-derived pro-inflammatory cytokine IL-6 have been reported, as well as increased levels of both the high affinity p55-soluble TNF-α receptor (sTNFR-I) and p75 sTNFR-II, supporting a pro-inflammatory response in the elderly. These findings are of clinical relevance, as heightened production of IL-6 is considered a robust marker for subsequent risk of atherosclerosis.

The production of these pro-inflammatory cytokines depends on intact signalling via Toll-like receptors (TLR), a family of pattern recognition receptors present on innate immune cells that recognise pathogen-specific molecules including lipopolysaccharide (LPS, recognised by TLR-4). Production of cytokines in response to TLR ligation provides a crucial link between the innate and adaptive immune systems.

**Natural killer cells**

Natural killer (NK) cells are cytotoxic lymphocytes that play a major role in controlling viral infections and in anti-tumour immunity. Phenotypically, NK cells are identified as CD3– CD56+ lymphocytes, with two main subsets that are functionally different; the majority of peripheral blood NK cells are CD56dim CD16+ with a minor subset being...
CD56\textsuperscript{bright} CD16\textsuperscript{+} The major subset kills defective (e.g. virally infected or transformed) cells efficiently, both directly as well as via antibody-dependent cellular cytotoxicity (ADCC). The minor subset has little or no cytotoxic activity but produces cytokines, e.g. interferon-\(\gamma\) (IFN\(\gamma\)).

Similar to the observed increase in mature CD14\textsuperscript{+} CD16\textsuperscript{+} monocytes with age, some studies of NK cell phenotype report an increased proportion of mature, CD56\textsuperscript{dim} (CD16\textsuperscript{+}) NK cells in the elderly.\textsuperscript{68-70} Other studies have shown that the proportion of CD56\textsuperscript{dim} NK cells is unaltered in the elderly but that the cytokine-producing CD56\textsuperscript{bright} NK cell subset is significantly reduced.\textsuperscript{71} Production of IFN\(\gamma\) in response to IL-2 stimulation may be moderately impaired in the aged,\textsuperscript{79} although data in this area are sparse. HIV infection is associated with accumulation of activated NK cells expressing HLA-DR, whereas expression of CD57 (a marker of terminal differentiation) is also increased.\textsuperscript{72} Most prominently, however, HIV infected individuals have an accumulation of anergic, CD56-negative NK cells (defined as CD3 CD4 CD14 slan CD16\textsuperscript{+} lymphocytes.\textsuperscript{65,73} These cells are defective in cell killing but are able to secrete cytokines and chemokines.\textsuperscript{73}

NK cell cytotoxicity on a per cell basis is impaired in the elderly\textsuperscript{74} and the extent of this defect correlates with an increased risk of infection and death.\textsuperscript{75} Our recent data indicate that NK-mediated ADCC is also impaired in treatment-naïve HIV-infected individuals and only partially restored by suppressive cART.\textsuperscript{76} The basis of this reduced activity appears to be a defect in Fc\(\gamma\) receptor signalling, which remains impaired even in virologically suppressed individuals.

Taken together, these data suggest that both aging and HIV infection are associated with increased activation and maturation of NK cells and impaired NK cell function, and that in the context of HIV infection, these defects may persist despite fully suppressive cART.

**Dendritic cells**

Dendritic cells (DC) are the principal antigen presenting cells and are a critical link between the innate and adaptive immune systems. There are two main types of DC: myeloid DC (mDC) evolve from a myeloid precursor, secrete interleukin-12 (IL-12) and are most similar to monocytes, whereas plasmacytoid DC (pDC) are lymphoid in origin and produce large amounts of IFN\(\alpha\).

