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HIV Co-infection Augments EBV-Induced Tumorigenesis *in vivo*

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In most individuals, EBV maintains a life-long asymptomatic latent infection. However, EBV can induce the formation of B cell lymphomas in immune suppressed individuals including people living with HIV (PLWH). Most individuals who acquire HIV are already infected with EBV as EBV infection is primarily acquired during childhood and adolescence. Although antiretroviral therapy (ART) has substantially reduced the incidence of AIDS-associated malignancies, EBV positive PLWH are at an increased risk of developing lymphomas compared to the general population. The direct effect of HIV co-infection on EBV replication and EBV-induced tumorigenesis has not been experimentally examined. Using a humanized mouse model of EBV infection, we demonstrate that HIV co-infection enhances systemic EBV replication and immune activation. Importantly, EBV-induced tumorigenesis was augmented in EBV/HIV co-infected mice. Collectively, these results demonstrate a direct effect of HIV co-infection on EBV pathogenesis and disease progression and will facilitate future studies to address why the incidence of certain types of EBV-associated malignancies are stable or increasing in ART treated PLWH.

Keywords: Epstein-Barr Virus (EBV), human immunodeficiency virus (HIV), co-infection, replication, tumorigenesis, B cell lymphoma, humanized mice

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INTRODUCTION

Epstein-Barr Virus (EBV) is a ubiquitous virus infecting over 90% of adults in developed and developing countries (1). EBV is an oncogenic herpesvirus that primarily infects B cells (2–4). Primary infection is characterized by the rapid proliferation of infected B cells until an effective immune response is elicited and/or EBV-infected B cells differentiate into latently-infected memory-like cells establishing a life-long infection of the B cell compartment (2–4).

In most individuals, EBV maintains a latent infection with intermittent periods of subclinical virus reactivation and shedding (5). However, EBV can induce the formation of B cell lymphomas in immune suppressed individuals including people living with HIV (PLWH) (4, 6, 7). Most individuals who acquire HIV are already infected with EBV as EBV infection is primarily acquired during childhood and adolescence (1). Without any interventions, EBV positive PLWH have over a 60-fold higher risk of developing lymphomas compared to the general population

(8). While antiretroviral therapy (ART) use has significantly decreased the incidence of AIDS-associated malignancies in PLWH, the incidence of certain types of EBV-associated cancers has remained elevated in PLWH (9–11). HIV mediated immune dysfunction may contribute to lymphomagenesis (12). A better understanding of how HIV co-infection affects EBV replication and tumorigenesis would facilitate the development and testing of novel interventions to ameliorate EBV-associated pathologies in PLWH.

Here, a humanized mouse model of EBV infection was used to evaluate the effect of HIV co-infection on EBV replication and tumorigenesis *in vivo*. Results demonstrate that HIV co-infection enhanced systemic EBV replication *in vivo* resulting in higher levels of virus in the peripheral blood and tissues. HIV co-infection also resulted in an enhancement of EBV-mediated $CD8^+$ T cell activation. Importantly, data directly demonstrated that HIV co-infection augmented EBV-associated tumorigenesis *in vivo*. These results will facilitate future studies to address how HIV-associated enhancement of EBV replication and EBV mediated immune activation and tumorigenesis is impacted by suppressive ART and why the incidence of certain types of EBV-associated malignancies are stable or increasing in ART treated PLWH.

RESULTS

Humanized mice reconstituted with human B cells and T cells were first exposed to EBV (**Supplementary Table 1**). To evaluate the effect of HIV co-infection on EBV replication and tumorigenesis *in vivo*, a subset of mice was subsequently exposed to HIV. EBV infection was assessed in mice by measuring EBV-DNA levels in peripheral blood (cells and plasma) longitudinally and in tissues at necropsy (16 weeks post exposure or earlier if mice experienced 20% weight loss and/or appeared lethargic). HIV infection was monitored over time by measuring peripheral blood plasma HIV-RNA levels. HIV-RNA was detected in the plasma of all mice exposed to HIV and virus replication sustained over time (**Supplementary Figure 1A**). By six weeks post-exposure, plasma HIV-RNA levels were higher in EBV/HIV co-infected mice ($P = 0.0317$) compared to mice infected with HIV-only (**Supplementary Figure 1A**).

