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Immunosenescence in Humans: Changes to the Aged T Lymphocyte Population in Response to Persistent Cytomegalovirus Infection

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Abstract

Immunosenescence describes the decrease in immune function with advancing age, a phenomenon that is associated with changes in the B and T lymphocyte populations. $CD8^+$ T cells display the most dramatic phenotypical and functional changes within the T cell compartment whereby the cohorts of effector and memory T cells expand while the total population and diversity of naive T cells both decline. The cause of immunosenescence is unknown; however, the accumulation of antigen-specific T cells, in particular cytomegalovirus (CMV)-specific T cells, may be a contributing factor. CMV is a beta human herpes virus that infects an extensive section of the global human population in which it may produce a lifelong, latent infection. This stimulates a highly immunogenic response that comprises a significant proportion of T cells that display a high specificity to CMV. It is postulated that this massive T cell inflation and subsequent exhaustion renders the T cell population senescent, reducing the immune system's ability to combat pathogens as humans age. This review considers fundamental alterations in the immune system in the elderly, focusing on those changes within the $CD8^+$ T cell population, and assesses the contribution of chronic CMV infection to immunosenescence. We propose that memory T cell inflation of CMV-infected individuals and ageing influence the functional and proliferative properties of human CMV-specific T cells, making them less efficient at controlling CMV reactivation.

Keywords: Immunosenescence; T Lymphocyte; Cytomegalovirus; Infection; Ageing

Introduction

The twentieth century and the first years of the new millennium have witnessed a profound increase in ageing of the human population as a consequence of reduced birth rates coupled with an increasing lifespan. The World Health Organization reports that the proportion of elderly individuals, defined as aged 60+ years, will rise to 22% of the world population by the year 2050 [1]. This statistic highlights the importance of understanding the relationship between the aged immune system and disease-causing pathogens to which an ageing population may be exposed. The alterations that occur to the immune system in the elderly resulting from persistent viral infection require detailed examination. Here, the impact of chronic cytomegalovirus (CMV) infection on an ageing individual's immunity, and subsequent T lymphocyte responses to viral stimulation, are discussed. Other persistent viral infections, such as with Epstein-Barr virus, human herpes viruses, or Torque teno virus, may cause similar effects on their host's immune response but which to date are less well defined [2].

The dynamic changes that occur to the aged immune system are collectively termed 'immunosenescence' [3-5]. Pawelec *et al.* (2010) define immunosenescence as "the impairment in immunity as a result of age-associated changes in function in a variety of cells: it is a phenomenon of decreased function, involving changes to both innate and adaptive immunity and a dysbalance between the two arms" [6]. This brings about a continuous adaptation of the immune system as a result of the degenerative alterations that occur over time [6,7]. The complex changes that remodel the aged immune system are poorly understood as not all immune compartments age in an identical manner [8]. This deterioration of immune function leads increasingly to the elderly being susceptible to infections, latent viral reactivation and reduced vaccine effectiveness [9-11].

The pervasiveness of infectious diseases may severely affect the quality of life of an elderly person. The incidence of viral disease as a cause of death among the elderly has risen in recent years in Australia, representative of developed countries, highlighting the aged immune system's decline in functionality and the impact this has on morbidity and mortality [12]. Alterations of the immune system are considered a natural process during ageing but it is evident that persistent viral infections have severe consequences due to their ability to manipulate to their advantage the host immune system [13]. The factors and mechanisms involved in remodelling the immune response are diverse and it is for this reason that understanding the pathogenic alterations of immune functions caused by persistent viral infections may help to explain different manifestations of the general ageing process, including the development of immunosenescence [14].

The Immune System and Persistent Viral Infection

The effects of immunosenescence cause alterations to both innate and adaptive immunity [6]. The evolution of the innate immune system occurred early in the history of multicellular organisms. Innate immunity involves soluble factors that are essential for the detection of exogenous antigens to ensure either the elimination of pathogens or their containment until activation of the adaptive immune system [15]. The dysfunction of the innate immune system involves multiple cell types and correlates with aberrant outcomes following infection, leading to the development of chronic inflammation and many diseases associated with the elderly [15,16]. Activation of the innate cellular repertoire by interaction with viruses evokes a strong γδ T cell response. In addition, alterations in the natural killer (NK) memory cell profile are seen in response to antigen stimulation [17-19]. The outcome of these changes is well documented; however, the mechanisms by which they occur are poorly understood [16]. Therefore, additional research is needed to elucidate how ageing affects the innate immune system [16,20]. In contrast, studies in both humans and in mouse models have provided insight into the influences of ageing on the adaptive immune system. Found solely in vertebrates, adaptive immunity produces an antigen-specific immune response that may take up to several days to elicit. It consists of humoral and cell-mediated arms, orchestrated by B lymphocytes and T lymphocytes, respectively, in which each clone expresses cell surface receptors of a single specificity [21]. This discussion focuses on the adaptive immune system, centring on the cellular response to viral stimulation and considers how ageing and persistent infection by CMV together influence T cell programming, resulting in immune dysregulation [22].

