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B cells latently infected with murine gammaherpesvirus 68 (MHV-68) are present in the mouse thymus – a step towards immune evasion?

More than 95% of all adults are infected without symptoms with the human gammaherpesvirus Epstein-Barr virus (EBV) for their lifetime. EBV-specific T-cells are essential in controlling EBV infection in healthy individuals but they are insufficient in eradicating the life-long viral infection. As a consequence, EBV infection is associated with lymphomas and carcinomas if immunity is impaired [1]. To maintain its infection despite a vigorous immune response, EBV has evolved multiple immunoevasive strategies [2], but how EBV avoids elimination by the immune system is not completely understood. EBV-specific T-cells constitute a considerable fraction of the memory T-cell repertoire of latently infected humans, but they are directed against a surprisingly small set of EBV epitopes [3]. This is remarkable since EBV, when it infects non-dividing B-cells, transiently expresses many lytic genes prior to the establishment of the latent infection in which only few viral genes are expressed [4]. It thus appears as if T-cells are absent that should recognize many viral antigens which are clearly expressed in infected cells *in vivo* [3]. This limited spectrum of anti-viral T-cell responses and an apparent bias in the repertoire cannot be solely explained by EBV's known immunoevasive strategies.

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We have recently shown that thymic B-cells bear the potential to tolerize the nascent T-cell repertoire towards endogenously expressed self-antigens [5]. On this basis, we hypothesized that EBV-infected B-cells might migrate to the thymus, express viral epitopes and induce central T-cell tolerance, eliminating certain classes of T-cells with antiviral properties. To address whether latently infected B cells can indeed be found in the thymus, we made use of murine gammaherpesvirus 68 (MHV-68), a well-established mouse model to study the host control of gammaherpesviruses [6]. First, we analyzed both the spleen and the thymus of mice infected for 17 days, when latency is established [6], for the presence of latently infected B cells [7]. As shown in Fig. 1A, we detected MHV-68-infected B-cells both in the spleen and in the thymus in a standard reactivation assay (reactivation frequency 1:3357 and 1:39574 in spleen and thymus, respectively) (more details on the methods used in this study can be found in the Supporting Information). We also determined the viral load in these organs by qPCR, and found 19.35 and 0.98 viral genome copies per 1000 copies of cellular genomic DNA in splenic and thymic B-cells, respectively. Of note, the viral load was substantially lower in thymic B cells as compared to spleen B cells. One plausible explanation is that the thymic B cell compartment may have a rather low turnover and only slowly equilibrates with the peripheral B cell pool subsequent to infection. Along these lines, the thymic B cell pool not only contains immigrants from the periphery but also cells that derive from intrathymic B lymphopoiesis. The exact relative contribution of either subset remains to be established, but it is conceivable that intrathymically differentiating B cells are not accessible to the virus. Second, we used a genetic reporter approach making use of ROSA-tdRFP mice with a Cre-inducible tdRFP cassette. Infection with a recombinant MHV-68 expressing Cre-recombinase (MHV-68-Cre) should lead to RFP-expression in infected cells that can be detected by FACS analysis. As shown in Fig. 1B and 1C, RFP-positive B cells could be detected in the thymus 17 days after infection with MHV-68-Cre but not after infection with wildtype MHV-68.

Taken together, our data clearly indicate that latently infected B cells are present in the thymus. This principal finding is in accordance with recent reports suggesting that EBV can reside in thymic B-cells and express an unusual combination of viral genes ([8] and references cited therein), which is, however, a controversial finding [9;10]. What remains to be shown is whether infected B cells in the thymus express viral genes that can result in a functional T cell tolerance against certain viral epitopes. If this were the case, it would offer an explanation for the biased and "incomplete" spectrum of the antiviral T-cell repertoire against EBV. Future work is needed to precisely establish the spectrum of viral epitopes that are presented by infected B cells in the thymus. It is tempting to speculate that the process of "intrathymic licensing" may result in reactivation of the virus and hence expression and tolerogenic presentation of lytic antigens. Although we focused on gammaherpesviruses, it is conceivable that also antigens from other viruses or pathogens may be conveyed to the thymus for their immune evasion.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Legend to Figure 1

