Immunosenescence and aging in HIV

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Purpose of the review
During this era of unprecedented antiretroviral therapeutic efficacy, there is hope for successfully treated individuals to achieve a longevity approaching that of the general population. However, the recent identification of a higher incidence of cardiovascular, bone, metabolic, neurocognitive and other aging comorbidities is of major concern and may compromise that ability. The purpose of this review is to focus on the dynamic process of immune remodelling, known as immune senescence, which occurs during HIV infection, and how it impacts on long-term comorbidities.

Recent findings
Early aging in those with HIV appears to stem from persistent chronic inflammation and residual immune activation despite successful antiretroviral therapy. Multiple similarities exist between the T-cell-senescent phenotypes found in many chronic autoimmune and inflammatory conditions, including HIV disease, and the elderly. The immune risk phenotype is linked to poor clinical outcomes in the elderly and may also have clinical consequences in those with HIV.

Summary
Immune senescence results in functional impairments of immunity and a reduced ability to adapt to metabolic stress. Understanding the factors driving the development of immune senescence is critical for the development of strategies to prevent early aging.

Keywords
cardiovascular disease and immune senescence, cytomegalovirus and expansion of memory T cells, HIV comorbidities and accelerated aging, immune risk phenotype, immune senescence and immune remodelling

INTRODUCTION

Immune senescence refers to a dynamic process of immune remodelling that occurs progressively over time. It is associated with chronic inflammation and many of the major clinical consequences of aging. In the elderly, the remodelling results in functional impairments of immunity and a reduced ability to adapt to metabolic stress. Clinical manifestations include hypo-responsiveness to vaccination [1], an increased susceptibility to infection [2], as well as a higher incidence of cancer [3] and autoimmunity [4].

Immune senescence has recently come to the forefront in the field of HIV as a possible contributor to increased risk across ages for cardiovascular, bone, metabolic and neurocognitive comorbidities [4–7]. In humans, HIV infection is a major source of inflammation and substantial immune activation, both of which are linked to 'inflammaging' [8], a concept that attributes a pro-inflammatory milieu to the aging process. The association of HIV infection with early aging of the immune system is often made because of the persistence of inflammation and of residual chronic immune activation despite successful antiretroviral therapy. There is a pattern of abnormalities in T-cell-senescent phenotypes found in those with HIV that is similar to those of uninfected individuals several decades older. Furthermore, these phenotypes have been linked to some of the comorbidities observed in those with advanced age [9]. Notwithstanding the association with an aging immune profile, definitive proof that T-cell immune senescence in those with HIV causes an accentuated development of comorbidities is still lacking.

In spite of our ability to evaluate the aging of solid organs through their intrinsic remodelling, it is difficult to measure aging of the human immune system. It has long been recognized that the thymus, an important immune organ, dramatically involutes

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KEY POINTS

- Patients with HIV now live longer and develop age-related comorbidities.

- Immune senescence in addition to long-standing lifestyle-associated risk factors collectively contributes to the aging process.

- Immune senescence is a slow progressive process that defines biologic aging.

- The immune risk phenotype is a strong prognostic indicator of mortality in the elderly; it remains to be seen if it will have the same prognostic significance in those with HIV.

by the end of puberty. However, it is only decades later, in the very elderly, that immune function declines to the point of causing clinical sequelae [10]. Premature T-cell aging occurs in individuals thymectomized in early childhood [11]. Although splenectomy is a major predisposing factor for potentially fatal pneumococcal sepsis, little is known about aging and splenic function. $CD4^+$ T-cell-receptor repertoire diversity is compromised in spleens of aged mice [12]. Reduced T-cell-receptor diversity in the elderly is also an important cause of immune dysfunction [13]. In rats, changes in expression of genes involved in cell signalling and immune function occur in the spleen and thymus of older animals [14].

Time-dependent tissue remodelling is not specific to the immune system. In a varying degree, it occurs in all organs and best defines biologic aging. During the initial era of antiretroviral therapy, anthropomorphic changes including loss of subcutaneous fat and acquired visceral adiposity were observed. These changes in adipose tissue occur commonly in the elderly, and in non-HIV-infected populations, visceral adiposity is linked to systemic inflammation as well as immune senescence [15]. Using new diagnostic imaging modalities, premature remodelling of other organs is now frequently recognized in those with successfully treated HIV, identifying early onset of osteoporosis, atherosclerosis and neurodegenerative diseases [7,16].

