The impact of CMV infection on survival in older humans

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Dysregulated immunity, 'immunosenescence', in the elderly is thought to contribute to their increased susceptibility to infectious disease and to impact on mortality. Accepted hallmarks of human immunosenescence are low numbers and frequencies of naïve T cells and higher numbers and frequencies of memory T cells in the peripheral blood of the elderly compared to the young. The proportion of the population infected with CMV increases with age and markedly influences these parameters. Infection with this persistent β-herpesvirus may therefore indirectly impact on survival in the elderly. Recent evidence pertaining to this controversial proposal is reviewed here.

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Introduction

Infectious disease in elderly people is more frequent and more severe than in the young [1]. The increase in the occurrence of 'classical' age-associated cancers and cardiovascular diseases in the population tends to level off at around 75–80 years of age, but this is not true for infectious disease which seems to continue to rise unabated [2]. It is therefore likely that declining immune protection materially contributes to this state of affairs. Many studies over the last three decades have explored the differences in immune status between young and old people, mostly in cross-sectional studies with all their limitations. The current consensus is that the elderly generally possess more late-stage differentiated memory T cells and fewer naïve T cells than the young in their peripheral blood. This is often assumed to be due to a lifetime's exposure to infectious agents, which results in conversion of the naïve

cells to memory cells. In the absence of significant thymic function in old age and hence minimal generation of new naïve cells, memory cells accumulate and both the proportion and absolute numbers of naïve cells can become vanishingly small. As far as can be discerned from available data, these phenomena are not limited to humans but are seen in all mammals studied, regardless of their longevity [3,4]. Other age-associated differences in immune parameters include decreased numbers and frequencies of B cells and increased myeloid cells and NK cells (for review, see [5]). In this review, we will focus on the T cell compartment and the over-riding impact that infection with a common herpesvirus, HHV5 (CMV), has on what are commonly assumed to be 'age-associated changes' to immune parameters. Whether infection with CMV has any detrimental effects on human longevity as a result of its marked impact on peripheral T cell phenotypes, or whether it has any other deleterious effects for other reasons, remains controversial and will be the central question considered in this review.

Prevalence of CMV infection

In most developed countries, a significant proportion of the population is CMV-negative, but this fraction decreases with age, with infection occurring predominantly at younger ages but also with some transmission occurring in older populations [6]. Intriguingly, the only factor influencing age-associated increasing infection with this virus in the large US NHANES III study was found to be socioeconomic status [7]. However, by the age of around 75–80, the proportion of the population infected seemed to plateau at ca. 85–90%. In a way consistent with this socioeconomic effect, but also possibly associated with greater popularity of breast-feeding, surveys in less-developed countries such as India suggest that young populations are already 85–90% infected [8]. The same is true of Alaskan Eskimos [9]. Thus, in Western countries, studies of age-associated parameters could be materially influenced by the age-associated increased proportion of the population infected with CMV, whereas in developing countries, baseline CMV-positivity is likely to have eliminated this potentially confounding factor, as all will be affected by CMV. However, most published studies are of 'WEIRD' (Western, educated, industrialized, rich, democratic) populations, which may skew our viewpoint.

Impact of CMV on 'age-associated' changes to T cell phenotypes

Although not generally recognized by most human T cell immunologists, the effect that CMV serostatus has on the distribution of T cell phenotypes in the peripheral blood

was first reported many years ago [10]. In some studies, the hallmark age-associated low levels of naïve cells and high levels of memory cells are not seen in cohorts selected for CMV-negativity [11•,12•]. Although not reported in all populations, it is probably true to say that CMV infection at least accelerates the age-associated decrease of naïve cells and increase of memory cells, and that its effects may depend upon state of health (e.g. [13,14]), genetic background [12•] and/or many other factors in highly diverse human populations. Nonetheless, it is clearly the case that the late-stage differentiated memory T cells that accumulate in the elderly, especially the CD8+ cells, are frequently CMV-specific and that their eventual loss may be associated with incipient mortality [15•]. This suggests that although longitudinal studies implicated the accumulation of late-stage differentiated CD8+ T cells as part of an 'immune risk profile' (IRP) prognostic for mortality at 2, 4 and 6 year follow-up [16], such events may rather reflect a compensatory mechanism required for the crucial maintenance of CMV immunosurveillance. A similar phenomenon is also observed in CD4+ T cells, where CMV-associated accumulations of late-stage memory cells and reduced naïve cells have also been reported [17]. Many questions arise, perhaps the most important being whether CMV is causative of these changes and what impact do they have on health and longevity?

