A constant companion: immune recognition and response to cytomegalovirus with aging and implications for immune fitness

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Abstract Approximately 50% of individuals aged 6–49 years in the United States are infected with cytomegalovirus (CMV), with seroprevalence increasing with age, reaching 85–90% by 75–80 years according to Bate et al. (Clin Infect Dis 50 (11): 1439–1447, 2010) and Pawelec et al. (Curr Opin Immunol 24:507–511, 2012). Following primary infection, CMV establishes lifelong latency with periodic reactivation. Immunocompetent hosts experience largely asymptomatic infection, but CMV can cause serious illness in immunocompromised populations, such as transplant patients and the elderly. Control of CMV requires constant immune surveillance, and recent discoveries suggest this demand alters general features of the immune system in infected individuals. Here, we review recent advances in the understanding of the immune response to CMV and the role of CMV in immune aging and fitness, while highlighting the importance of potential confounding factors that

influence CMV studies. Understanding how CMV contributes to shaping “baseline” immunity has important implications for a host’s ability to mount effective responses to diverse infections and vaccination.

Keywords Cytomegalovirus · Aging · Immune fitness · Coinfection · Herpesvirus · Immune response

Introduction

Cytomegalovirus (CMV) is a member of the β -herpesvirus family and infects approximately 50% of individuals aged 6–49 years in the United States, with seroprevalence increasing with age, reaching 85–90% by 75–80 years (Bate and Dollard 2010; Pawelec et al. 2012). Risk of seroprevalence is associated with race, female sex, age, and a variety of demographic factors, such as household income and low household education (Bate and Dollard 2010). CMV transmission may occur via saliva, breastfeeding, blood transfusion, placental transfer, sexual contact, solid organ transplantation, and hematopoietic stem cell transplantation (Crough and Khanna 2009).

Herpesvirus infection occurs in three general stages: (1) initial, primary infection; (2) latency- defined here as the maintenance of herpesvirus genomes in the absence of infectious virus production; and (3) reactivation from latency. The CMV genome consists of a linear DNA chromosome, which is translocated to the host cell

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nucleus during infection, where it rapidly circularizes, associates with cellular histones, and is maintained as an episome (Nitzsche et al. 2008). A characteristic of herpesviruses is the temporal cascade of gene expression during lytic infection. Transcription begins with immediate-early (IE) genes which prime the environment for infection, followed by expression of early (E) genes required for genome replication, and finally viral late (L) genes which include structural proteins necessary for virion production. Expression of IE genes is controlled by the major immediate-early promoter, which is subject to histone modifications, and thought to be the switch between latency and reactivation (Murphy et al. 2002; Liu et al. 2008).

Control of CMV requires constant immune surveillance. This demand has important implications for immune status because persistent antigen exposure and presentation can lead to T cell exhaustion and persistent immune activation may have detrimental off-target effects (Mueller and Ahmed 2009; van de Berg et al. 2010; Botto et al. 2011; Zuniga and Harker 2012). Indeed, several studies have shown that infection with CMV (murine or human) is associated with changes in multiple facets of the immune profile, such as an increase in pro-inflammatory serum cytokine levels and accumulation of highly differentiated T cells. For example, an analysis of correlations of immune parameters between monozygotic CMV discordant (positive-negative) or concordant (negative-negative) twin pairs revealed that CMV serostatus significantly influenced correlations in 58% (119 of 204) of measurements, including, but not limited to, frequency of effector CD8⁺ T cells, $\gamma\delta$ T cells, serum IL-10 levels, serum IL-6 levels, and cell signaling responses to various STAT1 and STAT3 stimuli (Brodin et al. 2015). More discussion on CMV virology, epidemiology, and disease associations can be found in the reviews by Leng et al. and Aiello et al. in this issue.

A better understanding of how CMV affects our immune profile and the subsequent effects on the immune phenotype is imperative to developing therapeutic strategies and providing insight into the development and/or risks of a CMV vaccination protocol. Here, we review recent advances in the understanding of the immune response to CMV and the role of CMV in immune aging and fitness, while highlighting the importance of potential interactions between CMV and confounding factors.

Immune response to CMV

Innate immunity

Initiation of an innate immune response begins with pathogen recognition mediated by pathogen recognition receptors (PRRs), which recognize evolutionarily conserved components unique to pathogens or nucleic acids mislocated within the cell (e.g., DNA in the cytoplasm). PRRs involved in detection of CMV are displayed in Table 1. Downstream signaling from activated PRRs can lead to caspase activation and subsequent activation of pro-inflammatory cytokines, followed by activation of transcription factors IRF3/7 leading to expression of antiviral genes and NK cell activation.

