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Impact of CMV upon immune aging: facts and fiction

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Abstract

Aging is accompanied by significant defects in immunity and compromised responses to new, previously unencountered microbial pathogens. Most humans carry several persistent or latent viruses as they age, interacting with the host immune systems for years. In that context maybe the most studied persistent virus is Cytomegalovirus, infamous for its ability to recruit very large T cell responses which increase with age and to simultaneously evade elimination by the immune system. Here we will address how lifelong CMV infection and the immunological burden of its control might affect immune reactivity and health of the host over time.

Introduction

Cytomegaloviruses (CMV) are ubiquitous beta herpes viruses that has co-evolved with its mammalian hosts by acquiring the ability to evade immune clearance. Immune evasion has provided CMV with the ability to persist and remain latent for lifetime in different host tissues, and it is believed that the virus may devote up to 90% of its genome towards that goal. This persistent and latent phenotype is a consequence of unique mechanisms by which the virus and host immune system engage in a back-and-forth dance to restrain viral replication. This may occur at a potentially significant cost for the host as CMV positivity is known to manipulate a vast number of immunological parameters and accounts for >50% of immune system variability in human monozygotic twins [1]. Most humans get infected by age of 40 [2] meaning that most elderly people live with the virus for decades. This lifelong infection has been described as associated with accelerated immunosenescence, increased risk of cardiovascular disease in older adults and all-cause mortality [3]. However, there is also evidence that CMV infection can be beneficial to adult immunocompetent hosts. Murine Cytomegalovirus (MCMV) latently infected mice show increased resistance to bacterial infection [4] while human CMV (HCMV) seropositive young adults exhibit enhanced antibody responses to influenza vaccination [5]. Old MCMV infected mice have even exhibited broader TCR repertoire mobilization in response to a third-party infection,

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Conflict of interest

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with potentially enhanced heterologous immunity [6]. Thus, CMV seropositivity might be beneficial to the host in adulthood, whereas its effects in aging might be difficult to ascribe as net-negative or net-positive. The focus of this review will be on the impact of CMV infection on lifelong immunity under healthy normal aging as well as the impact of CMV on the immune response to secondary infections. We will also discuss the impact of CMV upon T cell clonal diversity and repertoire.

1. CMV and T cell memory inflation

One of the most obvious consequences described during CMV infection has been termed memory inflation, the expansion of antigen specific memory T cells with time, which was first described by Reddehase and colleagues [7] and since expanded upon by others [8–12]. Total HCMV-specific T cell responses in seropositive humans can be enormous, comprising on average 10% of both the CD4 and CD8 memory compartments in blood, and reaching up to 50% in certain individuals [13, 14]. This increase in CMV specific memory T cells leads to an increase in overall number of circulating memory T cells with age, which does not occur during aging in the absence of CMV infection [15].

At the most basic level memory inflation in response to CMV infection occurs as follows; initial lytic systemic viremia occurs over a period of two to four days followed by a significant expansion of CMV specific T cells. Following initial rounds of replication, the viral load is systemically reduced and viral replication restrained by multiple lymphocyte subpopulations such as NK cell, CD8 and CD4 T cells [16] as CMV contracts to defined anatomical locations in mouse and man. Salivary gland and lungs were initially considered the main reservoirs of latency studies but mouse studies detected viral genomes in spleen, bone marrow, heart and kidney upon resolution of primary infection [17, 18]. Latency in multiple organs has also been confirmed in humans samples [19, 20].

The cellular reservoirs of latency following initial phase of viremia have been demonstrated within multiple cell lineages but are believed to involve few cells of any type. These cells include CD34+ hematopoietic cell precursors [21–23], the CD14+ monocyte population [24], vascular endothelial cells, epithelial cells [25] and liver sinusoidal endothelial cells [26] (reviewed in detail in [27]).

After initial infection with MCMV and subsequent contraction of canonical effector T cells, memory inflation continues and is as follows; CD8 T cells possessing a central memory phenotype (CD44hi, CD62Lhi) traffic through the vasculature in search of cognate antigen. Upon recognition of antigen cells trafficking through the vasculature enter what are presumed to be infected tissues and are maintained with expression of CD69, to antagonize S1P1 receptor, and tissue specific markers such as CD103e [28]. Locations where this tissue residency of CMV specific T cells occurs has been described in the spleen and lungs of humans [29, 30], as well as the salivary gland [31–33], lungs [7, 19], liver [34], and most recently in our hands the adipose tissue (Contreras et al. 2019, submitted) of mice.

CMV infection in mouse and man elicits a broad array of T cell responses, both phenotypically and in antigen specificity. During acute infection in the C57BL/6 mouse

model it was very clearly shown that 18 epitopes elicited a response for greater than 50% of CD8 T cell splenocytes [35]. The diversity seen in this response is somewhat maintained in the inflationary phase of T cell responses with splenocytes responding against 5 of 19 peptide pools [12]. Canonical, or non-inflationary, CD8 T cells responding to CMV peptides possess a central memory phenotype, being CD62L+CD44+CD127+CD28+ [7], as briefly described above. By contrast, the non-canonical, inflationary, CD8 T cells possess an effector memory phenotype being CD62−CD44+KLRG1+CD28− as reviewed in [36]. These phenotypes speak to the nature of these cells and their anatomical location, loss of CD62L expression confers the ability of T cells to enter peripheral tissues and leave lymphoid organs. In human infection phenotypic differences are also seen in CMV specific T cells, especially in that of aged adults. Similar markers are seen on inflationary cells in humans as are in mice, being CD28−CCR7−IL7R−. However, human inflationary T cells can re-express CD45RA [37] which is not expressed in mice. HCMV specific CD8 T cells in humans recognize a variety of epitopes and display effector responses to the products of 11 HCMV open reading frames (ORFs) irrespective of the age of the donor [38].

