Published in final edited form as:

Curr Opin Immunol. 2017 October ; 48: 23–30. doi:10.1016/j.coi.2017.07.011.

Known unknowns: How might the persistent herpesvirome shape immunity and aging?

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Abstract

The microbial community that colonizes all living organisms is gaining appreciation for its contributions to both physiologic and pathogenic processes. The virome, a subset of the overall microbiome, large and diverse, including viruses that persistently inhabit host cells, endogenous viral elements genomically or epigenomically integrated into cells, and viruses that infect the other (bacterial, protozoan, fungal, archaeal) microbiome phylla. These viruses live in the organism for its life, and therefore are to be considered part of the aging process experienced by the organism. This review considers the impact of the persistent latent virome on immune aging. Specific attention will be devoted to the role of herpesviruses, and within them, the cytomegalovirus, as the key modulators of immune aging.

The microbial community that colonizes all living organisms is gaining appreciation for its contributions to both physiologic and pathogenic processes. The virome, a subset of the overall microbiome, is of enormous size and diversity, including viruses that persistently inhabit host cells, endogenous viral elements genomically or epigenomically integrated into cells, and viruses that infect the other (bacterial, protozoan, fungal, archaeal) microbiome phylla [1]. The density of the human virome has been difficult to quantify, and is likely different between individuals, as well as across tissues in each person [2,3]. As a rough estimate, the density of viral particles (mostly phages [4]) in human feces has been estimated as comparable to that of bacteria, $\sim 10^9$ per gram [5,6]. This measurement significantly underestimates viruses outside of the GI tract; others estimate the total number of viruses within the human body at $\sim 10^{15}$ [6].

Amongst the virome, persistent or chronic viruses hold a special place. Adult humans harbor somewhere between 5–10 persistent or chronic viruses, and the majority of these cause no outward disease in healthy individuals [3,7]. Many persistent viruses are members of the herpesvirus family, five of which are widespread in the human population: Herpes simplex

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virus -1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV, chicken pox and shingles), Epstein Barr virus (EBV, infectious mononucleosis) and cytomegalovirus (CMV). Although other persistent viruses, including papilloma viruses, polyomaviruses, anelloviruses, adenoviruses, circoviruses, HIV, HCV, and many more are clearly present in the virome [4–6], herpesviruses have been the most thoroughly studied for their ability to specifically influence the aging immune system. These lifelong infections, and specifically, their reactivations, are not null events. Constant immune surveillance, and subsequent low-level immune activation and inflammation could potentially have dramatic effects on the health of an individual over a lifetime. We will discuss these associations and highlight the questions that need urgent research attention in the field.

Aging with persistent herpesvirus infections, and the special case of CMV

The lifecycle of herpesviruses presents a unique challenge to the host immune system. Upon primary infection of the host, the virus enters and rapidly replicates within host cells, while the innate immune system tries to limit and control the infection. Briefly, innate detection of CMV leads to production of pro-inflammatory cytokines (IFN α β , IL-12, IL-18) that aid in the activation of NK cells, which are critical for early viral control. Not surprisingly, CMV encodes numerous proteins that attempt to interfere with both cytokine production and NK activation (innate response reviewed in [8]). Upon subsequent activation of adaptive immunity, significant immune pressure is put on the virus, and the virus enters into a state of latency within host cells, with different herpesviruses showing different cellular tropisms for latency: CMV - hematopoietic progenitor and myeloid cells (latent reservoirs), vascular endothelial cells and epithelial cells (smoldering/chronic reservoirs) [9–11]; HSV and VZV - sensory neurons [12,13]; and EBV - memory B cells [14,15].

CMV, EBV and VZV are strictly species-specific, having closely co-evolved with their respective hosts over millions of years. Of these three, CMV exerts the most profound influence on the aging immune system [16, *17, 18]. CMV is known to produce devastating developmental anomalies when infecting a developing embryo, causing deafness, blindness or cognitive impairments, with shortening of overall lifespan [19]. However, most individuals are infected postnatally. Evolutionary considerations for the role of CMV in human health usually consider the host component through the sexual reproductive window, and there are some noted advantages to being infected with CMV in youth. In children, CMV seropositivity is associated with resistance to EBV infection in vitro [20], and young CMV+ adults show greater responses to seasonal influenza vaccination compared to uninfected counterparts [*21]. However, in the elderly, CMV has been associated with increased risk of cardiovascular disease (CVD) [16], Alzheimer's disease (AD) [22], and frailty [23,24]. Although the age of acquisition of CMV is somewhat dependent on socioeconomic status and ethnicity (within the US), between 40–95% of people are CMV seropositive before age 40 [25], and therefore live with CMV for at least 25 years before they reach age 65. Mechanistically, it remains unclear whether CMV specifically contributes to the “diseases of aging” (CVD, AD, frailty, etc.), or whether these correlations in human studies are due to another factor that simultaneously predisposes the host for these diseases and for CMV infection [26].

