Cytomegalovirus in the Elderly: Impact of Cytomegalovirus Infection on Senescence of the Immune System

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Abstract

Many alterations have been described of both innate and acquired immunity in the elderly. Age-associated dysregulation of the immune function has been demonstrated in diverse species including humans. Immunosenescence contributes to morbidity and mortality due to the greater incidence or reactivation of infectious diseases and, possibly, autoimmunity and cancer. The most relevant modifications of immunosenescence affect the T-cell compartment, with decreasing naive cells and increasing numbers of cells with an effector/memory phenotype. In elderly individuals, T-cell responsiveness to antigen and cytokine production is altered when compared to young individuals. These alterations in the T-cell compartment of the elderly have been implicated in the impaired immune response to viral infections and low response to vaccines. One of the factors that affect T-cell function and differentiation is cytomegalovirus infection. Large expansions of cytomegalovirus pp65-specific T-cells that can lead to impaired responses to other immune challenges have been demonstrated in elderly individuals. Thus, persistent cytomegalovirus infections contribute to the age-related changes observed in the CD8 T-cell compartment, although chronic stimulation by other persistent antigens can also play a role in T-cell immunosenescence. A better understanding of immunosenescence and the mechanisms responsible for the detrimental changes is needed to maintain a healthy state in later life and for the design therapeutic interventions. (Trends in Transplant. 2010;4:86-92)

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Key words

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Introduction

Age-associated dysregulation of immune response and function has been demonstrated in elderly persons and in aged animals. It has been suggested that these detrimental changes contribute to morbidity and mortality due to infections and the low response to vaccines found in the elderly, as well as possibly autoimmune phenomena and cancer[1-4].

Immunosenescence is a complex series of alterations that affect most aspects of immunity, resulting in an increased susceptibility to infectious diseases and pathological conditions relating to inflammation (e.g. cardiovascular disease, Alzheimer's disease) or auto-reactivity (e.g. rheumatoid arthritis) in elderly people. The alterations in the immune response are thought to be due to physiological deterioration associated with chronologic ageing, although there are other individual factors that also contribute to immunosenescence, most notably the multi-factorial complexity of the immune system. Whereas the number and functional capacity of some cell subsets of the immune system are decreased by aging, other subsets are increased or show an increased or aberrant response[1,5,6].

Innate and adaptive components of the immune system undergo significant age-related changes, although most studies in humans and mice have focused on analysis of the severe deterioration of adaptive immunity with age. Thus, the T-cell immune response is the most dramatically affected by ageing, although age-associated alterations in the phenotype and function of other cells of the immune system have been demonstrated. It is well established that the percentage and the number of naive T-cells is lower in the elderly than the young. Age-associated thymic involution contributes materially to this phenomenon. Reciprocally, the percentage and numbers of memory and effector-memory cells are higher in the elderly, likely as a result of exposure to pathogens throughout life. In particular, ageing affects mainly CD8 T-cells that are characterized by the decrease of naive T-cells and the accumulation of memory cells with a phenotype characterized by the low expression of co-stimulatory molecules, such as CD27 and CD28, and the increased expression of molecules such as perforin and granzymes and natural killer (NK)-associated receptors involved in regulation of the cytotoxic function[7-10].

Moreover, the requirement to maintain effective immune surveillance against persistent viruses is believed to be disproportionally involved in the accumulation of effector-memory CD8$^+$ T-cells[4].

It has been suggested in many works that maintenance of appropriate immunity is essential for exceptional longevity and also for standard longevity[11]. While components of innate and acquired immunity change with age, the clinical impact of these changes is not clear. Moreover, the immunosenescence markers and mechanisms are controversial. In humans, cross-sectional study design raises many difficulties, i.e. when examining immune status in the current elderly we are observing the results when circumstances were different from those applicable at the present time. These differences, which cannot be controlled, include genetics, environment, nutrition, developmental variables, and pathogen load[12].

Cumulative evidences in the last years strongly support that cytomegalovirus (CMV) infection modulates the peripheral lymphoid pool in healthy donors and affects T-cell function and differentiation[13-15]. As the infection by CMV in otherwise healthy individuals increases with age, many of the alterations found in the T-cell compartment in elderly humans are associated to CMV infection rather than to chronological ageing.