2. CMV and cellular senescence in T lymphocytes

It has been proposed that CMV infection leads to replicative senescence and/or exhaustion of responding T cells. However, unlike in the case of chronic persistent infections, where the virus is continuously present at hundreds of thousands or more of copies/ml (e.g. in the case of HIV, SIV or HCV), CMV does not present the immunocompetent immune system with an onslaught of constant antigenic stimulation. Therefore, there has been no evidence that exhaustion occurs in CMV-specific cells, as measured by the presence of exhaustion markers such as PD-1, PD-1L, LAG, TIM or 2B4. This has been found in murine [39], non-human primate [40] and human models alike [41].

Evidence is ambiguous on the topic whether CMV is a major driver of replicative senescence in T cells. This virus clearly drives a large subset of virus-specific cells into advanced effector/effector memory (Te/em) differentiation. Many of these cells are of Temra type (T effector memory cells reexpressing CD45RA). A cardinal characteristic of all these subsets is that they are poorly proliferative and are highly cytotoxic, with secretion of large amounts of cytokines. Yet, it is difficult to argue that such cells are senescent, because the above is precisely what these cell types are supposed to do – eliminate microbial pathogens via cytotoxicity and cytokine secretion without much proliferation. As discussed before [42], these cells are perfectly equipped to control the virus in the situation where no further expansion of T cells is needed or desired (i.e. when memory inflation has already expanded a large number of virus-specific T cells). In the absence of specific and conclusive demonstration that such cells are somehow harmful to the host in the course of aging, the deleterious role of CMV in promoting their accumulation will remain speculative.

3. Impact of aging upon CMV spread, number of latently infected cells, reactivation frequency and virological vs immunological reactivation

Despite the clarity of the inflationary phenotype in circulation and non-lymphoid tissues we still lack a clear understanding of the impact that CMV has on aging immunity,

susceptibility to third party infections and the impact on T cell repertoire diversity. To discuss these issues, we will first address this regarding CMV tissue spread, latent cells infected, triggers of reactivation and the mechanism driving viral activity.

First, it is quite clear that primary CMV infection progresses in a typical lytic replication cycle manner, resulting in host-wide viremia. However, after control by the immune system, there are very few cells that maintain productive viral genomes [22]. During latent infection, viral genomes were detected in very small percentage (0.004 to 0.01%) of mononuclear cells from granulocyte colony-stimulating factor-mobilized peripheral blood or bone marrow from seropositive donors, at a copy number of 2 to 13 genomes per infected cell [43]. These estimates are very informative, but it is still unclear how the latent viral reservoir differs between individuals or even within different tissues and cell types and how this might be affected by occasional reactivation events. It is also not clear whether and to what extent reactivation events may be localized vs. systemic.

Despite the presumably small number of latently infected cells, massive clinical CMV reactivation can occur in immune compromised humans undergoing transplantation [44] or chemotherapy [45]. Massive reactivation in immunocompromised individuals suggests that the immune system is constantly engaged in keeping CMV infection in check. But is there evidence that minor stressors cause CMV microreactivation in immunocompetent individuals? During latent infection, CMV infected cells can express viral immediate-early (IE) genes without producing viral progeny [46, 47]. This is a source of constant antigenic stimulation thought to drive memory inflation [48]. In the event of microreactivation a small number of infected cells would initiate viral gene transcription at a specific tissue location. This would lead to antigen presentation on MHC molecules on the host-cell surface and in turn activation of CMV specific T cells. Historically, phenotypic and functional profiles of antigen specific CD8 T cells have been used as a proxy for viral activity [49–51], as they have been more sensitive and more easy to detect than any measurement of viral activity.

Effector cytokines produced by activated CMV specific T cells could than have significant local effects without necessarily being increased in the serum [52].

But what kind of stressors could drive such reactivations? In immunocompromised HIV-infected humans massive CMV reactivation can cause viremic disease [53]. But there is also evidence that third party infections in immunocompetent host might trigger local CMV reactivation [54]. Reactivation of latent CMV can be induced by pathogen associated molecular patterns such as lipopolysaccharide (LPS) [55] or by inflammatory cytokines such as TNF-α [56] [57]. Conversely local CMV reactivation might have subtle direct effects on innate immune cells responding to secondary infection and our unpublished results show an increase in expression of several inflammatory cytokines in secondary lymphoid tissues of MCMV latently infected mice responding to bacterial infection. In that sense, the prevailing rodent model to study lifelong CMV infection- specific pathogen free (SPF) mouse, may not be adequate to mimic real life CMV infection.