There is mounting evidence of commonality in immune mechanisms underlying inflammatory disorders with respect to comorbidities of the elderly. Patients with autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis [17,18], in addition to other chronic inflammatory conditions such as inflammatory bowel disease [19], multiple sclerosis [20,21] and HIV disease, are more likely than age-matched healthy persons to

have evidence of atherosclerosis [14,21–26]. At the population level, cardiovascular risk factors include environmental, lifestyle and genetic factors. At an individual level, disease susceptibility and age of onset vary substantially despite similar risks. Part of the inter-individual variation in susceptibility may relate to biologic aging and the presence or absence of immune senescence.

The review will focus on immune senescence and aging comorbidities with emphasis on atherosclerosis. It will also explore the association of HIV with the immune risk phenotype (IRP), which is a predictor of mortality and morbidity in the elderly.

THE IMMUNE RISK PHENOTYPE AND AGING

Immune senescence in the elderly is defined primarily by changes in T-cell-subset profiles, as well as alterations in function. These include increases in circulating CD8 T cells with an expansion of CD4 and CD8 memory cells, a phenomenon that has been termed 'memory inflation'. The predominant human memory clones are directed to cytomegalovirus (CMV) [27], a virus whose prevalence increases with age [28]. Currently, it is unknown whether developmental, functional or homeostatic $FOXP3^+$ T-regulatory (Treg) cell defects contribute to the development of the immune senescence profile.

The 'immune risk phenotype' (abbreviated as IRP) is a term used to define a phenotype that includes CMV IgG seropositivity, a low CD4 : CD8 ratio and an abnormally high frequency of circulating T cells that do not express CD28 on their surface. Although an inverted CD4:CD8 ratio is the hallmark of untreated HIV infection, it is also significantly associated with CMV IgG seropositivity [29■]. The IRP and telomere shortening are both markers of biologic aging and are independently associated with morbidity and mortality [29■,30,31].

The IRP is linked to poor outcomes in the elderly, yet there is limited information on the prevalence and clinical significance of the IRP in those with immune disorders including HIV. Chronic inflammation and aberrant immunity occur in patients with autoimmune diseases and in immune deficiency disorders, yet it is unknown whether these diseases have a common underlying mechanism leading to aging comorbidities.

THE ROLE OF CYTOMEGALOVIRUS IN T-CELL SENESCENCE

Cytomegalovirus infection leads to an age-associated T-cell senescence defined by the expansion of the $CD28^-$ T-cell subset, low CD4:CD8 T-cell ratio,

immune activation, shortened telomeres, poor T-cell proliferation and low IL-2 production [32,33]. CMV infection has a prevalence of approximately 50% among middle-aged adults, increasing to 85–90% in the elderly [27]. Among an elderly Latino population in the USA aged 60–100 years, 95% were CMV-infected. Those in the top quartile with respect to anti-CMV IgG antibody titres had higher all-cause mortality, cardiovascular mortality and higher pro-inflammatory cytokine levels for tumour necrosis factor (TNF)-α and interleukin (IL)-6 [34]. Higher antibody levels are thought to reflect more frequent CMV reactivation, more replication and higher pro-inflammatory cytokine levels leading to vascular damage. It is not clear if active CMV infection is a risk factor for atherosclerosis, but it is known that CMV can infect endothelial cells, which may be an early step in the cascade of events leading to plaque formation [35]. Very few human studies have been able to demonstrate a direct viral effect on vessels and CMV genes have not been consistently found in atheromatous plaques [36]. In contrast, both cellular and humoral responses to human CMV have been shown to be associated with atherosclerosis [35,37,38,39▪].

During CMV latency, stochastic episodes of transient viral gene expression occur and drive a phenomenon in which the majority of human memory T cells are specific for CMV [32,40], and have short telomeres indicative of T-cell senescence [33]. These terminally differentiated effector memory cells are CD57$^+$ and produce high levels of interferon (IFN)-γ [41].

HIV-infected individuals have a higher prevalence of CMV infection and are more likely to develop an IRP at an earlier age than do uninfected individuals. They also have an increased risk of acute myocardial infarction [42] and advanced subclinical cardiovascular disease [43] than age-matched uninfected persons. The mechanism of increased cardiovascular risk in HIV infection is probably multi-factorial and includes traditional risk factors, as well as dyslipidemias associated with antiretroviral treatment. Immune activation characteristic of HIV infection also appears to play a role. However, precisely how HIV infection, CMV infection and IRP lead to cardiovascular abnormalities is still incompletely understood. A study of approximately 600 HIV-infected and 100 uninfected women with a median age over 40 years found that anti-CMV IgG levels were associated with carotid artery stiffness as measured by carotid artery distensibility [44]. Markers of immune activation and markers of immune senescence such as the percentage of CD28$^-$CD57$^+$CD4$^+$ and CD8$^+$ T cells were also associated with arterial stiffness [45,46].