The Adaptive Immune System

Activation of adaptive immunity is characterised by the production in large quantities of diverse cells that are specific for a broad selection of antigens. While this would appear to be an inefficient, energy-intensive process, this approach conveys selective advantages for cells to reach maturity. As with the innate immune system, adaptive immunity is associated with immunosenescence resulting in degeneration of immune function. Upon antigenic challenge through infection or vaccination, specific clones are selected for expansion from the cell repertoire and memory cells are produced; however, this successful means of response may become compromised in people over 50 years of age [23]. Changes with age in the adaptive immune response have been noted most in the T cell population. It is proposed that the long-time proliferation of naive T cells results in cell-intrinsic dysfunction and a reduced clonal naive T cell population, as seen in elderly subjects, consequently limiting the immune system's ability to respond to a range of antigens [24-26]. The impairment of immunity has been difficult to determine in the elderly who often have an increased concentration of pre-existing antibodies which may act to mask a prevailing reduced responsiveness that appears to correlate with poor reactivity to vaccination [27,28]. With the advent of contemporary molecular profiling techniques a clearer picture of immune regulation is set to be established.

The factors that contribute to a decline of the immune system involve a reduction in the naive T cell population and a rise in the memory T cell population, particularly those displaying an effector memory phenotype. The naive T cell is a mature cell that has successfully undergone central selection within the thymus but has not yet encountered its cognate antigen in the periphery. Upon activation and proliferation triggered by a novel encounter with a pathogen, a naive T cell will produce an acquired immune response in which a reservoir of memory cells is retained for future exposure to the same pathogen [29]. The changes to the naive T cell population are connected to thymus involution whereby homeostatic proliferation occurs in order to sustain the population while naive T cell receptor (TCR) diversity is maintained until advanced age (Figure 1) [24,30,31]. This distortion of the naive T cell population that is caused by the immune system's endeavour to maintain homeostasis appears to contribute to age-related immune defects with notable changes occurring within the $CD8^+$ T cell compartment. Hence, the study of $CD8^+$ T cells aims to characterise these alterations in the aged immune system by assessing functional, phenotypic and proliferative responses across the human lifetime.

Functional Analysis to Assess Changes in the T Cell Population: Assessment of cytokine production by T cells is commonly utilised to determine their functional profile in response to antigenic stimulation. Cytokines are secreted in response to cellular activation and therefore are an important mediator of antiviral immunity [32]. Numerous cytokines are produced by $CD8^+$ T cells in response to stimulation by antigen. Levels of interferon (IFN)-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-2 and lysosomal-associated membrane protein 1 (also referred to as CD107a) have proven to be extremely reliable indicators of functional behaviour. Each may be measured at the level of protein expression, typically by enzyme-linked immunosorbent assay or flow cytometry, but increasingly commonly by more modern analytical approaches, such as gene expression profiling and mass cytometry. The extent of the $CD8^+$ T cell response is a marker used to determine the reactivity of T cells and therefore the selection of multiple cytokines is used since the profile of a single cytokine may underestimate the full functional potential of the T cell population [33]. It has been suggested that polyfunctional T cells that produce simultaneously IFN-γ, TNF-α, IL-2 and CD107a are critical to control of infection [34-38].

The roles of IFN-γ and TNF-α in clearance of microbial infection via induction of cellular antiviral proteins have been characterised in detail [39-43]. IFN-γ is expressed by activated $CD8^+$ T cells and binds to the IFN-γ receptor that is expressed on all nucleated cells. Its antiviral properties include induction of macrophage activation. Furthermore, IFN-γ induces surface molecule expression, influences the processing of endogenous antigens and is associated with apoptosis promotion or inhibition [39]. TNF-α is a pro-inflammatory cytokine that activates the signalling pathways for cell survival and differentiation, apoptosis, inflammatory responses and regulation of immune cells [32].