A groundbreaking study by researchers from University of Minnesota [58] examined immune homeostasis and reactivity in wild-caught mice, mice from pet stores and inbred,

laboratory SPF mice and compared it to neonatal and adult humans. SPF mice lacked effector memory cells and resembled neonatal humans while pet shop and wild mice more faithfully approximated human distribution of T cell subsets (with low levels of naïve and high levels of effector and tissue-resident memory cells). Even more importantly, the authors showed that these traits could be transferred to SPF mice by co-housing them with pet shop mice. We have applied this model to latent MCMV infection, and our preliminary data shows evidence of viral MCMV reactivation in blood and saliva of C57BL/6 mice exposed to dirty microbiome (Coplen et al. in preparation). Thus, the choice of the SPF mouse as a model to study latent CMV infection needs to be revisited. In addition to being almost entirely free of other infections laboratory mouse models also suffer from a lack of natural stressors, physical and psychosocial [59] that could potentially reactivate the virus.

Most studies so far, in both humans and rodents failed to account for variance in viral activity and spread in different individual hosts exposed to different environments [60], an important conceptual point needed to better understand the effect of lifelong CMV infection on immune aging.

4. Impact of CMV on T cell repertoire diversity

We and others have demonstrated an absolute loss of naïve T cells with age in multiple species [15, 61–63], Maintenance of diverse naïve T cell populations depends on homeostatic signals delivered to T cells in secondary lymphoid tissue and possible mechanisms leading to age related loss of naïve T cells with age have been discussed elsewhere [64]. Our research has shown that lifelong CMV infection doesn't further accelerate naïve T cell loss [65], but there are other ways through which CMV could affect T cell repertoire. T cell receptor repertoire diversity of memory T cell pool is estimated to be about 100 fold lower compared to naïve T cell pool [66, 67].

HCMV specific CD8 T cell clones can be greatly expanded in peripheral blood of CMV positive humans with up to 4% of total T cells specific for individual HCMV peptides [68, 69]. Thus an increase in absolute number of oligoclonal CMV specific memory T cells seen in lifelong CMV infection might have a constraining effect on overall T cell repertoire diversity [70], High-throughput Illumina sequencing of unfractionated T cells showed a roughly linear decrease in TCR diversity with age [71] but since this analysis was performed on total T cells from peripheral blood it may simply reflect a decrease in ratio of circulating naïve to memory T cells. Thus, studies using single cell sorted naïve and memory T cells would be more informative. However, such studies are constrained by the large number of TCR sequences (estimates form sampling human PBMCs and mouse splenocytes range from 10^6 to 10^8 unique TCRβ sequences [72]. Historically, analysis of TCR repertoire has thus been dominated by studies looking at elicited antigen-specific T cell response to vaccination or infection. In addition, high-throughput naïve T cell repertoire studies performed to date have only assessed TCRβ chain sequences, as paired TCRαβ analysis of individual cells would further increase complexity. Assessment of naïve T cell repertoire diversity in CMV-seronegative humans over 65 years old compared to those under 35 revealed a relatively modest two to fivefold decrease in diversity.

Repertoire richness contraction with age was even less pronounced for memory CD4 and CD8 T cells [67], CDR3 sequencing of naïve CD4+ and CD8+ T cells from secondary lymphoid tissues obtained from individual organ donors aged 2 months to 73 years also showed a very modest decrease in variability [73]. The Qi et al study was performed on CMV-negative subjects, whereas Thome et al. did not stratify human subjects into CMV seropositive and negative. Therefore, the effect of CMV infection on whole T cell repertoire is still not adequately studied.

By using MHCI tetramer technology to identify pathogen-specific populations in combination with single cell sorting, the TCR repertoire analysis can be simplified to interrogate the TCRβ sequences of individual T cells recruited into an ongoing immune response to a pathogen. Such studies, in aged mice, showed that there is a marked narrowing of the elicited CD8 effector TCRβ repertoire diversity following primary infection with influenza [74, 75] and Herpes Simplex Virus 1 [76]. Narrowing of the elicited effector TCRαβ repertoire could partially be explained by age-related decrease in number of naïve T cells but defects in antigen presentation function by dendritic cells and cytokine production might also contribute to reduced effector T cell expansion or early contraction. Although informative, studies of the elicited T cell repertoire in aged laboratory mice have not been concerned with potential impact persistent viruses like CMV might have on the repertoire of Ag specific response to third party infections. To assess the impact of lifelong CMV infection upon diversity of T cell responses against other infections, we infected young adult male C57BL/6 mice at 3 months with MCMV aged them to 21 months and infected them with *Listeria monocytogenes* (*Lm*) engineered to express chicken ovalbumin (*Lm-OVA*), We then analyzed the T cell receptor (TCR) repertoire of single cell sorted SIINFEKL tetramer positive cells [6]. In adult mice, no single clones dominated the whole repertoire, so that ~12 different clones were mobilized to make up 80% of the repertoire and these clones were shared at a rate of 30-40% between individual mice of the same group. Consistent with our previous results [76], old mice exhibited strong narrowing and homogenization of the elicited repertoire such that 3 highly dominant clones made up 80% of the repertoire. Surprisingly, old mice with lifelong MCMV infection exhibited very diverse TCR repertoires with 21 clonotypes making up 80% of the total repertoire.