DC from elderly subjects have increased expression of the maturation markers CD86 and CD83,\textsuperscript{77} consistent with a shift towards a more mature phenotype. pDC from HIV-infected individuals also show increased basal expression of CD86.\textsuperscript{78} Both the elderly,\textsuperscript{77,79,80} and HIV-infected individuals\textsuperscript{81,82} have a reduced number of circulating pDC. Recent data suggest that mDC from the elderly have impaired cytokine production following TLR1/2, -2/6, -3, -5 and -7/8 stimulation, whereas pDC show an impaired response to TLR-7/8 and -9 stimulation.\textsuperscript{79} Importantly, an impaired TLR response of mDC and pDC is linked to a poor effector response to influenza vaccination.\textsuperscript{79} In contrast, basal intracellular levels of pro-inflammatory cytokines are increased in mDC and pDC from the aged.\textsuperscript{79}

HIV infection is associated with reduced TLR responsiveness, with whole blood from HIV-infected individuals having diminished production of IFN\(\alpha\) and IL-12 following stimulation with TLR-9 and -7/8 ligands respectively.\textsuperscript{82,83} pDC from HIV-infected individuals also exhibit reduced upregulation of the activation marker CD83 (but not CD86 or CD40) following TLR-7 ligation, and reduced IFN\(\alpha\) production following stimulation with TLR-7 and -7/8 agonists as compared with HIV-uninfected individuals.\textsuperscript{72} Like the elderly, pDC from HIV-infected individuals express higher basal levels of IFN\(\alpha\).\textsuperscript{81}

Thus, both age and HIV infection appear to impair DC number and function, although mDC and pDC appear to be differently affected. The finding of reduced expression of certain TLR within DC from the elderly\textsuperscript{79} is an important step towards elucidating the mechanism of impaired DC function; however, more work is clearly required.

**Neutrophils**

The granulocytes neutrophils, basophils and eosinophils are involved in the early innate response to infection, and are central in mediating inflammation. Although age-related changes to basophils and eosinophils remain poorly defined,\textsuperscript{80} the decline in neutrophil function with age is well described and is linked with several age-related conditions (see references \textsuperscript{85, 86} for reviews). Advanced age is associated with reduced chemotaxis of neutrophils to sites of inflammation. Neutrophils from the elderly have reduced phagocytosis and superoxide production in vitro.\textsuperscript{85} Neutrophil phenotype is generally unchanged with age, with the exception of a reduction in CD16B expression, which may be responsible for impaired phagocytosis via this receptor.\textsuperscript{88} Neutrophil priming and responses to activation signals are impaired with age, as these cells have multiple defects in intracellular signalling pathways involved in oxidative stress responses, chemotaxis and apoptosis.\textsuperscript{89}

A limited number of studies indicate HIV infection may also be associated with impaired neutrophil function.\textsuperscript{90,91} Neutrophil chemotaxis, phagocytosis and bacterial killing are defective in HIV-infected individuals,\textsuperscript{92} with impairment of chemotaxis correlating with reduced CD4\textsuperscript{+} T-cell numbers.\textsuperscript{93} The fact that cART has a minimal impact on the incidence of bacterial infections raises important questions surrounding the mechanism of impaired neutrophil function in HIV-infected individuals (reviewed in reference \textsuperscript{94}).

Thus, even though the impact of age on innate immune function remains to be fully defined, HIV infection is associated with several alterations to innate immune cells that are similar to those observed in the elderly. Furthermore, increasing evidence suggests that many of these defects are not fully restored by cART, which has important implications for the management of chronic HIV infection.

**Mechanisms driving persistent inflammation and immune activation in HIV infection**

Chronic inflammation is typically driven by persistent antigenic stimulation or autoimmune reactions. However, in the elderly, there is an additional contribution via dysregulation of
inflammatory processes, described as ‘inflamm-aging’. Immune activation and chronic inflammation are consistently found together in HIV-infected individuals, even in those receiving cART with full virological suppression. These abnormalities are considered to be the mechanisms driving accelerated immune aging and inflammatory-mediated age-related co-morbidities in these individuals. Fig. 1 summarises the potential mechanisms driving premature immune aging and associated disease in HIV-infected individuals. What drives persistent inflammation and immune activation in the absence of overt viral replication remains to be fully elucidated. Given the central role played by innate immune cells in chronic inflammation and the inflammatory pathogenesis of subsequent age-related diseases, our discussion will focus on possible causes of premature innate immunosenescence in HIV-infected individuals.