CMV

The dramatic impact of CMV on human immune system aging was discovered nearly 20 years ago [27], and has been described in numerous studies, including in the reports from a recent workshop [28–31]. However, whether CMV is a driver of immune dysfunction in late life is far less clear. In human monozygotic twins, CMV infection can account for upward of 50% of immune system variability, indicating that this virus has a dramatic impact on host biology [32]. Decades of interactions between CMV and the immune system lead to an absolute accumulation of effector T cells specific for CMV (termed memory inflation) [33–37], sometimes with one or a few clonal populations occupying up to 25% of the entire CD8 T cell pool in elderly humans [17,38]. Of interest, in the absence of CMV infection, with aging there is no absolute increase in memory T cells in the blood [35]. In mouse models, there is some evidence that CMV-specific memory T cell inflation is the result of continued recruitment of T cells into the immune response [39], although that issue is not fully settled. In contrast to chronic infections where antigen load is high and T cells are driven to functional exhaustion (Hepatitis C virus, HIV; reviewed in [40]), the inflated CMV-specific memory T cell populations retain robust effector function for the life of the individual, and have been described as “the last man standing” in the sea of reduced immune responses to other, acute pathogens [41,42].

HSV

HSV infection shares many similarities with CMV. In mouse models, systemic HSV-1 infection results in comparable memory T cell inflation over time [43,44], suggesting that HSV can provoke low level immune activation over a lifespan. However, natural human HSV-1 and 2 infections are not systemic – they are typically localized to neural ganglia in the facial/cranial area and the anogenital tract, respectively (Fig. 1A), and the reactivation of these viruses involves localized eruption in the area enervated by sensory neurons of these ganglia. Consequently, fewer studies of immune system aging have found strong correlations between HSV serostatus, immune phenotypes and function, other diseases, or mortality, particularly as related to HSV-2. More recently, in humans affected by Alzheimer’s disease (AD), correlations have been found between seropositivity for *B. burgdorferi*, HSV-1, CMV, *H. pylori* and *C. pneumoniae*, relative to healthy controls [22]. Further, in people with AD, the higher the infectious burden history (as measured by seropositivity to multiple pathogens), the greater the serum amyloid β levels, and the worse the cognitive deficits [22]. Infection with HSV-1 has been long suspected as a risk factor, as it induces inflammation in the same brain areas that are commonly affected by AD [45]. Further, HSV-1 DNA can be found in the plaques of AD brains [46]. Again, much like disease associations with CMV, causality has not been established, and it is not clear whether the virus, virus-related inflammation or some other, spuriously associated process, may be at the root of these observations.

EBV

Persistent infection with EBV has had very few associations with diseases of the aged outside of B cell lymphomas in humans. However, animal studies indicate that gamma-herpesviruses such as EBV have profound effects on the immune system. Mice infected with

γ MHV-68 (an EBV-like virus) or murine CMV show heterologous protection against subsequent bacterial infection [47], although this effect appears to be transient, and has been attributed to higher proinflammatory cytokines in the host [48]. A similar approach has shown that γ MHV-68+ mice subsequently infected with LCMV have impaired DC maturation, and altered T cell priming to skew the population toward memory rather than effector subsets [49]. In mouse models, infection with γ MHV-68 exacerbates EAE (a model of Multiple Sclerosis) [50]. In contrast, comparable infection of NOD mice alters antigen presentation and protects against the development of type-I diabetes [51].

Thus, the influence of persistent herpesvirus infections on the aging immune system, and how these infections might intersect with diseases of aging, has been difficult to parse. Each herpesvirus has its own biological niche within the host, has different sites of latency and reactivation (Figure 1), and the effects of these sites and their abundance are likely to have very different impacts on the immune system over a lifespan. But it has been difficult to conclusively assign the biological differences between the impact of individual herpesviruses upon the aging immune system to differences in rates of reactivation and/or the abundance of viral latency niches (see Table 1). Below we will consider these issues in greater details, including technical (low sensitivity of detection of viral persistence and reactivation in time and space) and ethical/model difficulties (host species restriction of strains of CMV, EBV and VZV; impracticality of human lifelong longitudinal studies) that must be overcome to gain mechanistic insights into the above questions.