NK cell activity is regulated by activating and inhibitory germline-encoded receptors, which are comprised of multiple families, such as the C-type lectin-like NK receptors (activating NKG2C, inhibitory NKG2A) and the killer cell immunoglobulin-like receptors (KIRs; activating 3DS1, inhibitory 3DL1-3) in humans (Béziat et al. 2013; Bartel et al. 2013). In mice, studies have shown a direct interaction between murine cytomegalovirus (MCMV) and NK cells, where the activating NK cell receptor Ly49H recognizes the m157 glycoprotein, thereby activating the NK cell to produce cytokines important for antiviral immunity and/or to kill

Table 1 Innate detection of murine cytomegalovirus (MCMV) and human cytomegalovirus (HCMV) by PRRs

Virus	Sensor	Ligand	Reference
HCMV	TLR1/2	Envelope gB and gH	(Boehme et al. 2006)
MCMV and HCMV	TLR9	Unmethylated CpG DNA	(Krug et al. 2004; Varani et al. 2007; Zucchini et al. 2008)
MCMV and HCMV	AIM2	dsDNA	(Rathinam et al. 2010; Huang et al. 2017)
MCMV and HCMV	cGAS-STING	dsDNA	(Lio et al. 2016)
MCMV	TLR3	dsRNA	(Tabeta et al. 2004)
MCMV	TLR7	ssRNA	(Zucchini et al. 2008)
HCMV	DAI	dsDNA	(DeFilippis et al. 2010)
HCMV	IFI16	dsDNA	(Gariano et al. 2012)

the virus-infected cell through release of perforins and granzymes (Brown et al. 2001; Pyzik et al. 2011). Indeed, m157 deletion mutant MCMV fails to activate Ly49H+ NK cells and results in enhanced virulence (Voigt et al. 2003; Bubić et al. 2004). While an equivalent interaction has yet to be found in humans, several studies have shown that CMV induces expansion of specific NK cell subsets and a stable imprint in the NK cell receptor repertoire (Béziat et al. 2013; Muntasell et al. 2013; Bayard et al. 2016). Furthermore, a case report shows a genetic mutation resulting in expression of the inhibitory receptor KIR2DL1 on the entire NK cell population leads to recurrent CMV infections (Gazit et al. 2004). Additionally, during hematopoietic stem cell transplantation, a donor activating KIR profile is predictive for low risk of CMV reactivation (Zaia et al. 2009).

Recent evidence shows CMV infection induces clonal expansion of $V\delta 2^{\text{neg}}$ $\gamma\delta$ T cells, with a highly differentiated effector memory phenotype, and enhanced effector function, suggesting a role for $\gamma\delta$ T cells in CMV immunity (Roux et al. 2013; Alejenef et al. 2014; Khairallah et al. 2017). Moreover, adoptive transfer of MCMV-induced $\gamma\delta$ T cells confers protection against lethal MCMV challenge (Khairallah et al. 2017).

Adaptive immunity

CMV infection induces a robust CD8+ T cell response. The full size of the response to CMV in humans (represented by 213 protein-spanning peptide pools) can be extrapolated from the responses to 19 proteins, including 6 dominant CD4 and 15 dominant CD8 T cell targets (Sylwester et al. 2005). Periodic reactivation of latent CMV is thought to repetitively “tickle” the T cell compartment, gradually increasing its size over time (Holtappels et al. 2000; Karrer et al. 2003). Indeed, the number of human cytomegalovirus (HCMV)-specific $\alpha\beta$ T cells in an otherwise healthy individual has been shown to range from 10 to 40% (Sester et al. 2002; Sylwester et al. 2005; Klenerman and Oxenius 2016). Homeostatic regulation of the size of lymph nodes and spleen led researchers to believe an upper boundary exists for the total number of memory T cells. In mice, studies have shown that new memory T cells generated during heterologous infections lead to a decrease in memory CD8+ T cells specific for a prior infection, raising the concern that periodic herpesvirus reactivation and expansion of memory CD8 T cells over time