**HIV-infected individuals exhibit chronic endotoxaemia**

Early in the course of HIV infection, there is catastrophic destruction of CD4+ T lymphocytes in the gut-associated lymphoid tissue. The resulting breakdown in the mucosal barrier increases the passage of gut-associated bacterial products such as LPS across the mucosa and into the bloodstream (a process called microbial translocation), resulting in chronic, low-level endotoxaemia (reviewed in reference98). Despite an initial reduction following initiation of cART, chronic endotoxaemia persists in HIV-infected individuals when compared with levels in uninfected controls.97 LPS is a very potent inflammatory mediator that binds to the CD14–TLR-4 receptor complex on monocytes, macrophages, neutrophils and DC, thereby triggering intracellular signalling pathways that drive the synthesis and release of pro-inflammatory cytokines. Plasma LPS levels are significantly elevated in HIV-infected individuals and levels correlate with the disease state. Plasma LPS levels are also elevated within the elderly, possibly also due to increased microbial translocation across mucosal membranes. Thus endotoxaemia may drive inflammation and immune activation within both HIV-infected individuals and the elderly. Indeed, plasma LPS levels in HIV-infected individuals correlate with T-cell activation, plasma markers of inflammation and disease progression, but chronic endotoxaemia is also associated with significant activation of the innate immune system. HIV-infected individuals also have elevated levels of soluble CD14 (shed by monocytes and macrophages following LPS stimulation and a marker of monocyte activation) and LPS-binding protein (involved in the binding of LPS to TLR4), and both markers correlate positively with plasma LPS levels.43

**Altered response to TLR agonists is associated with chronic endotoxaemia**

Chronic endotoxaemia results in numerous biological and physiological sequelae. Repeated exposure of monocytes and macrophages to low levels of LPS induces tolerance both in vitro and in vivo rendering these cells refractory to further LPS stimulation. Endotoxin tolerance may be one of the contributing mechanisms to innate immune senescence. It is clear that responses to endotoxin depend on the integrity of signalling pathways in the cell that are triggered by the binding of LPS to surface CD14 and TLR-4. Activation of TLR-4

![Fig. 1. Overview of the potential mechanism of immunosenescence within HIV-infected individuals.](attachment:image.png)
signalling pathways is impaired in cells that are tolerant to LPS.\textsuperscript{99} Studies in mice suggest that production of pro-inflammatory cytokines in response to LPS stimulation is reduced with age, which may be due to decreased expression of several proteins involved in LPS-signalling such as p38 and Jun N-terminal kinase mitogen-activated protein (JNK MAP) kinase.\textsuperscript{100} Impaired LPS-responsiveness due to defective signalling may contribute to impaired immunity associated with immunosenescence.

Observations of a cohort of Kenyan sex workers with chronic endotoxaemia found that plasma LPS concentrations correlated inversely with TLR-4 mRNA levels, indicating potential tolerance of cells to LPS stimulation.\textsuperscript{101} Surprisingly, this relationship was absent in HIV-infected individuals.\textsuperscript{101} Furthermore, pre-incubation of peripheral blood mononuclear cells with TLR-8 agonists such as HIV RNA enhances TLR4-responsiveness,\textsuperscript{101,102} suggesting that interaction between TLR signalling pathways may occur. Factors such as minimally oxidised low-density lipoprotein, which are often elevated by HIV infection, can also potentiate the effects of LPS, inducing increased production of pro-inflammatory cytokines from macrophages.\textsuperscript{103} The degree of impaired response of untreated HIV-positive individuals to TLR-9 and TLR-7/8 agonists within whole-blood assays correlates with plasma LPS concentrations,\textsuperscript{104} suggesting chronic endotoxaemia may have a widespread impact on TLR response. As chronic vireaemia and chronic endotoxaemia are both present in HIV-infected individuals, and these factors have opposing effects on TLR-4 biology, the additional consideration of LPS tolerance as well as innate immune aging confound the interpretation of data from HIV-infected individuals. Thus, the effects of HIV-associated, chronic, low-level endotoxaemia \textit{in vivo} remain to be fully defined, and may vary depending on interactions with other factors both directly (e.g. HIV vireaemia) and indirectly (e.g. levels of inflammatory mediators) associated with HIV infection.

