Immune senescence and cardiovascular morbidity as a result of chronic cytomegalovirus infection

Abstract
Asymptomatic chronic cytomegalovirus (CMV) infection in otherwise healthy individuals may have important implications for their long-term health. Recent research suggests that CMV may play a critical role in determining human longevity through the remodelling of vasculature and the ageing of the immune system. Immune ageing is known as immune senescence.

Acute CMV infection typically occurs in the setting of severe immune suppression, end-stage human immunodeficiency virus infection, post organ transplantation or autoimmune disease. In these instances, CMV infection is severely debilitating and often fatal. On the other hand, chronic CMV infection is mostly indolent, and affects the majority of the world’s population without clinically evident morbidity. This review focuses on the role of CMV in immune senescence and its relationship to cardiovascular mortality and morbidity. In the elderly, CMV seropositivity has been recently linked to immune senescence. The mechanisms by which CMV alters the function and architecture of the body’s immune system and promotes a state of chronic inflammation are only now becoming apparent. Much more research is needed to fully understand the broader impact of this virus. It is proposed that CMV-mediated immune processes are responsible for the development of inflammation-driven disorders – including diabetes, osteoporosis, cognitive dysfunction and cardiovascular disease – in the elderly.

Chronic CMV infection elicits recurrent effector T cell responses that may generate collateral damage. Over the course of decades, the host response to chronic CMV infection leads to the development and accumulation of apoptosis-resistant CD28- T cell clones that fail to respond to normal T cell proliferative responses. The cells that are present in atheromatous plaques release cytokines that generate a pro-inflammatory milieu in the intima of arteries, increasing the risk of acute coronary syndromes. In the elderly, the presence of CD28- T cell clones is also associated with weak neo-antigen responses and poor clinical outcomes.
Introduction
Cytomegalovirus (CMV) is a double-stranded DNA virus within the family Herpesviridae. This virus was originally thought to be clinically relevant only in the immune suppressed. Acute CMV infection accounts for severe and often fatal organ damage in the immune compromised. In a chronic setting, it also appears that host responses to CMV infection contribute to atherosclerosis, hypertension and immune system ageing and dysfunction in the elderly, whose seroprevalence exceeds 70%.1

As life expectancy increases in the Western world, research regarding immune senescence has become even more relevant. Ongoing advances in therapeutics and sanitation and declining fertility rates have led to a dramatic increase in the overall age of the human population. There are currently 650 million people worldwide greater than 60 years of age, and this figure is expected to reach two billion by 2050.2 With age, sequential changes to the human immune system occur, including thymic atrophy and the replacement of thymic and bone marrow parenchyma with adipose tissue.3 Recently, CMV has been shown to play a role in immune senescence by altering lymphocyte receptor profiles and causing the decline of humoral immunity and the unbalanced release of pro-inflammatory cytokines, resulting in immune deterioration and dysfunction.4 These CMV-mediated amplified inflammatory responses contribute to the progression of atherosclerotic disease, a major cause of cardiovascular morbidity and mortality in the elderly.5 The current dogma on the pathogenesis of cardiovascular disease is that its development is mediated by chronic inflammatory processes.6 The fibrous cap, the hallmark lesion of atherosclerosis, is generated as a consequence of immune-mediated events, beginning with endothelial dysfunction. Dysregulation of endothelial cells accommodates leukocyte adhesion and the migration of monocytes into the intimal layer. There, macrophages take up oxidised low-density lipoproteins by scavenger receptor-mediated phagocytosis and transform into foam cells. The pro-inflammatory milieu is maintained by the presence of activated T cells and encourages the accumulation of foam cells, fibrin and collagen. This is followed by the migration of smooth muscle cells to create a fibrous atheromatous plaque.6 Vascular remodelling is followed by plaque destabilisation. During this phase T-helper lymphocytes release interferon-γ (IFN-γ), which activates lipid-laden foam cells to release metalloproteases, tissue factor and other mediators that lead to atheroma instability and plaque rupture. The ensuing intravascular thrombotic events lead to myocardial infarction or other cardiovascular events.7

There is ongoing debate about the presence of CMV antigens in the endothelial wall. Should it be the case that CMV antigen is present, it would explain the documented presence of activated IFN-γ-producing T cells within the coronary or carotid arteries. This review will thus consider the host response to chronic CMV infection that leads to accelerated immune senescence, consequent immune dysfunction and possible progression of cardiovascular disease.