This is the first evidence that MCMV infection results in broader T cell recognition of a secondary infection in aged mice, raising the possibility that beneficial effects of CMV seen in vaccinated adult humans [5] may even extend to aging under certain circumstances.

5. Is CMV affecting T cell immune responses in “trans”, by crowding the new immune responses?

Given the expansion of CMV specific cytotoxic T cells in circulation it was hypothesized that this population of cells could interfere with control of secondary infections in an aged immune system [77, 78], Since latent CMV infection recruits very large CD8 T cell responses over time (up to 50% of all CD8 T_M cells), it is possible that this commitment of the immune system resources to control reactivation of latent CMV infection might negatively affect immune responses to other infections. We and others have shown that Ag-

specific T cell responses to viral [79, 80] and bacterial [62] challenge were decreased in the secondary lymphoid organs of MCMV-infected old mice and this decrease inversely correlated to number of CD8 T_{EM} in the blood. However, while Ag specific responses were somewhat reduced in lifelong MCMV infection, this did not translate into increased mortality in response to infectious challenge [62, 79, 81]. Recently we investigated whether reduced T cell immunity in CMV+ old mice may have been caused directly by inhibition of new immune responses by CMV-specific T_{EM} cells (Jergovic et al. 2019, submitted). To test this, we have investigated presence of T_{EM} CMV-specific CD8 T cells in lymph nodes of lifelong-CMV-infected mice and found that these cells do not accumulate at the sites of initiation of new primary immune responses in a significant manner (Jergovic. et al, 2019, submitted). This was similar to the results that human CMV-specific T cells do not accumulate in human tonsils [82] or peripheral lymph nodes (M. R. Betts, personal communication). Moreover, we have sorted T_{EM} CD8 T from lifelong latently infected mice, transferred them into adult MCVM-negative mice and challenged them with another virus (West Nile Virus) or bacteria (Listeria monocytogenes). However, adoptive transfer of T_{EM} cells from MCMV positive mice had no effect on the response to secondary infection in recipient mice implying that the inverse correlation between the number of T_{EM} cells in the blood and magnitude of naïve response to superinfection may not be causal. We therefore conclude that MCMV-specific T_{EM} cells are unlikely to directly interfere with an immune response against superinfection in the secondary lymphoid tissues. This does not come as a surprise as our results, as well as other studies, show that MCMV-specific cells are enriched in blood and bone marrow but do not accumulate in the secondary lymphoid organs of latently infected mice [83] or humans [19, 84].

Conclusion

Overall, while CMV has a massive impact on the T cell compartment and the immune system regardless of age, the initial hypotheses reviewed in [42, 77] that CMV may drive immune senescence, loss of TCR diversity and, directly, or indirectly, reduced responsiveness to third-party antigens/infections have either not been substantiated in critical, incisive studies, or have been rendered less likely by the available evidence. By contrast, data has been emerging on positive impact of CMV upon heterologous immune responsiveness, including that in aging. Therefore, while CMV remains perhaps the strongest modulator of the T cell compartment known, its association with immune aging is more nuanced and more difficult to describe in simple terms, and will have to await the next round of studies precisely correlating, and, hopefully, mechanistically linking, CMV viral activity/reactivation with immune activation, inflammation and aging of the entire organism (including specific organ systems, chiefly cardiovascular).