Impact of cytomegalovirus infection on immunosenescence

Human CMV infection in immunocompetent individuals is normally asymptomatic, but can be a major cause of morbidity in immunosuppressed individuals. Once established, the infection persists, and its containment becomes a priority for the immune system, which is unable to eliminate it completely. Therefore, after primary infection, the virus persists throughout life in a latent form in a variety of tissues[16]. In immunosuppressed and, occasionally, immunocompetent persons, CMV can be reactivated, and in these situations the presence of CMV-specific CD8 T-cells, which are not producing interferon gamma (IFNγ) and are therefore potentially anergic or *in vivo* exhausted, is frequent[17].

Ageing is associated with changes in the immune system with substantial alteration in T lymphocyte subsets. Cytomegalovirus infection modulates the peripheral lymphoid pool in healthy donors[13,14]. It also affects the functionality of T-cells, and the differentiation and large expansion of CMV-specific T-cells have been associated with impaired responses to other immune challenges[15]. Moreover, clonal expansions of CMV-specific T-cells may reduce the available repertoire for other antigens and contribute to the increased incidence of infectious diseases in the elderly[18-21].

Longitudinal studies in healthy elderly individuals above the age of 80 or 90 have defined an immune phenotype that is predictive of a significant decrease of two-, four- and six-year survival[4,22]. The "immune-risk profile" is based on the results of the OCTO/NONA studies of people over 85 years of age[4,12,23,24]. It was originally described for a cluster of simple immune parameters, including poor T-cell proliferative responses to mitogens, low numbers of B-cells, an inversion of the CD4/CD8 ratio caused by an accumulation of CD8 cells, and low lymphoproliferative response to mitogens[22,25,26]. Subsequent studies also indicated that the immune-risk profile was associated with other parameters such as the increased proportion of highly differentiated CD8+CD28− T-cells, a very restricted T-cell repertoire, elevated levels of proinflammatory cytokines, and CMV seropositivity (but not Epstein-Barr or herpes simplex virus)[22,25,26]. The accumulated CD8 cells, representing an essential hallmark of the immune-risk profile, were commonly specific for CMV antigens. Therefore, immunosurveillance against CMV is very important in elderly people. Recent studies show that individuals infected with CMV also have higher levels of C-reactive protein, indicating that they are more likely to suffer "inflamm-aging", itself correlated with increased occurrence of diabetes and other inflammatory diseases, as well as general frailty and increased mortality.

The infection with other persistent herpesviruses, such as Epstein-Barr, herpes simplex, or vesicular stomatitis virus, does not appear to have a similar effect[4,12,24,27]. The exclusivity of CMV in this context remains unknown. An explanation may reside in the cell types acting as CMV reservoirs and their intimate interactions with immune cells (i.e. antigen-presenting cells, such as dendritic cells, as well as endothelial cells)[28-30]. Therefore, immune signatures are indeed informative for "immunosenescence", which predicts mortality, but they are considerably influenced by CMV infection[14]. Taking this fact into account, immunogerontological studies should consider the CMV status of elderly individuals.

