Correction

MICROBIOLOGY

The authors note that the Acknowledgments section appeared incorrectly. It should instead appear as: “We thank Professor Young-Hyeh Ko at Samsung Medical Center, a board member of the Korean Society of Hematologic Pathology, for helpful comments. This study was supported by Grants HI09C1552 and A110637 from the Korea Health Technology Research and Development (R&D) Project through the Korea Health Industry Development Institute, funded by the Ministry for Health & Welfare, Republic of Korea; Grant 1120010 from the National R&D Program for Cancer Control, Ministry for Health and Welfare, Republic of Korea; and Grants NRF-2011-0012393, NRF-1997-D00214, and NRF-2015-R1A2A2A010064659 from the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.”

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Effects of lymphocyte profile on development of EBV-induced lymphoma subtypes in humanized mice

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Epstein-Barr virus (EBV) infection causes both Hodgkin’s lymphoma (HL) and non-Hodgkin’s lymphoma (NHL). The present study reveals that EBV-induced HL and NHL are intriguingly associated with a repopulated immune cell profile in humanized mice. Newborn immunodeficient NSG mice were engrafted with human cord blood CD34+ hematopoietic stem cells (HSCs) for a 8- or 15-wk reconstitution period (denoted by 8wN and 15wN, respectively), resulting in human B-cell and T-cell predominance in peripheral blood cells, respectively. Further, novel humanized mice were established via engraftment of hCD34+ HSCs together with non-autologous fetal liver-derived mesenchymal stem cells (MSCs) or MSCs expressing an active notch ligand DLL1, resulting in mice skewed with human B or T cells, respectively. After EBV infection, whereas NHL developed more frequently in B-cell–predominant humanized mice, HL was seen in T-cell–predominant mice (P = 0.0013). Whereas human splenocytes from NHL-bearing mice were positive for EBV-associated NHL markers (hBCL2+, hCD20+, hKi67+, hCD20+/EBNA1+, and EBER−) but negative for HL markers (LMP1−, EBNA2−, and hCD30−), most HL-like tumors were characterized by the presence of malignant Hodgkin’s Reed–Sterberg (HRS)-like cells, lacunar RS (hCD30+, hCD15+, Igl+/EBER/hCD30+, EBNA1+/hCD30+, LMP+/EBNA2+, hCD68+, hBCL2+, hCD20−/weak, Phospho STAT6+), and mummified RS cells. This study reveals that immune cell composition plays an important role in the development of EBV-induced B-cell lymphoma.

Epstein–Barr virus | humanized mice | Non-Hodgkin’s lymphoma | Hodgkin’s lymphoma | Reed–Sterberg cell

Epstein Barr virus (EBV) infects human B lymphocytes and epithelial cells in >90% of the human population (1, 2). EBV infection is widely associated with the development of diverse human disorders that include Hodgkin’s lymphoma (HL) and non-Hodgkin’s lymphomas (NHL), including diffused large B-cell lymphoma (DLBCL), follicular B-cell lymphoma (FBCL), endemic Burkitt’s lymphoma (BL), and hemophagocytic lymphohistiocytosis (HLH) (3).

HL is a malignant lymphoid neoplasm most prevalent in adolescents and young adults (4–6). Hodgkin/Reed–Sterberg (HRS) cells are the sole malignant cells of HL. HRS cells are characterized by CD30+/CD15−/BCL6+/CD20−/− markers and appear large and multinucleated owing to multiple nuclear divisions without cytokinesis. Although HRS cells are malignant in the body, surrounding inflammatory cells greatly outnumber them. These reactive nonmalignant inflammatory cells, including lymphocytes, histiocytes, eosinophils, fibroblasts, neutrophils, and plasma cells, compose the vast majority of the tumor mass. The presence of HRS cells in the context of this inflammatory cellular background is a critical hallmark of the HL diagnosis (4).

Approximately 50% of HL cases are EBV-associated (EBVaHL) (7–11). EBV-positive HRS cells express EBV latent membrane protein (LMP) 1 (LMP1), LMP2A, LMP2B, and EBV nuclear antigen (EBNA) 1 (EBNA1), but lack EBNA2 (latency II marker) (12). LMP1 is consistently expressed in all EBV-associated cases of classical HL (13, 14). LMP1 mimics activated CD40 receptors, induces NF-kB, and allows cells to become malignant while escaping apoptosis (15).

