**Abstract**

“Immunosenescence” is a loosely descriptive designation for age-associated alterations to most measures of immunity, which can be seen in all mammals examined in any detail. Both innate and adaptive immunity are affected in a manner assumed to be deleterious, but often the clinical consequences of the assessed changes are unclear or not even investigated. The mechanisms accounting for these changes, and biomarkers of immunosenescence, are currently the subject of intensive research. Cross-sectional studies have established hallmark age-associated differences between adaptive immune factors in young and old people, particularly a lower number and percentage of naïve T cells, especially CD8 + T cells, and accumulations of late-differentiated CD8 + T cells. The latter but not the former is strongly affected by infection with the persistent ß-herpesvirus HHV5 (cytomegalovirus, CMV). Only limited longitudinal studies have so far investigated whether these differences actually reflect age-associated changes at the individual level. The Swedish OCTO/NONA-Immune studies identified a set of immune parameters including infection with CMV which predicted survival in people over 85 at baseline. Moreover, the Leiden 85+ study showed that T cell-mediated pro-inflammatory specific for CMV antigen was enriched in very old survivors, suggesting the overarching necessity of maintaining effective immunosurveillance of this virus. Here, the disparate impact of CMV on “immunosenescence” and survival in human populations under different condition is reviewed.

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**1. Introduction**

Although non-communicable diseases, particularly cardiovascular, diabetes, Alzheimer’s and cancer are now the main public health concern in industrialized countries, they all share if not an inflammatory etiology at least a strong and possibly direct involvement of immune and inflammatory processes. However, although clearly age-associated, death rates from these diseases tend to plateau at very advanced age, presumably due to a selection process. This is not the case with infectious disease, the incidence of which continues to accelerate in late life presumably due to a selection process. This is not the case with infectious disease, the incidence of which continues to accelerate in late life, especially due to compromised immune against infectious disease, suggesting that the continuing acceleration (for example, see Horiuchi and Wilmoth, 1997). Immunity protects against infectious disease, but often the clinical consequences of the assessed changes are unclear or not even investigated. The mechanisms accounting for these changes, and biomarkers of immunosenescence, are currently the subject of intensive research. Cross-sectional studies have established hallmark age-associated differences between adaptive immune factors in young and old people, particularly a lower number and percentage of naïve T cells, especially CD8+ T cells, and accumulations of late-differentiated CD8+ T cells. The latter but not the former is strongly affected by infection with the persistent ß-herpesvirus HHV5 (cytomegalovirus, CMV). Only limited longitudinal studies have so far investigated whether these differences actually reflect age-associated changes at the individual level. The Swedish OCTO/NONA-Immune studies identified a set of immune parameters including infection with CMV which predicted survival in people over 85 at baseline. Moreover, the Leiden 85+ study showed that T cell-mediated pro-inflammatory specific for CMV antigen was enriched in very old survivors, suggesting the overarching necessity of maintaining effective immunosurveillance of this virus. Here, the disparate impact of CMV on “immunosenescence” and survival in human populations under different condition is reviewed.

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**2. Defining immune parameters relevant to health: the OCTO/NONA immune studies**