Peak EBV Viremia Is Enhanced by HIV Co-infection

EBV-DNA was detected in the peripheral blood of all mice exposed to EBV only and 6/7 mice (86%) exposed to EBV and HIV (**Figure 1A**). No significant difference in EBV acquisition, as measured by the presence of detectable EBV-DNA in peripheral blood, was observed between groups of mice ($P = 0.5007$). While cell-free (plasma) and cell-associated EBV-DNA was detected in the peripheral blood of all EBV-positive mice, peak levels of cell-free and cell-associated EBV-DNA were 15-fold and 5-fold higher ($P = 0.0082$ and $P = 0.0350$, respectively) in EBV/HIV co-infected mice (**Figures 1B–D**). These results demonstrate that HIV co-infection enhanced EBV viremia.

Systemic EBV-Induced $CD8^+$ T Cell Activation Is Amplified by HIV Co-infection

EBV infection induces a $CD8^+$ T cell response in peripheral blood that is characterized by the expansion and activation of $CD8^+$ T cells and acquisition of a memory phenotype (13). At necropsy, regardless of HIV co-infection status, $CD8^+$ T cell levels were significantly higher in the peripheral blood of EBV positive mice (baseline: 4.5 ± 0.7% s.e.m.; necropsy: 29.6 ± 6.5% s.e.m.; $P = 0.0009$) compared to baseline pre-exposure levels (**Supplementary Table 2**). In addition, the frequencies of memory (baseline: 43.5 ± 6.9% s.e.m.; necropsy: 94.3 ± 2.2% s.e.m.; $P < 0.0001$) and activated (baseline: 12.9 ± 2.9% s.e.m.; necropsy: 47.1 ± 7.2% s.e.m.; $P = 0.0002$) $CD8^+$ T cells in peripheral blood were also significantly higher at necropsy compared to baseline pre-exposure levels (**Supplementary Table 2**). At necropsy, no significant differences in the levels of $CD8^+$ T cells in peripheral blood and tissues were observed between mice infected with EBV only or co-infected with EBV and HIV (**Figure 2A**; **Supplementary Table 2**). Notably, the vast majority of $CD8^+$ T cells in EBV positive mice expressed a memory phenotype in peripheral blood and tissues at necropsy (**Figure 2B**; **Supplementary Table 2**). However, the levels of activated $CD8^+$ T cells were significantly higher in the peripheral blood ($P = 0.0087$), spleen ($P = 0.0221$), liver ($P = 0.0140$), and lung ($P = 0.0047$) of EBV positive mice co-infected with HIV (**Figure 2C**; **Supplementary Table 2**). In the peripheral blood of mice infected with HIV only, the levels of activated $CD8^+$ T cells only transiently increased peaking at 2 weeks post-exposure indicating that EBV and HIV contribute to the higher levels of $CD8^+$ T cell activation observed in EBV/HIV co-infected mice (**Supplementary Figure 1B**). Collectively, these results suggest that HIV co-infection enhanced EBV-mediated immune activation.

HIV Co-infection Augments Systemic EBV Replication

To evaluate the effect of HIV on systemic EBV replication, EBV-DNA levels were measured in the peripheral blood, spleen, lymph nodes, bone marrow, liver, lung, and brain of EBV and EBV/HIV co-infected mice at necropsy. Significantly higher levels of cell-free and cell-associated EBV-DNA were observed in peripheral blood of EBV/HIV co-infected mice ($P = 0.0022$). EBV-DNA was detected in 39/41 tissues samples analyzed from mice infected with EBV only and in 42/42 tissue samples analyzed from EBV/HIV co-infected mice (**Supplementary Table 3**). EBV-DNA levels were significantly higher in the spleen ($P = 0.0047$), lymph nodes ($P = 0.0140$), bone marrow ($P = 0.0350$), liver ($P = 0.0082$), and lung ($P = 0.0221$) of EBV/HIV co-infected mice (**Figure 3**; **Supplementary Table 3**). Notably, EBV-DNA levels were also 60-fold higher in the brain of EBV/HIV co-infected mice (**Figure 3**, **Supplementary Table 3**).