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References

1. Brodin P, Jojic V, Gao T, et al. (2015) Variation in the human immune system is largely driven by non-heritable influences. Cell 160:37–47. https://doi.Org/10.1016/j.cell.2014.12.020 [PubMed: 25594173]
2. Bate SL, Dollard SC, Cannon MJ (2010) Cytomegalovirus Seroprevalence in the United States: The National Health and Nutrition Examination Surveys, 1988–2004. Clin Infect Dis 50:1439–1447. 10.1086/652438 [PubMed: 20426575]
3. Aiello AE, Chiu YL, Frasca D (2017) How does cytomegalovirus factor into diseases of aging and vaccine responses, and by what mechanisms? GeroScience 39:261–271. https://doi.Org/10.1007/s11357-017-9983-9 [PubMed: 28624868]
4. Barton ES, White DW, Cathelyn JS, et al. (2007) Herpesvirus latency confers symbiotic protection from bacterial infection. Nature 447:326–329. https://doi.Org/10.1038/nature05762 [PubMed: 17507983]
5. Furman D, Jojic V, Sharma S, et al. (2015) Cytomegalovirus infection enhances the immune response to influenza. Sci Transl Med 7:. https://doi.Org/10.1126/scitranslmed.aaa2293
6. Smithey MJ, Venturi V, Davenport MP, et al. (2018) Lifelong CMV infection improves immune defense in old mice by broadening the mobilized TCR repertoire against third-party infection. Proc Natl Acad Sci 115:. 10.1073/pnas.1719451115
7. Holtappels R, Pahl-Seibert M-F, Thomas D, Reddehase MJ (2002) Enrichment of Immediate-Early 1 (m123/pp89) Peptide-Specific CD8 T Cells in a Pulmonary CD62Llo Memory-Effector Cell Pool during Latent Murine Cytomegalovirus Infection of the Lungs. J Virol 74:11495–11503. 10.1128/jvi.74.24.11495-11503.2000
8. Karrer U, Sierro S, Wagner M, et al. (2019) Memory Inflation: Continuous Accumulation of Antiviral CD8 + T Cells Over Time. https://doi.Org/10.4049/jimmunol.170.4.2022
9. Komatsu H, Sierro S, V Cuero A, Klenerman P (2003) Population analysis of antiviral T cell responses using MHC class I-peptide tetramers. Clin Exp Immunol 134:9–12 [PubMed: 12974748]
10. Morabito KM, Ruckwardt TJ, Bar-Haim E, et al. (2018) Memory Inflation Drives Tissue-Resident Memory CD8+ T Cell Maintenance in the Lung After Intranasal Vaccination With Murine Cytomegalovirus. Front Immunol 9:1861 https://doi.Org/10.3389/fimmu.2018.01861 [PubMed: 30154789]
11. Smith CJ, Turula H, Snyder CM (2014) Systemic hematogenous maintenance of memory inflation by MCMV infection. PLoS Pathog 10:e1004233 10.1371/journal.ppat.1004233 [PubMed: 24992722]
12. Munks M, Cho K, Pinto A, et al. (2006) Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection. J Immunol 177:450–458. https://doi.Org/10.4049/jimmunol.177.1.450 [PubMed: 16785542]
13. Sylwester AW, Mitchell BL, Edgar JB, et al. (2005) Broadly targeted human cytomegalovirus-specific CD4 $^{+}$ and CD8 $^{+}$ T cells dominate the memory compartments of exposed subjects. J Exp Med 202:673–685. 10.1084/jem.20050882 [PubMed: 16147978]
14. Klenerman P, Oxenius A (2016) T cell responses to cytomegalovirus. Nat Rev Immunol 16:367 [PubMed: 27108521]
15. Wertheimer AM, Bennett MS, Park B, et al. (2014) Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. J Immunol 192:2143–55. https://doi.Org/10.4049/jimmunol.1301721 [PubMed: 24501199]
16. Polic B, Hengel H, Krmpotic A, et al. (1998) Hierarchical and redundant lymphocyte subset control precludes cytomegalovirus replication during latent infection. J Exp Med 188:1047–1054. https://doi.Org/10.1084/jem.188.6.1047 [PubMed: 9743523]
17. Reddehase MJ, Balthesen M, Rapp M, et al. (1994) The conditions of primary infection define the load of latent viral genome in organs and the risk of recurrent cytomegalovirus disease. J Exp Med 179:185 LP–193. https://doi.Org/10.1084/jem.179.1.185 [PubMed: 8270864]
18. Reddehase MJ, Podlech J, Grzimek NKA (2002) Mouse models of cytomegalovirus latency: overview. J Clin Virol 25 Suppl 2:S23–36