Although endotoxaemia and its sequelae are the most well studied consequences of increased gut permeability, HIV-induced gut permeability can result in translocation of many other bacterial and yeast products that stimulate TLR activity including bacterial DNA (TLR-9), flagellin (TLR-5), ssRNA (TLR-7), ds RNA (TLR-3,) and di- (TLR-2/6) and tri- (TLR-1/2) acylated lipopeptides. Plasma concentrations of bacterial DNA generally parallel those of plasma LPS in HIV-infected individuals and both variables predict chronic immune activation.\textsuperscript{105} Further work is required to fully explore the impact of chronically elevated levels of LPS and other bacterial products on TLR responsiveness in the context of both aging and HIV infection.

\textbf{HIV infection is associated with increased oxidative stress}

HIV infection is associated with oxidative stress, which is defined as an imbalance in the generation and detoxification (by antioxidants such as glutathione, GSH) of reactive oxygen species (ROS). The presence of intracellular ROS damages cell proteins, lipids and DNA. Oxidative stress is a well recognised driver of aging and disease.\textsuperscript{106–108} The mechanism of HIV-associated oxidative stress remains to be defined and may be complicated by the ability of LPS to increase intracellular ROS.\textsuperscript{109}

ROS concentrations are increased in HIV-infected individuals and levels of GSH are decreased, irrespective of antiretroviral treatment status.\textsuperscript{110–113} In fact, cART may even exacerbate oxidative stress, with treated HIV-infected individuals exhibiting increased serum oxidant and decreased serum antioxidant levels as compared with untreated controls.\textsuperscript{112} The protease inhibitor (PI) drugs indinavir, nelfinavir (NFV), lopinavir (LPV) and ritonavir, and the NRTI drugs stavudine (d4T) and zidovudine (AZT) have been shown \textit{in vitro} to increase ROS.\textsuperscript{114–116} Interestingly, the PI drugs amprenavir and atazanavir and the NRTI abacavir (ABC) showed no effect. Macrophages cultured \textit{in vitro} with NFV, LPV, AZT or d4T increased ROS production, and this is associated with increased production of the pro-inflammatory cytokines TNFα and IL-1β and increased expression of CCR2 (the receptor for the monocyte chemoattractant protein MCP-1).\textsuperscript{117} Furthermore, treatment of macrophages \textit{in vitro} with several PI drugs increased production of the inflammatory chemokines MCP-1 and MIP-1, but the NRTIs tested (AZT, d4T and ABC) showed no effect.\textsuperscript{118} In addition to increasing ROS levels, d4T and AZT have further been shown to induce premature cellular senescence,\textsuperscript{115} and the same study found no such effect with the drugs ABC, didanosine, lamivudine and tenofovir. The increased ROS production associated with some antiretroviral therapy drugs largely results from inhibition of mitochondrial function.\textsuperscript{115,116} A study by Masia \textit{et al.} showed that plasma peroxide concentrations (a marker of oxidative stress) were significantly higher in HIV-infected patients treated with a PI-based regimen as compared with a non-NRTI-based regimen and further showed that plasma peroxide concentrations predicted cardiovascular risk in HIV-infected individuals.\textsuperscript{117} As these data suggest that individual PI and NRTI have differential effects on ROS production, careful selection of cART may minimise the potentially detrimental effects of antiretroviral therapy on ROS production. Clinical studies will be needed to substantiate this approach, and further investigations are warranted to determine the toxicity of newer antiretroviral therapy drugs and their effects on premature immune senescence.

