Advances in cytomegalovirus (CMV) biology and its relationship to health, diseases, and aging

Janko Nikolich-Žugich · Luka Čicin-Šain · Donna Collins-McMillen · Sarah Jackson · Annette Oxenius · John Sinclair · Christopher Snyder · Mark Wills · Niels Lemmermann

Abstract Cytomegalovirus (CMV) is one of the largest and most ubiquitous latent persistent viruses. Most humans are infected with CMV early in life, and all immunocompetent humans spend several decades living with CMV. In the vast majority of the hosts, CMV does not cause manifest disease, and CMV therefore can be considered part of normal aging for 50–90% of the human population worldwide. Experimental, clinical, and epidemiological studies suggest that CMV carriage can have nuanced outcomes, including both potentially harmful and potentially beneficial impacts on the host. We here present a summary of the 7th International Workshop on CMV and Immunosenescence, covering various aspects of the interplay between CMV and its mammalian hosts in the context of virus spread, immune evasion, antiviral immunity, as well as the impact on health span and aging.

Keywords Cytomegalovirus · Immunity · Aging · Latency · Immune evasion

CMV, immunosenescence, and chronic infections

The impact of persistent and chronic infections on human and animal health has intrigued biomedical scientists and clinicians alike. Among them, cytomegalovirus (CMV) has garnered exceptional attention for several...
Progress in understanding CMV latency regulation

To properly understand the impact of CMV on immunity, aging, health, and disease, it is essential to address virus biology, which has been elusive and at times frustrating in the case of CMV. After resolution of acute CMV infection, life-long latency is established and maintained at defined organ sites. In this state, the virus can sporadically reactivate in response to external stimuli, and viral progeny can shed from the asymptomatic host. The molecular mechanisms used by CMV to establish latency and persist for the lifespan of the host remain poorly understood. Several groups presented recent progress toward understanding how HCMV regulates virus latency and how the sophisticated use of different viral promoters allows the infected cell to pivot between infectious states of latency and reactivation.

US28, a G protein-coupled receptor homologue encoded by HCMV, was recently described as essential for the establishment and maintenance of latency in CD34+ myeloid progenitor cells (Humby and O’Connor 2015) (Krishna et al. 2017). It has been shown that US28 has multifunctional signaling capacity during lytic infection (reviewed in Krishna, Wills, and Sinclair 2019), but during latency its function is not yet clear (Zhu et al. 2018). John Sinclair (Cambridge, UK) presented new data that US28 is essential to prevent lytic infection in experimentally infected CD14+ monocytes but that it changes its downstream signaling during differentiation of these cells. Notably, US28 activity leads to modulation of a large number of cellular signaling pathways, including the ERK1/2 pathway, which is known to be activated during differentiation of myeloid cells treated with IL-4, GM-CSF, and LPS. Differentiation of infected cells following treatment with IL-4/GM-CSF/LPS led to virus reactivation in a manner which required the 19-bp repeat elements within the major immediate-early promoter (MIEP). ERK signaling and IFI16 repression were needed to prevent MIEP activation in undifferentiated monocytes. J. Sinclair also discussed the potential for utilizing proteins expressed during HCMV latency to design novel therapeutics to target virus from either bone marrow or solid organ transplants (Krishna et al. 2017; Krishna, Wills, and Sinclair 2019). Specifically, as CD34+ progenitor cells in bone marrow were defined as important sites of HCMV latency in humans (Mendelson et al. 1996), it is important to understand the HCMV-specific immune responses in this compartment and whether they differ from those in peripheral organs.

Differentiation of CD34+ myeloid progenitor cells to CD14+ monocytes and further to macrophages and dendritic cells plays an important role in CMV replication and dissemination in the periphery. Whereas the secretome of latently infected CD34+ cells has been shown to recruit suppressive CD4+ T cells (Mason...
et al. 2012), Sarah Jackson (M. Wills group, Cambridge, UK) presented evidence that the secretome of latently infected CD14+ monocytes attracted activating rather than suppressive immune cells, via the chemokine CXCL10. The recruited cells upregulated CXCR3 and were able to induce HCMV reactivation from CD14+ monocyte by activation of the ERK signaling pathway. This observation is pertinent from the standpoint of aging. Specifically, it is well established that there is an increase in inflammation with aging, including inflammatory cytokines IL-6, TNFα, and IL-1β (Franceschi and Campisi 2014), such that there might be a link between the inflammatory environment, activated T cells, and induction CMV reactivation in older people, leading to low-level viremia, which has been observed in older people (Stowe et al. 2007; Furui et al. 2013).

