JVI Journals.ASM.org

Immune Protection against Virus Challenge in Aging Mice Is Not Affected by Latent Herpesviral Infections

Thomas F. Marandu,[a,c] Jennifer D. Oduro,[a] Lisa Borkner,[a] Iryna Dekhtiarenko,[a] Jennifer L. Uhrlaub,[f] Anja Drabig,[a] Andrea Kröger,[e] Janko Nikolich-Zugich,[d,f] Luka Cicin-Sain[a,b]

Department of Vaccinology and Applied Microbiology, Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany[a]; Institute for Virology, Medical School Hannover, Hanover, Germany[b]; Dar es Salaam University College of Education, Dar es Salaam, Tanzania[c]; Oregon Health and Science University (OHSU), Portland, Oregon, USA[d]; Research Group Innate Immunity and Infection, HZI, Braunschweig, Germany[e]; Department of Immunobiology and the Arizona Center on Aging, University of Arizona College of Medicine, Tucson, Arizona, USA[f]

Latent herpesvirus infections alter immune homeostasis. To understand if this results in aging-related loss of immune protection against emerging infections, we challenged old mice carrying latent mouse cytomegalovirus (CMV), herpes simplex virus 1 (HSV-1), and/or murine gammaherpesvirus 68 (MHV-68) with influenza virus, West Nile virus (WNV), or vesicular stomatitis virus (VSV). We observed no increase in mortality or weight loss compared to results seen with herpesvirus-negative counterparts and a relative but not absolute reduction in CD8 responses to acute infections. Therefore, the presence of herpesviruses does not appear to increase susceptibility to emerging infections in aging patients.

The vast majority of people carry a combination of latent herpesviruses which may cause severe disease and death if they reactivate upon immune suppression (1–4). It has been proposed that human cytomegalovirus (HCMV) infection may be a major environmental factor accelerating immune senescence in older people (5–10).

Studies in the mouse cytomegalovirus (MCMV) model of infection and immunity recapitulated the key aspects of cellular immunity to HCMV (11–14). More recently, we showed that MCMV induces permanent changes of the CD8 T-cell compartment (15), consistent with the changes observed in elderly CMV-seropositive people (5, 16). Furthermore, responses to emerging virus (e.g., lymphocytic choriomeningitis virus [LCMV], influenza virus, or West Nile virus [WNV]) were reduced in aging mice infected with MCMV, although the CD8 response to *Listeria monocytogenes* was not affected by latent MCMV or herpes simplex virus 1 (HSV-1) infection (17). Independent studies have shown that infectious influenza virus titers are elevated in old mice carrying latent MCMV infection (18), albeit immune protection against superinfections was improved in young mice carrying latent virus (19, 20).

Therefore, whether herpesviruses impair T-cell-mediated immune protection against viral infections of older hosts remains unclear. To address this issue, we performed a series of animal experiments at Oregon Health and Science University (OHSU) following IACUC protocol 0724 or at Helmholtz Centre for Infection Research (HZI) in compliance with LAVES permit number 33.9-42502-04-11/0109. DBA2xBALB/c F1 mice (6, 12, 16, or 20 months of age) were intraperitoneally infected with 2×10^5 PFU MCMV or 10^6 PFU Western Reserve vaccinia virus (VACV) or mock infected and were challenged with 50 PFU of WNV at 22 months of age, as detailed previously (15). A nonsignificant increase in mortality over mock controls was observed in MCMV-infected mice ($P = 0.092$) but also in VACV-infected mice ($P = 0.085$) (Fig. 1A). Therefore, within the limits of our experiment, we observed no MCMV-specific effects on immune protection of aging hosts against WNV. To validate this finding, we compared weight loss kinetics upon sublethal influenza virus challenge in 129Sv mice, challenged with 300 50% egg infective doses (EID^{50}) of influenza virus (PR/8/34 strain). Levels of weight loss were not statistically different between mice infected with MCMV and those infected with VACV for 5 months prior to challenge and the mock-infected controls (Fig. 1B); if anything, the level of weight loss was slightly lower in the MCMV group, in line with observations in young mice (19). Similar results were observed in BALB/cxC57BL/6 mice (not shown). Finally, to test the effect of latent infection by representatives of all herpesvirus families, we latently infected DBA2xC57BL/6 F1 mice with HSV-1 strain 17, MCMV (21), or murine gammaherpesvirus 68 (MHV-68) (22) or with all three viruses together and challenged the mice with VSV at 15 months of age, as detailed previously (23). We observed no significant differences in levels of weight loss (Fig. 1C) or survival (Fig. 1D) compared to mock- or VACV-infected mice. Hence, our results argue that herpesviral infections do not impair immune protection against viral challenges.