One of the first attempts at the type of study referred to above has provided seminal information on immune parameters associated with mortality (Wikby et al., 1994). The OCTO and then NONA longitudinal studies were primarily biobehavioural studies from the Institute of Gerontology in Jönköping, Sweden, onto which some quite simple measurements of immune parameters were bolted. OCTO included only on...
free-living people over 85 who were in extremely good health (Ferguson et al., 1995; Wikby et al., 1998). Later waves of recruitment focused on a representative sample including only about 10% in such good health, but it was found that this did not influence the outcome as far as the simple immune parameters and survival were concerned (Nilsson et al., 2003). These studies identified a set of factors consisting of high absolute numbers and percentages of CD8+ CD28-T cells (causing a decrease rather than the more common age-associated increase) in the CD4:8 ratio and resulting in poor T cell proliferative responses to mitogens, consistent with the fact that late-differentiated T cells undergo little further clonal expansion. Together with low numbers of B cells, these factors constituted a risk profile associated with greater all-cause mortality at 2-, 4- and 6-year follow-up. This was designated the “Immune Risk Profile” (IRP) in a discussion paper considering biomarkers of immune health and aging (Pawelec et al., 2001). In addition to the B and T cell parameters, infection with CMV as assessed by seropositivity also clustered with the IRP despite the high penetrance of this virus (ca. 85%, as would be expected from the literature). This was because 100% of the individuals in the IRP at baseline were CMV-seropositive (Olsson et al., 2000), accounting for about 15–20% of subjects surveyed. Mortality of individuals with an IRP after 4 years reached ca. 70% in these studies, in contrast with around 40% in the majority. Thus, the IRP as defined in this manner was an important predictive factor, but still a very blunt tool requiring improvement. Although CMV-seropositivity clustered with the T and B cell factors to define the IRP, it was not a predictive factor by itself in these studies (Pawelec et al., 2009). As CMV is essentially ubiquitous, it may be necessary to determine the individual’s immune response to the virus at both the cellular and humoral levels in order to establish a more nuanced predictive contribution of CMV to immune health, morbidity and mortality. Some of these concepts are further discussed below.

3. Impact of CMV on immune status and mortality

Consensus hallmark signs of human immunosenescence are accumulations of CD8+ memory T cells and a paucity of CD8+ naïve T cells. Data not only from cross-sectional studies, but also a few longitudinal studies, have mostly confirmed that the major age-associated differences are seen in the CD8 rather than the CD4 subset. Several studies have now shown that lower percentages and absolute numbers of naïve CD8+ T cells are seen in all elderly subjects whereas the accumulation of very large amounts of CD8+ late-stage differentiated memory cells is seen only in a majority but not in all elderly. The major difference between the majority of subjects with such accumulations of memory cells and those without is that the former are infected with CMV (Almanzar et al., 2005; Chidrawar et al., 2009; Derhovanessian et al., 2010) (but not other persistent herpesviruses, see Derhovanessian et al., 2011). Why only CMV has this effect remains a puzzle. In the Swedish OCTO/NONA studies, the defining factor for whether an individual was assigned to the IRP group was the absolute number and percentage of late-stage differentiated CD8+ T cells lacking both CD27 and CD28 costimulatory receptors. Naïve cells were not part of the IRP. Many or most of these late-stage memory cells are known to be or are likely to be specific for CMV antigens (Hadrup et al., 2006; Khan et al., 2002; Ouyang et al., 2003a,b,c). Moreover, they are likely to accumulate due to apoptosis resistance, possibly related to the low avidity of their T cell receptors (Griffiths et al., 2013). Other factors including any CD4 phenotype or other CD8 phenotypes did not contribute to the IRP, but this may have been due to the unsophisticated methodology employed at the time. The application of modern polychromatic flow cytometry and CyTOF analysis longitudinally on appropriate cohorts should help to improve the definition of the IRP.