A hallmark of HCMV latency in myeloid cells is suppression of immediate-early (IE) gene expression, while the viral genome is maintained. During productive infection, the viral replication cycle is initiated by activation of the MIEP (Stinski and Isomura 2008). Its activity regulates transcription of the immediate-early (IE) genes IE1 and IE2, which influence the cellular environment for replication and transactivate the downstream early genes of the replication cycle. The MIEP is epigenetically silenced in myeloid cells, resulting in repressive marks on the histones associated with the MIEP – and this is critical for latency in this cell type. The repressive chromatin state at the MIEP is mediated by a number of cellular and viral factors. New insights into the switch from latency to reactivation and the complex regulatory structure within the HCMV major immediate-early (MIE) gene locus were presented by Donna Collins-McMillen (F. Goodrum laboratory, Tucson, AZ, USA) (Collins-McMillen et al. 2019). New viral promoter sequences were identified within intron A of the MIE locus which were not required for productive infection in fibroblasts but seemed to dominate MIE promoter activity during viral reactivation after monocyte differentiation. Transcripts encoding the IE proteins within hematopoietic cells were derived from these alternative promoters and were potently induced when cellular differentiation and viral reactivation were triggered by treatment with phorbol esters in THP-1 monocytic cells. By contrast, MIEP-derived transcripts were detected only sporadically and at low levels, if at all. The intronic promoter regions were important for efficient IE protein expression following differentiation and reactivation in THP-1 cells and were required for efficient reactivation in CD34+ hematopoietic progenitor cells when reactivation was triggered by stimulation with IL-6, G-CSF, and GM-CSF. Although these results do not preclude involvement of the MIEP in reactivation from latency, they demonstrate a mechanism to reinitiate MIE transcription from the latent genome in cells where the MIEP is silenced. Taken together, these findings suggest that the molecular requirements of HCMV reactivation following different stimuli merit further investigation. It is of interest to determine how transcription factor binding sites distal to the intronic promoters, and therefore attributed to control of the MIEP, might affect activation of the intronic promoters.

Thomas Stamminger (Ulm, Germany) presented data suggesting that HCMV US27 also may be a molecular switch utilized by HCMV. The presented data indicate that US27 could activate NF-κB under certain conditions in the cell and that certain cellular proteins regulated this function. In the absence of cellular regulation, US27 activation of NF-κB resulted in the production of chemokines and cytokines from the infected cell, which might foster viral dissemination.

Advances in understanding immune evasion by CMV

CMV employs a complex array of immune evasion mechanisms, which manipulate many immune processes and cell signaling pathways. It is becoming apparent how finely tuned these viral mechanisms are, allowing CMV to avoid some, but not all immune pathways, or acting as molecular switches that “sense” the cellular environment. Multiple investigators described ongoing work dissecting CMV’s immune evasion mechanisms and the ability of the immune system to control the virus, and the phrase “it’s a complicated virus” was heard multiple times in these sessions.

Some of CMV’s complexity was illustrated by work showing the varied array of targets and mechanisms employed by individual CMV proteins. Anne Halenius (Freiburg, Germany) presented data defining two different molecular mechanisms used by HCMV US11 to disrupt expression of HLA-A subfamilies A2 and A3 molecules. Remarkably, these mechanisms involved two different domains of US11 with two different target sites on HLA-A*02 and HLA-A*03 molecules, illustrating one way that HCMV tackles the diversity of MHC molecules that are present in the human population. Elena Muscolino (Wolfram Brune laboratory, Hamburg, Germany) described a 2-step mechanism by
which MCMV M45 induces the destruction of both RIPK1 and NEMO to block cell death and NF-κB activation, respectively. Most interestingly, M45 used a novel strategy that employed autophagy, and some data was presented that a similar strategy may be used by other herpesviruses as well. Finally, Melanie Brinkmann (Braunschweig, Germany) presented data indicating that HCMV UL35 and MCMV M35 utilize different molecular mechanisms to block interferon (IFN) production. Her data further indicated that MCMV m152 blocks IFN production after stimulator of interferon genes (STING) activation, but without limiting STING activation of NF-κB (Stempel et al. 2019). MCMV m152 is better known as an antagonist of cellular MHC-I and retinoic acid-inducible-1 (RAE-1) protein, indicating that this single protein has control over the production of Type I IFN and activation of NF-κB upon infection, as well as the activation of NK cells and T cell recognition of infected cells.