Importantly, frequencies of CD8 T cells specific for an H-$2K^b$-restricted VSV peptide (RGYVYQGL) were reduced in latently infected mice (Fig. 2A), consistent with our previous report on CD8 T cells responding to WNV or influenza virus in latent MCMV infection (15). However, the absolute counts of RGYVYQGL-specific CD8 T cells were similar in all groups (Fig. 2B). Similar effects were observed by measuring functional cytokine responses (data not shown) and in DBA/2xC57BL/6 F1 mice.

Received 11 August 2015 **Accepted** 26 August 2015

Accepted manuscript posted online 2 September 2015

Citation Marandu TF, Oduro JD, Borkner L, Dekhtiarenko I, Uhrlaub J, Drabig A, Kröger A, Nikolich-Zugich J, Cicin-Sain L. 2015. Immune protection against virus challenge in aging mice is not affected by latent herpesviral infections. J Virol 89:11715–11717. doi:10.1128/JVI.01989-15.

Editor: K. Frueh

Address correspondence to Luka Cicin-Sain, luka.cicin-sain@helmholtz-hzi.de.

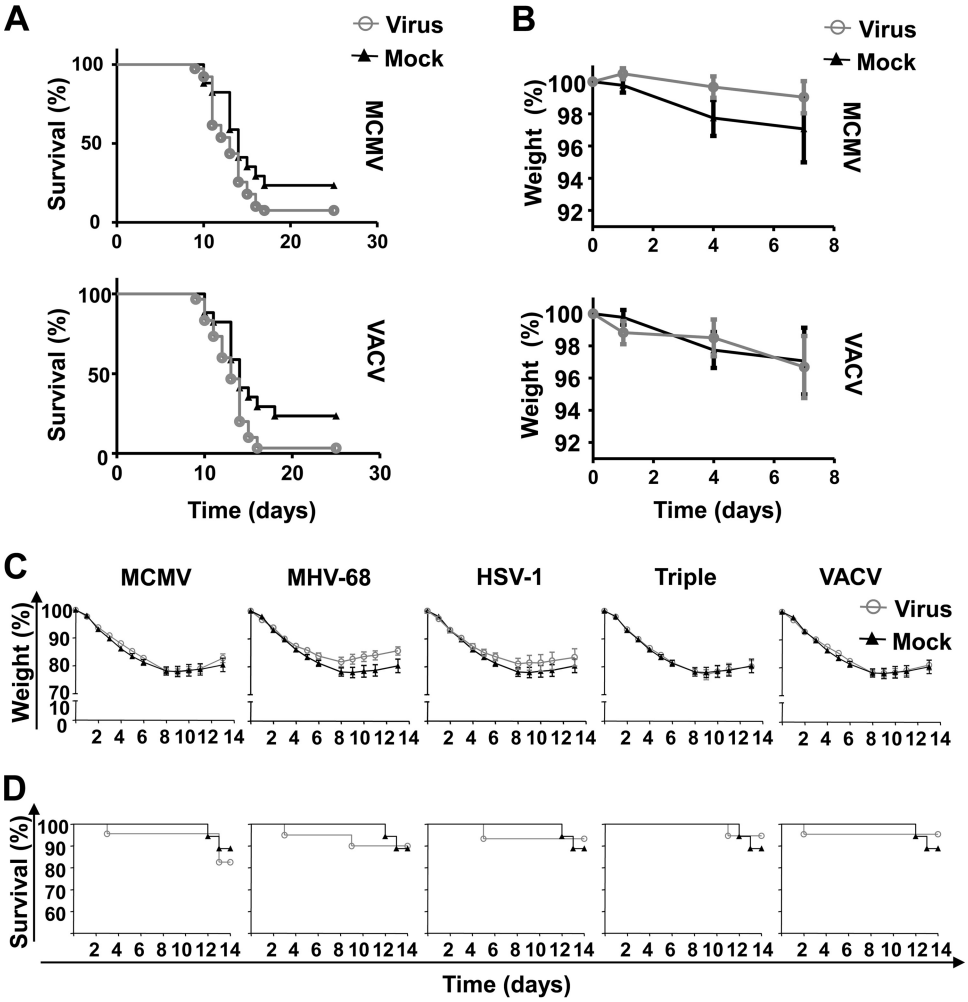

FIG 1 Herpesvirus infections do not impair immune protection. (A) DBA2xBALB/c F1 mice were mock infected ($n = 17$) or infected with MCMV ($n = 39$) or VACV ($n = 31$) for 2 to 16 months prior to West Nile virus challenge. Survival rates upon challenge are shown. (B) Year-old 129Sv mice were mock infected ($n = 5$) or infected with MCMV ($n = 9$) or VACV ($n = 10$) and challenged with flu at 17 months of age. Weights on indicated days are displayed as group averages (± standard error) relative to the weight at