Epidemiological studies on CMV infection and health outcomes

The conventional view of CMV infection thus far has mostly been that although this virus can be a powerful pathogen, infection is usually symptom-free in an immunocompetent host and clinical problems arise only in immune-deficient individuals. However, there are some indications that even apparently healthy, younger individuals may indeed suffer symptoms of CMV infection potentially requiring hospitalization [18,19]. CMV-reactivation in non-immunosuppressed, but critically ill individuals, has also been demonstrated in numerous studies and has been associated with all-cause mortality, prolonged hospital and/or ICU stay and increased nosocomial infections in these patients [20]. Nonetheless, the indication for developing the only CMV vaccine so far was for CMV-negative mothers, who, if infected immediately *post-partum*, pass on the virus to the baby, with disastrous clinical consequences [21•]. Interestingly, despite the huge commitment of immune resources to controlling CMV in healthy infected people, this vaccine can nonetheless boost anti-CMV responses further in already-infected women [22••]. In other clinical settings, particularly transplantation and HIV, CMV can be a major life-threatening pathogen. But does it have any deleterious effects on the general population?

Emerging epidemiological data suggest that it may well have unanticipated detrimental effects on frailty and longevity. Again taking data from the NHANES III study, it was concluded that together with higher than median levels of the inflammatory marker CRP, and taking confounding factors into account, all-cause and cardiovascular mortality was significantly greater over 10-year follow-up in CMV+ versus CMV-negative subjects [23]. Earlier data indicated an association between CMV and cognitive decline [24], the latter also being associated with plasma inflammatory marker levels (IL 6) and mortality in a completely different longitudinal study [25]. It is important to point out that these effects and many other observed effects were associated with CMV infection but not with infection by another persistent herpesvirus, HSV. This is also reflected at the level of the changes to T cell immune phenotypes, where CMV but not HSV is associated with this phenomenon [26]. The association of CMV infection with increased markers of inflammation also ties in with many earlier observations on associations between 'inflammaging' and frailty and mortality in the elderly [27]. This state probably represents not only dysregulated innate immunity but also both positive and negative interactions with the adaptive immune system [28]. Complex system interactions resulting in important alterations to health status, such as the ability to respond to vaccination, clearly require further dissection in extended human studies before they can be fully understood and modulated for the benefit of the patient [29].

The earlier link with cardiovascular disease is probably the strongest evidence for a deleterious effect of CMV, and has been reported from many other studies. Not only the CMV serostatus of patients but their anti-CMV IgG titer has also been implicated as a marker predicting mortality, for example in Finnish men with stable cardiovascular disease [30•]. This may be related to frequency and degree of CMV-reactivation but even this is poorly explored in the elderly. One study suggests that reactivation may be more frequent in healthy older people than in the young [31], although the neutralizing antibody response to CMV seems to be unaffected by age, at least in women [32]. There is also the question of the rather unusual property of CMV to reinfect seropositive healthy individuals, presumably by a different strain, which might impact even more heavily on immune resources and trigger yet greater levels of inflammatory mediators [33]. It is still unclear whether IgG antibody levels to CMV are stable or increase with age. Therefore, further studies examining whether IgG increases with age and whether this reflects an increase in reactivation with age or other changes in the immune systems response to CMV are needed.

Several other prospective studies that have emerged over the last two years have also examined correlations with antibody levels rather than CMV infection per se, since the way the host deals with CMV infection may be more

important than the fact of infection (at least evolutionarily speaking, given that the feral situation is probably that everyone is CMV-positive). In the Baltimore WHAS study, CMV infection and especially high antibody titers as a result were strongly associated with frailty and mortality in women 70–79 years of age [34•]. In the SALSA study, 10-year follow-up revealed, again, that highest quartile CMV antibody titers were more strongly associated with all-cause and cardiovascular mortality. It was concluded that this effect of CMV was mostly mediated through the increased levels of IL 6 and TNF [35]. A Spanish study concluded that the functional decline of 100 elderly individuals in a nursing home was associated with the presence of the IRP and that the level of the response to CMV was a contributory factor [36••]. However, similar results have not been obtained in all published studies of different cohorts. Thus, in the BELFRAIL study, it was found that CMV seropositivity was inversely associated with frailty [37]. This may have been a selection effect, as this cohort was older than those previously studied (although younger than the Swedish population in which the IRP was originally defined). Nonetheless, high CMV IgG titers did tend to be associated with functional impairment even in this analysis.

The timing of acquisition of CMV infection has important implications for studying the epidemiology of CMV in aging populations. Specifically, the duration of CMV infection starting from acquisition in young age to old age may need to be assessed and considered in models examining risk of CMV on development of chronic health conditions in adults. Inconsistent epidemiological associations between CMV and chronic disease in adult populations may be explained by measurement error related to an inability to properly account for duration of infection in these populations. In addition, aging may explain social gradients in CMV antibody response and the link between infection and chronic disease development. For example, CMV antibody response may represent earlier acquisition, life-long carriage, and greater likelihood of reactivation of CMV among individuals who are of lower versus higher socioeconomic status. Further studies examining timing of infection, life-long carriage, and reactivation are needed to specify the pathways by which CMV may impact development of chronic disease.