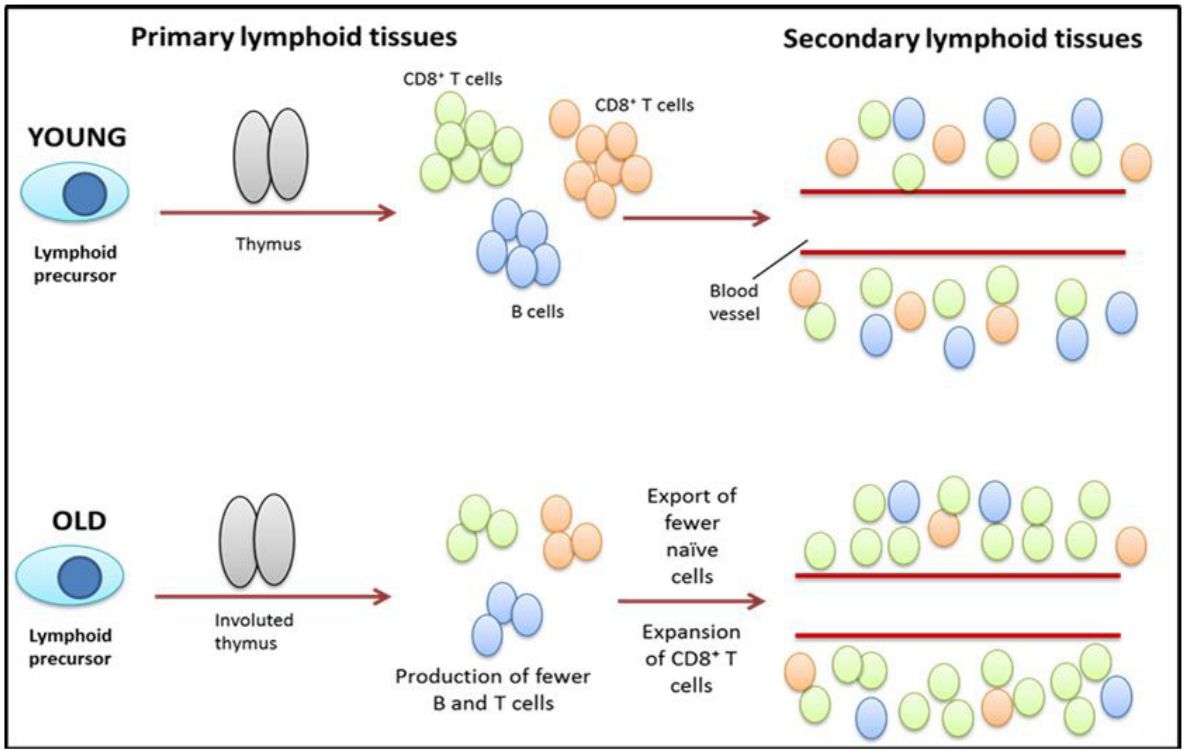

Figure 1: The effects of ageing on lymphocytes production and distribution. T cell population is decreased due to thymic involution causing fewer naive T cell to be exported. The distortion of the T cell repertoire and an increased number of terminally differentiated memory $CD8^+$ T cell expansion. Modified from Dorshkind *et al.*, 2009 [127].

IL-2 is secreted following autocrine activation of T cells via antigen binding and $CD28^+$ co-stimulatory signals. IL-2 functions as a T cell chemoattractant that stimulates activated leucocytes to proliferate and promotes $CD8^+$ T cell differentiation. IL-2 is also an important factor involved in self-tolerance and homeostasis [32]. CD107a is located in the cytotoxic granule membrane of activated $CD8^+$ T cells, and which, upon cell activation and granule exocytosis, is expressed transiently, thus making this protein ideal as a marker of degranulation [44,45].

Phenotypic Analysis to Characterise Changes in the T Cell Population: Assessment of T cell phenotypic markers have produced reliable information to define different antigen-specific $CD8^+$ T cell populations and to identify T cell differentiation in humans [46]. However, owing to the inherent limitations of conducting clinical trials, much of what has been learned is owed to the experimental mouse model. Studies in mice have shown that functionally exhausted T cells display multiple co-stimulatory molecules [47]. In order to assess the phenotype of T cells, programmed cell death protein 1 (PD-1), T cell immunoglobulin mucin domain-3 (Tim-3), lymphocyte activation gene -3 (LAG-3) and CD244 are consistent determinants.

PD-1 and Tim-3 are linked with $CD8^+$ T cell exhaustion throughout chronic viral infections, influencing their fate and/or differentiation. Upon antigen-specific activation, both are up-regulated on the surface of $CD8^+$ T cells and transmit inhibitory signals that reduce proliferation, thereby controlling the accumulation of these T cells. Blockade of the PD-1 pathway in CMV-infected mice has been shown to restore the function of exhausted $CD8^+$ T cells and therefore to reduce viral load. In addition, blocking of the Tim-3 pathway increases proliferation and cytokine secretion by $CD8^+$ T cells significantly in response to antigen stimulation [48-52]. The inhibitory signalling molecule LAG-3 is involved directly in the negative regulation of T cell expansion and homeostasis [53-55]. $CD8^+$ T cells express LAG-3 at low levels; however, further to antigenic stimulation, LAG-3 expression increases dramatically [55]. Lastly, CD244 is a member of the group of signalling lymphocyte activation molecules (SLAM) which are expressed on virus-specific $CD8^+$ T cells. Cross-linking of CD244 can promote the duel function of occupation and activation of antigen-specific T cells, depending on the level of TCR expression [56].

Proliferative Analysis to Characterise Changes in the T Cell Population: Intracellular expression of Ki67 as a marker of *in vivo* cycling and the proportion of CD69 expression are often measured to investigate the antigen-specific proliferation of T cells following antigenic exposure. Ki67 is associated with cellular proliferation and is a marker used frequently for determining the growth fraction of a cell population due to its presence in all active phases of the cell cycle and mitosis [57-59]. CD69 is an early activation marker that is detectable within an hour of ligation of the TCR complex and is a T cell activation and proliferation co-stimulatory molecule [60]. CD69 is expressed only on activated T cells and not upon those in a resting state [61]. The comparison of cell populations after antigen stimulation on sequential days indicates the proliferative capacity of antigen-stimulated T cells. Suppression of T cell proliferation may also be measured *in vitro* by a variety of standard assays [62].