\textbf{The impact of HIV on telomere length}

As discussed above, telomere shortening is strongly linked with immune senescence as well as a range of age-related morbidities. HIV infection has been shown to accelerate telomere shortening in CD8\textsuperscript{+} and CD4\textsuperscript{+} T-cells and although there has been a similar report in B-cells from HIV infected individuals,\textsuperscript{119} the effect of HIV infection on telomere length in other cell types, including innate immune cells, has not been explored. Telomere length in innate immune cells such as monocytes\textsuperscript{115} and NK cells\textsuperscript{120} may shorten with age, albeit at a rate slower than that observed for T-cells. Telomere length in mature NK cells is also reduced compared with immature NK cells,\textsuperscript{120} although the impact of HIV on telomere length in these cells is unknown.
Telomerase is an enzyme expressed within highly replicative cells such as T-cells and haematopoietic stem cells, and is responsible for repairing telomere attrition and thus preserving proliferative potential. HIV infection can significantly downregulate the activity of telomerase in peripheral blood lymphocytes\textsuperscript{121} and CD34\textsuperscript{+} haematopoietic progenitor cells,\textsuperscript{122} thereby accelerating telomere shortening and inhibiting cellular replicative potential. The observation of impaired telomerase activity in CD34\textsuperscript{+} haematopoietic stem cells that were not infected with HIV suggests that the effect is an indirect one. Many innate immune cells such as monocyte and macrophages do not proliferate in the periphery and are not thought to express telomerase, but continual cell turnover due to HIV-associated chronic immune activation and the observed downregulation of telomerase within bone marrow progenitor cells could result in accelerated telomere shortening and thus premature senescence within these cells.

Residual viraemia may contribute to cellular senescence
Although cART effectively suppresses viral replication to levels below the detection limit of most commercial viral load assays (20–50 RNA copies per mL plasma), up to three-quarters of cART-treated individuals exhibit residual viraemia with levels between 1 and 50 RNA copies per mL.\textsuperscript{123,124} Even these persistently low levels of HIV in plasma may contribute to immune activation and inflammation. The source of this residual virus is unknown, but it is thought to come from proviral reservoirs of HIV cDNA that persist in long-lived cells such as memory T-cells, and monocytes and macrophages despite effective cART. A recent study investigating intensification of cART with the integrase inhibitor raltegravir supported the hypothesis that residual viral replication drives immune activation, as further inhibition of HIV replication via cART intensification significantly reduced T-cell activation.\textsuperscript{125} This important finding suggests residual viraemia may contribute significantly to the immune activation which occurs within cART treated individuals.

Extra-chromosomal HIV cDNA
In addition to integrated (proviral) HIV cDNA, several intracellular, extra-chromosomal by-products of HIV replication are also generated during infection. Extra-chromosomal HIV cDNA is detected within the majority of HIV-infected individuals even in the absence of detectable plasma HIV RNA and it persists for many years.\textsuperscript{126} These forms of HIV cDNA are relatively short-lived in dividing cells such as T-cells but can persist for up to 2 months in macrophages.\textsuperscript{127} In vitro data suggest that unintegrated HIV may be transcriptionally active; nef, tat, env and gag-pol mRNA is produced from unintegrated HIV in macrophages\textsuperscript{128} and production of early viral proteins such as Tat and Nef from macrophages and T-cells has been reported.\textsuperscript{128–130} Unintegrated HIV cDNA can modulate T-cell activity\textsuperscript{131} and induce pro-inflammatory cytokine production from macrophages.\textsuperscript{128} Production of early HIV proteins such as Nef can downregulate CD4 expression, induce cell activation and promote HIV replication within T-cells.\textsuperscript{132} Furthermore, these proteins can have a trans effect on surrounding cells. Tat protein can activate transcription from latent HIV reservoirs,\textsuperscript{133} induce IL-6 production from macrophages and modulate the responsiveness of macrophages to LPS.\textsuperscript{134} Tat has also been shown in vitro to hyperactivate T-cells via increasing the expression of NF-κB-responsive genes,\textsuperscript{135} which may potentiate immune activation. Incomplete reverse transcription products, which accumulate within the cytoplasm of non-productively infected resting T-cells, were able to elicit a pro-inflammatory response within an ex vivo infection model.\textsuperscript{136} Thus, although extrachromosomal HIV cDNA does not support active HIV replication, it may stimulate immune activation and inflammation in virologically suppressed HIV-infected individuals.