Hallmark features of immunosenescence as represented by the IRP are now being found in other populations (Huppert et al., 2003; Peres et al., 2003); there are also indications that its presence in younger people may be informative (Turner et al., 2013). It may be absent in the extreme elderly, perhaps as a result of selection for survival at advanced age (Derhovanessian et al., 2013a; Strindhall et al., 2007). Without studying associations with mortality, the real implications of these immune signatures of course remain unclear. The original IRP cluster was also based on a small number of relatively unsophisticated analyses, which need to be extended to include more parameters that might enable closer associations between the risk profile and mortality. The accumulations of late-differentiated CD8+ T cells which are a hallmark of immunosenescence and the IRP were also being seen in many circumstances; this phenomenon is sometimes referred to as “memory inflation” as defined by the presence of CD28-negative, CD57+, CD85j+ cells, or with other markers of late-stage differentiation (Kern et al., 1999; Khan et al., 2002; Merino et al., 1998; Northfield et al., 2005; Ouyang et al., 2003c; Trzonkowski et al., 2004). The nature of these cells has been intensively investigated. Very early studies initiated as soon as MHC/antigen multimers became available (“tetramers”) suggested that, at least in people who were HLA-A2+, a high fraction of these late-differentiated CD8+ T cells seemed to be specific for CMV antigens (Ouyang et al., 2003c). This fraction could be so large that T cells with other specificities would be “crowded out” This was not the case for T cells reacting even to EBV, where accumulations were much smaller (Ouyang et al., 2003b) or HSV, which had no such effect at all (Derhovanessian et al., 2011). Similar results have been reported by others in cross-sectional studies, strengthening the notion that the age-associated accumulation of late-differentiated CD8+ T cells, primarily responsible for assignment into the IRP group, were actually driven by CMV infection (Almanzar et al., 2005; Khan et al., 2002; Vescovini et al., 2004). Our own studies first concluded that these late-stage differentiated IRP-dominant CD8+ T cells were dysfunctional, because we found that a smaller proportion of this population in the elderly than in the young produced interferon-γ (IFN-γ) when stimulated with CMV peptides in vitro (Ouyang et al., 2003a). Despite this, the overall production of IFN-γ was greater in the elderly than the young, because of their larger absolute numbers in the blood, thereby possibly contributing to an increased level of pro-inflammatory factors commonly seen in older people (Ouyang et al., 2003a). This is consistent with other reports of increased IFN-γ production in the elderly, when examining whole PBMC responses to CMV stimulation (Almanzar et al., 2004) and is consistent with more recent data which also encompassed production of TNFα, IL2, Granzyme B, Perforin, and mobilization of CD107a, by virus-specific late-stage differentiated CD8+ T cells (Lelic et al., 2012). To what extent some of these late-differentiated CD8+ T cells are truly “senescent” depends on how senescence is defined in the T cell context. If one accepts that fewer IFN-γ-producing cells define dysfunctionality, together with lack of CD28 expression, upregulation of CD57 and KLRG-1, having short telomeres, acquiring apoptosis resistance and proliferating poorly defines senescence, then these cells are indeed senescent (Effros et al., 2005; Vallejo, 2005). Alternatively, because these cells mediate many functions they could simply be viewed as a distinct differentiation state, being late-stage effector cells. Why CMV-specific CD8+ T cells in the blood should re-express CD45RA+ whereas most other viral effectors do not, remains unclear (Appay et al., 2002), although the “usual” central memory phenotype of CMV-specific cells may be found in the bone marrow (Lettsch et al., 2007). Illustrating their functionality as effectors, these CMV-specific CD8+ T cells contain granzyme and perforin, as well as expressing those negative costimulatory surface receptors which turn off a proliferative response. This could act to prevent unnecessary further clonal expansion thereby preventing apoptosis and replicative senescence, and thus safeguarding the ongoing chronic response (Ouyang et al., 2003a; Tarazona et al., 2000). The findings that turnover of cells with this phenotype, alone among all those studied in young and old volunteers, is slower in the elderly are consistent with this notion (Wallace et al., 2004). We know that despite mostly symptom-free cohabitation with its host, CMV is a powerful pathogen in the immunosuppressed, and suspect that it may not be
quite so innocuous as generally believed in younger people either. There must be a high cost to immune resources for maintaining CMV surveillance throughout life. Given that increasing proportions of the CMV-specific CD8+ T cell population may become dysfunctional with age, we could view the accumulations of late differentiation stages in the elderly as a compensatory increase required to maintain immunosurveillance against CMV, no matter what the cost. If such immunosurveillance fails with age, or due to immunosuppression, CMV disease might be triggered. In the very elderly, this may remain undiagnosed if followed by bacterial pneumonias as a cause of death. There is some but not much data in support of the idea that immunosurveillance may indeed be failing in the very elderly towards the end of life (Hadrup et al., 2006). These data are in sore need of reproduction and validation.