CD8⁺ T Cell Response to Viral Antigens

The importance of the contribution of CD8⁺ T cells to the immune response to pathogens is well-recognised [63]. Antigen-specific CD8⁺ T cells emerge in the peripheral blood early after infection and respond to the viral-encoded peptides presented by major histocompatibility complex (MHC) class I molecules on the surface of infected cells or antigen-presenting cells (APC) [64]. A peptide/MHC class I complex is formed within an infected cell and transported to the cell membrane, which is targeted by CD8⁺ TCR [65,66]. Naive T cells are activated in response to the viral antigens causing T cells to undergo expansion with the aim to control the viraemia. Recognition by CD8⁺ T cells of specific viral epitopes enables infected cells to be lysed via differing mechanisms [63]. Typically, after clearance of acute viral infection, there is significant contraction of the antigen-specific T cell population whereby a small percentage differentiate into memory T cells to enable recognition of virus-specific epitopes upon future exposure (Figure 2) [67]. However, persistent viral infections, of which CMV has been the most studied, drive the memory T cell pool into a further restricted repertoire in which the T cell population does not contract but remains to accumulate at a high frequency (Figure 3). This occurrence has been termed T cell memory inflation [68-70]. Memory inflation is observed in the elderly in whom repeated stimulation of T cells over a lifetime and maintenance of viral latency together contribute to the accumulation of memory T cells that comprises a significant component of the overall aged T cell repertoire [68]. As much as 50% of the available T cell pool may show activation towards a single viral epitope, indicative of the vast commitment of the immune system to managing this one pathogen [69,70]. These antiviral T cells exhibit changes in the expression of genes associated with the cell cycle that may render virus-specific T cells less responsive to the proliferative signals required to induce cell division in response to exposure to antigen.

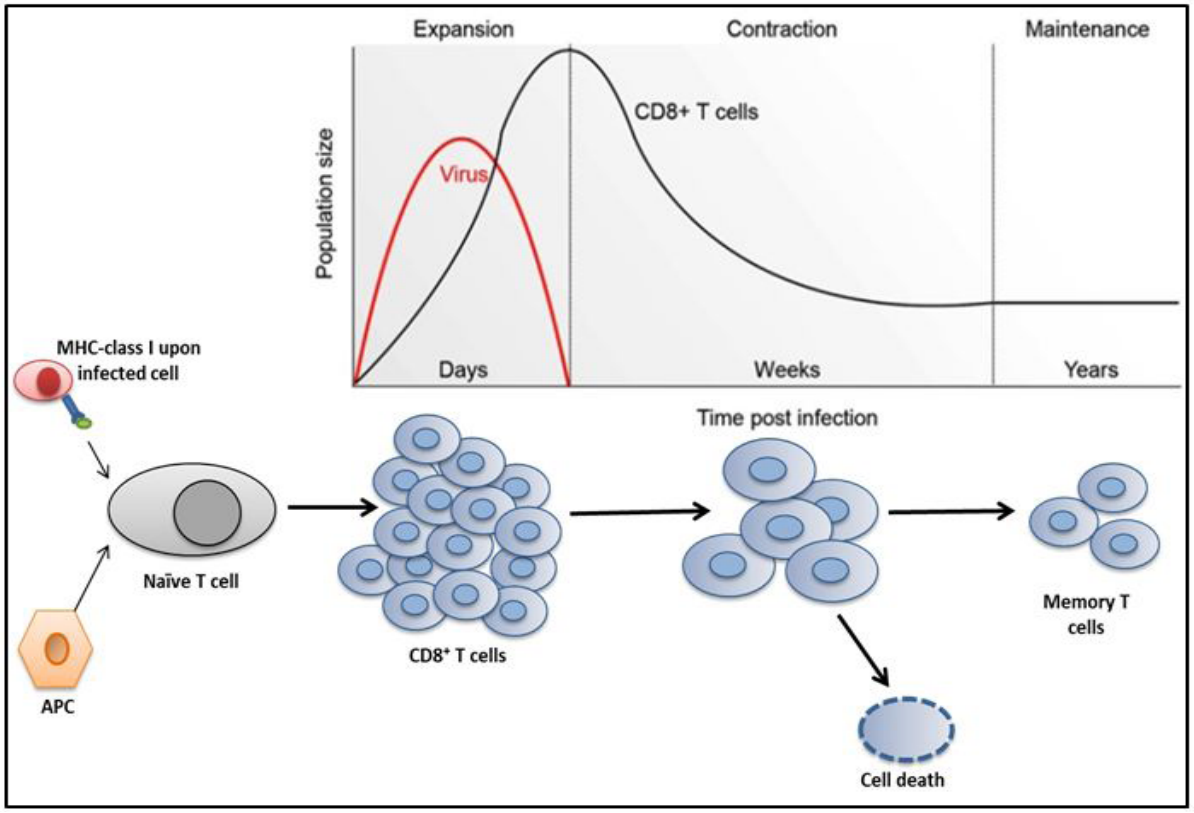

Figure 2: T cell activation and clonal expansion upon antigen stimulation. Modified from Russ *et al.*, 2013 [128].