Molecular latency vs. Organismal latency – implications for human aging

Herpesviruses are characterized by their ability to undergo latency within a host cell. Yet, infected cells can express viral immediate-early (IE) genes, without proceeding to full reactivation and production of viral progeny [52,53]. The question emerges: should that event be considered part of latency or part of reactivation? Perhaps latency, from the viral point of view, but clearly reactivation from the immune perspective. At an organismal level, it may be more useful to think of these viruses as persistent and smoldering, and that some degree of low-level reactivation is happening regularly. In other words, herpesvirus “latency” is not a silent state – latency at one location or within one cell does not mean latency everywhere. Rather, it is conceivable that “micro-reactivations” occur, where a single infected cell is initiating viral gene transcription, surrounded by uninfected, or latently infected cells (Figure 2). Measuring such an event within an organism has presented significant technical challenges to date. However, one study aimed at treating CMV in an incompletely reconstituted HIV population showed that valganciclovir treatment to target CMV activity reduced CD8+ T immune activation [54]. The consequences of these subclinical herpesvirus reactivation events remain unknown.

Massive herpesvirus, and in particular, CMV, reactivation is clinically evident in immune compromised individuals such as those infected with HIV [55], immunosuppressed for the purposes of transplantation [56,57] or by chemotherapy [58], and in otherwise healthy trauma patients [59]. Collectively, this suggests that the immune system is constantly engaged in controlling CMV. As a proxy for evidence of micro-reactivations, we can make inference from the slow and steady memory inflation of T cell populations specific for CMV

and HSV. Punch biopsy images from the genital tract of HSV-1 infected humans demonstrated frequent viral reactivation in the tissue, but that resident memory T cells can prevent lesion development if present at a high enough density [**60].

If continuous micro-reactivations are occurring through the organism, the immune surveillance and highly localized, cytokine production could produce low level inflammation that is not necessarily measurable by the systemic serum cytokine profiling, which has been the standard assessment in human subjects (and even animal models). Idiopathic systemic inflammation has been correlated with numerous “Western” age-related diseases – diabetes, cardiovascular disease, metabolic syndrome, and immune senescence. CMV specifically has been implicated in heightened inflammation and an increased risk of vascular disease and all cause mortality [16,24,61,62]. Again, solid mechanistic and quantitative dissection of both the phenomena and the causality have been difficult to achieve.

Human CMV infection over the lifespan – more questions than answers

Let us examine the key questions and current limitations in knowledge regarding the herpesvirome/aging intersect, using the example of CMV as the most potent modulator of immunity and healthspan with aging. Using a viromic approach, whole genome sequencing from the blood of uninfected donors readily detects multiple human herpesvirus strains [63]. The frequency of cells carrying latent CMV has been estimated at ~ 1 in 10^{4-5} peripheral blood mononuclear cells (PBMCs), with ~ 10 viral copies per latently infected cell [64]. Although this is a useful calculation from healthy adult donors, it is unclear whether the latent viral reservoir may be different within different cell types (CD34+ progenitors, monocytes, endothelial cells) or even within different cells of the same type in the same individual. Further, it is unclear what the latent viral load means - does it relate to immune resources devoted to suppressing the virus? Individuals can be infected with more than one strain of CMV [65,**66]. Do different strains of CMV reactivate at different levels? Do they establish different latent viral loads? We speculate that the answer to both of these questions is yes. What determines the frequency of these reactivations – the virus? The host? Here, we would venture that systemic and local stress probably play a key role, but that requires further investigation. Are some viruses more efficient at reactivation (such that IE gene expression leads to fully blown production of infectious virus)? If the virus can't sense immune pressure until it commits to reactivation, what provokes it to leave latency? Is this a random and stochastic process, or one driven by some change in the host cell? Does the rate of these micro-reactivations change over a lifespan, and does that differ from individual to individual? Why do individuals show such different magnitude of memory T cell inflation? Do some individuals have more CMV-infected cells (a higher latent load) so that the odds of reactivation are just higher? Or is the immune system in some individuals simply better able to keep the virus at bay, perhaps using fewer resources for that task (lower memory inflation, lower antiviral antibody titers)? Some evidence of this scenario emerged from the Leiden longevity study. Specifically, the offspring of long-lived individuals behaved as “elite controllers” of CMV, unlike their domestic partners, assumed to have been exposed to CMV under similar circumstances [**67]. This suggests a genetic component to controlling CMV with aging. Or is the latent virus quieter in some hosts and more active in others irrespective of virus load?