19. Gordon CL, Miron M, Thome JJC, et al. (2017) Tissue reservoirs of antiviral T cell immunity in persistent human CMV infection. J Exp Med jem.20160758 https://doi.Org/10.1084/jem.20160758
20. Goodrum F (2016) Human Cytomegalovirus Latency: Approaching the Gordian Knot. Annu Rev Virol 3:333–357. 10.1146/annurev-virology-110615-042422 [PubMed: 27501258]
21. Mendelson M, Monard S, Sissons P, Sinclair J (1996) Detection of endogenous human cytomegalovirus in CD34+ bone marrow progenitors. J Gen Virol 77:3099–3102. https://doi.Org/10.1099/0022-1317-77-12-3099 [PubMed: 9000102]
22. Hahn G, Jores R, Mocarski ES (2002) Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. Proc Natl Acad Sci 95:3937–3942. https://doi.Org/10.1073/pnas.95.7.3937
23. von Laer D, Meyer-Koenig U, Serr A, et al. (1995) Detection of cytomegalovirus DNA in CD34+ cells from blood and bone marrow. Blood 86:4086 LP–4090 [PubMed: 7492764]
24. Soderberg-Naucler C, Nelson JA, Allan-Yorke J, et al. (2002) Reactivation of Latent Human Cytomegalovirus in CD14+ Monocytes Is Differentiation Dependent. J Virol 75:7543–7554. https://doi.Org/10.1128/jvi.75.16.7543-7554.2001
25. Jarvis MA, Nelson JA (2007) MINIREVIEW Human Cytomegalovirus Tropism for Endothelial Cells : Not All Endothelial Cells Are Created Equal . 81:2095–2101. 10.1128/JVI.01422-06
26. Seckert CK, Kuhnapfel B, Krause C, et al. (2009) Liver Sinusoidal Endothelial Cells Are a Site of Murine Cytomegalovirus Latency and Reactivation. J Virol 83:8869–8884. https://doi.Org/10.1128/jvi.00870-09 [PubMed: 19535440]
27. Reddehase MJ and Lemmermann NAW (2019) Cellular reservoirs of latent cytomegaloviruses. MMIM-D-19-00049R1 in press
28. Thom JT, Oxenius A (2016) Tissue-resident memory T cells in cytomegalovirus infection. Curr Opin Virol 16:63–69. https://doi.Org/10.1016/j.coviro.2016.01.014 [PubMed: 26855038]
29. Turner DL, Bickham KL, Thome JJ, et al. (2014) Lung niches for the generation and maintenance of tissue-resident memory T cells. Mucosal Immunol 7:501–510. https://doi.Org/10.1038/mi.2013.67 [PubMed: 24064670]
30. van Aalderen MC, Remmerswaal EBM, ten Berge IJM, van Lier RAW (2014) Blood and beyond: Properties of circulating and tissue-resident human virus-specific αβ CD8 $^{+}$ T cells. Eur J Immunol 44:934–944. 10.1002/eji.201344269 [PubMed: 24448915]
31. Thom JT, Weber TC, Walton M, Torti N (2015) The Salivary Gland Acts as a Sink for Tissue-Resident Memory CD8 + T Cells, Facilitating Protection from Local Cytomegalovirus Infection. Cell Rep 13:. https://doi.Org/10.1016/j.celrep.2015.09.082
32. Farrell HE, Lawler C, Tan CSE, et al. (2016) Murine Cytomegalovirus Exploits Olfaction To Enter New Hosts. MBio 7:e00251–16. 10.1128/mBio.00251-16 [PubMed: 27118588]
33. Arens R, Wang P, Sidney J, et al. (2008) Cutting edge: murine cytomegalovirus induces a polyfunctional CD4 T cell response. J Immunol 180:6472–6 [PubMed: 18453564]
34. Popovic B, Golemac M, Podlech J, et al. (2017) IL-33/ST2 pathway drives regulatory T cell dependent suppression of liver damage upon cytomegalovirus infection. PLOS Pathog 13:e1006345 https://doi.Org/10.1371/journal.ppat.1006345 [PubMed: 28448566]
35. Munks MW, Gold MC, Zajac AL, et al. (2006) Genome-wide analysis reveals a highly diverse CD8 T cell response to murine cytomegalovirus. J Immunol 176:3760–6. https://doi.Org/10.4049/JIMMUNOL.176.6.3760 [PubMed: 16517745]
36. O'Hara GA, Welten SPM, Klenerman P, Arens R (2012) Memory T cell inflation: Understanding cause and effect. Trends Immunol 33:84–90. https://doi.Org/10.1016/j.it.2011.11.005 [PubMed: 22222196]
37. Derhovanessian E, Larbi A, Pawelec G (2009) Biomarkers of human immunosenescence: impact of Cytomegalovirus infection. Curr Opin Immunol 21:440–445. https://doi.Org/10.1016/j.coi.2009.05.012 [PubMed: 19535233]
38. Jackson SE, Mason GM, Okecha G, et al. (2014) Diverse Specificities, Phenotypes, and Antiviral Activities of Cytomegalovirus-Specific CD8^{+} T Cells. J Virol 88:10894 LP–10908. 10.1128/JVI.01477-14 [PubMed: 25008941]
39. Lang A, Brien JD, Nikolich-Zugich J (2009) Inflation and Long-Term Maintenance of CD8 T Cells Responding to a Latent Herpesvirus Depend upon Establishment of Latency and Presence of Viral

Antigens. J Immunol 183:8077–8087. https://doi.Org/10.4049/jimmunol.0801117 [PubMed: 20007576]