would lead to significant memory T cell attrition and impaired immune responses (Selin et al. 1996, 1999; Liu et al. 2003). However, recent evidence suggests this is not what occurs in humans. A study of CD8+ T cells in peripheral blood and lymph nodes shows that HCMV-specific T cells express high levels of CX3CR1 and accumulate in the blood but not in the lymph nodes, contrary to influenza and Epstein Barr virus-specific T cells which occur in similar frequencies in both locations (Remmerswaal et al. 2012). Moreover, analysis of CD8+ T cells following primary HCMV infection in renal transplant recipients shows the accumulation of HCMV-specific CD8+ T cells over time occurs with an increase in total CD8+ T cell numbers (van Leeuwen et al. 2006). These studies suggest periodic reactivation and subsequent memory inflation do not consume limited space in secondary lymphoid organs; rather, the CD8 T cell compartment is flexible and may expand in response to HCMV infection.

T cell phenotypes

The accumulation of CMV-specific memory T cells may be maintained through a continuous replacement of short-lived, functional T cells and/or accumulation of apoptosis-resistant late-stage differentiated or “senescent” T cells (Van Lier et al. 2003; Ouyang et al. 2004; Snyder et al. 2008). In mice, it has been shown that viral inoculum dose impacts the degree of CMV-specific memory T cell inflation and the phenotype of memory subsets (Redeker et al. 2014). Of note, the studies to date that explore the relationship between immunosenescence and the size of the CMV-specific T cell response in humans have considered mainly interferon- γ (IFN- γ) secretion in response to a restricted number of target proteins, especially UL83 or UL123. Despite these limitations, there is a consensus regarding CMV-specific T cell phenotypes. In both young and old participants, the bulk of the CMV-specific T cell response arises from the T effector memory (CD45RA $^{-}$, CD27 $^{-}$, TEM) compartment in CD4+ T cells and, to a similar extent, from the TEM and T effector memory RA+ (CD45RA+, CD27 $^{-}$, TEMRA) compartments in CD8+ T cells. However, in the eldest group (“survivors”), a large contribution to the CD8 T cell response size and the greatest contribution to the CD4 T cell response size originated from the T central memory (CD45RA $^{-}$, CD27+, TCM) compartment (Bajwa et al. 2017).

Breadth of the T cell response to CMV

A recent study assessed T cell responsiveness to a wider range of proteins than previously studied, utilizing multiple functional response readouts. Using protein-spanning peptide pools for CMV antigen-specific stimulation, the authors demonstrated that the older group had, on average, larger T cell responses than the young, confirming the accumulation of CMV-specific T cells over time (Kern et al. 2000). The eldest group recognized more proteins on average than the other groups and had even bigger T cell responses than the old group with a significantly larger central memory CD4 T cell component (Bajwa et al. 2017).

Importantly, this work shows that age-related expansions of the CMV-specific T cell response is best assessed by using a broader range of proteins than is commonly employed, combined with several functional readouts. Indeed, response size differences due to age were easier to detect when the analysis was restricted to particular single-effector readouts (e.g., IFN- γ , TNF- α), but more difficult to measure when multiple readouts were combined.

T cell polyfunctionality and CMV responses

Polyfunctional T cells, capable of the simultaneous production of multiple cytokines, have been associated with improved immunological control across many acute and chronic viral infections, including CMV (Betts et al. 2006; Darrah et al. 2007; Krol et al. 2011; Sauce et al. 2016). Recently, Chiu et al. described that a higher degree of cytotoxicity-associated polyfunctionality was positively correlated with a larger total CMV-specific response size, both for CD8+ and CD4+ CMV responses (Chiu et al. 2016). In contrast, IL-2-associated polyfunctionality did not follow the same trend. Thus, despite an extended period of antigen presentation and a failure to clear CMV, the responding T cells do not take on a functionally exhausted phenotype, even in older adults (Lelic et al. 2012). Similar data has been obtained in mice and non-human primates (Cicin-Sain et al. 2011; Lang and Nikolich-Zugich 2011). The correlation with the magnitude of the CMV cellular response extended to a positive correlation with the humoral response (Chiu et al. 2016). However, these results contrasted somewhat with another study, in which the level of anti-CMV antibody was found to be positively associated with

viral load and negatively associated with T cell receptor repertoire diversity (Wang et al. 2012).

