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Letter to the Editor

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To the Editor

Murine gammaherpesvirus 68 (MHV-68) escapes from NK-cell-mediated immune surveillance by a CEACAM1-mediated immune evasion mechanism

In a paper published in the *European Journal of Immunology*, Usherwood et al. [1] showed that the control of murine gammaherpesvirus 68 (MHV-68), a virus that is frequently used to investigate gammaherpesvirus pathogenesis in a small animal model [2], is apparently independent of NK cells [1]. This finding, which was also confirmed by others [3], was very surprising to the community since NK cells have been shown to be critical for the defense against several herpesviruses including the human gammaherpesvirus Epstein–Barr virus (EBV; reviewed in [4]). It was even more surprising because the MHV-68 mK3 protein has been shown to downregulate MHC class I [5], which would be expected to activate NK cells, in particular since MHV-68 encodes no known MHC class I decoy receptors or NK inhibitory molecules [2]. A potential explanation would be that MHV-68 somehow evades NK-cell-mediated cytotoxicity by a hitherto unknown mechanism.

We recently described that infection with MHV-68 upregulates CEACAM1 on alveolar epithelial cells in lungs of WT mice (see Fig. 7 in [6]). CEACAM1 is an inhibitory receptor expressed by a variety of cells including NK cells [7]. Interestingly, compared to WT mice, Ceacam1$^{-/-}$ mice showed enhanced viral clearance in the lung (see Fig. 1 in [6]). For human NK cells, it has been shown in vitro that homophilic CEACAM1 interactions can inhibit NK-cell cytotoxicity against tumor cells [8, 9]. Thus, we hypothesized that a similar mechanism might be operative in vivo during MHV-68 infection: in WT mice, the upregulation of CEACAM1 blocks NK-cell cytotoxicity, while the absence of CEACAM1 in Ceacam1$^{-/-}$ mice releases the block in NK-cell cytotoxicity, resulting in the observed enhanced viral clearance. To prove our hypothesis, we depleted NK cells in Ceacam1$^{-/-}$ mice and analyzed the effect of this depletion on virus titers in the lungs 6 days after infection, the time point at which viral titers usually reach a peak. As shown in Figure 1, virus titers were significantly higher in the lungs of NK-cell-depleted Ceacam1$^{-/-}$ mice when compared with those of NK-cell-nondepleted Ceacam1$^{-/-}$ mice. Consistent with previous reports [1, 3], we did not observe any effect on virus titers in the lungs of MHV-68-infected WT mice when NK cells were depleted (4.12 ± 0.18 log PFU/lung and 4.15 ± 0.15 log PFU/lung in nondepleted and NK-cell-depleted WT mice, respectively; mean ± SD; $n = 3$), strongly suggesting that the effect of NK cells on acute MHV-68 replication is dependent on the presence or absence of CEACAM1. If the presence of CEACAM1 would indeed affect NK-cell killing, we assumed that YAC-1 cells, classical targets of mouse NK cells, should become more resistant to killing by NK cells when engineered to express a ligand for CEACAM1. This ligand could be CEACAM1 itself, due to its homophilic adhesion property. To test this assumption, we generated YAC-1 cells stably expressing either CEACAM1–2s

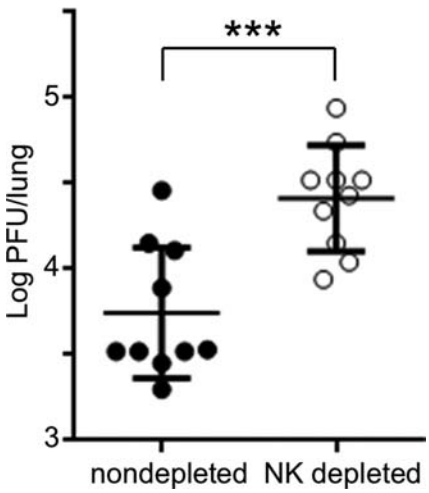

Figure 1. Depletion of NK cells results in higher virus titers in the lungs of MHV-68-infected Ceacam1$^{-/-}$ mice. To deplete NK cells, mice were injected i.p. with 250 μg of anti-NK1.1 monoclonal antibody (PK136) at days −2 and +3 relative to the infection. Mice were i.n. infected with 5×10^4 PFU, and lytic replication was analyzed 6 days after infection by determining virus titers in lung homogenates by standard plaque assay. Each symbol represents an individual mouse and the bars represent the mean. The data are compiled from two independent experiments. ***$p \leq 0.001$ (unpaired, two-tailed Student's *t*-test).

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or CEACAM1–4L (Supporting Information Fig. 1A). NK-cell activation, as determined by degranulation assay, was not influenced by CEACAM1 expression on YAC-1 cells (Supporting Information Fig. 1B). However, CEACAM1-expressing YAC-1 cells (Supporting Information Fig. 1C and 1D) showed increased resistance against NK-cell killing, independent of the expressed CEACAM1 isoform. Since cytotoxicity but not degranulation of NK cells was inhibited by CEACAM1 expression on YAC-1 cells, we speculate that the cytotoxic activity in this system is not mediated by cytotoxic granule release but rather via the Fas-FasL or TNF-TNFR pathway. Importantly, while CEACAM1-expressing YAC-1 cells were more resistant to killing by WT NK cells, both WT YAC-1 cells and CEACAM1-expressing YAC-1 cells were equally sensitive to killing by Ceacam1$^{-/-}$ NK cells (Supporting Information Fig. 1D), demonstrating that for effective NK-cell killing/cytotoxicity, CEACAM1 needs to be present on both target and effector cells.

In summary, we show here that in Ceacam1$^{-/-}$ mice, NK cells significantly contribute to the control of acute MHV-68 infection. Furthermore, we demonstrate that YAC-1 target cells, engineered to express different CEACAM1 isoforms, become more resistant to killing by NK cells, providing a mechanistic explanation for the observed phenomenon. We want to note, however, that YAC-1 cells expressing CEACAM1 are only a substitute for physiological target cells, and that this system has limitations as it is only indicative. Clearly, better models need to be established to test CEACAM1-mediated inhibition of NK-cell function by MHV-68-infected cells. While a CEACAM1-mediated immune escape mechanism was previously demonstrated in vivo for tumor cells [10], ours is the first report showing that a CEACAM1-mediated immune escape mechanism has also an impact on viral infections in vivo. Thus, our novel findings resolve the paradox that in contrast to other gammaherpesvirus infections, the control of MHV-68 infection appears to be independent of NK cells.

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