The etiologic role of EBV in numerous disorders has been studied in humanized mouse models in diverse experimental conditions. Humanized mouse models recapitulate key characteristics of EBV infection–associated disease pathogenesis (16–24). Different settings have given rise to quite distinct phenotypes, including B-cell type NHL (DLBCL, FBCL, and unspecified B-cell lymphomas), natural killer/T cell lymphoma (NK/TCL), nonmalignant lymphoproliferative disorder (LPD), extremely rare HL, HLH, and arthritis (16–24). Despite considerable efforts (16–24), EBVaHL has not been properly produced in the humanized mouse setting model, owing to inappropriate animal models and a lack of in-depth analyses. After an initial report of infected humanized mice, HRS-like cells appeared to be extremely rare in the spleens of infected humanized mice; however, the findings were inconclusive (18). Here we report direct evidence of EBVaHL or HL-like neoplasms in multiple humanized mice in which T cells were predominant over B cells. Our study demonstrates that EBV-infected humanized mice display additional EBV-associated pathogenesis, including DLBCL and hemophagocytic lymphohistiocytosis (16, 17).

Results

NHL in B-Cell–Predominant 8wN–EBV Mice. In the first experimental trial, 13 newborn NSG mice were engrafted with hCD34+ HSC for 8 wk (denoted by 8wN), after which components of the human immune system (HIS) (e.g., hCD45, hCD3, hCD19) were

Significance

The mechanism of how Epstein-Barr virus (EBV) contributes to the development of two distinct lymphomas remains unknown. Intriguingly, EBV-associated Hodgkin’s lymphoma was seen exclusively in mice with activated T-cell conditions, whereas EBV-associated non-Hodgkin’s lymphoma was developed in mice with suppressed T-cell conditions, in which immature B cells were predominant at the time of EBV infection. This distinct association provides new insight into the pathogenesis of specific types of EBV-induced lymphomas.


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www.pnas.org/cgi/doi/10.1073/pnas.1407075112
Fig. 1. Experimental schemes. (A) Four experimental settings were used to establish humanized mice and EBV infection. In the first and second trials, newborn mice (1 d old) were injected i.p. with busulfan for bone marrow ablation, then 24 h later intrathymatically transplanted with 2 × 10^6 human cord blood CD34^+ HSCs. The mice were housed for 8 wk or 15 wk for reconstitution, then infected with EBV B95.8 virus or PBS through the tail vein. The mice were examined at 5 wpi by flow cytometry immune cell profiling (gray triangle), and housed for the indicated time or until moribund. In the third trial, the protocol for the second trial was followed, except that HSCs were engrafted along with non-autologous MSCs or MSCs expressing DLK1. ^{15}W/N denotes an hNSG (N) mouse reconstituted for 8 wk (8w) with hCD34^+ cells. (B and C) The number of mice with a predominance of B or T cells in PBMCs in experimental settings at 0 wpi (B) and 5 wpi (C). Note that four ^{8w}hN mice and five ^{15w}hN mice remained EBV-noninfected control in C (Table 1 and SI Appendix, Table S1). The fractions of hCD45 in PBMCs, CD45^+ gated CD19 cells, and CD45^+ gated CD3 cells were determined by flow cytometry.

**A** Scheme

<table>
<thead>
<tr>
<th>8w hN</th>
<th>15w hN</th>
<th>15w hNSG-MSC</th>
<th>15w hNSG-DLK1</th>
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<tbody>
<tr>
<td>1d</td>
<td>1d</td>
<td>1d</td>
<td>1d</td>
</tr>
<tr>
<td>Birth</td>
<td>Busul</td>
<td>15w</td>
<td>15w</td>
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<tr>
<td></td>
<td>EBV</td>
<td>15w</td>
<td>15w</td>
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<tr>
<td>8w</td>
<td>22w</td>
<td>13w</td>
<td>13w</td>
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**B** 0 wpi

<table>
<thead>
<tr>
<th>3</th>
<th>12</th>
<th>11</th>
</tr>
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**C** 5 wpi

<table>
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<th>21</th>
<th>3</th>
<th>13</th>
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Fig. 2. Profiles of reconstituted human immune cells in PBMCs in humanized mice. (A) HCD45, HCD45-gated hCD19 (HCD19^{CD45^+}), and hCD3^+ fractions in the 8-wk reconstitution group (^{15}hN). (B) The 15-wk reconstitution group (^{15}hN). (C) ^{15}hNSG mice coimplanted with MSCs (^{15}hNSG-MSC). (D) ^{15}hN mice coimplanted with MSCs expressing DLK1 (^{15}hN-M-DLK1). Data are mean ± SEM.
HSCs to develop preferentially into T cells, whereas MSCs alone skew HSCs to develop preferentially into B cells (26, 27).