In addition to conventional subset-by-subset analysis, Bajwa et al. used a novel polyfunctionality index (PI) which facilitates analysis of the “degree and variation of polyfunctionality and enables comparative and correlative parametric and non-parametric statistical tests” (Larsen et al. 2012). Their results confirmed published reports indicating that age per se is not linked to reduced polyfunctionality of CD8+ T cell responses (Bajwa et al. 2017). Of note, results in the eldest group demonstrated decreased polyfunctionality, with an overall reduced PI of both CD4+ and CD8+ T cell responses. The fact that the oldest age group had a lower PI score may indicate that polyfunctionality is not required to successfully control CMV at an extreme age (Boyd et al. 2015). Since cells with greater polyfunctionality also produce larger and potentially more harmful quantities of effector cytokines, the level of pathogen control must be balanced against the risk of collateral tissue damage, and it might actually be an advantage for long-term survival to achieve reduced polyfunctionality of CMV-specific T cell responses in very old people (Lachmann et al. 2012). However, the link between polyfunctionality and clinical outcomes remains poorly defined and significantly more research is warranted, given the growing interest in the possible role of CMV in a range of age-associated pathologies (Solana et al. 2012; Terrazzini et al. 2014).

Immune aging and CMV

A complex set of age-related changes, collectively termed immunosenescence, includes alterations in both the innate and adaptive immune systems, in the extracellular microenvironments where immune cells develop or reside, and in soluble factors that guide immune homeostasis and function, resulting in dramatic impairment of immune function, leaving older adults more susceptible to infectious diseases (Nikolich-Zugich et al. 2012; Shaw et al. 2013; Moreau et al. 2017).

One characteristic of immunosenescence is a decrease in the naïve CD8+ T cell population and an increase in highly differentiated memory CD8+ T cells. This change is partially due to thymic involution and reductions in hematopoietic stem cells committed to lymphoid lineage development, but also results from large expansions of CMV-specific T cells observed in

older people, creating the paradigm of CMV-induced T cell “memory inflation” (Khan et al. 2002; Klenerman and Hill 2005; Whiting et al. 2015). Of interest, in the absence of CMV infection, aging alone does not result in increased absolute numbers of memory T cells in the blood (Wertheimer et al. 2014). This imprint of CMV infection on the memory T cell population is so robust that CMV infection status can be determined for human samples by sequencing the total T cell repertoire from peripheral blood—the frequency of common CMV-specific T cell receptors is so frequent and shared across HLA-disparate individuals (Emerson et al. 2017). In contrast to chronic infections where antigen load is high and T cells are driven to functional exhaustion (hepatitis B/C viruses, HBV/HCV; human immunodeficiency virus, HIV), the inflated CMV-specific memory T cell populations retain robust effector function for the life of the individual (Cicin-Sain et al. 2011; Riddell et al. 2015; Wherry and Kurachi 2015). However, previous studies of late-differentiated memory CMV-specific T cells in older adults have shown impaired immune competence, such as limited replicative capacity (Fletcher et al. 2005; Hadrup et al. 2006). Memory inflation, immune dysfunction, and skewing of the T cell receptor repertoire have been associated with the immunosenescent phenotype and an inverted CD4/CD8 ratio ($CD4/CD8 < 1$), a parameter in the immune risk profile (IRP; associated with earlier mortality) (Olsson et al. 2000; Khan et al. 2002; Pawelec et al. 2004; Hadrup et al. 2006; Pourghesari et al. 2007). However, this index remains to be validated in other studies.

A large-scale study of the immune system during normal human aging confirms that age, followed by sex and CMV status, has the greatest impact on the immune system (Whiting et al. 2015). This study determined that CMV contributes to shaping the immune profile and function during normal human aging, and highlights the importance of assessing potential interactions with age. Indeed, immune cell subsets in late middle-aged (50–65) males and females are differentially impacted by CMV, with aging CMV-positive men showing more pronounced changes to numerous lymphocyte subsets than either CMV-positive females or uninfected individuals (van der Heiden et al. 2016).

The high prevalence of CMV worldwide makes it important to understand the consequences of CMV associated changes with respect to basal immune status and fitness during daily encounters with pathogens. As

new tools and technologies develop, more studies are needed to further assess the relationship between CMV and the aging immune system.