Historically, seropositivity has been used to stratify human subjects into CMV antibody (Ab) + or Ab– groups. Occasionally, studies will further subdivide the CMV+ cohort based on quartiles of Ab titer. However, what does titer mean? Do all individuals control CMV with a particular set of immune resources? Surprisingly, very little work has been done looking at global immune responses to CMV within the same individual (i.e. T, B and NK responses). Do some people generate a higher Ab titer and a lower T cell response, and vice versa? Is one strategy more effective at controlling viral reactivation than another? Does a high Ab titer always come with a similarly high T cell response? Does this indicate poor viral control, and greater reactivation from latency within that person? Does viral reactivation actually drive titer, or are titer values relatively stable? In healthy individuals, the CMV serologic titer remains pretty constant over years to decades, suggesting that this control is relatively efficient. When people undergo chemotherapy, titer spikes, and then resolves back to the previous set point [58]. In a longitudinal study of 11 women aged 70–79, most subjects' IgG CMV titers remained remarkably stable over 12 years [68], although a larger sample size is needed to confirm this finding, and studies in men should be included, as the overall immune system in middle-aged men is much more prominently affected by CMV carriage [69].

Conclusions

Overall, it is clear that we have many more questions than answers at this moment, related to the role of CMV and the persistent herpesvirome in immune aging and healthspan (main ones are summarized in Table 1). High-resolution molecular, reporter and imaging studies would be of great help to quantify the above phenomena, along with high-throughput and high-resolution immune monitoring techniques, to resolve this tantalizing issue of considerable importance to human healthy aging.

Acknowledgments

Supported by NIA R01 AG048021 and NIAID Contract N01-AI-000017 (to JNZ) from the National Institutes of Health.

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Highlights

- Five different herpesviruses persist widely in the human population: HSV-1, HSV-2, VZV, EBV and CMV and as such, play a potential role in the aging of the organism.
- Subclinical herpesvirus reactivations occur throughout the lifespan, and require ongoing immune attention to control these viruses, thus influencing immune aging.
- Each herpesvirus exhibits a different host cell tropism and viral reservoir, so immune control will require different levels of immune resources, and this may change over the lifespan.
- Of the five, CMV infection specifically has been associated with numerous “diseases of aging”, yet recent results show benefits of CMV infection, highlighting a nuanced interaction between CMV and the aging host.

Figure 1. Differences in host cell tropism would influence the frequency or distribution of herpesvirus reactivation events

As most herpesviruses have limited host cell reservoirs for latency, the inflammatory impact of viral reactivations would be localized. For example, HSV latency (left) is limited to craniofacial and anogenital sensory neurons for HSV-1 and -2, respectively, thus reactivation would occur only within these specific anatomic sites. In contrast, CMV reservoirs include hematopoietic stem cells, myeloid cells, vascular endothelial and epithelial cells. CMV reactivations can therefore occur throughout the entire body, and the resulting inflammation could impact numerous other tissues/organs.

Figure 2. Predicted immune consequences of CMV latency, micro-reactivation, and full reactivation

In latency (top panel) cells harboring virus would predominantly produce latency-associated transcripts (red), while the few cells attempting to reactivate may also produce lytic viral transcripts (green). There would be little to no antigen presentation by MHC to either circulating memory T cells, or tissue-resident memory T cells. During a micro-reactivation event (middle panel), one infected cell may produce lytic transcripts (green) that are translated into protein, resulting in antigen presentation (green) within the MHC molecules on the host cell surface. This will lead to antigen recognition by both circulating and resident-memory CMV-specific T cells, leading to their release of effector cytokine molecules targeted to the reactivating cell. However, the majority of these cytokines would remain local, or would be released into circulation at such a low level that they would not be detected by serum analysis. Full viral reactivation (bottom panel) would result in large-scale production of lytic viral proteins, high levels of antigen presentation by MHC molecules, and massive activation of circulating and resident-memory CMV-specific T cells. This would result in massive secretion of proinflammatory effector cytokines that would be detectable by serum analysis.

Table 1

Known “Unknowns” of Human CMV Infection

1. How many different strains of CMV are most individuals infected with? Does this impact the size of the immune resources devoted to CMV control (T and B cells)?
2. Do different CMV strains have different rates of reactivation? Does this change as a person ages? What controls frequency of reactivations from person to person?
3. Is serologic titer an appropriate proxy measurement for CMV-specific T and NK responses within an individual?
4. Do any measureable immune parameters in PBMCs (Ab titer, T cell response) provide insight into CMV reactivation frequency within an individual?
5. Does lifelong, low-level CMV reactivation within an individual contribute to localized inflammation? Does this influence the development of “diseases of aging” (CVD, AD, frailty)?