challenge. (C and D) DBA2xC57B/6 mice were mock infected ($n = 18$) or infected with MCMV ($n = 23$), HSV-1 ($n = 15$), MHV-68 ($n = 20$), all three herpesviruses (Triple; $n = 19$), or VACV ($n = 22$) for a minimum of 9 months prior to challenge with VSV at the age of 15 months. (C) Weight loss was monitored daily and is displayed as average (± standard deviation) weight relative to the weight at challenge. (D) Survival of mice latently infected with the indicated viruses upon VSV challenge. Mock controls were injected with phosphate-buffered saline (PBS) when young but were VSV challenged in parallel at 15 months of age.

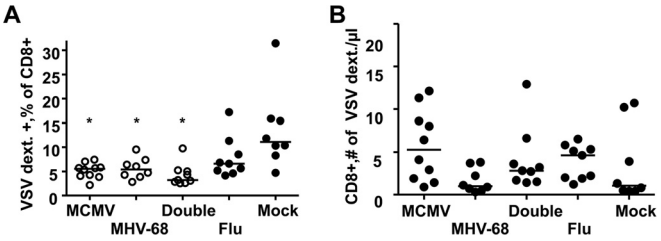

FIG 2 Reduced frequencies but maintained counts of CD8 T cells responding to VSV. 129SVxBALB/c F1 mice infected with MCMV, MHV-68, a combination of MCMV and MHV-68 (Double), or influenza virus (Flu) or mock infected at 9 months of age were challenged with VSV 9 months later. At 7 days postchallenge, blood cells were stained with a previously described antibody panel (15) or allophycocyanin (APC) dextramers (dext. +) against the VSV peptide RGYVYQGL and analyzed by flow cytometry. (A) Percentages of CD8 T cells responding to RGYVYQGL. (B) Absolute counts of CD8 T cells responding to RGYVYQGL as established in an Accuri cell counter. Each dot represents a mouse; lines represent group medians. Infected groups were compared to mock controls by the Kruskal-Wallis test followed by Dunn's postanalysis. Empty dots and asterisks denote groups with $P < 0.05$.

