Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

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

Janko Nikolich-Zugich[1], Felicia Goodrum[1], Kenneth Knox[1,2] and Megan J Smithey[1]

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, and 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.

Addresses
[1] Department of Immunobiology and the Arizona Center on Aging, University of Arizona College of Medicine-Tucson, Tucson, AZ 85724, United States
[2] University of Arizona College of Medicine-Phoenix, Phoenix, AZ 85004, United States

Corresponding authors: Nikolich-Zugich, Janko (nikolich@email.arizona.edu), Smithey, Megan J (smithey@email.arizona.edu)

Current Opinion in Immunology 2017, **48**:23–30

This review comes from a themed issue on **Host pathogens**

Edited by **Elizabeth Hartland** and **Marc Pellegrini**

http://dx.doi.org/10.1016/j.coi.2017.07.011

Introduction

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 and 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 virus-1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV, chicken pox and shingles), Epstein Barr virus (EBV, infectious mononucleousis) 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 (IFNαβ, 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 and 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) [33–36,37••], 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 (Figure 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 *Borrelia burgdorferi*, HSV-1, CMV, *Helicobacter pylori* and *Chlamydophila 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 γMHV-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 γMHV-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 γMHV-68