Cytomegalovirus-specific CD8 T-cells are expanded in the elderly

It has been proposed that the abnormal T-cell subset distribution in the elderly is due to chronic antigen stimulation by latent viruses like CMV[4,31,32]. The importance of CMV in this process is highlighted by the demonstration
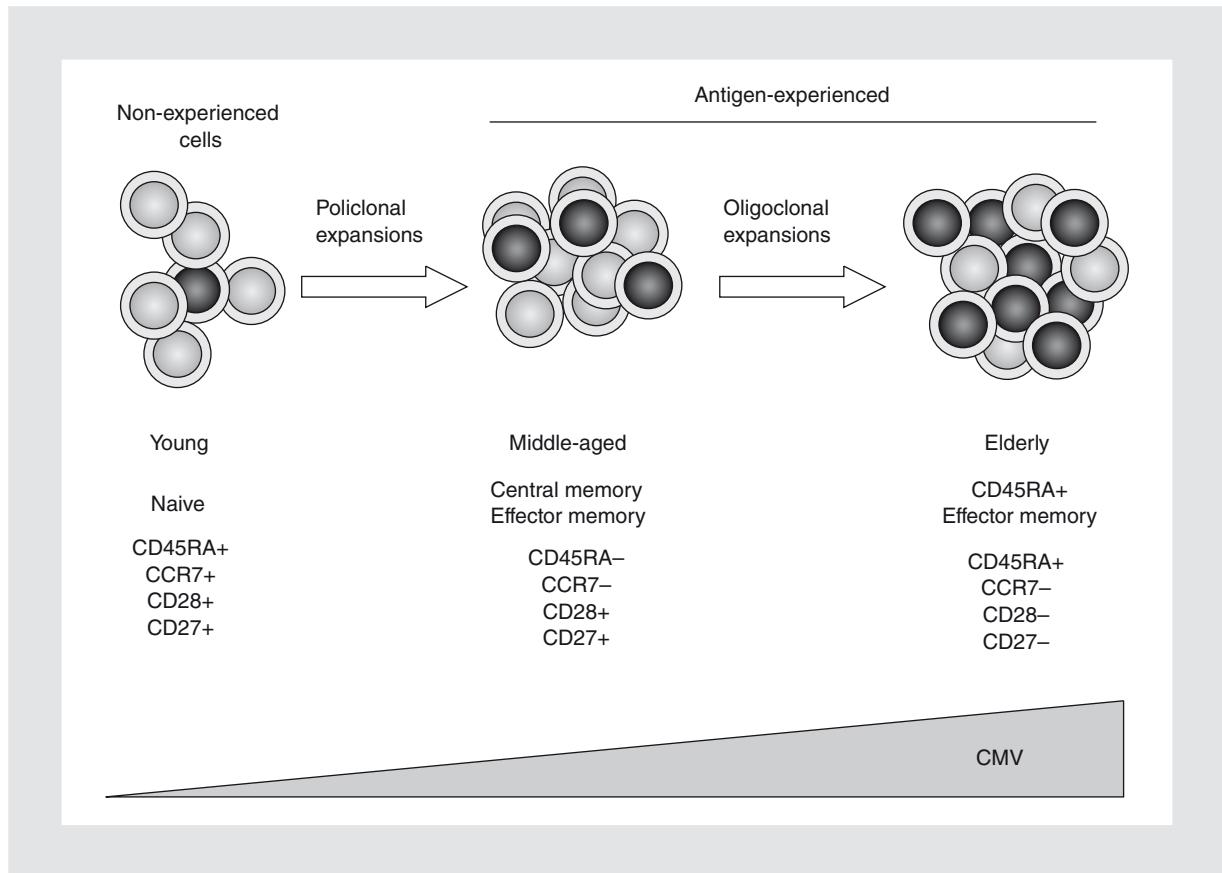
Figure 1. *Oligoclonal accumulation of senescent T-cells, and repertoire shrinkage in cytomegalovirus (CMV)-positive aged individuals. In young and middle-aged individuals, CMV infection triggers repeated cycles of expansion and apoptosis. In the elderly, chronic stimulation of CD8 T-cells by CMV leads to large dysfunctional oligoclonal populations of CMV-specific T-cells* (adapted from Gayoso, et al. 2007[2]).

of oligoclonal expansion of CMV-specific CD8 T-cells (Fig. 1). In CMV-seronegative elderly individuals, the response against other viruses, such as Epstein-Barr, can induce clonal expansions similar to those found in CMV infection[22].

Host defense against infection by CMV is ensured to a great extent by cytotoxic CD8 T lymphocytes directed against the tegument protein pp65[33]. In aged people, the CMV phosphoprotein pp65 (UL83) is the major antigen recognized by T lymphocytes targeting functionally efficient T-cell effector responses, with massive production of T-helper 1 cytokines and exhibition of CD107a degranulation marker[34]. Phenotypic analysis of CMV-specific CD8 cells has demonstrated that the proportion of cells expressing CD27 and/or CD28

is strongly decreased in the elderly when compared with younger individuals. Phenotypic analysis of the CMV-specific CD8 cell differentiation stages, defined by cells' combined use of CCR7 and CD45RA, has demonstrated that, in elderly individuals, these CMV-specific CD8 cells are mainly effector-memory cells that do not express CCR7. The expression of CD27, CD28, and CD45RA in these effector-memory cells allows the identification of subsets that are not still well defined in their detailed function and differentiation stage. The CD45RA+ effector-memory cells, which express high levels of perforin and granzyme, express NK-associated receptors, and are considered primarily cyanotypes, are increased in the elderly (Fig 1), whereas in young individuals, a significant proportion of CMV-specific CD8 cells are included in the naive subpopulation

(CCR7^{+}CD45RA^{+})[21,35]. In addition, CMV-specific CD8 cells have an increased NK-associated receptor expression[12,21]. Furthermore, all cells in the elderly are killer cell lectin-like receptor subfamily G member 1 (KLRG1)-positive, while in the young, the number of KLRG1-positive cells is significantly lower. The expression of KLRG1 on CD28[null] cells can be considered a marker of end-stage differentiation and apoptosis resistance, whereas, on the contrary, CD28^{+} cells are still capable of proliferation despite the expression of KLRG1[36]. Ageing also modifies the functional capacity of CMV-specific T-cells; these cells do not respond to antigenic stimulation[37-39]. This phenotype associated with ageing appears to be restricted to CMV-specific T-cells[2,21].