There was also a discussion of how CMV may target restriction factors known to be targeted by other viruses. Renate König (Langen, Germany) described the function of cellular SAMHD1, which is a known restriction factor for HIV. Most of the described work detailed the mechanisms of SAMHD1 inhibition of HIV and the role of phosphorylation in regulating SAMHD1 function. However, SAMHD1 has also been described to restrict the replication of DNA viruses, and evidence was presented that HCMV UL97 may phosphorylate SAMHD1, thereby similarly inhibiting its function (Kim et al. 2019).

Finally, data was presented on the virus’ ability to avoid or shape the immune response to allow dissemination and persistence. Christopher Snyder (Philadelphia, USA) presented work indicating that evasion from T cells and NK cells protected MCMV during dissemination in hematopoietic cells after intranasal infection. These data argued that viral evasion of T cell responses was critical for viral fitness early in infection as the virus moved from sites of entry to sites of shedding. Rafaela Holtappels (Mainz, Germany) showed evidence that MCMV infection induced MCMV-specific T cells with regulatory function that could suppress the proliferation of other T cell populations. Her work may suggest that these regulatory T cells limit the efficacy of antiviral T cell responses, possibly promoting viral persistence. Mark Wills (Cambridge, UK) showed evidence that T cells responding to latency-associated HCMV antigens are more likely to produce IL-10 rather than IFN-γ (Jackson et al. 2017a, b). These results may suggest that HCMV promotes a fundamentally different type of immune response during latency, one that is less capable of clearing the infected cells from the host.

Together, these studies highlighted both the specificity and breadth of CMV’s immune evasion mechanisms, as well as the subtlety of viral immune manipulation. While the presented data represent a significant step forward in our understanding of this complex virus, they also alert us to the enormous diversity of mechanisms employed by CMV to antagonize host immune responses and the intricacy of outcomes after immune recognition of infected cells. Future work will need to both broaden and deepen our understanding of this complicated virus, including investigations of the networks of interactions and the functions of specific viral proteins over time and how they promote viral growth or latency and/or shape the immune response to allow viral persistence.