40. Cicin-Sain L, Sylwester AW, Hagen SI, et al. (2011) Cytomegalovirus-Specific T Cell Immunity Is Maintained in Immunosenescent Rhesus Macaques. J Immunol 187:1722–1732. https://doi.Org/10.4049/jimmunol.1100560 [PubMed: 21765018]
41. Vieira Braga FA, Hertoghs KML, van Lier RAW, van Gisbergen KPJM (2015) Molecular characterization of HCMV-specific immune responses: Parallels between CD8+T cells, CD4+T cells, and NK cells. Eur J Immunol 45:2433–2445. https://doi.Org/10.1002/eji.201545495 [PubMed: 26228786]
42. Nikolich-Zugich J (2008) Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. Nat Rev Immunol 8:512–22. 10.1038/nri2318 [PubMed: 18469829]
43. Slobedman B, Mocarski ES (1999) Quantitative analysis of latent human cytomegalovirus. JVirol 73:4806–4812 [PubMed: 10233941]
44. Ljungman P, Hakki M, Boeckh M (2011) Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients. Hematol Oncol Clin North Am 25:151–169. https://doi.Org/10.1016/J.HOC.2010.11.011 [PubMed: 21236396]
45. Kuo CP, Wu CL, Ho HT, et al. (2008) Detection of cytomegalovirus reactivation in cancer patients receiving chemotherapy. Clin Microbiol Infect 14:221–227. https://doi.Org/10.1111/j.1469-0691.2007.01895.x [PubMed: 18070129]
46. Kurz SK, Reddehase MJ (1999) Patchwork pattern of transcriptional reactivation in the lungs indicates sequential checkpoints in the transition from murine cytomegalovirus latency to recurrence. J Virol 73:8612–8622 [PubMed: 10482614]
47. Tervo H-M, Holtappels R, Bohm V, et al. (2006) CD8 T Cells Control Cytomegalovirus Latency by Epitope-Specific Sensing of Transcriptional Reactivation. J Virol 80:10436–10456. 10.1128/jvi.01248-06 [PubMed: 16928768]
48. Seckert CK, Simon CO, GrießI M, et al. (2012) Viral latency drives 'memory inflation': a unifying hypothesis linking two hallmarks of cytomegalovirus infection. Med Microbiol Immunol 201:551–566. 10.1007/s00430-012-0273-y [PubMed: 22991040]
49. Wertheim-van Dillen PME, Yong S-L, ten Berge IJM, et al. (2014) The Size and Phenotype of Virus-Specific T Cell Populations Is Determined by Repetitive Antigenic Stimulation and Environmental Cytokines. J Immunol 172:6107–6114. https://doi.Org/10.4049/jimmunol.172.10.6107
50. Souquette A, Frere J, Smithey M, et al. (2017) A constant companion: immune recognition and response to cytomegalovirus with aging and implications for immune fitness. GeroScience 39:293–303. 10.1007/s11357-017-9982-x [PubMed: 28647907]
51. Thimme R, Appay V, Koschella M, et al. (2005) Increased Expression of the NK Cell Receptor KLRG1 by Virus-Specific CD8 T Cells during Persistent Antigen Stimulation. J Virol 79:12112–12116. https://doi.Org/10.1128/JVI.79.18.12112-12116.2005 [PubMed: 16140789]
52. Nikolich-Žugich J (2018) The twilight of immunity: emerging concepts in aging of the immune system. Nat Immunol 19:10–19. 10.1038/s41590-017-0006-x [PubMed: 29242543]
53. Springer KL, Weinberg A (2004) Cytomegalovirus infection in the era of HAART: Fewer reactivations and more immunity. J Antimicrob Chemother 54:582–586. https://doi.Org/10.1093/jac/dkh396 [PubMed: 15282241]
54. McGuinness BJ, Ferguson RM, Zhang Y, et al. (2002) Intra-abdominal Bacterial Infection Reactivates Latent Pulmonary Cytomegalovirus in Immunocompetent Mice. J Infect Dis 185:1395–1400. https://doi.Org/10.1086/340508 [PubMed: 11992273]
55. Cook CH, Trgovcich J, Zimmerman PD, et al. (2006) Lipopolysaccharide, Tumor Necrosis Factor Alpha, or Interleukin-1 Triggers Reactivation of Latent Cytomegalovirus in Immunocompetent Mice. J Virol 80:9151–9158. 10.1128/JVI.00216-06 [PubMed: 16940526]
56. Simon CO, Seckert CK, Dreis D, et al. (2005) Role for tumor necrosis factor alpha in murine cytomegalovirus transcriptional reactivation in latently infected lungs. J Virol 79:326–340. https://doi.Org/10.1128/JVI.79.1.326 [PubMed: 15596827]