Less information is known regarding the prevalence of CMV positivity in other chronic inflammatory conditions compared with age-matched controls. Although several studies have found a weak though not significant association between CMV and risk of multiple sclerosis, it is unknown whether patients with autoimmune disorders or primary immune deficiency having CMV infection as part of an IRP are more likely to have evidence of cardiovascular disease [47].

ROLE OF THE EXPANDED EFFECTOR MEMORY T-CELL POOL

The frequency of circulating CD4$^+$CD28$^-$ T cells is increased in patients with many autoimmune conditions, including type II diabetes [48], polycystic ovary disease [49], rheumatoid arthritis [49–51], systemic lupus erythematosis [52], multiple sclerosis [53,54], inflammatory bowel disease [55], and immunodeficiencies including HIV infection and common variable immune deficiency [56].

In normal individuals, without immune-mediated diseases, having recurrent ischaemic coronary episodes, the CD4$^+$CD28$^-$ T-cell subset has prognostic significance for myocardial infarction or death [57]. It is of interest that statins lower blood lipids and reduce morbidity and mortality primarily in those with high circulating CD4$^+$CD28$^-$ T-cell levels [58].

CD4$^+$CD28$^-$ cells differ from conventional CD4$^+$CD28$^+$ cells in several ways. CD4$^+$CD28$^-$ cells are terminally differentiated and have pro-inflammatory functions; they secrete large amounts of IFN-γ, TNF-α and IL-2 [59,60]. They express granzyme B and perforin allowing them to kill endothelial and smooth muscle cells [61,62]. They express NKG2D and other natural killer (NK)-cell receptors belonging to the killer immunoglobulin-like receptor (KIR) family and the receptor for fractalkine (CXCR1) [61–64]. These receptors may allow CD4$^+$CD28$^-$ cells to respond to as yet undefined environmental signals that lead to their activation and survival [65]. They resist suppression by natural Treg cells, do not undergo Fas-mediated apoptosis and are unable to provide help to B cells since they do not express CD40 [54,59,65,66]. They can be isolated from ruptured atherosclerotic plaques, a finding that has been interpreted as evidence that they are involved in the development of unstable plaques [60].

Patients with atherosclerosis and age-matched healthy individuals differ from each other, the former having an expanded circulating CD4$^+$CD28$^-$ T-cell subset [61]. In healthy individuals, the frequency of these cells is between 0.1 and 2.5% of the

circulating CD4 T cells [67] and less than 50% of CD8$^+$ T cells. These subsets expand with aging, during persistent viral infections and in the setting of chronic inflammation, where median CD4$^+$CD28$^-$ T-cell frequencies are 10-fold higher [50,54,68].

An increased frequency of CD4$^+$CD28$^-$ cells is an independent predictor of future coronary events [57]. These cells invade unstable but not stable atherosclerotic plaques [57]. CD4$^+$CD28$^-$ cells isolated from patients with atherosclerosis kill endothelial cells *in vitro* and are thought to facilitate intraplaque haemorrhage and plaque rupture [61,69]. These findings underlie the notion that selective homing of CD4$^+$CD28$^+$ T cells to atherosclerotic plaques may be one of the factors that leads to plaque destabilization.

CARDIOVASCULAR DISEASE AND IMMUNE SENESCENCE

The elderly have the highest incidence of stroke and myocardial infarction. The Strategies for the Management of Antiretroviral Therapy (SMART) study was the first to reveal a high risk of cardiovascular events in HIV patients that were not maintained on sustained antiretroviral therapy [70,71]. Atherosclerosis is a life-long inflammatory process that occurs progressively with aging. It can begin as early as in late puberty [72], has a strong genetic component, is influenced by lifestyle, and characterized by stiffness and progressive narrowing of the arterial vasculature. Chronologic age is the most important contributor of overall cardiovascular risk [73].

