

Epstein-Barr Virus-induced Human B-Cell Lymphomas in SCID Mice Reconstituted with Human Peripheral Blood Leukocytes¹

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Abstract

Epstein-Barr virus (EBV) infection is associated with immunoblastic B-cell lymphomas in immunosuppressed or human immunodeficiency virus-infected individuals and in SCID mice reconstituted with human peripheral blood leukocytes (hu-PBL-SCID) from EBV-seropositive donors. The risk of tumors appearing in the hu-PBL-SCID mice differs among EBV-seropositive donors. Four different outcomes have been noted: (a) no tumors appear (no incidence donors); (b) tumors appear in a fraction of hu-PBL-SCID mice with a 10–20 week latent period (low- and intermediate-incidence donors); or (c) tumors appear in all hu-PBL-SCID mice within 6–10 weeks (high-incidence donors). The latter category of rapidly appearing tumor invariably involved activation of EBV replication, whereas more slowly growing tumors rarely activated EBV. The results indicate that prospective screening of high-risk individuals in the hu-PBL-SCID model may predict the risk of EBV-associated lymphoma development.

Introduction

EBV³ is a common DNA herpesvirus that infects the majority of the human population. It is associated with three malignant diseases [endemic Burkitt's lymphoma (1), nasopharyngeal carcinoma (2), and immunoblastic B-cell lymphomas (3, 4)]. The B-cell lymphomas occur with high frequency in patients with primary, secondary, or iatrogenic immunodeficiency (5–7), and are thought to reflect inadequate immune surveillance of B-cells latently infected with EBV.

Acute clinical infection with EBV results in active viral replication (lytic cycle) in both B-cells and epithelial cells of the oropharynx. Following the development of EBV-specific immunity, there is low-level persistent viral replication in the oropharynx, and small numbers of latently infected B-cells persist in the peripheral blood and lymphoid organs. In addition, normal B-lymphocytes can be transformed *in vitro* by EBV, and only latent gene expression is required for transformation. These latent genes include several EBV nuclear antigens (EBNA-1, -2, -3a, -3b, -3c, -4, or leader protein) and two membrane proteins (LMP-1, LMP-2) (8).

There are two animal models for EBV induction of lymphomas. EBV infection of cottontop tamirins leads to development of B-cell lymphomas (9). In a more recently developed model, xenotransplantation of human peripheral blood lymphocytes derived from EBV-seropositive donors to SCID (severe combined immune deficient) mice leads to the onset of human B-cell immunoblastic lymphomas (10–14). Using the hu-PBL-SCID model, we have performed studies to assess the incidence of these lymphomas when different EBV-seropositive PBL donors are used, and to evaluate the risk factors that may distinguish between donors.

Materials and Methods

All of the pertinent techniques have been summarized elsewhere (10, 11). The Southern blot analysis of tumor DNA for replicating *versus* episomal genomes was performed by using the *Bam*NJ probe (15), which detects *Bam*HI restriction site heterogeneity created by the fusion of the terminal repeats of the EBV genome.

Results

Tumors generated in hu-PBL-SCID mice appear within 20 weeks of transferring 25–50 × 10⁶ PBL from EBV-seropositive donors *i.p.* to SCID recipients. We analyzed the overall incidence of tumor formation and the latency period from PBL injection to tumor detection in 85 hu-PBL-SCID mice derived from 10 healthy donors with IgG anti-viral capsid antigen titers ranging from 1:80 to 1:640. Two of 10 donors failed to produce any tumors in SCID mice. The results for the remaining 8 of 10 donors are shown in Fig. 1.

These donors fell into three categories: high-incidence donors who gave tumors in 100% of SCID recipients within 10 weeks, intermediate-incidence donors who gave rise to tumors in 40–80% of SCID mice in 10–13 weeks, and low-incidence donors who produced tumors in <40% of mice in 13–20 weeks.

Tumor cells from lymphomas derived from each category of donor were compared for surface antigen expression. The results are summarized in Table 1, and show that all tumors had the same phenotype. The tumors differed from lymphoblastoid cell lines established from the same donors by *in vitro* transformation with B95–8 strain EBV by showing lower expression of EBNA-2 and CD23, the latter a B-cell activation marker. These changes in EBNA-2 and CD23 expression appear to result from the environment in which the EBV-transformed B-cell resides, however, since *in vitro* growth of tumors resulted in up-regulation of both markers, and passage of lymphoblastoid cell lines to SCID mice resulted in down-regulation of both markers.

DNA from tumors from each category of PBL donors was analyzed for viral replication *versus* latency.

The results of this analysis are summarized schematically in Fig. 2. These results show that tumors arising from low-incidence donors were mono- or biclonal, and did not involve activation of EBV replication (episomal forms only). By contrast, tumors derived from intermediate- and high-incidence donors showed evidence of viral replicative intermediates, and tended to be more multiclonal. The clonality of the tumors was confirmed by Southern blot analysis of Jh rearrangements at the immunoglobulin H-chain locus.

