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Drabig, A., Kröger, A., Nikolich-Zugich, J., Cicin-Sain, L.**

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

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

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

**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. DBA/2xBALB/c F1 mice (6, 12, 16, or 20 months of age) were intraperitoneally infected with  $2 \times 10^5$  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<sub>50</sub>) 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 DBA/2xC57BL/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<sup>b</sup>-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.

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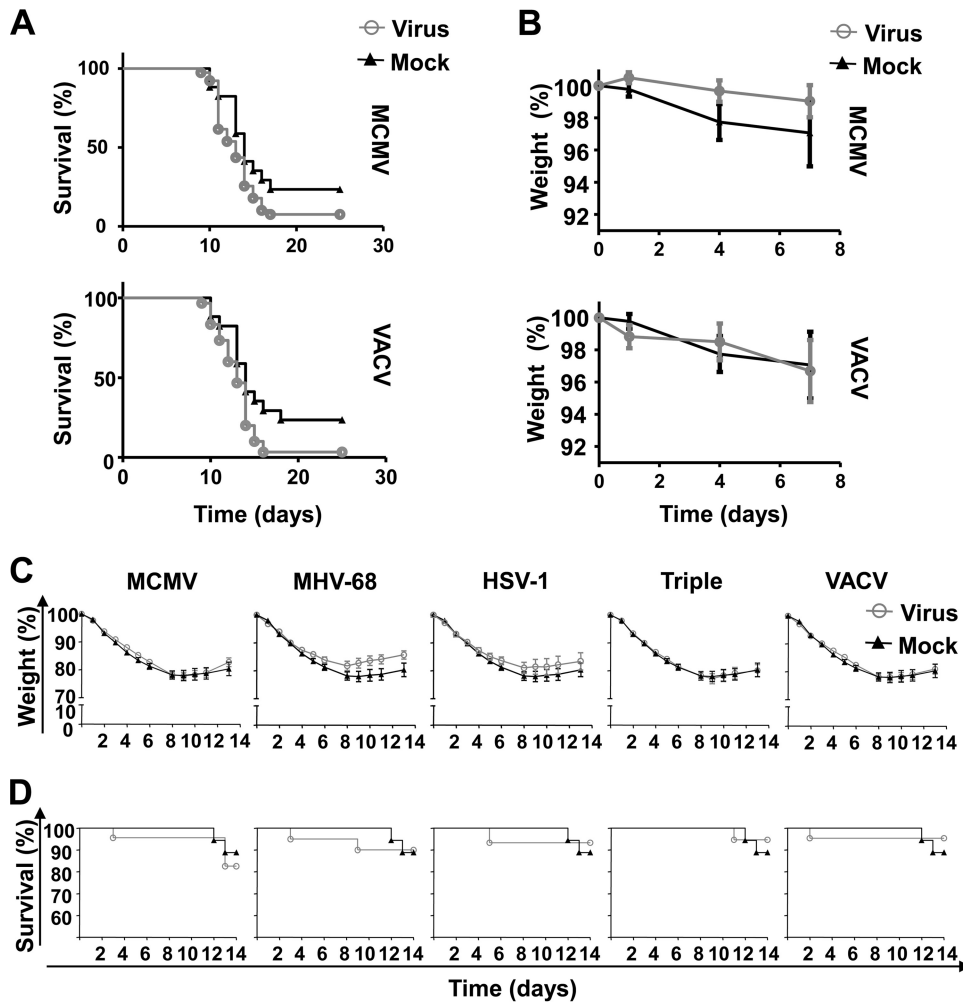
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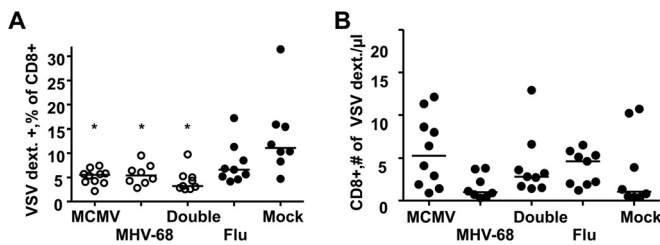
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Address correspondence to Luka Cicin-Sain, luka.cicin-sain@helmholtz-hzi.de.

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**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 ( $\pm$  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 ( $\pm$  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.

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