Immune senescence in the elderly
Lymphoid cells have a biologic clock and their survival is determined by an interplay of cell cycle regulation, cellular senescence and response to apoptotic cues. The thymus is critical for proper lymphoid differentiation as it hosts T cell maturation. Thymic architecture involutes with age, resulting in a concurrent dampening of function.8 Changes begin with atrophy of the epithelial space in the first year of life and progress to involution of the thymus after puberty.9 Subsequently, thymic epithelial tissue decreases by 3% annually until age 35-45, when the parenchyma becomes fully replaced by adipose tissue.8 These histological changes to the parenchyma lead to a reduction in naïve T cells and an increase in memory T cells, which do not express the surface molecule CD28 (CD28-).9 Logically, this results in a decreased naïve:memory T cell ratio. A decrease in circulating naïve T cells has direct implications for the ability of the host to respond to neo-antigens. Normally, after stimulation by an antigen, CD4+ and CD8+ T cells commence a cell proliferation sequence that ultimately leads to the formation of memory cells. The presence of co-stimulatory molecules – such as CD28 – are critical for the initial immune responses, such as the recognition of antigens, and for T cell activation and proliferation sequences.9 Over time, T memory cells with viral specificity – mostly to CMV, HIV or EBV – progressively lose CD28 cell surface expression.9 For the most part, these cells are terminally differentiated and produce large amounts of perforin and IFN-γ. These CD28- T cells accumulate due to their decreased susceptibility to activation-induced cell death following antigenic stimulation, a compensatory mechanism as the body attempts to adapt to the age-associated loss of naïve T cells.10 The number of CD28- memory T cells is proportional to age –
20% of T cells are CD28- in those less than 40 years of age, 40% in 40- to 60-year-olds and 60% in those older than 60 years.11 Paradoxically, the lack of CD28 in immune senescence does not render the T cells inactive; instead, the cells become deregulated and release high levels of pro-inflammatory cytokines. For example, the levels of IFN-γ may triple their normal range, whereas cytokines responsible for T cell differentiation, such as interleukin-4, remain within normal limits.8

Individuals with chronic viral infections such as CMV are unable to maintain normal homeostatic immune function.12 This derangement in homeostasis may later on lead to higher rates of morbidity and mortality in the elderly. Furthermore, by the replacement of bone marrow parenchyma with fat, less growth hormone and other mediators that normally stimulate haematopoietic stem cell proliferation are synthesised, thus leading to a decrease in haematopoiesis with age.13 Humoral immunity is also affected by immune senescence due to the decline in function of circulating T lymphocytes, changes in primary lymphoid tissues and age-related atrophy of secondary lymphoid organs. The lack of costimulatory ligand expression on CD28- T cells inhibits B cell maturation, activation, class switching and immunoglobulin secretion.8,14

Thus, the exhaustion of the naïve T cell pool, the decrease in capacity to generate a diversity of antigen-specific T effector cells, the promotion of a pro-inflammatory state by CD28- T cells, a general decrease in haematopoietic processes and the inhibition of B cell maturation all work to make the immune system of the elderly less capable of responding to and eradicating new pathogens.