On evaluation, although the MSC and MSC-DLK1 methods reconstituted the total leukocyte population with comparable efficiency (hCD45+, 16.73% vs. 11.9%) (Fig. 2 C and D and SI Appendix, Fig. S1), the three MSC-engrafted humanized mice displayed B-cell dominance (average, hCD19+, 39.3% vs. hCD3+, 8.9%) over T cells in PBMCs (Fig. 2 C and Table 1). Two of the three B-cell–predominant mice developed NHL. In contrast, all three MSC-DLK1–engrafted humanized mice displayed T-cell dominance (average, hCD3+ 61.8% vs. hCD19+, 3.3%) over B cells, which persisted for the next 5 wk after engraftment (Figs. 1 B and 2D). As a result, all three T-cell–predominant mice developed HL (Table 1 and SI Appendix, Figs. S3 and S4).

Taken together, the results of the three independent experimental trials suggest that B-cell predominance before EBV infection may have predisposed to NHL, whereas T-cell predominance was associated with, but not a prerequisite for, HL ($P = 0.0013$, Fisher’s exact test). In other words, NHL more frequently (but not exclusively) developed in B-cell–predominant mice, and all cases of HL were developed in mice with T-cell predominance at the time of EBV infection. Immune cell evolution appeared to not be associated with phenotypic neoplasm (SI Appendix, Table S1).

**Characteristics of Experimental EBV-Associated Lymphoma.** Of note, after EBV infection, although eight of the nine *hN* mice had NHL, the 13 *hN* mice with mostly T-cell predominance before EBV infection developed HL-like tumors ($n = 5$), NHL ($n = 4$), or both NHL and HL-like tumors ($n = 3$), indicating that T-cell predominance does not necessarily lead to the development of HL-like tumors (Table 1 and SI Appendix, Table S1 and Figs. S3–S5). HL-like splenocytes showed mostly type II latency (LMP1* and EBNA2* by IHC). In neoplasms of T-cell–predominant 15*H*–EBV mice, atypical, transformed large HRS cells surrounded by abundant nonmalignant lymphocytes were consistently encountered (average 24

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**Table 1. Summary of EBV-infected humanized mice**

<table>
<thead>
<tr>
<th>Phenotype*</th>
<th>EBV-noninfected in</th>
<th>EBV-infected in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B cells ($n = 4$)</td>
<td>T cells ($n = 5$)</td>
</tr>
<tr>
<td>NHL</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HL-like</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None$^6$</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Evolution$^7$</td>
<td>T(4)</td>
<td>T(5***)</td>
</tr>
</tbody>
</table>

NA, not applicable.

* NHL, EBV-associated non-Hodgkin’s-like lymphomas (EBV+ BL, DLBCL, FBCL, and unspecified B-cell lymphoma), HL-like, EBV-associated Hodgkin's lymphoma (HL), or HL-like [hRS cells with hCD30+/EBNA1+ (EBER+), HCD15*].

1 B-cell predominant mice with T-cell development suppressed at the time of EBV infection.

2 T-cell predominant mice with B-cell development suppressed at the time of EBV infection.

3 Two-sided Fisher’s exact $t$ test.

4 Three mice showing both HL and NHL were included.

5 No specific neoplasm.

6 Evolution of immune cell dominance by T or B cells for 5 wk after EBV infection or PBS (SI Appendix, Table S1 and Fig. 1).