HIV Co-infection Augments EBV-Induced Tumorigenesis *in vivo*

The presence of tumors in EBV and EBV/HIV infected mice was also analyzed at necropsy. HIV co-infection enhanced

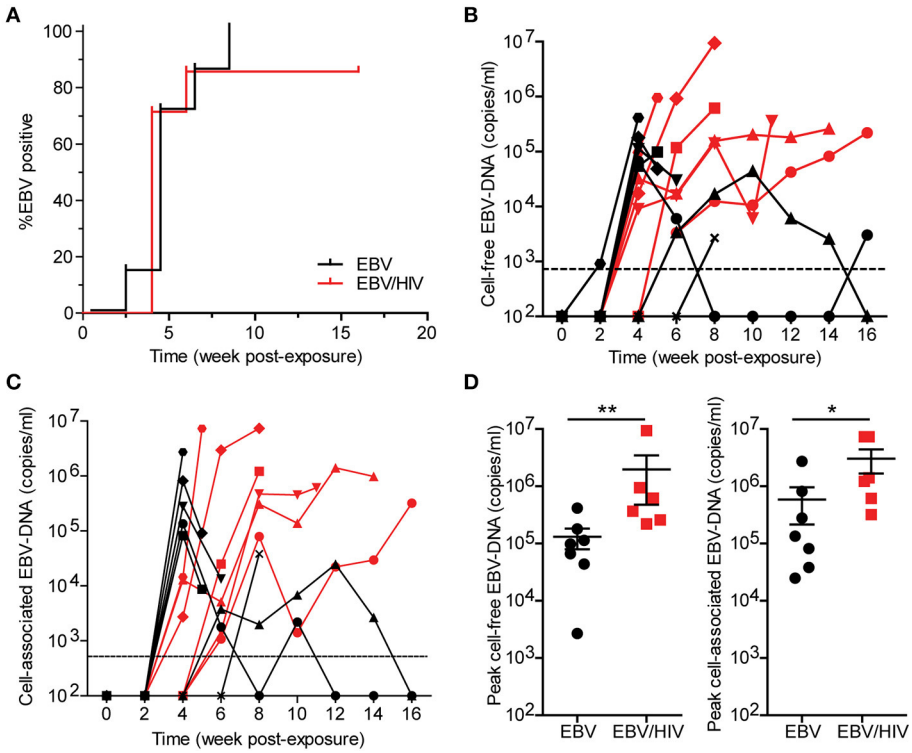

FIGURE 1 | HIV co-infection enhances EBV viremia. Humanized mice were inoculated intraperitoneally with EBV B95.8 ($n = 14$). Following EBV exposure, a group of mice ($n = 7$) were subsequently inoculated intravenously with HIV-1 LAI. EBV-DNA levels were monitored longitudinally in peripheral blood plasma (cell-free EBV-DNA) and cells (cell-associated EBV-DNA) of mice with real-time PCR. **(A)** A Kaplan Meier plot depicts the proportion of mice inoculated with EBV (black) or EBV/HIV (red) that became systemically infected with EBV as determined by the presence of EBV-DNA in peripheral blood. The **(B)** cell-free and **(C)** cell-associated EBV-DNA levels for individual mice systemically infected with EBV ($n = 7$ mice, black symbols) or EBV/HIV ($n = 6$ mice, red symbols) are shown. A dashed line indicates the assay limit of detection. **(D)** Peak cell-free and cell-associated EBV-DNA levels in the peripheral blood of EBV ($n = 7$ mice, black circles) and EBV/HIV ($n = 6$ mice, red squares) infected mice. The mean and standard error mean EBV-DNA levels are shown. Peak EBV-DNA levels between EBV and EBV/HIV infected animals were compared with a two-tailed Mann-Whitney test (*$p < 0.05$ and **$p < 0.01$).