The promotion of a senescent immune phenotype by CMV

The elderly have high rates of CMV exposure, with 87% of octogenarians being CMV-IgG seropositive, compared to 55% of those in middle age.15 Recurrent antigenic challenges by viruses that cause chronic infection in humans, particularly CMV, lead to an altered immune composition characterised by the presence of CD57+ CD28- T cells – a senescent phenotype. In CMV-seropositive elderly adults, the majority of CD28- T cells are generated in response to the chronic infection.15 In individuals older than 50 years of age, the level of CD28- T cell clones increases dramatically because of a decrease in activation-induced apoptosis. Mortality rates in women infected with CMV are almost three times greater than age-matched CMV seronegative elderly women.15

These findings were confirmed by the OCTO study, which found that mortality rates rose in proportion to decreases in naïve:memory T cell ratio, and correlated significantly with CMV seropositivity.16 The skewed T cell phenotype ratio, where more memory T cells are present than naïve ones, observed in these individuals, seems to be irreversible, and continues to decline with age.16

Importantly, in these studies, every patient with a skewed naïve:memory T cell ratio was CMV seropositive.14-16 These findings demonstrate a correlation between CMV seropositivity and a senescent immune phenotype in the elderly. Altered lymphocyte phenotypes with marked naïve:memory T cell ratio changes can occur in all age groups following the initial stages of CMV infection, but are most pronounced in the elderly.17 CMV-specific CD8+ T cell clonal expansion in the elderly is attributed to the effects of chronic infection. These clones are present in the seropositive elderly in greater numbers than any other virus-specific clone by several orders of magnitude.18 CMV-specific T cells can make up more than 30% of the T cell repertoire in CMV-seropositive individuals. Virus re-activation increases CMV IgM levels and is associated with extensive CD8+ T cell activation.18

Chronic CMV infection and cardiovascular disease

CMV-mediated modulation of host immune function plays a major role in the acceleration of atherogenesis. Increased release of pro-inflammatory cytokines, specifically interleukin-6, as a result of chronic CMV infection has been associated with an increase in arterial blood pressure and an increased risk of myocardial infarction, coronary artery disease (CAD) and stroke.19 Moreover, an elevated C-reactive protein (CRP) level, which is induced by states of inflammation, is an independent risk factor for CAD. The subset of a population at highest risk of CAD consists of individuals that are both CMV-seropositive and have an elevated CRP level.20

The initiation of atherogenesis is mediated by membrane co-factor protein-1 (MCP-1), which interacts with inflammatory cells in atheromatous plaques and within the intima of blood vessels.3 MCP-1 is overexpressed in CMV patients, thereby promoting inflammation in vascular tissues and allowing for leukocyte adhesion to occur.3

CMV infection further promotes leukocyte adhesion to damaged endothelial cells through a virus-mediated increase in the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).5 Moreover, CMV infection leads to the increased production of pro-inflammatory cytokines via increased macrophage activation.20 CMV also contributes to hypertension independent of high cholesterol, diet and atherosclerosis.21 The virus induces the aforementioned pro-inflammatory cytokine release, which contributes to an increase in arterial blood pressure. Importantly, it has been demonstrated that chronic CMV infection influences the renin-angiotensin aldosterone system (RAAS) by increasing circulating levels of renin and angiotensin II.21 Activation of the RAAS increases the secretion of antiuretic hormone from the posterior pituitary and aldosterone from the adrenals and increases sodium and water retention. Moreover, RAAS acts directly on the blood vessel, increasing smooth muscle contraction in the arterial wall.21
These and other studies have demonstrated the correlation between the CMV-induced pro-inflammatory state and cardiovascular morbidity and mortality, and between CMV status and the incidence of hypertension. In conjunction with other cardiovascular risk factors, such as family history of heart disease, dyslipidaemia, smoking, obesity and a sedentary lifestyle, chronic CMV infection should be considered a significant factor in the progression of cardiovascular disease.

**Conclusion**

The clinical manifestations associated with acute CMV infection have been well documented; however, the consequences of chronic asymptomatic CMV infection are less certain. It has been proposed, with considerable evidence supporting this claim, that CMV seropositivity is associated with an increased risk of cardiovascular disease through the promotion of a pro-inflammatory state, the destabilisation of atheromatous plaques and the activation of hypertensive host responses.18-20 Further investigation of the possible sequelae of asymptomatic chronic CMV infection is needed. Verification and elucidation of the molecular mechanisms at play in CMV-associated cardiovascular disease are required, with new management paradigms for cardiovascular disease and ageing. This knowledge may guide the creation of new prevention strategies such as the development of CMV vaccines or anti-CMV drug treatment guidelines.

**References**