Associations between immune profile, status, fitness, and CMV infection

It is estimated that the three mammalian subfamilies of herpesviruses arose approximately 180–220 million years ago, and recent speciation within subfamilies occurred in the last 80 million years, likely driven, at least in part, by co-evolution with the host (McGeoch et al. 1995). Taking into account that at least 90% of the human population is infected with at least one herpesvirus, and herpesvirus infection is largely asymptomatic in immunocompetent individuals, it is likely that herpesviruses provide some benefit to the host (Virgin et al. 2009). It was initially thought that CMV latency was a transcriptionally quiescent phase; however, recent evidence shows several proteins and at least one non-coding RNA are transcribed during latency, and serve a variety of functions including maintenance of latency, immune evasion (discussed in more depth in the review by Jackson et al. in this issue), and genome maintenance (Sinclair and Reeves 2013). For example, HCMV encodes two homologs of human interleukin-10, a cytokine which exhibits immunosuppressive functions such as decreased costimulatory molecule expression and impaired antigen presentation. The two viral IL-10 homologs are a result of alternative splicing of the UL111A region transcript; one homolog is expressed during lytic infection (cmvIL-10), the other during latent infection (LAcmvIL-10). Recent evidence shows that both cmv-IL10 and LAcmvIL-10 can mimic the immunosuppressive function of human IL-10 via downregulation of MHCII on granulocyte-macrophage progenitors and monocytes. However, these homologs have functional differences in their ability to manipulate dendritic cell maturation. In response to LPS stimulation, lytic cmvIL-10 inhibited the expression of DC costimulatory molecules CD40, CD80/86, and maturation marker CD83. Additionally, cmvIL-10 inhibited the secretion of IL-1 α , IL-1 β , IL-6, and TNF- α . Conversely, LAcmvIL-10 treatment did not alter DC maturation or cytokine production. These data demonstrate “conditional” immune suppression by HCMV and perhaps reflect an evolved mechanism to optimize HCMV replication during lytic infection via more

comprehensive immune suppression, but limit immune modulation during latency to facilitate latency maintenance, while allowing for “normal” immune responses to other antigens/infections encountered on a daily basis (Jenkins et al. 2008). Given CMV’s ability to manipulate immunity during all phases of its life cycle and the wide variety of altered immune parameters associated with CMV serostatus, it is important to understand how these changes may subsequently affect the immune phenotype during co-infection with an unrelated virus or following vaccination.

Co-infection with CMV

When assessing whether herpesvirus co-infection alters the immune phenotype, there are several factors that may influence the observed effects. The most important is, of course, the type of secondary infection. While latent herpesvirus infection is beneficial during bacterial and/or viral challenge (T_H1 dominant), helminth co-infection (T_H2 dominant) exacerbates disease and induces reactivation of murine γ -herpesvirus 68 (MHV68; a murine version of human γ -herpesviruses) from latency (Barton et al. 2007; Reese et al. 2014; Ančicová et al. 2015). Thus, the following sections will be divided into “Acute Co-infection” and “Chronic Co-infection”, and additional potential interacting factors will be highlighted throughout.

Acute co-infection

Generally, acute infections are controlled by the immune system and eventually cleared. Herpesviruses are different from common acute infections because infection generally lasts for life, during which the herpesvirus persists in a latent state, with periodic reactivation. Indeed, studies have shown that the state of herpesviruses matters when assessing the effects during co-infection. MCMV reactivation leads to decreased bacterial clearance and exacerbates *Staphylococcus aureus*-induced pneumonia (Hraiech et al. 2017). Conversely, latent MCMV and MHV68 infections confer protection against lethal bacterial challenge with *Yersinia pestis* and *Listeria monocytogenes*. Protection is mediated through increased levels of IFN- γ and TNF- α , resulting in increased activation of macrophages (Barton et al. 2007). Recent work in a mouse model shows that infection with lymphocytic choriomeningitis virus (LCMV) followed by MCMV reduces inflation of MCMV-

specific memory CD8 T cells, augments MCMV viral load, and alters immunopathology, whereas infection with MCMV followed by LCMV results in an increase in the overall magnitude of the CD8 T cell response to LCMV (6-weeks post MCMV infection), and a robust CD8 T cell response to a normally subdominant LCMV epitope, $L_{2062-2069}$, suggesting sequence of infection is also an important factor. This shift in immunodominance occurs in approximately 20% of mice and is facilitated by cross-reactivity with a newly defined MCMV epitope, $M57_{727-734}$ (Che et al. 2017). In addition, the context of antigen encounter may lead to different outcomes. Analysis of activated virus-specific and total CD8 T cells in 50 patients with acute HBV, dengue, influenza, and adenovirus infections show that HCMV- and EBV-specific CD8 T cells are activated, proliferate, and have increased production of IFN- γ during heterologous infection (Sandalova et al. 2010). Conversely, vaccination for yellow fever or vaccinia virus did not induce activation or proliferation of EBV- and HCMV-specific CD8 T cells, despite robust CD8 T cell responses (Miller et al. 2008).