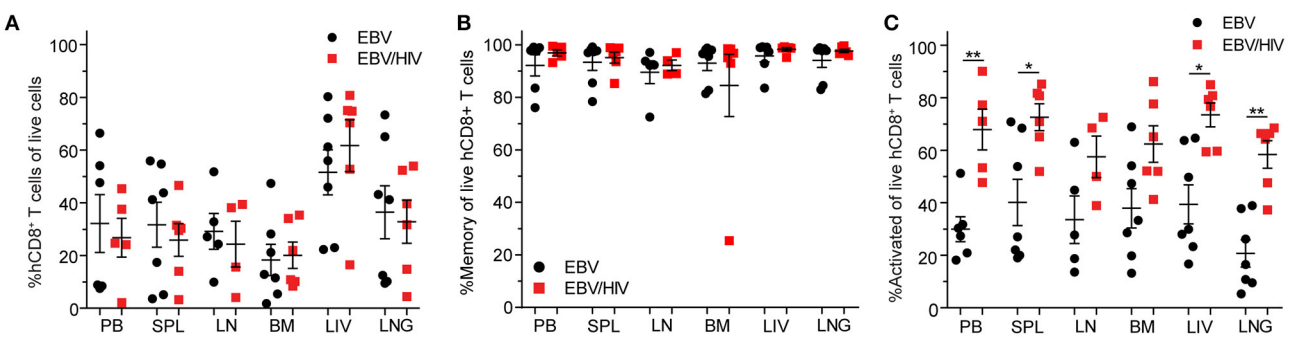

FIGURE 2 | Systemic EBV-induced $CD8^+$ T cell activation is amplified by HIV co-infection. **(A)** $CD8^+$ T cell, **(B)** memory ($CD45RA^{neg}$) $CD8^+$ T cell and **(C)** $CD8^+$ T cell activation ($HLA\text{-}DR^+CD38^+$) levels as determined by flow cytometric analysis in the peripheral blood (PB) and spleen (SPL), lymph node (LN), bone marrow (BM), liver (LIV) and lung (LNG) of EBV (SPL, BM, LIV, LNG: $n = 7$ mice, PB: $n = 6$ mice, LN: $n = 5$ mice, black circles) and EBV/HIV (SPL, BM, LIV, LNG: $n = 6$ mice, PB: $n = 5$ mice, LN: $n = 4$ mice, red squares) infected mice at necropsy. The mean and standard error mean are shown. $CD8^+$ T cell, memory $CD8^+$ T cell, and $CD8^+$ T cell activation levels in the PB and tissues of EBV and EBV/HIV infected animals were compared with a two-tailed Mann-Whitney test (*$p < 0.05$ and **$p < 0.01$).

EBV-induced tumorigenesis *in vivo*. Macroscopic tumors were readily observed in 7/7 EBV/HIV co-infected mice (**Figure 4A**; **Supplementary Table 3**). In contrast, only 4/7 mice infected with EBV only had macroscopic tumors (**Figure 4A**; **Supplementary Table 3**). Tumors were located in a greater number of distinct anatomical sites per mouse in EBV/HIV

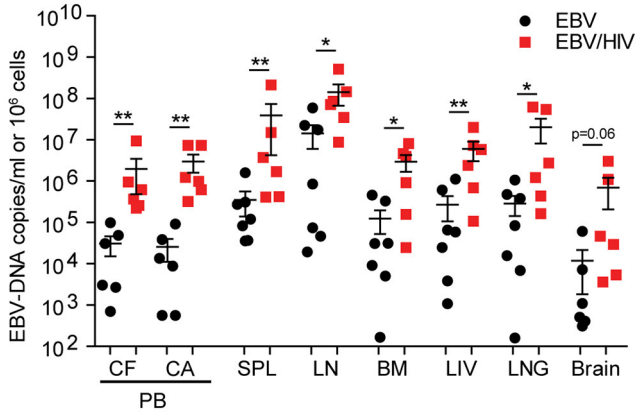

FIGURE 3 | HIV co-infection augments systemic EBV replication. EBV-DNA levels in the peripheral blood (PB, CF, cell-free; CA, cell-associated) and tissues (SPL, spleen; LN, lymph nodes; BM, bone marrow; LIV, liver; LNG, lung and brain) of EBV ($n = 7$ mice, black circles) and EBV/HIV ($n = 6$ mice, red squares) infected mice at necropsy. The mean and standard error mean EBV-DNA levels are shown. EBV-DNA levels between EBV and EBV/HIV infected animals were compared with a two-tailed Mann-Whitney test (*$p <$ 0.05 and **$p < 0.01$).