Consequences of Immunosenescence

The altered T cell compartment and the resulting functional consequences in the elderly appear to reflect in the reduced ability of the aged immune system to respond to vaccines and defend against pathogens. Several studies have documented that the immune response is low following influenza immunisation with an inactivated vaccine in the elderly (30-50% protection) in comparison to the young (65-80% protection) where poor response correlates with the expanded CD8⁺ T cell population [71-73]. It is therefore reasonable to speculate that the distortions in the T cell population reveal an immune system with functional impairment, particularly the CD8⁺ T cell compartment.

Age-Related Changes to CD8⁺ T Cells: A diverse repertoire of T cells is maintained by continual replenishment in the thymus from where naive T cells are exported upon reaching maturity. The thymus involutes with age due to the changes in both the thymic microenvironment and T cell progenitor population but continues to remain active, albeit in a limited capacity, until advanced age [30]. However, the repertoire of naive T cells is compromised and in the absence of sufficient, pristine T cells exported from the thymus, existing T cells increasingly proliferate (sometimes changing phenotype to memory cells), which depletes further the naive T cell compartment [30,74]. In addition, exposure of the ageing host to antigens during their lifetime expands further the memory

T cell pool. Lastly, among memory cells, a great many clonally expanded T cells form over time that eventually come to dominate the T cell memory pool, thereby restricting further cell diversity [75,76]. Such behaviour collectively contributes to the decline in diversity of the $CD8^+$ T cell repertoire. Consequently, $CD8^+$ T cell responses become inadequate upon encountering pathogens to which the person has not had prior exposure.

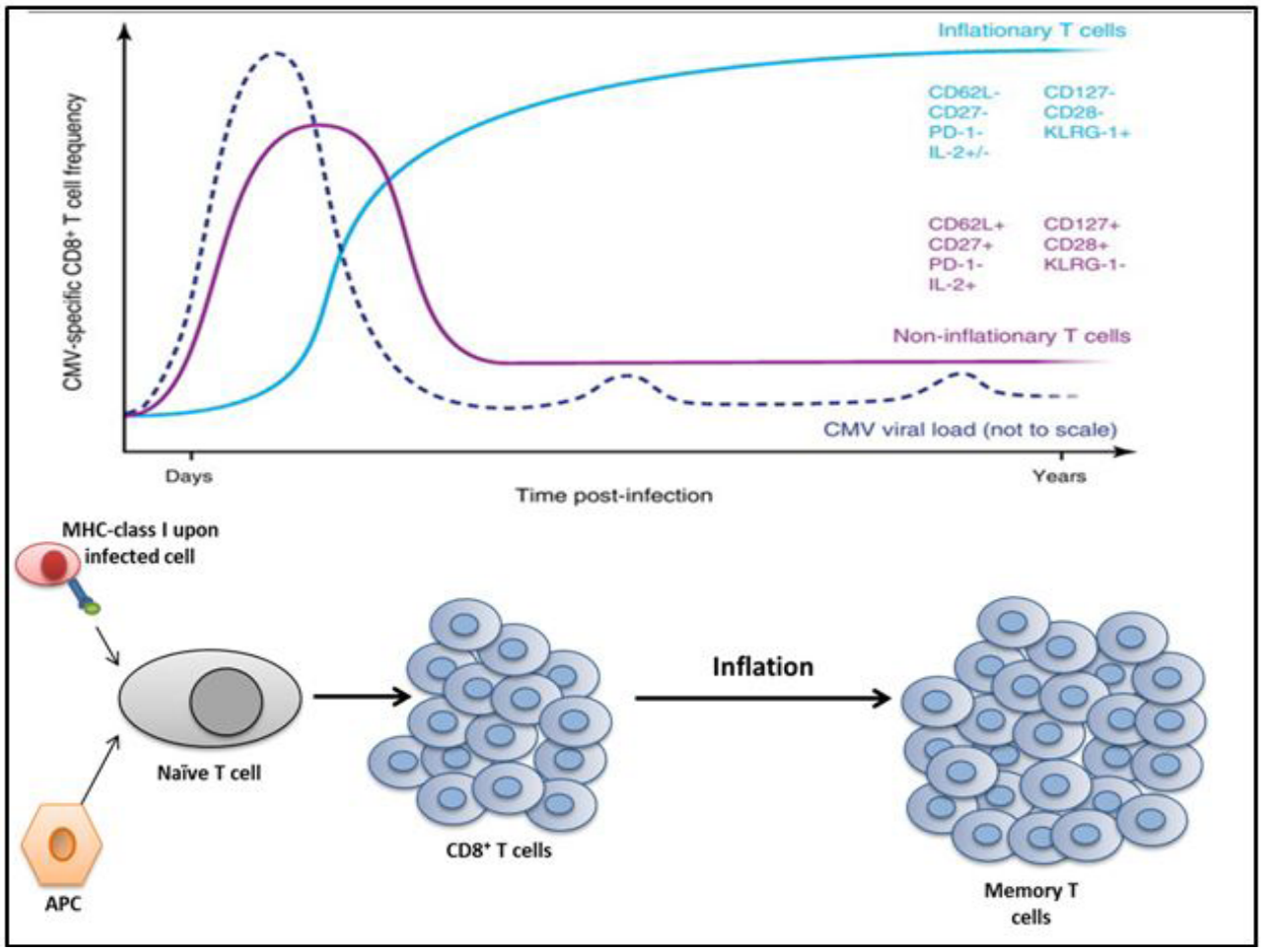

Figure 3: The development of CMV-specific T cells characterised by the lack of memory cell contraction and accumulation of cells over time. Modified from O'Hara *et al*., 2012 [129].