CMV and vaccination
Despite the important public health significance of influenza infection and death in the elderly and the well-known relative inefficacy of seasonal vaccination in this age group, plus the fact that an early study reported a relationship between high anti-CMV antibody titer and poor responses [38], this issue remains controversial and little has been published over the past two years. Indirect evidence continues to accumulate that more immune functionally impaired elderly, who might be considered as having a 'frailty syndrome' of which CMV seropositivity

is an integral part, do indeed respond less well to seasonal influenza vaccination [36••]. In contrast, one recent published study reported similar influenza antibody titers after vaccination of residents of long-term care facilities whether they were seropositive for CMV or not [39]. However, in this latter paper, antibody responses to only one of the three influenza strains were reported, namely H3N2, and blood samples were taken over 10 years previously, at a time when H3N2 had been the dominant strain for 3 decades. This, coupled with the poor state of health of the subjects, may have contributed to the finding of an apparent lack of influence of CMV infection on outcome. This finding may also reflect the limitations of antibody responses as correlates of protection against A/H3N2 strains in community-dwelling older people [40,41]. Given the effect of persistent CMV infection on the accumulation of CD8+CD28− T cells, high levels of CMV antibody may be expected to predict a poor CD8+ T cell response to vaccination and loss of protection against influenza A/H3N2 strains in the over 65 population.

Influenza-specific CTL are the key effectors responsible for clearing virus from infected tissues and it has been recently shown in young adults that CD4+ CTL clear virus upon influenza challenge [42]. Virus-specific killing is mediated by granzymes contained in granules within CTL, which migrate to the 'immune synapse' and are transported into the cytoplasm of the virus-infected target cell to trigger enzymatic cascade that leads to apoptotic cell death. T cell responsiveness including the IFNγ:IL-10 ratio and GrzB activity in peripheral blood mononuclear cells responding to live influenza A/H3N2 challenge appear to be better correlates of protection than the antibody response to these vaccine strains [41]. Thus, the decline in the CD8+ T cell response to live influenza challenge following vaccination [43] in association with the accumulation of CD8+CD28− T cells was postulated to be associated with persistent CMV infection. Indeed T cells with a late-stage or terminally differentiated phenotype were shown to express high levels of GrzB activity, in the absence of perforin, with an ∼3-fold increase in GrzB levels in unstimulated T cells (so-called 'bGrzB') in older adults who are CMV+ compared to those who are CMV− [29]. Our preliminary results indeed suggest that CMV seropositivity and high levels of bGrzB predict a poor T cell response to the A/H3N2 strains of influenza in the population over age 65.

Bosch and colleagues recently presented a significant association between increased CMV antibody levels and poor influenza vaccination response in university students, suggesting that the relationship between CMV antibody levels and influenza vaccine response is apparent even among younger populations (J. Bosch, presented at the 3rd CMV & Immunosenescence Workshop, Cordoba, Spain, 14–16 March, 2012). In addition to mechanistic studies, further research examining whether

the relationship between influenza vaccination response and CMV antibody response is consistent across populations and age groups is needed.

Conclusions

The past two years has seen something of a shift away from the view that CMV is essentially harmless in healthy adults to willingness to at least consider the possibility that indirect consequences of CMV infection may include many age-associated disease syndromes. If we consider that the 'wild-type' situation is that everyone is infected with CMV early in life, we may ask why immunity has not developed ways to eliminate the virus over evolutionary time. One answer might be 'antagonistic pleiotropy' often seen in many organisms, that is, what is good for you when you are young is bad for your when you are older, but post-reproductive evolutionary forces are too weak to select against the latter. There is one animal model demonstrating a survival advantage to CMV-infected mice, by way of increased IFN-γ production and enhanced macrophage activation (in young mice; the animals were not tested as they aged) [44••]. This could be the case in humans too; studies in hunter-gatherer populations in Africa suggest that a pro-inflammatory phenotype confers a survival advantage to younger individuals, but probably at a price in later life [45••]. This implies that efforts to eliminate CMV would be beneficial if vaccination programs were successful in providing sufficient herd immunity to control infectious disease without the necessity for enhancing a generally higher pro-inflammatory status causing collateral damage over the years and decades of the human lifespan. Epidemiological studies are needed to assess whether CMV infection earlier in life is a risk factor for more rapid oligoclonal expansion of T cells specific for CMV resulting in a greater imbalance between mature and naive T cells and ultimately earlier development of chronic diseases.

Conflicts of interest

JEM and GP declare University of British Columbia Provisional Patent Application 11-098 for bGrzb.

Acknowledgements

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