Figure 1. A) B cells reactivating latent virus are detectable in the thymus and the spleen by an ex vivo reactivation assay. C57BL/6 mice were intranasally inoculated with wildtype MHV-68. At day 17

after infection, thymi and spleens were harvested. Single cell suspensions were prepared, B cells were isolated by MACS and analyzed in the ex vivo reactivation assay or DNA was isolated for real-time PCR analysis. The dashed line indicates the point of 63.2% Poisson distribution, determined by nonlinear regression, which was used to calculate the frequency of cells reactivating lytic replication. Data are from a single experiment representative of two experiments with pooled B cells from both thymi and spleens of 3 mice per experiment. **B) and C) RFP-positive B cells are present in the thymus 17 days after infection with MHV-68-Cre.** ROSA26-RFP reporter mice were intranasally inoculated with either wildtype MHV-68 or MHV-68-Cre. At day 17 after infection, thymi were harvested, single cell suspensions were prepared and thymic B cells analysed by flow cytometry. In panel B, an example for the frequency of RFP positive cells in thymic B cells is shown (for gating strategy see Supplementary Figure 1). Panel C depicts the summary of all mice tested (n=7 for MHV-68-wt; n=9 for MHV-68-Cre). Each symbol represents a mouse. Results are compiled from three independent experiments and are shown as mean ± SD (* denotes p=0.008; two-tailed, unpaired Student's t-test).

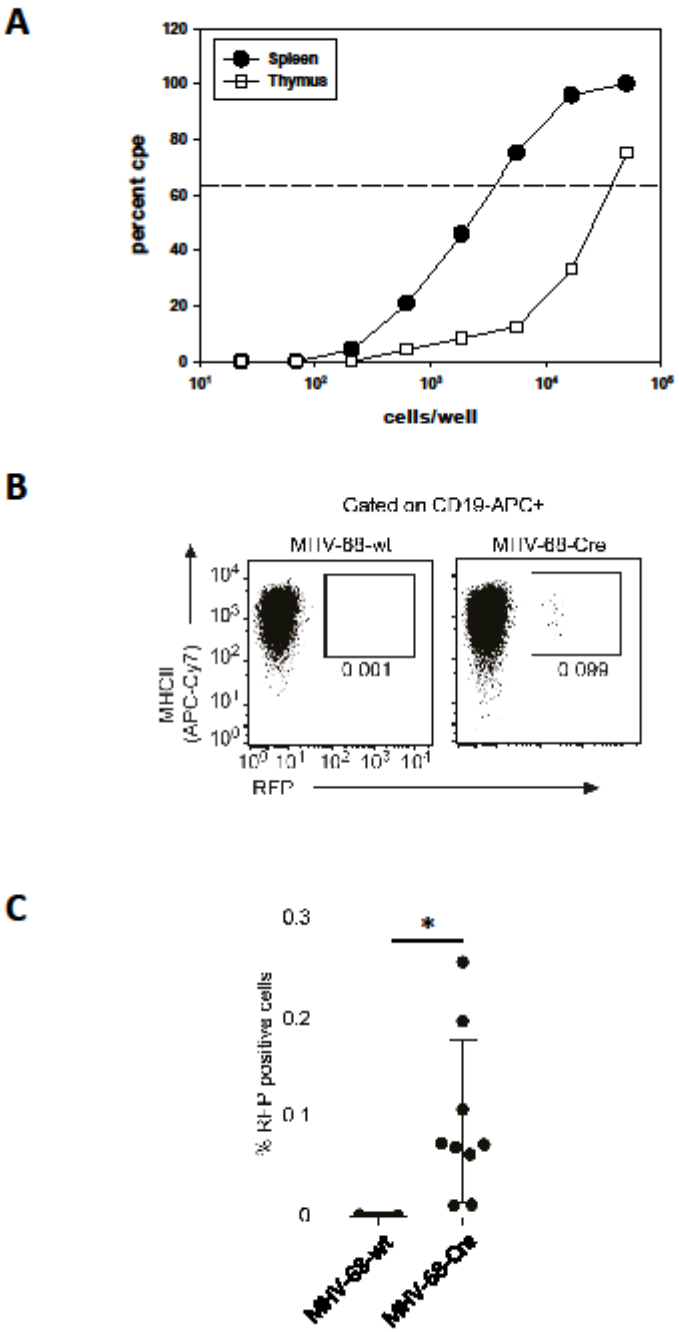

Figure 1

We show that latently gammaherpesvirus-infected B cells are present in the thymus. This could result in a functional T cell tolerance against certain viral epitopes. It is conceivable that also antigens from other viruses or pathogens may be conveyed to the thymus for their immune evasion.