What strategies may inhibit the deregulated innate immune response and prevent non-AIDS comorbidities in the cART era?
Treatment strategies currently being used or investigated in the elderly to reduce immunesenescence such as anti-inflammatory agents and inhibitors of microbial translocation may be applicable to prevent age-associated comorbidities in HIV-infected individuals. Chronic inflammation is present in both the elderly and HIV-infected individuals, and is intrinsically involved in diseases associated with aging such as CVD. The anti-inflammatory drug aspirin, which is widely used to prevent recurrent cardiovascular events, is currently being assessed to determine if long-term therapy can prevent other age-related conditions such as frailty and dementia in healthy aging populations (the ASPirin in Reducing Events in the Elderly (ASPREE) trial). However, the dose of aspirin required to inhibit chronic inflammation (higher than the levels required to reduce CVD by inhibiting platelet aggregation) is possibly associated with too many side-effects to be clinically beneficial.

The clinical benefit of minimising immune activation induced by microbial translocation products is currently being assessed within the AIDS Clinical Trials Group using chloroquine, which blocks TLR-mediated responses. Trials are also underway to determine whether antibiotics such as rifaximin and norfloxacin and the drug 21-aminosteroid U74389G can prevent microbial translocation and subsequent immune activation in mice, primates and humans. Studies of centenarians show gross alterations in gut microbiota,\textsuperscript{137} suggesting that these alterations, the immune response to antigens produced by gut microbiota or both may be intrinsically linked to inflammation and aging. The association of oxidative stress with cellular aging and senescence also suggests a potential benefit of antioxidant therapy.

Efforts to reduce the size of the HIV reservoir by, for example, cART intensification, may minimise residual viral replication and thus immune activation.\textsuperscript{138} Reducing and eliminating viral production from the proviral reservoir is currently the subject of intense investigation, with many strategies such as the use of histone deacetylase inhibitors showing early promise.\textsuperscript{125} Whether these drugs have a role in eradication of HIV from cells of the innate immune system remains to be explored. A better understanding of the
mechanisms driving inflammation, immune activation and subsequent immunosenescence within HIV-infected individuals will assist identification of optimal strategies to minimise age-related comorbidities.

Summary

Increasing evidence suggests that HIV-infection is associated with accelerated immune aging of both the adaptive and the innate immune systems. Like the adaptive immune system, innate immune aging is characterised by increased markers of activation and impaired functionality, and HIV infection accelerates many of these age-associated changes. The mechanisms which drive many of these changes within both the aged and HIV-positive individuals remain to be fully defined, but chronic endotoxaemia, increased pathogen burden due to viral persistence or reactivation and associated immune activation and inflammation are likely to be key contributors. The observed correlation between markers of inflammation and immune activation and age-related comorbidities within both groups highlights the significance of these processes in disease development. However, more work is required to delineate immune biomarkers which contribute to disease pathogenesis from those which are merely associated with or a consequence of disease. If non-AIDS related comorbidities are to be prevented, future treatment strategies for HIV infection will need to incorporate supplementary therapeutics to prevent the premature immune decline which drives these diseases.

Conflicts of interest

None declared.

References

HIV and innate immune aging

Sexual Health


A. C. Hearps et al.
HIV and innate immune aging