Therefore, the VSV response was not reduced in absolute terms, but only relatively, likely due to the doubling of the blood CD8 compartment in latent MCMV infection (15 and not shown). In conclusion, our data strongly argue that herpesvirus infections (including MCMV infections) do not exert massive adverse effects upon functional immune responses and protection against emerging pathogens in aging patients, alleviating concerns about the MCMV-induced decline in the immune response in aging populations.

ACKNOWLEDGMENTS

We thank Byung Park, Julia Holzki, Franziska Dag, Klaus Schughart, Paulina Blazejewska, Adrien Weingärtner, Linda Ebermann, and Rosaely Casalegno for expert technical advice and Ilona Bretag, Ayse Barut, Inge Hollatz-Rangosch, Nils Hapke, Oliver Hartmann, and Jennifer Wolf for excellent technical support.

This work was supported by the Helmholtz Association through grants NG-VH-638 and VI-VH-424 to L.C.-S.

REFERENCES

1. **Goedert JJ, Cote TR, Virgo P, Scoppa SM, Kingma DW, Gail MH, Jaffe ES, Biggar RJ.** 1998. Spectrum of AIDS-associated malignant disorders. Lancet **351:**1833–1839. http://dx.doi.org/10.1016/S0140-6736(97)09028-4.
2. **Ocheni S, Kroeger N, Zabelina T, Sobottka I, Ayuk F, Wolschke C, Muth A, Lellek H, Petersen L, Erttmann R, Kabisch H, Zander AR, Bacher U.** 2008. EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. Bone Marrow Transplant **42:**181–186. http://dx.doi.org/10.1038/bmt.2008.150.
3. **Kameyama T, Sujaku C, Yamamoto S, Hwang CB, Shillitoe EJ.** 1988. Shedding of herpes simplex virus type 1 into saliva. J Oral Pathol **17:**478–481. http://dx.doi.org/10.1111/j.1600-0714.1988.tb01320.x.
4. **Russell MY, Palmer A, Michaels MG.** 2011. Cytomegalovirus infection in pediatric immunocompromised hosts. Infect Disord Drug Targets **11:**437–448. http://dx.doi.org/10.2174/187152611797636686.
5. **Olsson J, Wikby A, Johansson B, Lofgren S, Nilsson BO, Ferguson FG.** 2000. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. Mech Ageing Dev **121:**187–201.
6. **Pawelec G, Akbar A, Caruso C, Solana R, Grubeck-Loebenstein B, Wikby A.** 2005. Human immunosenescence: is it infectious? Immunol Rev **205:**257–268. http://dx.doi.org/10.1111/j.0105-2896.2005.00271.x.
7. **Akbar AN, Fletcher JM.** 2005. Memory T cell homeostasis and senescence during aging. Curr Opin Immunol **17:**480–485. http://dx.doi.org/10.1016/j.coi.2005.07.019.
8. **Le Saux S, Weyand CM, Goronzy JJ.** 2012. Mechanisms of immunosenescence: lessons from models of accelerated immune aging. Ann N Y Acad Sci **1247:**69–82. http://dx.doi.org/10.1111/j.1749-6632.2011.06297.x.
9. **Koch S, Larbi A, Ozcelik D, Solana R, Gouttefangeas C, Attig S, Wikby A, Strindhall J, Franceschi C, Pawelec G.** 2007. Cytomegalovirus infection: a driving force in human T cell immunosenescence. Ann N Y Acad Sci **1114:**23–35. http://dx.doi.org/10.1196/annals.1396.043.
10. **Pawelec G, Ouyang Q, Colonna-Romano G, Candore G, Lio D, Caruso C.** 2002. Is human immunosenescence clinically relevant? Looking for 'immunological risk phenotypes'. Trends Immunol **23:**330–332. http://dx.doi.org/10.1016/S1471-4906(02)02255-X.