co-infected mice compared to mice infected with EBV only ($P = 0.0023$) (**Figure 4B**). Tumors were identified in one or two different tissues in mice infected with EBV only. In contrast, tumors were identified in up to five different tissues per mouse in mice co-infected with EBV and HIV (**Figure 4B**). In mice infected with EBV only tumors were only observed on the spleen and/or liver (**Figures 4C,D**; **Supplementary Table 3**). However, in EBV/HIV co-infected mice tumors could be observed on the spleen, liver, kidney, gastrointestinal tract, and/or salivary glands (**Figures 4C–E**; **Supplementary Table 3**). An analysis of viral gene expression in tumor samples collected from mice demonstrated that all tumors analyzed from EBV infected mice regardless of HIV co-infection status expressed LMP1 and EBNA2 (**Figure 4F**). This is characteristic of type III latency gene expression. Although HIV co-infection enhanced systemic EBV replication and EBV-induced tumorigenesis, no significant difference in survival ($P = 0.8372$) was observed between mice infected with EBV only or co-infected with EBV and HIV (**Supplementary Figure 2**).

DISCUSSION

Using a humanized mouse model of EBV infection (14–17), we directly demonstrated that HIV co-infection enhances systemic EBV replication, immune activation, and EBV-induced tumorigenesis *in vivo*. These data are consistent with studies reporting increased detection of EBV-DNA in the peripheral blood and saliva and the increased incidence of certain types of EBV-associated cancers in PLWH without any interventions (6–8, 18–21). In mice infected with EBV only, macroscopic tumors were only observed on the spleen and liver. In EBV/HIV co-infected animals, tumors were observed on the liver, spleen, kidneys, gastrointestinal tract, and salivary glands. These results suggest that HIV co-infection facilitates the spread of EBV-associated lymphomas and/or the simultaneous development of tumors at multiple different sites. Similar results were recently observed by another laboratory in humanized mice during a study focused on evaluating the effect of EBV infection on the cellular tropism of HIV (22). Several types of EBV positive lymphomas on extra nodal sites have been observed in PLWH including Hodgkin's lymphoma and diffuse large B cell lymphomas (6, 8). While we did not define the type of malignancies present, expression of EBV latency genes LMP1 and EBNA2 were detected in all tumors analyzed from humanized mice albeit at different levels. Co-expression of LMP1 and EBNA2 are indicative of type III latency (e.g. immunoblastic diffuse large B cell lymphomas) (4, 6).

Suppressive antiretroviral therapy (ART) reduces the incidence of AIDS-associated malignancies in PLWH (9, 10). The effect of ART on EBV reactivation and replication is less clear. For example, one study reported that incidence of EBV reactivation (as measured by the detection of EBV-DNA in saliva) was higher in PLWH compared to HIV negative controls regardless of ART use. However, the incidence of EBV reactivation was reduced in PLWH (ART +/–) with high $CD4^+$ T cell counts (23). Despite ART, the incidence of Hodgkin's lymphoma in PLWH has not decreased (9, 11). Most Hodgkin's lymphomas in PLWH are associated with EBV (7). While ART efficiently suppresses systemic HIV replication in PLWH, systemic immune activation and dysfunction persist presumably in part due to residual HIV replication and gut dysbiosis (24). Chronic immune activation and dysfunction in ART-suppressed PLWH may impair the formation of a robust immune response allowing for the formation of EBV-associated malignancies. Humanized mice could be used in the future to directly evaluate the effect of ART on systemic EBV replication and EBV-associated tumor formation and correlations between markers of chronic immune activation (e.g. LPS, sCD14, CRP, neopterin, TNFα, IL-6, IL-10, IFNγ, etc.) and EBV-associated disease.

Most PLWH acquire EBV infection prior HIV infection (1). Therefore, our study focused on the effect of HIV co-infection on EBV pathogenesis. However, EBV may influence the course of HIV infection. Here, we observed higher levels of HIV-RNA in the plasma of EBV/HIV co-infected mice compared to mice infected with HIV only by six weeks post-exposure. Even in healthy individuals, EBV periodically reactivates in the body which could stimulate local immune activation, potentially enhancing HIV replication (5). Studies in PLWH have attempted to evaluate associations between herpesvirus shedding (as determined by detectable virus in peripheral blood, throat washes, urine, stool, and/or semen) and HIV viral loads, however, given the high prevalence of other herpesviruses in PLWH, it is difficult to directly determine the effect of EBV on HIV replication and pathogenesis (25, 26). Humanized mice could serve as a model to directly evaluate the effect of EBV infection on HIV replication, pathogenesis and latency *in vivo*.