Discussion

The heterogeneity in EBV-seropositive donors with regard to their capacity to produce lymphomas in hu-PBL-SCID mice might be due to differences in levels of immunity to EBV latent- or replication-stage antigens, or to differences in the transforming capacity of naturally occurring EBV strains. No differences in antibody levels to EBV have been noted, but T-cell immunity to EBV remains to be evaluated. EBV recovered from these

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³ The abbreviations used are: EBV, Epstein-Barr virus; hu-PBL-SCID, SCID mice reconstituted with human peripheral blood leukocytes.

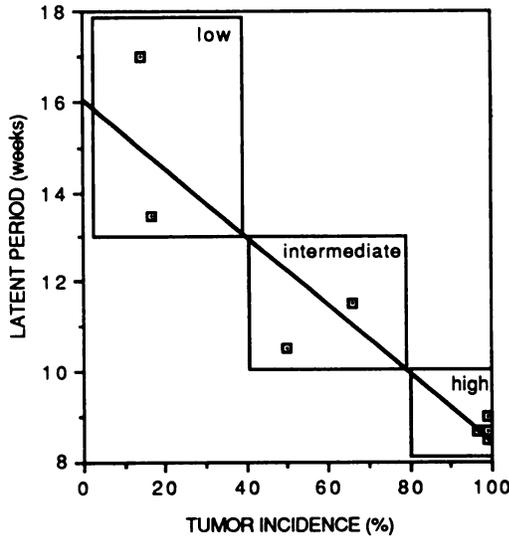


Fig. 1. Mean tumor incidence (percentage of injected hu-PBL-SCID mice developing tumors) plotted versus mean latent period to tumor detection (in weeks) in 4-9 mice/donor. Each symbol represents the mean values for 1 of 8 donors. Virtually identical results were obtained in a second experiment utilizing donors representing each incidence category.

Table 1 Phenotype of B-cell lymphomas in hu-PBL-SCID mice

Levels of expression are indicated by + (low) to +++ (high) as measured by flow cytometry or intensity on Western blot (EBV antigens).

Donor	Expression of hu-PBL-SCID lymphomas			
	LCL ^a	High	Intermediate	Low
B-cell markers				
CD10	-	-	-	-
CD19	+++	++	++	++
CD20	+++	+++	+++	+++
CD23	+++	+	+	+
CD39	+++	++	++	++
EBV antigens				
EBNA-1	+++	+++	+++	+++
EBNA-2	+++	±	±	±
LMP	+++	+++	+++	+++
Cell adhesion molecules				
LFA-1	+++	++	++	++
ICAM-1	+++	++	++	++
LFA-3	+++	++	++	++
MHC molecules				
Class I	+++	+++	+++	+++
Class II	+++	++	++	++

^a LCL, lymphoblastoid cell line; MHC, major histocompatibility complex.

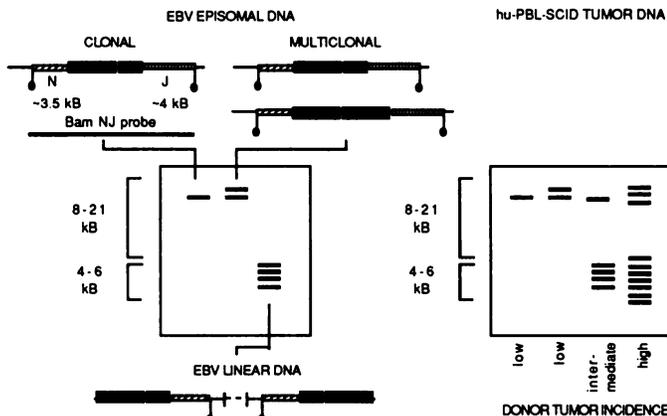


Fig. 2. Southern blot analysis of EBV genomes recovered from hu-PBL-SCID tumors. BamHI restricted DNA was probed with the BamNJ probe, which distinguishes between circular episomal DNA and linear replicating DNA, as illustrated on the left side of the figure. Results are summarized schematically on the right side of the figure.

tumors has all been the more highly transforming type A (1) strain by EBNA-2 typing, but some differences in transforming capacity between virus isolated from high-incidence tumors and low-incidence tumors has been noted. We believe that both immunity and viral variation contribute to our results.

The development of the hu-PBL-SCID model provides a novel experimental approach to assessing the risk for human malignancies. How broadly it can be applied to non-Hodgkin's lymphomas in general cannot be predicted until more studies are done. We do suggest that these studies summarized here indicate that it is likely to be useful in predicting risk for the development of post-transplant or acquired immunodeficiency syndrome-associated B-cell lymphomas.

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