In summary, the changes that occur to the aged immune system are: (i) due to the host's immunological history differentiated and memory T cells accumulate in larger numbers; (ii) due to persistent antigen exposure the naive T cell population decreases; (iii) due to the physical limit of T cells in the body, there is an imbalance in the ratio of memory to naive T cells as daughter memory cells outnumber their parental naive cells.

Role of Latent Infections in Driving Immunosenescence

Immunosenescence presents as a multifactorial process that imposes a pressure on the body's homeostatic mechanisms to maintain a continuous pool of T cells for the majority of adulthood that is potentially responsible for their exhaustion and functional impairment. The consensus of opinion among researchers is shifting towards the view that persistent antigenic stress, and in particular exposure to chronic infections including CMV, plays a vital role in directing the exhaustion of T cells and induction of senescence [3,4]. CMV influences immunity by driving the accumulation of late-stage differentiated $CD8^+$ T cells, i.e. by the process of memory inflation. As these cells remain latent in the host, they may be detected long after the virus has been controlled [69,70]. It has been proposed that the continued and lengthy stand-by status of the host's T cells demanded by persistent CMV infection exhausts the naive T cell reserve, hence leading to reduced clonal T cell diversity and impaired T cell functionality [77,78]. Thus, prolonged challenge with CMV may accelerate immunosenescence, as well as a person's biological ageing processes. Deficiencies in antigen processing and presenting, cytokine environment and maintenance of memory lymphocyte populations give rise to inadequate responses to newly evolving pathogens and a reduced efficacy in protection against infection via vaccination. These changes are poorly understood immunologically and from a clinical perspective have profound implications for the quality of life of elderly individuals [14].

Cytomegalovirus (CMV)

CMV is a ubiquitous beta human herpes virus that can infect a wide range of human tissues with subsequent secretion of the virus in bodily fluids in persons both young and old. CMV is highly species-specific and its evolutionary host adaptation is reflected in the notable number of host-tailored genes, which are usually non-essential for viral replication but that may play a role in virus-host interactions [79]. It is estimated that CMV infects 50-90% of a given population, depending on socioeconomic status, geographical location and hygiene levels [80,81]. In Australia, the seroprevalence rate is approximately 72% over the age of 50 years; however, in certain ethnic groups, seroprevalence approaches 90% of an infected population [82,83]. Transmission occurs through direct contact with infectious bodily fluids, whereby sexual contact and interaction with children are common types of transmission [84,85]. CMV infection is acquired during childhood with disease prevalence generally increasing with age [85]. The changes in demographics will be significant if it is found that CMV is an important driving factor for immunosenescence.

CMV Infection

During primary challenge to a host, CMV targets a broad range of cell types. This establishes an asymptomatic, latent, lifelong infection on which a large fraction of the immune system of the elderly is focused. Endothelial and epithelial cells are primary targets for infection, with particular predilection for the cells of the salivary glands and kidneys that are shed throughout body fluids during a person's lifetime [86]. The ensuing CMV infection generates two outcomes; infection of host fibroblasts leading to extensive viral replication and subsequent infection and/or latent infection resulting from CMV transcription programme suppression in undifferentiated myeloid cells [87]. The persistent nature of CMV infection is possibly related to latency and the ability of the virus to escape the host's immune surveillance by differing mechanisms through which the virus remains at a low level of lytic replication [87,88]. Recurrence occurs by reactivation of the endogenous virus within a seropositive individual [3,89]. Changes to the distribution of immune cells and to the selection of memory cells are rapid during primary infection, yet the precise sites where viral latency and reactivation are established have not been determined [90].

Establishment of Viral Latency and Reactivation

A common feature of CMV is to establish lifelong viral latency from which the viral genome is able to reactivate under favourable conditions. However, the extent to which latency is a mechanism for viral persistence is not known. Reactivation of CMV in adults is usually asymptomatic, making the virus difficult to identify and study. There is little evidence regarding the frequency of reactivation and the consequences of this on the ageing immune system. While the viral genome (genomic viral DNA) of CMV is detected within various cells types, the principal site of latency appears to reside in cells of the myeloid lineage [88]. The nature of the cellular environment that CMV encounters plays a critical role in determining the characteristics of infection and viral reactivation from latency [91]. Three possible pathways to enable establishment of CMV viral latency have been proposed. Following entry into an individual: (i) after viral attachment to a host cell, the virus enters latency, lacking *de novo* viral gene expression; (ii) productive infection is interrupted prematurely; and (iii) genes that are unrelated to replication but are necessary for successful latency are activated [63,91-94].