Limitations to our study include no uninfected control animals to serve as a comparison for the changes in the $CD8^+$ T cell compartment observed in EBV-infected mice. However,

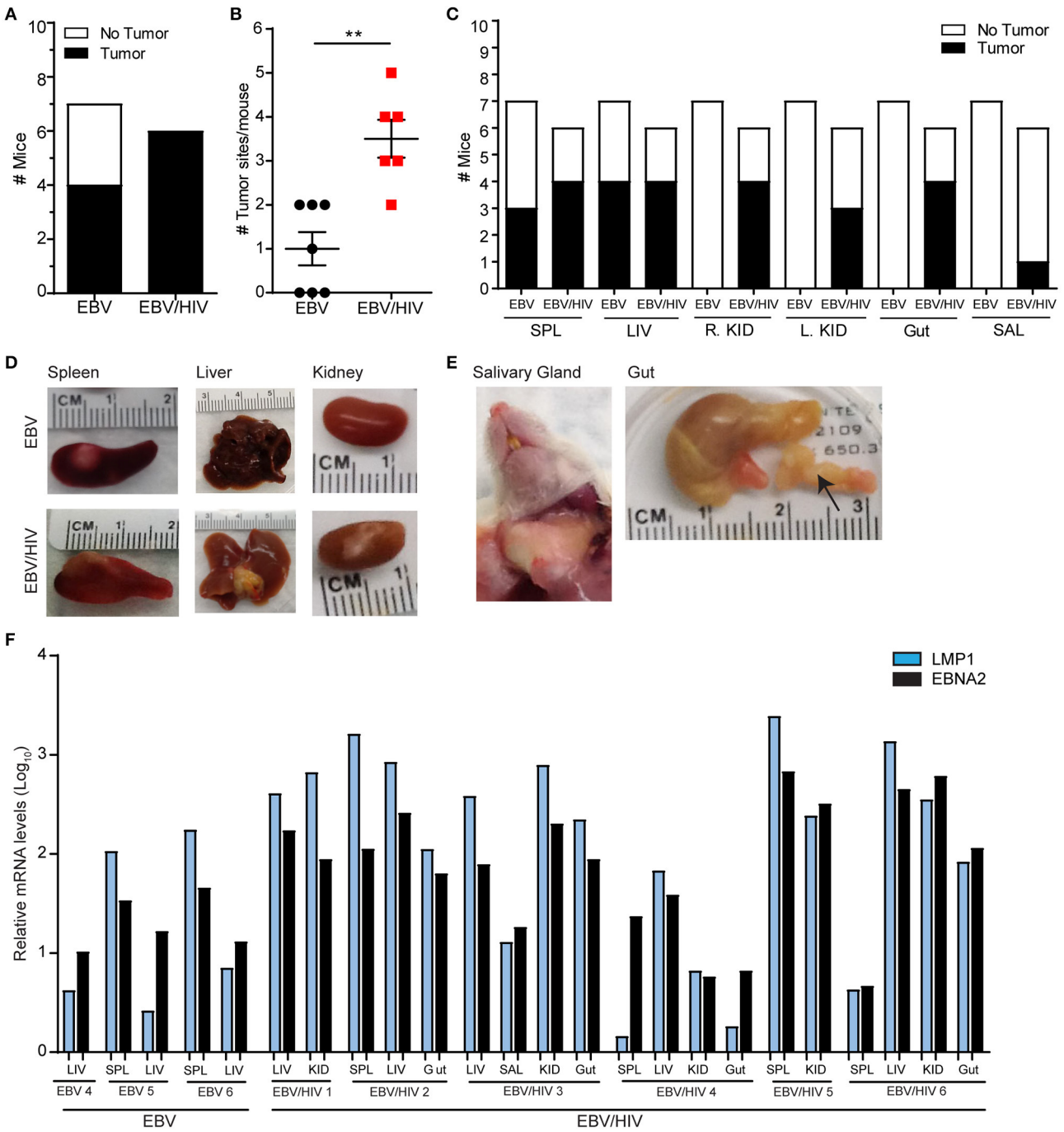

FIGURE 4 | HIV co-infection augments EBV-induced tumorigenesis *in vivo*. The presence of macroscopic tumors in the organs of EBV ($n = 7$ mice) and EBV/HIV ($n = 6$ mice) infected animals were noted at necropsy. **(A)** Tumor incidence in EBV and EBV/HIV infected mice (white: no tumors detected, black: tumors detected). **(B)** The number of different tissues with macroscopic tumors for each EBV (black circles) and EBV/HIV (red squares) infected mouse. The mean and standard error mean are shown. The number of tumor sites detected in EBV and EBV/HIV infected mice was compared with a two-tailed Mann-Whitney test (**$p < 0.01$). **(C)** Tumor incidence in the spleen (SPL), liver (LIV), right kidney (R.KID), left kidney (L.KID), gut and salivary glands (SAL) of EBV and EBV/HIV infected mice (white: no tumors detected, black: tumors detected). **(D)** Representative images of the spleen, liver, and kidney harvested from EBV and EBV/HIV infected mice. **(E)** Images of tumors detected in the salivary glands and gut (noted with an arrow) of EBV/HIV infected mice. **(F)** LMP1 and EBNA2 mRNA levels in tumors isolated from the spleen (SPL), liver (LIV), kidney (KID), gut and salivary glands (SAL) of EBV and EBV/HIV infected mice. Relative LMP1 (blue bars) and EBNA2 (black bars) mRNA levels were quantified using β-actin as an internal reference.

the effect of EBV infection on the $CD8^+$ T cell compartment including expansion, activation, and acquisition of a memory phenotype, has been well-documented in humans and in multiple humanized mouse models when compared to baseline levels and/or uninfected controls (13–15, 27–30). Furthermore, it has been shown that while the levels of human hematopoietic cells ($hCD45^+$) in the peripheral blood of mice is stable between 16 and 32 weeks post engraftment, the percentage of T cells that are $CD8^+$ decreases over time which is the opposite of what was observed in EBV-infected humanized mice (31). In addition, the short lifespan of humanized mice compared to humans requires experimental timelines to be condensed. Despite the shortened