7 Presumed dominance in one mouse included.

8 B-cell predominance was maintained in 5 of 14 infected mice, likely owing to EBV-mediated B-cell proliferation.

9 Three of five mice maintaining B-cell predominance developed NHL.

10 Eight of nine mice converted to T-cell predominance developed NHL.

11 T-cell predominance remained unchanged in all 11 mice.
HRS cells per spleen). Most of these transformed cells were consistent with EBV-associated HL-like phenotypes (e.g., hCD20+, hCD15+, hCD20+/−, Igλ, hCD20+/EBNA1+, hCD30+/EBER1+, LMP1+, EBNA2+, phospho-STAT6+), but were negative for the NHL markers hBCL2 in the hCD20− background cells (Figs. 3 and 4B and SI Appendix, Figs. S3–S13). In H&E and immunophenotyping analyses, each spleen best diagnosed as HL-like had phenotypic hallmarks of HL based on updated Revised European-American Lymphoma (REAL)/World Health Organization (WHO) criteria (28): numerous atypical malignant EBV-positive HRS-like cells, lacunar-type HRS cells, and mummified HRS cells in association with predominant T cells or hCD68+ histiocytes (29) (SI Appendix, Figs. S3 and S4). In addition, in accordance with the fact that STAT6 is constitutively phosphorylated in >80% of HRS cells of classical HL (30), phosphor-STAT6+ was positive in all four HL-like tumors tested but negative in all three NHL tumors tested (SI Appendix, Fig. S12), supporting a diagnosis of HL-like tumors in this humanized setting. This activated the STAT6 signaling pathway, which most likely was activated by the cytokines IL-4 and IL-13, could induce EBV LMP1 even in the absence of EBNA-2, implicating type II EBV latent gene expression in EVBaNHL (31) (SI Appendix, Fig. S3).

Costaining revealed colocalization of EBNA1 with hCD30 in HL-like cells. The lack of HIC-validated non-mouse hCD15 antibody for double IHC (note that EBNA1 Ab for IHC was a mouse monoclonal Ab) hindered the double staining in HRS cells. Instead, additional EBER and hCD30 double staining further demonstrated EBER+/hCD30+ colocalization in HRS-like cells (Fig. 4B and SI Appendix, Fig. S3). EBNA1 was costained with hCD20 in B-cell–high NHL tissue (Fig. 4), which was supported by an abundance of hCD20+ cells in NHL. In contrast, EBNA1+ HRS cells were mostly negative for hCD20 (SI Appendix, Figs. S3 and S6), which is also consistent with the fact that HL cells are hCD20− based on the REAL/WHO criteria. Numerous human hCD20+ cells were present in uninfected spleen. In support of these data, EBNA1 was clearly negative in these uninfected cells by qPCR (SI Appendix, Figs. S6 and S13). qPCR revealed hCD30 expression in HL-like tissues, but not in NHL or uninfected spleen (SI Appendix, Fig. S13). In addition, there were frequent somatic hypermutations (SHMs) in cDNA encoding the Ig heavy-chain variable region (V_{H}) of spleen DNA of HL-like and NHL tumors from EBV-infected humanized mice (SI Appendix, Fig. S14). In contrast, B-cell–predominant mice from the 5thn-EBV group exhibited EBVaNHL-like neoplasms (e.g., DLBCL, BL) that showed extensive or complete distortion of splenic architecture with malignant immuno-lymphoproliferation. In keeping with the criteria for NHL (e.g., hBCL2+, hCD20+/−), these cancerous lesions were consistently positive for EBV-associated NHL markers (hCD20+, hCD20−, hKi67+, EBER+, EBNA1+, and hCD20+/EBNA1+) but mostly negative for type II/III latency markers (EBNA2+ and LMP1−) and HL markers (hCD30− and hCD15−) (Fig. 4A and SI Appendix, Figs. S2 and S6–S12). These data suggest that infection of 5thn with EBV results in NHL-type neoplasms with predominant type I latent infection. Analytical qPCR analyses for spleen cDNA confirmed the result of type I latency in the 5thn-EBV mice (SI Appendix, Fig. S13).