A study in healthy young (20 to 35 years old) and elderly (60 to >89 years old) individuals found that young CMV-positive individuals have a general elevation in immune function, such as increased IFN- γ and IL-13 (T_H1 and T_H2 cytokines), enhanced CD8 T cell responses, and greater expression of genes involved in antigen processing, MHC molecules, and NK cell cytotoxicity. Moreover, during co-infection with influenza virus, mice latently infected with MCMV exhibited reduced influenza viral titers and increased magnitude of influenza virus-specific CD8 T cell responses (Furman et al. 2015). However, studies in aging mice show that latent MCMV, herpes simplex virus 1–2, and/or MHV68 did not confer protection during influenza virus, West Nile virus, or vesicular stomatitis virus infection (Marandu et al. 2015). This apparent discordance is also observed in studies examining whether CMV infection alters influenza vaccine responses and highlights the need for further investigation into interacting factors, such as age. The age of an individual is a unique factor in the context of CMV co-infection because it alters three aspects of infection control and immunity: (1) the normal aging of the immune system and age-associated effects independent of CMV status; (2) the chronic immune profile associated with CMV (e.g., memory inflation); and (3) potential independent associations with the etiologic agent of the secondary

infection (e.g., younger individuals can have more severe influenza virus infection and exhibit hyperinflammatory immune responses) (Oshansky et al. 2014; Wertheimer et al. 2014; Furman et al. 2015; Whiting et al. 2015).

A study comparing influenza vaccine responsiveness in elderly nursing home residents (age range 65–99 years old) versus staff (19–40 years old) found that the immune system of non-responders, in both age groups, could be characterized by increased pro-inflammatory cytokines, increased CMV IgG antibody levels, and elevated frequencies of CD57+ CD8+ T cells (Trzonkowski et al. 2003). In contrast, a different study of elderly residents (age interquartile range 78–88, median age 83) in long-term care facilities showed no difference in influenza vaccine response based on CMV serostatus (den Elzen et al. 2011). Another study comparing the immune response to influenza vaccination between adults (18–59 years old) and the elderly (>60 years old) found that CMV infection was associated with poor humoral responses only in adults >60. Higher frequencies of late-differentiated CD4+ (CD45RA+ CCR7– CD27– CD28–) T cells also associated with lower responses to the influenza vaccine (Derhovanessian et al. 2013). Importantly, recent evidence has identified 35 single nucleotide polymorphisms associated with the frequency of CD4+ CD28– T cells in humans, many of which are located near genomic loci in the human leukocyte antigen (HLA) region (Furman et al. 2015). These results suggest a role for genetics and polymorphisms in modulation of T cell phenotypes during CMV infection. This study also found increased antibody responses to influenza vaccination in young CMV-positive individuals, but no difference in the elderly (Furman et al. 2015).

Chronic co-infection

Herpesviruses are unique even amongst chronic infections such as HIV and HBV/HCV. While each of these are persistent infections, herpesviruses establish latency with periodic reactivation events, or produce virus at extremely low levels (“smoldering infections”), resulting in often undetectable virus production; in stark contrast, HIV and HBV/HCV never establish latency and consistently produce large amounts of virus. The difference in viral load has important implications for the immune system and is reflected in the degree of disease severity. In an immunocompetent host,

herpesvirus infections are usually asymptomatic or mildly symptomatic and can be resolved without drug treatment. Conversely, HIV and HBV/HCV are symptomatic, capable of overwhelming and/or impairing the immune system, are overtly detrimental to the host, and require therapeutic interventions. The consequence of CMV co-infection in these scenarios is largely dependent on the secondary infection.

