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Immune protection against virus challenge in aging mice is not affected by latent herpesviral infections

Running title: Herpesviruses do not impair immune protection in aging

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Abstract

Latent herpesvirus infections alter the immune homeostasis. To understand if this results in aging-related loss of immune protection against emerging infections, we challenged old mice carrying latent mouse CMV, HSV-1 and/or MHV-68 with Influenza, WNV or VSV. We observed no increase in mortality or weight-loss over herpesvirus-negative counterparts and a relative, but no absolute reduction in CD8 responses against acute infections. Therefore, herpesviruses do not appear to increase susceptibility to emerging infections in aging.

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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 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 the cellular immunity to HCMV (11-14). More recently we showed that MCMV induces permanent changes of the CD8 T-cell compartment (15), consistent with those observed in old CMV-seropositive people (5, 16). Furthermore, responses to the emerging viruses [e.g. LCMV, influenza 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 type-1 (HSV-1) infection (17). Independent studies have shown that infectious influenza titers are elevated in old mice carrying latent MCMV infection(18), albeit the 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 question, we performed a series of animal experiments at OHSU following the IACUC protocol #0724 or at HZI in compliance with the LAVES permit number 33.9-42502-04-11/0109. Six, 12, 16, or 20 months old DBA2xBALB/c F1 mice were intraperitoneally infected with $2x10^5$PFU MCMV, 10^6PFU Western Reserve Vaccinia virus (VACV) or mock-infected, and challenged with 50 PFU of WNV at 22 months of age, as detailed previously (15). A non-significant increase in mortality over mock controls was observed in MCMV

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(p=0.092) but also in VACV-infected (p=0.085) mice (Fig. 1A). Therefore, within the limit of our experiment, we observed no MCMV-specific effects on immune protection of aging hosts against WNV. To validate this finding, we compared the weight loss kinetic upon sub-lethal influenza challenge in 129Sv mice, challenged with 300 EID^{50} of influenza PR/8/34 strain. Weight loss was not statistically different between mice infected with MCMV or VACV for 5 months prior to challenge and the mock-infected controls (Fig. 1B;); if anything, it was slightly less pronounced 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), MHV-68 (22), or all three viruses together and challenged them with VSV at 15 months of age, as detailed previously (23). We observed no significant differences in weight loss (Fig. 1C) or in survival (Fig. 1D) as 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 in latent MCMV infection (15). However, the absolute count of RGYVYQGL-specific CD8 T-cells was 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. 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).

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In conclusion, our data strongly argue that herpesvirus infections (including MCMV) do not exert massive adverse effects upon functional immune responses and protection against emerging pathogens in aging, alleviating concerns about MCMV-induced immune decline in aging.

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157 **Figure Legends**

158 **Figure 1: Herpesvirus infections do not impair immune protection.** (A)
159 DBA2xBALB/c F1 mice were mock-infected (n=17), or infected with MCMV (n=39) or
160 VACV (n=31) for 2-16 months prior to West-Nile virus challenge. Survival upon
161 challenge is shown. (B) Year-old 129Sv mice were mock-infected (n=5), infected with
162 MCMV (n=9) or VACV (n=10), and challenged with flu at 17 months of age. Weights on
163 indicated days are displayed as group averages (+/- standard error) relative to the
164 weight at challenge. (C, D) DBA2xC57B/6 mice were mock-infected (n=18) or infected
165 with MCMV (n=23), HSV-1 (n=15), MHV-68 (n=20), all three herpesviruses (Triple,
166 n=19) or VACV (n=22) for a minimum of 9 months prior to challenge with VSV at the
167 age of 15 months. (C) Weight loss was monitored daily and is displayed as average (+/-
168 Standard deviation) of the weight relative to the weight at challenge. (D) Survival of
169 mice latently infected with indicated viruses upon VSV challenge. Mock controls were
170 injected with PBS when young, but VSV challenged in parallel at 15 months of age.
171 **Figure 2: Reduced frequency but maintained counts of CD8 T-cells responding to**
172 **VSV.** 129SvXBALB/c F1 mice, infected with MCMV, MHV-68, a combination of MCMV
173 and MHV-68 (double), Flu or mock infected at 9 months of age were challenged with
174 VSV 9 months later. 7 days post-challenge, blood cells were stained with a previously
175 described antibody panel (15), APC-dextramers against the VSV peptide RGYVYQGL,
176 and analyzed by flow cytometry. (A) Percentages of CD8 T-cells responding to
177 RGYVYQGL. (B) Absolute counts of CD8 T-cells responding to RGYVYQGL as
178 established in an Accuri cell counter. Each dot represents a mouse; lines represent
179 group medians. Infected groups were compared to mock controls by the Kruskal-Wallis