Discussion

In the present study, humanized mice recapitulated many of key characteristics of EBV infection-associated disease pathogenesis. EBV-associated disorders in humanized mice, including post-transplantation lymphoproliferative disorder, NHL (DLBCL, FBC1, HLH, arthritis, and chronic active EBV infection, have been reproduced. The outcome should depend on different inputs (e.g., strain, age of recipient, donor cells, duration of transplant lymphoproliferative disorder, NHL (DLBCL, FBC1, HLH, arthritis, and chronic active EBV infection, have been reproduced. The outcome should depend on different inputs (e.g., strain, age of recipient, donor cells, duration of infection), which is consistent with the fact that HL cells are hCD20− based on the REAL/WHO criteria. Numerous human hCD20+ cells were present in uninfected spleen. In support of these data, EBNA1 was clearly negative in these uninfected cells by qPCR (SI Appendix, Figs. S6 and S13). qPCR revealed hCD30 expression in HL-like tissues, but not in NHL or uninfected spleen (SI Appendix, Fig. S13). In addition, there were frequent somatic hypermutations (SHMs) in cDNA encoding the Ig heavy-chain variable region (V_{H}) of spleen DNA of HL-like and NHL tumors from EBV-infected humanized mice (SI Appendix, Fig. S14). In contrast, B-cell–predominant mice from the 5thn-EBV group exhibited EBVaNHL-like neoplasms (e.g., DLBCL, BL) that showed extensive or complete distortion of splenic architecture with malignant immuno-lymphoproliferation. In keeping with the criteria for NHL (e.g., hBCL2+, hCD20+/−), these cancerous lesions were consistently positive for EBV-associated NHL markers (hCD20+, hCD20−, hKi67+, EBER+, EBNA1+, and hCD20+/EBNA1+) but mostly negative for type II/III latency markers (EBNA2+ and LMP1−) and HL markers (hCD30− and hCD15−) (Fig. 4A and SI Appendix, Figs. S2 and S6–S12). These data suggest that infection of 5thn with EBV results in NHL-type neoplasms with predominant type I latent infection. Analytical qPCR analyses for spleen cDNA confirmed the result of type I latency in the 5thn-EBV mice (SI Appendix, Fig. S13).
reconstitution, dose of EBV, depth of analyses) (16-18, 20–23). IHC staining of spleen sections for EBV latent gene markers identified EBV infection in these humanized mice (SI Appendix, Figs. S8–S11). To our knowledge, this study is the first to describe EBVaHL-like disorder in multiple humanized mice with supportive evidence. The malignant HL-like tissues in this study were characterized by atypical EBV-infected HRS or HRS-like cells. Similar to typical HRS, where 90% HRS of human HL tissues in situ is hCD20−/−, the HL-like tissues in this study were clearly hCD20−/−, also consistent with the updated REAL/WHO criteria (28) and thus strongly suggestive of experimental HL-like neoplasms (12–14, 28, 32, 33). T-cell preexpansion does not prevent EBV lymphomagenesis, because T-cell–dominant mice developed lymphoma; rather, preconditioned mice with B-cell predominance (or T-cell suppression) developed NHL after EBV infection (Table 1 and SI Appendix, Table S1). Most of the NHL neoplasms in this study were characterized by hBCL2+, hKi67+, hCD30+, EBER+, EBNA2+, and LMP expression. Three mice were found to have both NHL and HL-like tumors.

Watanabe et al. (34) previously reported that a significant number of B-cell progenitors accumulate in the spleen in human cord blood-derived hCD34+ HSC-reconstituted humanized NSG (or NOG) mice. Numerous other groups also have reported obvious immature B-cell predominance in the periphery and spleen, especially in humanized mice reconstituted for short periods (<12 wk) (18, 34–38). Therefore, the predominant B cells before EBV infection in the hN mice in this study are believed to be immature B cells, which will differentiate to develop mature B-cell and T-cell fractions. In this regard, the development of NHL primarily in the hN mice after EBV infection (hN-EBV; eight of nine mice) suggests that a significant fraction of immature B cells at the time of EBV infection are likely associated with NHL development, although our study does not provide direct evidence of this. Despite apparent normal T-cell development, hCD3+ T cells could have functional abnormalities in central or peripheral lymphoid organs (34), related mainly to the lack of human thymic tissues normally required for thymic education during functional T-cell development. Inappropriate or incomplete immune function should affect malignant transformation after EBV infection. Nevertheless, many of the HL-like tumors in these mice displayed multiple HRS and lacunar–type cells with broken spleen architecture, the usual lack of germinal center, frequent—but not always (or entirely)—loss of lymphoid follicles (LFs) or LFs replaced with immature cells, frequent enlargement of white pulp or periarteriolar lymphoid sheaths (PALS), and often atrophy in red pulp. Therefore, the malignant HL-like tumor cells in this study were defined as atypical giant cells within or near abundant immature lymphocytes that replace follicles or disrupt splenic normal architecture such as follicles and germinal centers. Besides coexisting NHL or infectious mononucleosis (IM) in certain mice, almost all of the tumors in T-cell–dominant conditioned mice in this study certainly contained subsets of HL-like tumors, because they displayed multiple criteria of HL (i.e., frequent HRS cells with J chain−, CD30−, CD15−, EBER−, EBNA2−, hBCL2−, and phospho-STAT6+) (SI Appendix, Fig. S12) (30). The activated STAT6 signaling pathway could induce EBV LMP1 in absence of EBNA-2, implicating type II EBV latent gene expression in EBVaHL (30, 31). The majority of HL-like tumors exhibited type 2 latency; five of eight HL-like tumors displayed type 2a latency (LMP1+/EBNA2−), whereas the remaining three showed apparent type 3 latency. Despite having multiple molecular characteristics of HL, the apparent type 3 latency in these three so-called “HL-like” tumors is not typically observed in human HL. This is suggestive of an atypical HL that may occur in certain conditions, such as an experimental animal model. On the other hand, given that the “markers” (such as CD30) used to define HL are not absolutely specific for HL, and that the EBV latency type is not that seen in human HL, it also is possible that the tumors in this T-cell–predominant model may be atypical HL-like tumors rather than true representatives of HL.