timeline between EBV and HIV exposures, our experimental data supports the clinical observation that the incidence of EBV-associated malignancies is higher in PLWH compared to the general population (6–8). In addition, while we anticipate that infection with a CCR5 or CXCR4 tropic strain of HIV would enhance EBV-associated tumorigenesis, a CXCR4 strain of HIV was chosen for this study to accelerate the development of HIV-associated pathogenesis.

In summary, we have implemented a model that permits the analysis of the effect of HIV infection on EBV. Our results demonstrate that HIV co-infection enhances EBV replication and the formation of EBV-associated malignancies. This work will facilitate future studies using humanized mouse models to address why the incidence of certain types of EBV-associated malignancies are stable or increasing in ART treated PLWH in contrast to the decline observed for many types of AIDS-associated malignancies.

MATERIALS AND METHODS

Preparation of Humanized Mice

Humanized mice were constructed by transplanting 12–15-week-old female and male irradiated (200 rads) NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice (NSG; The Jackson Laboratory) with 2–3.5 x 10^5 human $CD34^+$ hematopoietic stem cells intravenously. Reconstitution of animals with human hematopoietic cells was monitored longitudinally with flow cytometry as previously described (14). Animals were maintained by the Division of Comparative Medicine at the University of North Carolina-Chapel Hill.

Production of Virus and Infection of Humanized Mice

Infectious EBV was reactivated by transfection of BZLF1 and gp110 into 293 cells harboring a B95.8 EBV bacmid (32). Cell supernatant fluid was passed through a 0.4 uM filter and concentrated with an Amicon ultra 100-kDa-molecular-mass-cutoff filter (Millipore). EBV stocks were titered on Raji cells as previously described (33). Stocks of HIV-1_{LAI} were generated by transient transfection of 293T cells and titered on TZM-BL indicator cells as previously described (34–40). Mice (16–24 weeks post-transplant) were exposed to 1–1.2 x 10^5 green Raji units (GRU) EBV via intraperitoneal injection. On the same day and following EBV exposure, a group of mice was also exposed intravenously to 3 x 10^4 TCIU HIV-1_{LAI} via tail vein injection.