11. **Munks MW, Cho KS, Pinto AK, Sierro S, Klenerman P, Hill AB.** 2006. Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection. J Immunol **177:**450–458. http://dx.doi.org/10.4049/jimmunol.177.1.450.
12. **Karrer U, Sierro S, Wagner M, Oxenius A, Hengel H, Koszinowski UH, Phillips RE, Klenerman P.** 2003. Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. J Immunol **170:**2022–2029. http://dx.doi.org/10.4049/jimmunol.170.4.2022.
13. **Holtappels R, Pahl-Seibert MF, Thomas D, Reddehase MJ.** 2000. Enrichment of immediate-early 1 (m123/pp89) peptide-specific CD8 T cells in a pulmonary CD62L(lo) memory-effector cell pool during latent murine cytomegalovirus infection of the lungs. J Virol **74:**11495–11503. http://dx.doi.org/10.1128/JVI.74.24.11495-11503.2000.
14. **Komatsu H, Sierro S, Cuero AV, Klenerman P.** 2003. Population analysis of antiviral T cell responses using MHC class I-peptide tetramers. Clin Exp Immunol **134:**9–12. http://dx.doi.org/10.1046/j.1365-2249.2003.02266.x.
15. **Cicin-Sain L, Brien JD, Uhrlaub JL, Drabig A, Marandu TF, Nikolich-Zugich J.** 2012. Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. PLoS Pathog **8:**e1002849. http://dx.doi.org/10.1371/journal.ppat.1002849.
16. **Wikby A, Johansson B, Olsson J, Lofgren S, Nilsson BO, Ferguson F.** 2002. Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. Exp Gerontol **37:**445–453. http://dx.doi.org/10.1016/S0531-5565(01)00212-1.
17. **Smithey MJ, Li G, Venturi V, Davenport MP, Nikolich-Zugich J.** 2012. Lifelong persistent viral infection alters the naive T cell pool, impairing CD8 T cell immunity in late life. J Immunol http://dx.doi.org/10.4049/jimmunol.1201867.
18. **Beswick M, Pachnio A, Lauder SN, Sweet C, Moss PA.** 2013. Antiviral therapy can reverse the development of immune senescence in elderly mice with latent cytomegalovirus infection. J Virol **87:**779–789. http://dx.doi.org/10.1128/JVI.02427-12.
19. **Furman D, Jojic V, Sharma S, Shen-Orr SS, CJLA, Onengut-Gumuscu S, Kidd BA, Maecker HT, Concannon P, Dekker CL, Thomas PG, Davis MM.** 2015. Cytomegalovirus infection enhances the immune response to influenza. Sci Transl Med **7:**281ra43. http://dx.doi.org/10.1126/scitranslmed.aaa2293.
20. **Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, Virgin HW, IV.** 2007. Herpesvirus latency confers symbiotic protection from bacterial infection. Nature **447:**326–329. http://dx.doi.org/10.1038/nature05762.
21. **Jordan S, Krause J, Prager A, Mitrovic M, Jonjic S, Koszinowski UH, Adler B.** 2011. Virus progeny of murine cytomegalovirus bacterial artificial chromosome pSM3fr show reduced growth in salivary glands due to a fixed mutation of MCK-2. J Virol **85:**10346–10353. http://dx.doi.org/10.1128/JVI.00545-11.
22. **Adler H, Messerle M, Koszinowski UH.** 2001. Virus reconstituted from infectious bacterial artificial chromosome (BAC)-cloned murine gammaherpesvirus 68 acquires wild-type properties in vivo only after excision of BAC vector sequences. J Virol **75:**5692–5696. http://dx.doi.org/10.1128/JVI.75.12.5692-5696.2001.
23. **Marandu TF, Finsterbusch K, Kroger A, Cicin-Sain L.** 2014. Mouse CMV infection delays antibody class switch upon an unrelated virus challenge. Exp Gerontol **54:**101–108. http://dx.doi.org/10.1016/j.exger.2014.01.017.