Atherosclerosis is a consequence of chronic inflammation and reshaping of the intimal layer of arteries through the development of atherosclerotic plaque. Formation of plaque involves a variety of adhesion molecules, cytokines, monocytes, foamy macrophages and activated lymphocyte [74]. Clinical outcomes associated with arterial remodelling are acute cardiovascular syndromes and stroke. Both these events result from the sequential occurrence of atherosclerotic plaque destabilization, plaque rupture, intravascular thrombosis and ultimately arterial lumen compromise [75]. A cascade of interactions between antigen-presenting cells and lymphocytes is thought to drive the destabilization of atherosclerotic plaque. Key to this process are activated T cells and those with a senescent phenotype [76].

In addition to chronologic age, there are other risks factors associated with the development of atherosclerosis including hypercholesterolemia, hypertension, diabetes, smoking, and male sex [77]. These are very prevalent in the HIV population.

Cholesterol crystals, high serum glucose and possibly smoking can directly trigger innate immunity through activation of the inflammasome and the production of pro-inflammatory cytokines, maintaining chronically activated auto-inflammatory pathways [78]. In the initial stages of the atherosclerotic process, the immune system facilitates changes in the arterial intima. The creation of atherosclerotic plaque requires monocyte–macrophage involvement in generating foam cells through the uptake of oxidized low-density lipoprotein (oxLDL) [79–82]. Atheroma development thus involves both the innate and adaptive arms of the immune systems [83–85]. Advanced and unstable atherosclerotic plaques usually take years to develop and present as clinical syndromes, yet appear to occur at an earlier age in HIV patients [86]. The most common clinical presentations of coronary artery disease are acute coronary syndromes that result from the rupture of unstable atherosclerotic plaque, the formation of a thrombus leading to acute narrowing or occlusion of an artery. They present as unstable angina, myocardial infarction or sudden death [79].

T cells in atherosclerosis lesions are activated to produce IFN-γ-stimulating smooth muscle cells such that they lose their ability to produce collagen and to proliferate [87]. This cytokine also triggers macrophages, resident in the intima, to produce downstream mediators that include metalloproteases, collagenases, tissue factor and other prothrombotic elements collectively impacting the stability of atherosclerotic plaques and thrombus formation [88]. The inflammatory cascade is triggered by adaptive host responses to specific antigens such as oxLDL and heat shock proteins, or via innate mechanisms involving pattern recognition by Toll-like receptors, which are abundant in atherosclerotic lesions [85,89]. The antigenic trigger for the initial step in the development of atherosclerosis is not known. There is evidence that atherosclerosis may be an autoimmune response to oxLDL and that heat shock protein may also be involved [90–92]. T cells appear to play a dominant role. The most abundant cell types in the plaque infiltrate are CD4$^+$ T cells that produce pro-inflammatory cytokines such as IFN-γ, and TNF [93]. NKT cells, a heterogenous group of T cells that share properties of both T cells and natural killer (NK) cells, are also present [94]. There is evidence that CD8 T cells may exacerbate plaque inflammation and disease [95]. Tregs, in contrast, can suppress atheroma formation, but are not able to control CD4$^+$CD28$^-$ T cells.

Epidemiological studies suggest that acute coronary events are preceded by a decade of activated

innate and adoptive immunity in the context of long-term inflammation [96–99]. It is reasonable to hypothesize that the chronic triggering of T cells may stem from chronic latent viral disorders such as HIV, CMV, and other viruses that undergo intermittent reactivation. The expanded CD8$^+$CD28$^-$ and CD4$^+$CD28$^-$ T-cell subsets may be markers as well as drivers of chronic immune activation, and a low CD4:CD8 ratio may be a consequence of chronic immune activation and CD8 memory cell expansion.

CONCLUSION

The relationship of chronic inflammation, aberrant lymphocyte function and phenotype as it relates to biologic aging is still incompletely understood. Areas for further exploration include B- and T-cell regulation and signalling, changes in stability of FOXP3$^+$ Treg function and phenotype, the role of NK-cell markers on CD4$^+$CD28$^-$ cells and the importance of Toll-like receptors. Large-scale studies in those with HIV will be required to prove that T-cell immune senescence, as defined within the immune risk profile, contributes significantly in the development of atherosclerosis and other comorbidities of aging. If confirmed, then the immune risk profile could be used as a surrogate marker for subclinical atherosclerosis and a useful tool for the benchmarking of biological age rather than chronologic age in risk assessment. Furthermore, strategies to reduce the incidence of the IRP could then be initiated. These would include much earlier initiation of HIV therapy as well as attempts to comprehensively screen and control latent viral infections.

Conflicts of interest

The author is a member of Merck and Abbott Advisory Boards on antiretrovirals.

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