Evidence suggests that viral latency is a highly dynamic state, the mechanisms for which are remarkably varied. However, it is increasingly apparent that a significant event during latency is the suppression by cellular transcription factors of the major early promoter. This occurs in addition to post-transcriptional modifications of histone that promote and maintain a transcriptionally inactive state [95,96]. Recent studies have concluded that during latent infection expression of numerous viral gene products and production of cytokines, in particular TNF-α, may orchestrate substantial changes in latently infected cells and their surrounding environment, thereby facilitating the promotion of viral reactivation [84,91,97].

CMV-Specific $CD8^+$ T Cell Response

During infection, multiple components of the host immune system interact with CMV including NK cells, antibodies, $CD4^+$ T cells and $CD8^+$ T cells [98]. The most notable changes to the aged adaptive immune response to CMV take place in the $CD8^+$ T cell compartment. Effector functions of CMV-specific $CD8^+$ T cells include cytotoxicity and cytokine production, each of which has been shown to be important to control of infection. Cytotoxicity follows activation and causes apoptosis of the target cell. This occurs via both granule-dependent and-independent pathways. The former mechanism utilises lytic granules located within the cell cytoplasm and upon $CD8^+$ TCR activation, lysosomal-associated membrane proteins (LAMP) including CD107a and CD107b are exposed. Cytokines are secreted after $CD8^+$ T cell activation, which include IL-2, IFN-γ and TNF-α. Quantification of CMV-specific $CD8^+$ T cell function has clinical relevance and is therefore integral to monitoring CMV activity [46,68,99-102].

Cell Inflation and Exhaustion: The T cell response to CMV involves a large pool of $CD8^+$ T cells that remains in circulation during latency. These apparently resting $CD8^+$ T cells contain cytotoxic granules and potentially produce IFN-γ while awaiting viral reactivation [102]. The majority of CMV-specific $CD8^+$ T cells have a terminally differentiated effector memory (TEMRA) T cell phenotype displaying reduced CD28, CD27, CCR7 and CD62, increased CD57 and re-expression of the surface marker CD45RA [103,104]. An initial study to define changes in the $CD8^+$ T cell compartment showed that $CD4^+$ $CD28^-$ and $CD8^+$ $CD27^-$ T cell levels increased two-to-three fold in CMV-infected older individuals and that the changes were specific to CMV [105]. This finding was confirmed by several reports showing an expansion of the $CD4^+$ $CD28^-$ compartment, but in particular a massive rise in the $CD27^-CD8^+$ T cell population in CMV-positive older individuals with fewer naive T cells [106-109]. Comparable results were at-

ained by studying a cohort of CMV-positive elderly individuals (60+ years of age) who displayed approximately 20% more T cells in comparison to CMV-negative individuals [110]. In addition, the elderly displayed a 41% increase in $CD8^+$ T cells associated with an increased memory pool in comparison to the younger cohort. A heightened TEMRA profile following CMV infection may contribute to the so-called 'inflamm-aging' phenomenon, upregulation of the inflammatory response that occurs with advancing age [111,112]. Although the naive T cell pool diminishes naturally with age, it is particularly low in CMV-positive individuals [110]. The resulting net increase of $CD8^+$ T cells reduces the CD4:CD8 ratio, a hallmark of immunosenescence. CMV has additionally proven to drive oligoclonal expansion in the aged individual. Studies of the TCR of CMV-specific $CD8^+$ T cells indicated that clonal populations increase with age. Expansion results in approximately 25% of the T cell compartment and increases in the CMV-positive elderly [111,112]. Along with the size of response, the TEMRA T cell phenotype makes the CMV-specific T cell population unique [103].

The ageing process and a reduction in T cell diversity are associated and CMV infection drives the T cell population further towards a restricted repertoire. Although it appears that senescence is displayed at the level of the individual cell, the total number of cells is massive and therefore overall reactivity is enhanced and not reduced [113]. This indicates that the traits of immunosenescence are significant contributors to CMV infection and supports the concept that CMV is a causal factor in the age-associated accumulation of late-differentiated $CD8^+$ T cells, which places the elderly into an at-risk group [113]. It has been suggested that these cells are dysfunctional since a smaller proportion of T cells from aged donors respond to antigenic stimulation *in vitro* than that from young donors. However, as mentioned, the response of the cell population in the elderly is enhanced because of T cell inflation. This raises the question as to whether the 'senescent' cells that remain in circulation are deleterious or necessary to control infection, and, if these changes are not triggered specifically by CMV, are they associated exclusively with the ageing process? It has been postulated that as a consequence of the combined effects of a massive T cell expansion, loss of circulating naive T cells and thymus involution as seen during CMV infection in the elderly, the $CD8^+$ T cell pool becomes exhausted, causing a functional loss of memory cells [102,104]. During persistent viral infections, there are substantial alterations to $CD8^+$ T cell phenotype and functionality due to the possibility of a failure of virus-specific T cells to differentiate into fully functional memory T cells. This is indicative of a progressive loss of $CD8^+$ T cell function (termed 'exhaustion') [114]. In turn, this implies that the exhaustion of both naive T cells and age-dependent, replicative memory $CD8^+$ T cells is accelerated by CMV infection, especially in the absence of an adequate renewal of naive T cells from the thymus as a consequence of involution [115]. The process generates the characteristic phenotype of CMV-specific T cells, in particular the loss of the CD28 surface marker. CD28 interacts with CD80 and/or CD86 (which are presented on activated APC), providing stimulus for T cell activation and differentiation. CD28 receptor signalling overcomes the pathway for auto-inhibition of sustained T cell activation and production of IL-2. In addition, a decrease in telomerase activity due to the loss of CD28-mediated Akt signalling (involved in cell proliferation and transcription) contributes further to the exhaustion of T cells [116].