57. Forte E, Swaminathan S, Schroeder MW, et al. (2018) Tumor necrosis factor alpha induces reactivation of human cytomegalovirus independently of myeloid cell differentiation following posttranscriptional establishment of latency. MBio 9:1–15. https://doi.Org/10.1128/mBio.01560-18
58. Beura LK, Hamilton SE, Bi K, et al. (2016) Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature 532:512–6. 10.1038/nature17655 [PubMed: 27096360]
59. Rector JL, Dowd JB, Loerbroks A, et al. (2014) Consistent associations between measures of psychological stress and CMV antibody levels in a large occupational sample. Brain Behav Immun 38:133–141. https://doi.Org/10.1016/j.bbi.2014.01.012 [PubMed: 24472683]
60. Leng SX, Kamil J, Purdy JG, et al. (2017) Recent advances in CMV tropism, latency, and diagnosis during aging. GeroScience 39:251–259. 10.1007/s11357-017-9985-7 [PubMed: 28681110]
61. Vescovini R, Fagnoni FF, Telera AR, et al. (2014) Naïve and memory CD8 T cell pool homeostasis in advanced aging: impact of age and of antigen-specific responses to cytomegalovirus. Age (Dordr) 36:625–40. 10.1007/s11357-013-9594-z [PubMed: 24318918]
62. Smithey MJ, Li G, Venturi V, et al. (2012) Lifelong Persistent Viral Infection Alters the Naive T Cell Pool, Impairing CD8 T Cell Immunity in Late Life. J Immunol 189:5356–5366. https://doi.Org/10.4049/jimmunol.1201867 [PubMed: 23087407]
63. Cicin-Sain L, Messaoudi I, Park B, et al. (2007) Dramatic increase in naive T cell turnover is linked to loss of naive T cells from old primates. Proc Natl Acad Sci U S A 104:19960–5. https://doi.Org/10.1073/pnas.0705905104 [PubMed: 18056811]
64. Thompson HL, Smithey MJ, Surh CD, Nikolich-Žugich J (2017) Functional and homeostatic impact of age-related changes in lymph node stroma. Front Immunol 8:1–8. https://doi.Org/10.3389/fimmu.2017.00706 [PubMed: 28149297]
65. Nikolich-Zugich J, Li G, Uhrlaub JL, et al. (2012) Age-related changes in CD8 T cell homeostasis and immunity to infection. Semin Immunol 24:356–364. https://doi.Org/10.1016/j.smim.2012.04.009 [PubMed: 22554418]
66. Casrouge A, Beaudoing E, Dalle S, et al. (2000) Size Estimate of the αβ TCR Repertoire of Naive Mouse Splenocytes. J Immunol 164:5782 LP–5787. 10.4049/jimmunol.164.11.578267. [PubMed: 10820256]
67. Qi Q, Liu Y, Cheng Y, et al. (2014) Diversity and clonal selection in the human T-cell repertoire. Proc Natl Acad Sci 111:13139 LP–13144. 10.1073/pnas.1409155111 [PubMed: 25157137]
68. Weekes MP, Carmichael AJ, Wills MR, et al. (1999) Human CD28-CD8+ T cells contain greatly expanded functional virus-specific memory CTL clones. J Immunol 162:7569–77. https://doi.Org/10.4049/jimmunol.168.1.29 [PubMed: 10358214]
69. Gillespie GMA, Murphy M, Moss PAH, et al. (2002) Functional Heterogeneity and High Frequencies of Cytomegalovirus-Specific CD8+ T Lymphocytes in Healthy Seropositive Donors. J Virol 74:8140–8150. 10.1128/jvi.74.17.8140-8150.2000
70. Khan N, Shariff N, Cobbold M, et al. (2019) Cytomegalovirus Seropositivity Drives the CD8 T Cell Repertoire Toward Greater Clonality in Healthy Elderly Individuals. 10.4049/jimmunol.169.4.1984
71. Britanova OV, Putintseva EV, Shugay M, et al. (2019) Age-Related Decrease in TCR Repertoire Diversity Measured with Deep and Normalized Sequence Profiling. https://doi.Org/10.4049/jimmunol.1302064
72. Robins HS, Campregher PV, Srivastava SK, et al. (2009) Comprehensive assessment of T-cell receptor β-chain diversity in αβ T cells. Blood 114:4099 LP–4107. https://doi.Org/10.1182/blood-2009-04-217604 [PubMed: 19706884]
73. Thome JJC, Grinshpun B, Kumar BV, et al. (2016) Long-term maintenance of human naïve T cells through in situ homeostasis in lymphoid tissue sites. Sci Immunol 1 :eaah6506 https://doi.Org/10.1126/sciimmunol.aah6506 [PubMed: 28361127]
74. Yager EJ, Ahmed M, Lanzer K, et al. (2008) Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. J Exp Med 205:711–723. 10.1084/jem.20071140 [PubMed: 18332179]

75. Quinn KM, Zaloumis SG, Cukalac T, et al. (2016) Heightened self-reactivity associated with selective survival, but not expansion, of naïve virus-specific CD8+ T cells in aged mice. Proc Natl Acad Sci U S A 113:1333–8. 10.1073/pnas.1525167113 [PubMed: 26787864]
76. Rudd BD, Venturi V, Davenport MP, Nikolich-Zugich J (2011) Evolution of the Antigen-Specific CD8+ TCR Repertoire across the Life Span: Evidence for Clonal Homogenization of the Old TCR Repertoire. J Immunol 186:2056–2064. https://doi.Org/10.4049/jimmunol.1003013 [PubMed: 21248263]
77. Pawelec G, Akbar A, Caruso C, et al. (2004) Is immunosenescence infectious? Trends Immunol 25:406–410. 10.1016/j.it.2004.05.006 [PubMed: 15275638]
78. Tu W, Rao S (2016) Mechanisms underlying T cell immunosenescence: Aging and cytomegalovirus infection. Front Microbiol 7:1–12. 10.3389/fmicb.2016.02111 [PubMed: 26834723]
79. Cicin-Sain L, Brien JD, Uhrlaub JL, et al. (2012) Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. PLoS Pathog 8:e1002849 10.1371/journal.ppat.1002849 [PubMed: 22916012]
80. Mekker A, Tchang VS, Haeberli L, et al. (2012) Immune Senescence: Relative Contributions of Age and Cytomegalovirus Infection. PLoS Pathog 8:. https://doi.Org/10.1371/journal.ppat.1002850
81. Marandu TF, Oduro JD, Borkner L, et al. (2015) Immune protection against virus challenge in aging mice is not affected by latent herpesviral infections. J Virol 89:JVI.01989-15. 10.1128/JVI.01989-15
82. Hislop AD, Buckley CD, Alan B, et al. (2005) Tonsillar homing of Epstein-Barr virus – specific CD8 + T cells and the virus-host balance Find the latest version : Tonsillar homing of Epstein-Barr virus – specific CD8 + T cells and the virus-host balance. 115:2546–2555. https://doi.Org/10.1172/JCI24810.2546
83. Sierro S, Rothkopf R, Klenerman P (2005) Evolution of diverse antiviral CD8+ T cell populations after murine cytomegalovirus infection. Eur J Immunol 35:1113–1123. 10.1002/eji.200425534 [PubMed: 15756645]
84. Jackson SE, Sedikides GX, Okecha G et al. (2019) Generation , maintenance and tissue distribution of T cell responses to Human Cytomegalovirus in lytic and latent infection. MMIM-D-19-00031 in press