The proportion of the population infected with CMV increases with age, dependent on socioeconomic (Dowd et al., 2009), genetic (Derhovanessian et al., 2010) and other conditions. Hallmark age-associated differences in immune parameters may thus more reflect the age-associated increased likelihood of becoming infected with CMV (Looney et al., 1999) on a background synthesizing all the other factors influencing the impact of CMV on immunity and physiology in general. How far the lower levels of naïve T cells commonly seen in the elderly is dependent on CMV infection is unclear, but CMV-negative donors do still seem to have less naïve cells, as one would expect from their exposure to many other pathogens throughout life. As the thymus ceases to produce large numbers of naïve cells at puberty, due to thymic involution that could be exacerbated by changes at the hematopoietic stem cell level (Waterstrat and Van Zant, 2009), maintenance of peripheral T cell homeostasis is thought to take place by peripheral expansion most obviously of the cells that accumulate as late-stage memory cells and fill the “immunological space” (Franceschi et al., 2000). This could then prevent the export even of the greatly reduced numbers of naïve cells from the old thymus (Gruver et al., 2007). Whether this negative feedback is really important is not known, but some data (in mice) suggest that the “immunological space” may be more malleable than previously appreciated (Vezys et al., 2009).

The results discussed above can be interpreted as supporting the hypothesis that CMV “stresses” the immune system in a manner which exacerbates the aging processes that are associated with mortality. Indeed, some epidemiological studies are indicating that CMV infection itself might have a negative impact on survival (Dowd et al., 2009; Simanek et al., 2011). Moreover, data on health issues related to antibody levels in CMV-seropositives are beginning to accumulate, for example, showing an inverse correlation with survival in stable cardiovascular disease patients (Strandberg et al., 2009). This could be related to a local pro-inflammatory effect of CMV-reactive cells, not necessarily reflected in systemic levels of inflammatory mediators. Findings in free-living not overtly sick subjects also suggest a major contribution of CMV to health problems. A 5-year follow-up of women aged 70–79 yr with the highest CMV seroconverters–vs–uninfected women showed that there was significantly more frailty and 3-fold more mortality in the former (Wang et al., 2010). What different antibody titers actually reflect is unclear so far; possibly, a correlation with the frequency of reactivation of the virus, which is not known. CMV may reactivate more readily in older people (Stowe et al., 2007), but this is under-researched. Higher titers could also reflect superinfection with a different viral strain, but it is not known whether this occurs in humans or only in monkeys (Hansen et al., 2010). Certainly, the magnitude of cellular anti-CMV responses increases with age, and it has been reported that some T cell clones can be found in the elderly but not in the young, the TCR of which use CDR3 sequences imbuing the receptor with a higher avidity in the elderly (Schwaminger et al., 2008). These data are compatible with a scenario whereby ever more immune resources are required to keep CMV in check, and with an eventual exhaustion of this defense capacity presaging death in the very elderly (Hadrup et al., 2006). Middle-aged people already dedicate as much as 10% or more of their peripheral CD4+ and CD8+ T cell repertoire to recognizing many different antigens from CMV (Sywester et al., 2005). This increases with age, as does the commitment of humoral resources, as anti-CMV antibody increases with age (Hamprecht, Derhovanessian et al., our unpublished results). Hence, a notable but occult public health and human cost may be associated with CMV infection, hitherto mostly considered “harmless” except in the context of transplantation or other instances of immunosuppression. The notion that variations in hospital admissions impacting materially on health care costs may be caused by unrecognized exacerbations of CMV re- or neo-infection is beginning to be explored (e.g. see Jones, 2013).