Shortened Telomere Length: Telomeres form a complex protein-DNA structure at the ends of chromosomes that protects them from degradation. Changes in the structure and function of telomeres are thought to play a role in malignant transformation and cellular senescence [117]. Recent studies have investigated the length of telomeres of CMV-specific $CD8^+$ T cells [118-120]. Infection with CMV results in a large pool of $CD8^+$ T cells that are terminally differentiated. As a consequence, the lymphocyte pool produces a $CD8^+$ T cells subset with shortened telomere lengths that is maintained for three years post-infection [121]. Although a shortening of telomere length correlates with age, it is apparent that CMV infection exacerbates this effect. In one study, individuals who are CMV-positive displayed a loss of 94 base pairs per year in comparison to CMV-negative individuals who showed a loss of 74 base pairs per year; this difference correlated with the degree of $CD8^+$ T cell differentiation [120]. This finding supports the notion that infection with CMV accelerates, or at least assists, the development of immunosenescence.

CMV, Immunosenescence and Immune Risk Profile

It is becoming evident that the interaction of CMV with the aged immune system drives production of dysfunctional T cells, leading to the development of immunosenescence. In the elderly, the response of $CD8^+$ T cells can be pronounced and is possibly responsible for the characteristic T cell profile of the aged immune system. However, the relationship between changes in the T cell compartment and the definition of functional immunosenescence remains unclear. The link was first investigated by Swedish longitudinal and subsequent OCTO/NONA-immune studies, from which the mortality predictive biomarker of immune health termed the 'immune risk profile' (IRP) was established [122,123]. This study ascertained that IRP was associated strongly with CMV seropositivity in addition to inflated $CD8^+$ T cell populations. These were noted to cause an inversion of the CD4:CD8 T cell ratio, constituting a profile aligned to increased mortality at 2, 4 and 6 years' follow-up of people 85 years old at baseline [90]. This immune profile has since been shown to exist in hexagenerians, but significantly more so in men than in women [124]. The role of immunity in survival and the impact of lifetime exposure to pathogens raise the issue of whether immunological age is a better biomarker than chronological age. Hence, the influence of CMV in driving immunosenescence is a focus of continuing research.

Future Research Directions

The ageing process has a profound effect on human physiology and is associated with the decline of some of the biological functions of the body [73,125]. Thus, it is important to distinguish between the consequences of a healthy ageing process and ageing due to interactions with pathogens. While it is recognised that dysregulation of the immune system that results in increased susceptibility

to disease is linked with ageing, the causes have not been determined. As discussed, CMV appears to be a driver behind the observed phenomenon of $CD8^+$ T cell differentiation with ageing. The intensity of the immune response by the immune system to CMV, which appears to increase with age, represents an enormous commitment of resources to controlling a single virus, suggesting that CMV modulates the immune ageing process and therefore could have an impact on survival. The questions arise as to whether CMV is a causative factor for immunosenescence and to what extent infection influences longevity. In order to assess the immune response to CMV, examining the differences in T cell responses, polyfunctionality and phenotypical characterisation of antigen-specific $CD8^+$ T cells will aid dissecting the effects of impaired immune capacity in the elderly.

Conclusion

Why and how $CD8^+$ T cells memory inflation occurs in some people but not others, and if this inflation is associated with defects in T cell programming that renders them less efficient in controlling CMV, remains poorly defined. To date, there is no effective CMV vaccine available and although antiviral therapy is prescribed, drug delivery is difficult and the drugs themselves have a dose-dependent toxicity. In addition, drug-resistant CMV can occur [85,126]. Future investigations should focus on the functional and proliferative properties of human CMV-specific T cells that may provide detailed information regarding the complexities of interaction between the aged immune system and CMV infection.

Understanding the pathogenic alterations of immune functions may help to explain different characteristics of the ageing process, including development of age-associated disorders. This indicates that the immune response to a single pathogen may compromise $CD8^+$ T cell diversity and the capacity of the host cell to counter challenge by further pathogens. It may be hypothesized that memory T cell inflation of CMV-infected individuals and ageing together influence the functional and proliferative properties of human CMV-specific T cells, thereby rendering them less efficient at controlling virus reactivation.

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The authors declare no competing interests.

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