**Health relevance of CMV: a good or a bad actor?**

The impact of CMV infection on the memory T cell compartment and NK cells is most apparent in cases of comorbidity. While in many of these incidences, infection with CMV may be coincidental to the presence of disease, it cannot be out of hand excluded that the presence of the virus and the effects it has on the immune memory compartment may exacerbate the primary disease. Evidence from large population studies in both Europe and the USA points to an association of CMV seropositivity with increased morbidity and mortality from cardiovascular diseases (Savva et al. 2013) (Simanek et al. 2011; Pachnio et al. 2016; Olson et al. 2013; Firth et al. 2016; Martin-Ruiz et al. 2020). The impact of CMV infection on cardiovascular and related diseases in human studies was discussed by Paul Moss (Birmingham, UK), who described an association between CMV infection, CMV-specific T cells, and detrimental changes to the cardiovascular system. Specifically, he recapped his group’s results that CMV-specific CD4+ T cells express molecules that target vascular endothelium (Pachnio et al. 2016), that CMV infection is associated with increased systolic blood pressure in the elderly (Firth et al. 2016), and that the expansion of cytotoxic CD4+ T cells in CMV infection is associated with increased arterial stiffness in patients with autoimmune anti-neutrophil cytoplasmic antibody (ANCA).
alterations in the total T cell pool, this did not appear to
Berg et al. 2018). Also discussed was the effect of
the quality of the influenza vaccine response (van den
showed that CMV infection did not have an impact on
investigate the effect of CMV coinfection on responses
to influenza vaccination and natural infection with con-
licting results. Debbie van Baarle (Bilthoven, the Nether-
lands) presented work looking at the responses to the
pandemic influenza vaccine delivered in 2009, to inves-
tigate the effect of CMV on influenza responses, in-cluding the influence that
CMV seropositivity had on this baseline, along with interactions with an individual’s genetics, was discussed by Aisha Souquette (P. Thomas lab, Nashville, USA). She suggested that herpesviruses and host genetics can independently and interactively affect baseline immuni-
CMV infection and reactivation also appears to exert an influence on the ability of patients to mount adequate responses to other infections, both bacterial and viral. It has been noted that critically ill patients in intensive care units often reactivate CMV, and this results in increased ventilation times and sometimes increased mortality rates (Kalil and Florescu 2011). Charles Cook (Boston, USA) presented an overview of the role CMV reactiva-
tion plays in aggravating disease in sepsis patients, presenting data from a murine model of sepsis which allows comparison with healthy controls (Mansfield et al. 2016). The work presented showed that the lungs were an important site for CMV reactivation in these patients and that the presence of the virus alters the immune microenvironment, including changes to T cells and neutrophils in particular. A number of studies have investigated the effect of CMV coinfection on responses to influenza vaccination and natural infection with con-
inguine model of congenital CMV, Ilija Brizić (S. Jonjić lab, Rijeka, Croatia), presented data suggesting that NK cell generation in the bone marrow is impaired by CMV infection. In the context of aging and human infection, Luca Pangrazzii (B. Grubeck-Loebenstein group, Innsbruck, Austria) showed that in bone marrow samples obtained from hip replacement operations of CMV seropositive individuals, there is an increased expression of proin-
flammatory T cells (Pangrazzii et al. 2017a, b). Meanwhile, Nico Contreras (J. Nikolich-Zugich lab,
Tucson, USA) presented data that CMV infects murine adipose tissue (potentially for life) and that this is accompanied by a lifelong accumulation of activated virus-specific resident memory CD8+ T cells and by subclinical metabolic dysregulation at the level of glucose elevation and reduced insulin sensitivity (Contreras et al. 2019).

By contrast to these clear functions of CMV as a pathogen, stand the results from Janko Nikolich-Zugich (Tucson, USA), with evidence that latent MCMV infection broadens the TCR clonality of CD8+ T cell responses to listeria challenge (Smithey et al. 2018), continuing on the theme of the prior meeting in this series (Nikolich-Zugich and van Lier 2017; Sansoni et al. 2014) that CMV seems to exercise great care to help its host survive, if not thrive, to ensure prolonged viral life within itself. Finally, Edward Mocarski (Atlanta, USA) discussed the impact of CMV-mediated block in apoptosis and necroptosis via different pathways that almost always involve IE genes (Feng et al. 2019). He concluded that while the benefits for the virus are great, the benefits for the host are also substantial, typically with improved survival in the face of potentially lethal insults such as LPS and TNF shock.

Memory inflation and anti-CMV T cell responses

Memory inflation is a hallmark of CMV infection and, at least in mice (situation may be more nuanced in humans), is characterized by an age-related accrual of antigen-specific cells responding to defined immunodominant viral epitopes that maintain a terminally differentiated CD8+ T cell phenotype over prolonged periods of time. The speakers focused on several inconsistencies in the published literature and offered hypotheses to explain the counterintuitive observations from their own work or those of their colleagues.

As already discussed, M. Wills pointed out that several CD8 T cell responses target CMV epitopes expressed during virus latency in myeloid cells (e.g., LUNA, UL138, US28), rather than epitopes from lytic genes (e.g., pp65, IE1, IE2, gB). This is unexpected, because immune targeting of latently expressed genes should offer little respite to CMV and chase the virus from the organism, yet this is (obviously) not consistent with clinical and experimental observations of CMV persistence in the host. This conundrum might be resolved in light of the Wills group data showing that CD8+ T cells targeting lytic HCMV genes respond predominantly by IFNγ, whereas those that target the latent epitopes show a robust increase in IL-10 secretion (Jackson et al. 2017a, b). Hence, an immunosuppressive response to antigens derived from latency-associated CMV genes would be consistent with virus maintenance, in the face of robust and inflationary CD8 responses.