Analysis of HIV Infection

HIV-RNA levels were measured longitudinally in the peripheral blood plasma of mice using real-time PCR as previously described (34–40).

Analysis of EBV Infection

EBV-DNA levels were measured longitudinally in peripheral blood and in tissues at necropsy using a real-time PCR assay as previously described (14). Flow cytometry was used to evaluate levels of $CD8^+$ T cells, memory ($CD45RA^{neg}$) $CD8^+$ T cells and $CD8^+$ T cell activation (HLA-DR+CD38+) in peripheral blood and tissues as previously described (14). Live cell gates were determined by forward and side scatter. Plasma was collected from peripheral blood following centrifugation (375 x g, 5 min). Cells were isolated from the peripheral blood, spleens, lymph nodes, bone marrow, livers, lungs and brains collected from mice for analysis by real-time PCR and flow cytometry as previously described (34–40). Liver, lung, and brain mononuclear cells were purified with a Percoll gradient. Mice were euthanized at 16 weeks post-exposure or earlier if they experienced 20% weight loss and/or appeared lethargic and a necropsy performed to assess the presence of tumors. Tumors were harvested, snap-frozen and stored at −80C for subsequent nucleic acid extraction and gene expression analysis. Tissue and tumor images were taken with an iSight color camera and the brightness adjusted in Adobe Photoshop CS6.

EBV Gene Expression Analysis

RNA was isolated from 100 mg of tissue from tumor samples using the TRIzol reagent (*Invitrogen*) according to manufacturer's instructions. Total RNA quantity and quality was determined using a Nanodrop 1000 (ThermoScientific). Total RNA (1 ug) was converted to cDNA using the iScript cDNA kit (BioRad) and random primers according to manufacturer's guidelines. qPCR was performed using iTaq Universal SYBR Green Supermix (BioRad) and the QuantStudio6 Real-Time PCR system (Applied Biosystems) under standard conditions. Samples were monitored for LMP1, EBNA2 and B-actin with the following primers: LMP1, 5′-AATTTGCACGGACAGGCATT-3′; (forward) and 5′-AAGGCCAAAAGCTGCCAGAT-3′ (reverse); EBNA2, 5′-GCTTAGCCAGTAACCCAGCACT-3′ (forward) and 5′-TGCTTAGAAGGTTGTTGGCATG-3′ (reverse), B-Actin, 5′-GTCTGCCTTGGTAGTGGATAATG-3′ (forward) and 5′-TCGAGGACGCCCTATCATGG-3′ (reverse). Relative levels of LMP1 and EBNA2 were quantified using β-actin as an internal reference.

Statistical Analyses

Statistical analyses were performed in Prism, version 6 (Graph Pad). A Log-rank Mantel-Cox test was used to compare the rates of EBV-DNA detection in the peripheral blood and survival between EBV and EBV/HIV exposed mice. A two-tailed Mann-Whitney U test was used to compare the peak peripheral blood cell-associated and cell-free HIV-DNA levels, tissue EBV-DNA levels, levels of $CD8^+$ T cells, memory $CD8^+$ T cells, and $CD8^+$ T cell activation in peripheral blood and tissues, and the number of tumor sites per mouse between EBV and EBV/HIV exposed mice.

A two-tailed Mann-Whitney U test was also used to compare $CD8^+$ T cells, memory $CD8^+$ T cells, and $CD8^+$ T cell activation levels in the peripheral blood of EBV infected mice (regardless of HIV co-infection status) at baseline and at necropsy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

AUTHOR CONTRIBUTIONS

CW produced and titered stocks of EBV, performed the analysis of EBV latency gene expression, contributed to the experimental design, data interpretation, and manuscript writing. MR longitudinally monitored mouse health and survival, processed samples from EBV-exposed mice, performed flow cytometric analyses, performed the real-time PCR analysis of EBV-DNA levels in peripheral blood, tissue samples, and analyzed data. AT longitudinally monitored mouse health, survival and processed samples from EBV-exposed mice. RS performed and supervised the real-time PCR analysis of EBV-DNA. JP contributed to the experimental design and data interpretation. AW conceived, designed experiments, coordinated the study, contributed to data interpretation, data presentation, and manuscript writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fviro.2022.861628/full#supplementary-material

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