At the present time, the most clearly demonstrated and least controversial, but not completely uncontroversial, impact of immunosenescence on public health is seen in vaccination programs for influenza and possibly pneumococcus (Chen et al., 2009; Grubeck-Loebenstein et al., 2009; McElhaney et al., 2012). The criteria usually used to establish vaccine efficacy (for influenza) were standardized in younger people and have not been properly assessed in the elderly (Gravenstein et al., 1994). Some studies suggest that the currently practiced influenza vaccination may be ineffective in protecting the elderly and reducing mortality (Simonsen et al., 2007). Several lines of evidence suggest that CMV-seropositive elderly respond poorly to influenza vaccine relative to uninfected elderly (Trzonkowski et al., 2003). The accumulations of CD8+ CD28–negative cells, part of the IRP and mostly caused by responses to CMV infection, were shown to correlate with depressed influenza vaccine responses (Goronzky et al., 2001; Saurwein-Teissl et al., 2002). “Inflammaging” in the elderly, increasing their chronic pro-inflammatory status, as reflected in increased serum IL-6, is also associated with poorer influenza vaccine responses (Trzonkowski et al., 2009) but whether CMV contributes to this remains unknown. T cell responses to influenza may be better predictors of protection than antibody titers alone; these are clearly different in CMV-seropositive-vs-seronegative elderly donors (Derhovanessian et al., unpublished results). Furthermore, in an assessment of antibody titer after vaccination with a formulation specifically licensed for use in the elderly, subjects infected with CMV were found to respond less well to the vaccine (Derhovanessian et al., 2013b). These reports and additional data from several investigators implicate CMV infection in hindering the response to influenza vaccination in the elderly, and suggest that responses to other vaccines could also be compromised.

4. Should public health policy aim to eradicate CMV?

In light of the above, it would appear that CMV may represent a usually occult threat to public health, but could be causing unrecognized problems, in which case eradication would be a desirable aim. Nonetheless, it remains possible that CMV might actually be in some way beneficial in early life, or at least that might have been the case in the evolutionary past. At a time when infectious disease was the major killer, as was the case globally until recently, and is still the case in many countries, infection with CMV might have some protective effects. There is one reported animal model, consistent with this idea, in which mice infected with murine CMV had increased serum IFN–γ relative to uninfected mice. Under certain pathogen challenge conditions, the uninfected mice died but the infected mice survived due to increased macrophage activity stimulated by the IFN–γ (Barton et al., 2007). There is some evidence, as a kind of variant of the hygiene hypothesis, that specifically CMV (and to some extent EBV) infection in childhood may protect against allergies in later life (White et al., 2012). There may be certain similarities in human populations exposed to a high pathogen load under natural circumstances—the price of the extra protection against pathogens due to high levels of pro-inflammatory factors could be survival to later life but then with increased disease and frailty as a result (Kuningas et al., 2009). If this argument is correct, the benefit to modern populations, in hygienic conditions and protected by vaccines, would presumably outweigh any disadvantages associated
with CMV eradication. Some partially effective vaccines have recently been developed for protecting CMV-negative pregnant women (for example, see Pass et al., 2009) but these could probably be used for other indications as well. Pharmaceutical and biotechnological antiviral agents could also conceivably be developed, and one might consider therapeutic vaccination in the elderly, perhaps in combination with these drugs and/or immunomodulators. Clearly, there would have to be compelling reasons to treat elderly humans in any of these ways unless protocols without side effects could be developed. Such speculations remain entirely without experimental basis so far.

On the other side of the equation, there are still some possible conditions under which even the elderly might even benefit from being infected with CMV. There is some evidence that elderly CMV-seropositive kidney recipients who have more CD8 + CD28-negative T cells with short telomeres suffer less acute rejection (Trzonkowski et al., 2010). This might correlate with their responsiveness to immunodominant I-E antigens (Nickel et al., 2009). Finally, it has also been reported that the “alternative” T cell lineage, the γδ T cells, are stimulated and expanded by CMV (Dechanet et al., 1999)—these cells can exert anti-cancer activity with measurable clinical benefit (Couzi et al., 2010). We have found that such γδ T cells, or at least the Vδ2-negative major subset thereof, also accumulate in the elderly—but, as with the late-stage differentiated CD8 + T cells, this only happens to a marked degree in CMV-positive subjects (Wistuba-Hamprecht et al., 2013). The eradication of CMV from the population, even if it could be achieved, would come with some disadvantages. Better perhaps to aim for a slightly more realistic goal, therapeutic vaccination.

Conflict of interest

The authors have no conflicts of interest.

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