Given that IM has no evidence of crippling SHMs in the V\textsubscript{H} gene (43), whereas HL has nonsense or deletion mutation in the V\textsubscript{H} gene, resulting in loss of the correct reading frame in ~30% of cases (44), the presence of SHMs in the HL-like tumors in this study further supports a pathological diagnosis of HL-like malignancy. SHMs were identified in all tumors examined, regardless of the presence of IM-like lesions. This is because malignant cells were present in all tissues examined. In addition to hCD30+/hCD15+/EBNA1+/EBER+, the presence of phospho-STAT6+ HRS-like cells and a V\textsubscript{H} nonsense mutation strengthened the diagnosis of EBVaHL-like tumors (Fig. 4). The coexistence of occasional type III IM cells and type II HL-like cells accounts for the apparent type III latency in some of the HL-like tumor-bearing spleens. Along with the coexisting NHL or IM, almost all tumors in the T-cell–dominant conditioned mice in this study also contained subsets of HL-like tumors. Of note, the results showing apparently fewer numbers of EBER-positive cells than EBNA1-positive cells shown in Figs. 3 and 4 and SI Appendix, Figs. S8 and S9 were confirmed by additional staining (SI Appendix, Fig. S15). The discrepancy is likely due to decreased EBER promoter activity by unphosphorylated active retinoblastoma tumor-suppressor protein during cell cycle phases G(0) and early G(1) (45, 46).

Materials and Methods

Ethics Statement. Human protocol (IRB file no. 2010-08-159) for human material was approved by the Institutional Review Boards of Samsung Medical Center. Animal protocol (no. 20100210001) was approved by the Institutional Animal Care and Use Committee (IACUC) of Samsung Biomedical Research Institute (SBRI) (see SI Appendix for detail). Cell preparation for engraftment, infection with EBV, and characterization of humanized NSG (hNSG) mice are described in detail in SI Appendix.

Preparation of Humanized Mice. Nonobese diabetic/severe combined immunodeficient mice with IL2R knockout (NOD/LtSz-scid/IL2Rnull), referred to herein as NSG, were purchased from The Jackson Laboratory (47, 48). Newborn progenies for transplantation experiments were obtained from inbred breeding and maintained under specific pathogen-free conditions. For reconstitution of the HIs in mice, 1-d-old newborn female NSG mice were injected i.p. with busulfan (Ben Eun Laboratories) at a dose of 15 mg/kg to ablate residual bone marrow (47, 48). At 24 h after busulfan injection, 2 × 10\textsuperscript{6} hCD34+ HSCs were injected intraperitoneally.

The mice were housed for 8 or 15 wk before characterization and EBV infection (Fig. 1A). NSG mice reconstituted with HIs components are referred to as hNSG mice. Reconstitution was evaluated as described previously (20, 24). Where necessary, humanized mice with skewed populations of B or T cells were generated as described above with the following modifications. B-cell–predominant NSG mice were coengrafted with cord blood hCD34+ HSCs and human FL-MSCs, which enhanced HSC engraftment and suppressed T-cell proliferation (49–52). This treatment resulted in B-cell–predominant humanized
mice. T-cell–predominant NSG mice were coengrafted with cord blood hCD34+ HSCs and NFl-MSC-DLK1 cells expressing an activated Notch ligand (DLK1) to suppress B-cell development (33). This treatment resulted in T-cell–predominant humanized mice. After 15 wk, mice were infected with EBV as described in SI Appendix, followed by investigation for 13 wk.

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