In a study of chronic viral hepatitis B and C patients, 52.3% and 36% were infected with HCMV, respectively. Although histology scores measuring necroinflammation and fibrosis were higher in HCMV-positive patients in HBV and HCV groups, intrahepatic HBV and HCV loads were decreased in HCMV-infected patients (Bayram et al. 2009). Additionally, a model of primary EBV infection in PBMCs from children 2–5 years old showed decreased EBV-induced expansion of IgD– CD27+ B cells in CMV-positive children, with elevated levels of IFN- γ and frequencies of CD8+ CD57+ T cells (Sohlberg et al. 2013). These studies suggest a beneficial role of CMV co-infection mediated by enhanced viral control. However, studies in patients infected with HIV demonstrate how a chronic pathogen may interact with CMV resulting in increased susceptibility to non-AIDS-related morbidities, such as cardiovascular disease and cognitive impairments. Research in HIV-positive males has shown increased CMV viral load is associated with increased HIV viral loads, independent of antiretroviral therapy (Gianella et al. 2013, 2014, 2016). Additional studies have found that CMV-positive ART-treated HIV patients have decreased CD4/CD8 T cell ratios, as a result of increased CD8 T cell counts, and higher levels of proinflammatory IP-10/CXCL10 (Freeman et al. 2016). Recent evidence also shows CMV co-infected HIV subjects have approximately a 50% increase in risk for severe non-AIDS related events/death, such as cerebrovascular and cardiovascular diseases, even after controlling for potential confounders (Lichtner et al. 2015).

Studies thus far show that immune changes associated with CMV may have significant impacts during co-infection and vaccination, but the extent of these modulations is highly dependent on other factors. There are three additional areas worth noting that remain understudied and should be included in future research designs to understand the immune response to CMV alone and the extent to which CMV may modulate the immune response in the context of co-infection: (1) diverse ethnic/ancestral backgrounds. CMV

seroprevalence increases in populations with lower socioeconomic status and/or from developing countries. For example, a study of 200 healthy donors in India found seroprevalence to be 95%, with no significant age stratification (Kothari et al. 2002). Populations such as this will have increased duration of CMV exposure which may result in enhanced CMV associated phenotypes and/or immune adaptation to the high demand of immune resources necessary to control CMV. (2) Multiple herpesvirus infections. Most adults have more than one herpesvirus infection, which may lead to different immune modulation signatures. Indeed, studies in mouse models of MCMV and MHV68 have shown unique alterations in host gene transcription based on single- vs. double-herpesvirus infection (White et al. 2010). Furthermore, studies in mice show more “human like” responses to vaccination following sequential infection with common pathogens, including MCMV and MHV68, suggesting that animal models may be improved by exposing them to pathogens commonly encountered in humans, including those which (e.g., CMV) may contribute to the immune phenotype (Reese et al. 2016). (3) The reciprocal effect of secondary infections on the immune response to CMV and how this shapes the anti-CMV response over time. A study in CMV-positive Tanzanian children, adolescents, and adults showed HIV co-infection and latent *Mycobacterium tuberculosis* infection altered the phenotype and function of CMV pp65-specific CD4 T cells (Portevin et al. 2015). More studies examining the relationship between CMV and each of these potential interacting factors are needed to gain a better understanding of the extent to which these variables contribute to shaping the immune system. These studies will provide valuable insights which can be used to develop more efficacious prophylactic therapies and/or post-exposure treatments to reduce disease severity in co-infections.

Conclusion

Although herpesviruses are characterized by their ability to enter into latency within host cells, latency is not transcriptionally quiescent. Rather, CMV can express viral immediate-early (IE) genes without proceeding to full reactivation and production of viral progeny (Taylor-Wiedeman et al. 1994; Kurz and Reddehase 1999). This low-level “smoldering” of CMV is not a

null event, but rather a source of ongoing antigenic stimulation to the host immune system.

The past and current studies reviewed here contribute to the growing evidence that this persistent immune activation plays an important role in shaping multiple facets of the immune profile and fitness in the context of co-infection and vaccination. The general consensus of these studies suggests that latent infection with herpesviruses is beneficial during childhood-adult years, but may contribute to immune dysfunction in the elderly. More studies are needed into the effects of aging, in combination with other demographic and infection parameters, to examine at what point the switch occurs from a beneficial to a detrimental host-virus interaction and the associated triggers. Throughout this review, we also highlighted the necessity for more studies into various factors that may interact with CMV and affect immune measures, such as genetics, sex, and multiple herpesvirus infections. Collectively, these studies provide important insights into host-virus interactions, demonstrating the significant impact CMV infection has in shaping the immune system and underscoring the need for more studies to determine the mechanism behind immune modulation and subsequent effects in the immune phenotype. Studies such as these may ultimately lead to new therapeutic targets and the development of more effective vaccines. Importantly, they will also shed light into potential consequences of CMV vaccination, and perhaps suggest a restricted vaccination protocol, strictly to those at risk for loss of CMV control.

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