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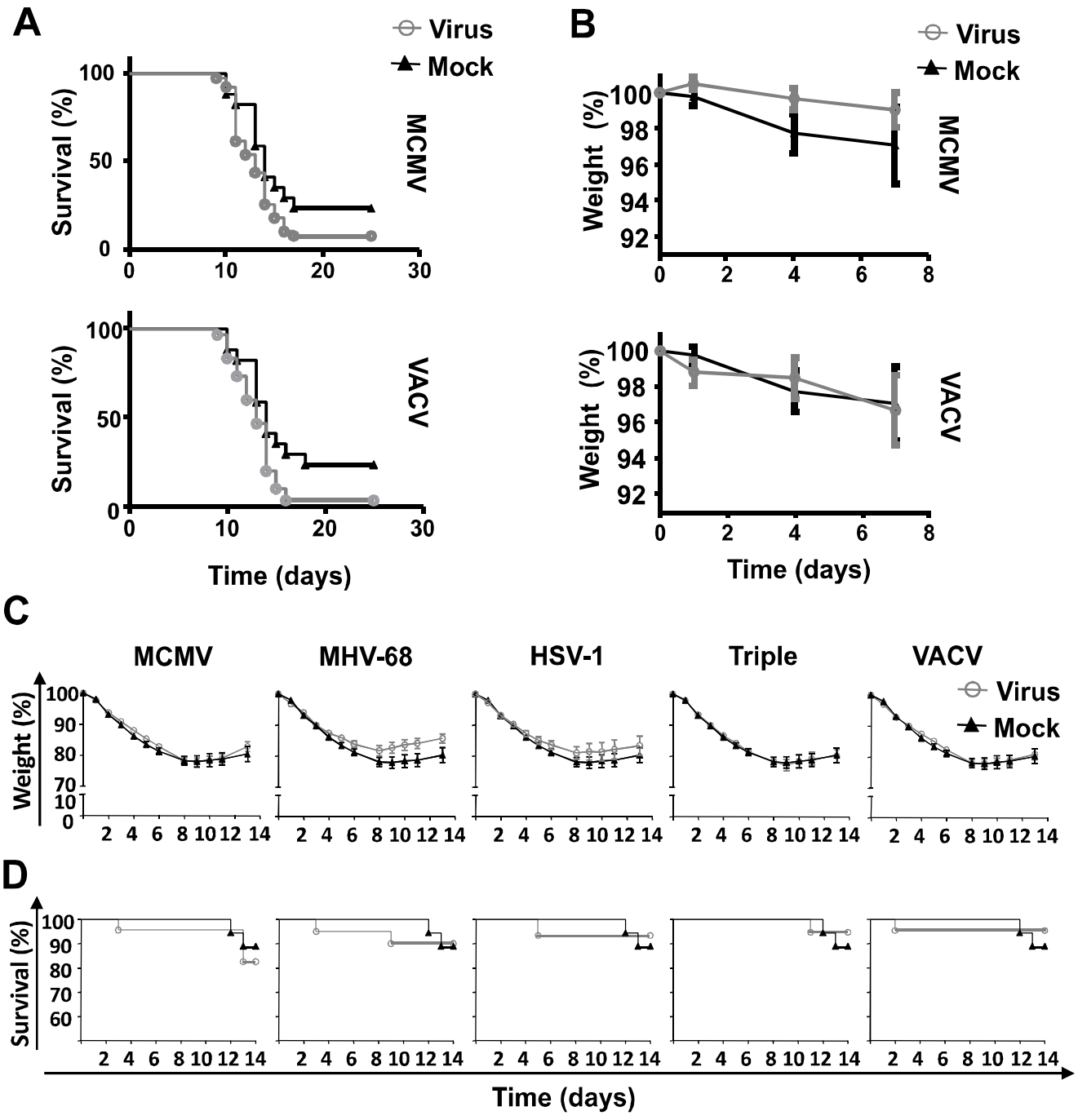
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test followed by Dunn's post-analysis. Empty dots and asterisks denote groups with $p<0.05$.

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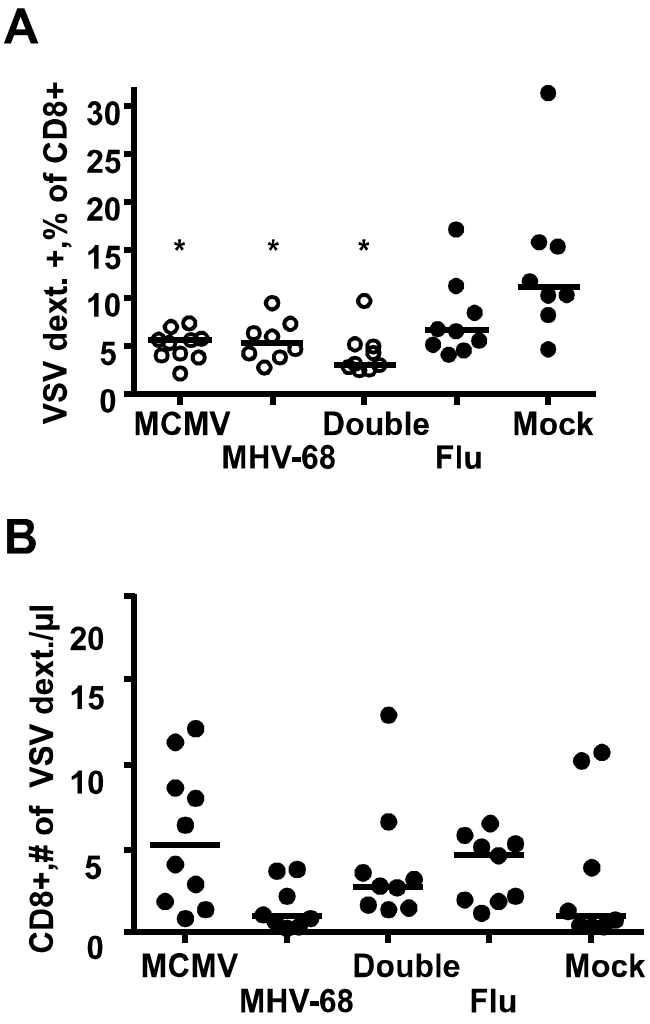
Figure 1



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Figure 2



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