In summary, latent infection with CMV could be considered a major factor contributing to the differentiation of CD8 T-cells to poorly functional senescent cells with an effector-memory phenotype.

Cytomegalovirus immunosenescence and solid-organ transplantation

Because of immunosuppression, there is a higher frequency of CMV reactivation in CMV-seropositive transplant recipients and of primary infection in CMV-seronegative recipients receiving a CMV-seropositive organ (D[+]/R[−])[40-42]. Replication of CMV is evident in approximately 50% of solid-organ transplant recipients and 10-50% will develop symptomatic CMV disease. The risk of developing CMV is usually estimated on clinical-risk markers such as the type of organ transplantation, donor and recipient CMV serostatus, type of immunosuppression, and coinfection with other herpesvirus, without considering other individual risk markers that might allow a personalized treatment strategy. Among these individual variables, CMV-specific T-cell response

is critical in the control of CMV. This CMV T-cell response is mainly dependent on previous contact with CMV, the patient's age (immunosenescence), or the immunosuppression used[43]. After transplantation, CMV replication induces the accumulation of CMV-specific CD8^{+} T-cells, which may account for as much as 10-30% of the total CD8^{+} T-cell pool[44,45]. As observed in CMV-seropositive elderly donors, these cell clones have a highly differentiated phenotype[2,46,47] and show similar functional changes[44,48,49]. In a cross-sectional study in patients who had received a solid organ transplant at least one year previously, we have studied the frequency and phenotype of human CMV-specific CD8^{+} T-cell population and its relationship to age and CMV replication posttransplantation. The results show that, in transplanted patients, CMV replication is associated to an increased frequency of CD27^{−}/CD28^{−} CMV-specific CD8^{+} T-cells in patients below 50 years, so the proportion of these subpopulations in patients below 50 years is similar to that observed in over-50 patients without replication, supporting that CMV replication may cause phenotypic changes in younger transplanted patients similar to those changes induced by age[50]. Although it cannot be ruled out that our results in patients below 50 years of age were the consequence of stimulation of the immune system by CMV in immunosuppressed patients, other hypotheses can also be proposed. The first is that the increase in expression of CD27^{−}/CD28^{−} CMV-specific CD8^{+} T-cells in younger transplanted patients corresponds to a form of accelerated immunosenescence at early ages, associated, among other possible factors, to CMV replication[46,48,51]. Another alternative hypothesis is that phenotypic changes are generated before or during transplantation (dialysis, immunosuppressants, etc.), which may facilitate viral replication. Clarifying these hypotheses requires conducting a prospective cohort study in patients with and without CMV replication, aimed at studying phenotypic and functional changes before and at

different points after transplantation. In a similar way, the expression of CD45RA is increased in CD27− and CD28− CMV-specific T-cells.

Conclusions and future prospects

In conclusion, immunosenescence comprises an intricate succession of alterations that depend on chronological ageing and on exogenous factors, like constant antigenic stress, which leads to chronic activation of the immune system. Ageing is associated with immunological changes in the T-cells, mainly owing to thymus involution ensuing in a shrinking production of naive cells. Furthermore, new evidences underline the suggestion that many alterations observed in the CD8 T-cell compartment can be explained by the chronic activation of the immune system by latent viruses such as CMV. In humans, CMV clearly has an enormous impact on key parameters of immunity and is associated with an immune-risk profile predicting mortality in elderly individuals. The exact mechanism, and whether it is applies generally to all human populations, is unclear. Many authors pointed out that the persistence of CMV as a chronic antigenic stressor is a major contributor to immunosenescence and associated mortality. Even if CMV does not cause immunosenescence, it seems to have a considerable impact on immune parameters in later life, and it might also contribute to increased morbidity and eventual mortality. On the other hand, it has been proposed that being CMV-positive could possibly have been an advantage in early life. The enhanced proinflammatory status on infected people might have had a protective effect against infection with other pathogens under conditions in the wild. This believed benefit produced by CMV infection in early life represents an example of "antagonistic pleiotropy", which seems to constitute one of the few general laws of ageing[52].

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