There is a clear evidence that memory inflation in mice is driven by MCMV-derived antigen expression in non-hematopoietic cells (Torti et al. 2011; Seckert et al. 2011). Where exactly this antigen exposure takes place is still ill defined (Torti et al. 2011; Smith, Turula, and Snyder 2014), but there is evidence that central memory cells specific for inflationary CMV epitopes respond to such antigen encounter on non-hematopoietic cells by clonal expansion and differentiation into effector memory cells. In support of this hypothesis, Annette Oxenius (Zurich, Switzerland) showed that the inflationary pool of MCMV-specific CD8 T cells in peripheral tissues (e.g., the lung) is stable in size and phenotype, yet undergoes a dynamic process of replenishment, as the half-life of this pool of peripheral inflationary CD8+ T cells is ~ 10–12 weeks (Baumann et al. 2018; Snyder et al. 2008). This relatively long half-life of effector-like inflationary CD8+ T cells is promoted by IL-15 signaling in peripheral tissues – mainly provided by non-hematopoietic cells in lung tissue (Baumann et al. 2018). So, what defines the size of the inflationary pool of MCMV-specific CD8+ T cells in presence of a defined latent viral load)? The size of established MCMV-specific memory precursor cells early after infection proved to correlate directly with the ensuing magnitude of the inflationary pool in peripheral tissues. Finally, Oxenius addressed the question whether there are anatomical and functional differences between inflationary CD8+ T cells and tissue-resident (T_Rm) CD8+ T cells in providing protection against a peripheral viral challenge. Based on the presented data, it seems that both populations of memory CD8+ T cells are equally able to provide protection against local viral rechallenge, despite their different prechallenge anatomical locations and despite different strategies of responding to the rechallenge, with T_Rm cells responding by local proliferation and redistribution and inflationary CD8+ T cells mainly responding by local redistribution.
Ramon Arens (Leiden, the Netherlands) showed that in experimental MCMV infection, the size of the virus inoculum determines and predicts the magnitude of inflationary T cell responses. While low-inoculum infection resulted in poor and weaker CD8+ T cell responses and inflation, high-inoculum infection drove a stronger response of T cells that exhibited a more differentiated phenotype (Redeker, Welten, and Arens 2014). Previous experiments in latently infected mice showed no effect of latent MCMV infection on survival after challenge with West Nile, influenza or VSV virus, and a relative decrease in the percentage of responding CD8+ T cells, but no decrease in their absolute counts (Marandu et al. 2015). This is interesting in light of the already mentioned data from Nikolich-Zugich (Tucson, USA) on the broadening of TCR repertoire specificities of CD8+ T cell responses to listeria challenge (Smithey et al. 2018). On the other hand, LCMV challenge of latently infected mice resulted an absolute decrease in CD8+ T cell responses and an increase in virus titers (Mekker et al. 2012). R. Arens further showed that an early challenge of latently infected mice with LCMV results in an increase in CD8+ T cell responses and improved control of the virus, whereas a long-term latency followed by an LCMV challenge in very old mice resulted in poor immune responses and control, but only if the initial MCMV inoculum was high. Mice that were infected with less than 10^7 PFU of MCMV were essentially indistinguishable from non-infected controls (Redeker et al. 2018). Therefore, he argued that not all CMV infections are created equal, because a robust infection, causing a robust latent load (Reddehase et al. 1994), may also contribute to immune deterioration later in life.

Luka Čičin-Šain (Braunschweig, Germany) stirred the pot by claiming that memory inflation is a misnomer. He clarified that inflation does not affect specifically the memory compartment, but rather the pool of terminally differentiated effector cells. Moreover, he showed how even this compartment does not expand slowly over time, but rather spikes in size rapidly upon infection and is maintained at elevated levels for the life of the latently infected host (Čičin-Šain et al. 2012). The kinetics of CD8+ T cell responses to inflationary epitopes is different, because it accrues slowly over time. This difference in kinetics therefore necessarily reflects focusing of the immune response toward T cell populations with higher TCR avidity for epitopes expressed by robust promoters that outcompete the less efficient ones (Farrington et al. 2013; Dekhtiarenko et al. 2013; Borkner et al. 2017). This is also consistent with the unpublished data shown by A. Oxenius, who pointed out that high-avidity TCRs expand more promptly in conditions of memory inflation. Therefore, the avidity of pMHC-TCR binding as well as promoter dominance in generating MHC-binding epitopes are the two likely selection criteria that define the winners of memory inflation, both on the side of viral antigens and on the side of host T cell clones.

**Time of infection and its physiological relevance**

Stipan Jonjić (Rijeka, Croatia) presented interesting data on congenital MCMV infection and viral spread into the brain. He showed that NK cells are the source of brain IFN-γ, which in turn was the cause of cerebellar developmental abnormalities, whereas CD8+ T cells were protective and established brain residency (Brizic et al. 2019). Leonore Pereira (San Francisco, USA) addressed human CMV infection during pregnancy (Pereira 2018). There is 20–40% fetal transmission, of which 5–10% is symptomatic. Of symptomatic cases, 20% are stillborn and 50–80% display developmental anomalies. HCMV replicates in the decidua (presumably in fetal macrophages and DC that infiltrate the trophoblast). About one third of all placentas contained both HCMV and bacteria, and another 5–6% had HCMV and HSV. CMV reactivation in the placenta was seen with coinfection, including with the Zika virus, and neutralizing Ab may be able to block infection, with FcR and ADCC being implicated as effector mechanisms. Stuart Adler (Richmond, USA) presented a review of older data from several laboratories (Reddehase, Arvin, and his own). He concluded that recurrence is a stochastic, focal event and discussed the age at which humans acquire CMV. He concluded that most humans acquire CMV by 2–5 yr of age and that population density matters. These presentations, and the one by C. Snyder discussed above, prompted discussion both at the session and at the round table on what is the most relevant time, dose, and infection route we should use in mice to best mimic natural human infection.

**Remaining questions**

Every meeting of this type is marked by new and exciting advances, as well as with frustrations that CMV has been able to sometimes evade even the most basic
understanding of its biology, particularly on the quantitative side. At the conference round table, the following advances and considerations emerged:

**New and Exciting information**

(i) Antigenic load and localization are critical determinant of the CMV footprint on the immune system in mice. Getting a handle on the same issue in humans remains elusive.

(ii) CMV seems to be able to change evasion strategies depending on the route of infection.

**Information that changes existing paradigms/beliefs**

(i) CMV status in the elderly really does not seem to impact responses to vaccination. Together with the increasing evidence of beneficial impact of CMV upon immunity, this suggests that CMV does not have a clear and measurable negative immune senescence impact.

**Still not known/clear**

(i) Natural (low-dose infection in childhood) infection is generally not well approximated in laboratory models (young adult infection with high dose, often via a non-physiological route). Related to this, we lack understanding of the viral/host factors that limit fast control of CMV infection in nasal mucosa and salivary gland.

(ii) What is the interaction between CMV and cardiovascular diseases (etiology) in humans and why is this not apparent in mice? How do we prove CMV relevance in this case?

(iii) Which cells drive memory inflation (MI) in humans and mice? Are there preferred MI epitopes in humans that drive it? A cautionary point with regard to measures for MI - physical methods (tetramer) vs. functional assays can give different answers.

(iv) Do we really look to the right cell type, when we analyze CD34+ cells in cell culture as state-of-the-art model for latency?

(v) What does the profile of HCMV shedding look like in healthy age-profiled humans? Viremia is almost never detected, saliva/urine shedding was reported, but is it of clinical utility in different patient populations? What happens in individuals with coinfection, or people with chronic diseases without infection?

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**Abbreviations** CMV, cytomegalovirus; HCMV, human CMV; MCMV, murine CMV; MIEP, major immediate-early promoter; STING, stimulator of interferon genes

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