**BHRF1, the Epstein-Barr Virus (EBV) Homologue of the BCL-2 Proto-oncogene, Is Transcribed in EBV-Associated B-Cell Lymphomas and in Reactive Lymphocytes**


BHRF1, one of many Epstein-Barr virus (EBV)-encoded proteins, shows strong functional homology to the human bcl-2 proto-oncogene product, a protein involved in the pathogenesis of a subset of B-cell lymphomas, ie, follicle center cell lymphomas (FCCL). We have investigated the presence of possible latent and lytic transcripts of BHRF1 using a reverse transcriptase-polymerase chain reaction (RT-PCR)-based assay in a group of EBV-associated B-cell lymphomas in patients with (N = 5) or without overt immunodeficiency (N = 4), in T-cell lymphomas (N = 6), and in cases of Hodgkin’s disease (N = 6). BHRF1 transcription was found consistently in EBV-associated (ie, diffuse EBER 1/2-positive) B-cell lymphomas in patients with or without immune deficiency, whereas in EBV-associated T-cell lymphomas or in EBV-associated Hodgkin’s disease, BHRF1 transcription was only detected in two T-cell lymphomas and one of Hodgkin’s disease, which also harbored EBER 1/2-positive reactive cells. Moreover, weak BHRF1 signals were found in two T-cell lymphomas where EBER 1/2 expression was detected mainly in sporadic reactive lymphocytes and in one reactive tonsil with sporadic EBER 1/2-positive lymphocytes. BHRF1 transcripts were found to be generated by the C or W promoter (associated with viral latency) and/or by the H promoter (associated with the virus lytic cycle). In all cases with H promoter-derived BHRF1 transcripts, transcripts encoding ZEBRA were also detected, suggesting a reactivation of the virus lytic cycle. Analysis of other EBV genes revealed transcription of BARFO in all tested EBV-harbouring tissues. Transcription of EBNA1 and LMP1 was usually detected, whereas EBNA2 transcription was found exclusively in B-cell lymphomas in immunocompromised patients. These data demonstrate that BHRF1 transcripts are exclusively found in EBV-associated B-cell lymphomas. When BHRF1 transcripts are detected in T-cell lymphomas or in Hodgkin’s disease, it is probably due to the presence of reactive EBV 1/2-positive lymphocytes. The consistent transcription of BHRF1 in EBV-associated B-cell lymphomas suggests a possible pathogenic role for this gene product in EBV-positive B-cell lymphomas analogous to bcl-2. © 1995 by The American Society of Hematology.

**EPSTEIN-BARR virus (EBV)** is the etiologic agent in mononucleosis infectiosa and has been implicated in the pathogenesis of certain malignancies. The virus can be detected in most, if not all, undifferentiated nasopharyngeal carcinomas (NPC), and, depending on histologic type, in 10% to 70% of cases of Hodgkin’s disease (HD).\(^3\) Moreover, EBV is detected in T-cell non-Hodgkin’s lymphomas (T-NHL) depending on site, ranging from 5% in nodal T-cell lymphomas up to 100% in nasal T-cell lymphomas.\(^4\) In B-cell lymphomas, EBV is classically associated with African Burkitt’s lymphoma and with most lymphoproliferative disorders in immunocompromised patients.\(^5\) In other B-cell lymphomas in patients without overt immune deficiency, EBV is only detected in approximately 5% of cases.\(^5\) This is remarkable, because the majority of healthy individuals are latently infected by the virus, and B lymphocytes are probably the host cells for this latent EBV infection.\(^6\) In biopsy material, EBV was shown to be present in morphologically malignant cells by either DNA in situ hybridization or by a more sensitive RNA in situ hybridization assay (RISH) using the abundantly transcribed noncoding EBER 1/2 RNAs.\(^7\) Lymphomas with EBER 1/2-positive staining in the large majority of neoplastic cells were called EBV-associated lymphomas.\(^8\)

In trying to identify the role of EBV in the pathogenesis of different types of malignancies, the expression of specific EBV genes has been studied. Three different patterns of EBV latent gene expression emerged: latency types I, II, and III.\(^4\) In Burkitt’s lymphoma, expression is restricted to the EBER 1/2 genes, EBNA1 and BARFO\(^5\) (latency type I). Latency type II (EBER 1/2, EBNA1, LMP1, BARFO, and often LMP2A and/or LMP2B) was detected in NPC, HD, and T-cell lymphomas.\(^7,11\) In large B-cell lymphomas in immunocompromised patients, transcripts of all known EBV latent genes were detected (EBER 1/2; EBNA1, 2, 3A, B, and C; LMP1; LMP2A; LMP2B; and BARFO): latency type III.\(^8,20,21\) Thus, in different types of malignancies, different EBV genes are expressed.

Interestingly, one of the EBV genes, the BHRF1 gene, shows partial sequence homology to the human bcl-2 proto-oncogene, a gene involved in the pathogenesis of a subset of B-cell lymphomas, ie, follicle center cell lymphomas (FCCL). Both BHRF1 and bcl-2 are members of a growing family of proteins involved in regulation of apoptosis. All members share two highly conserved domains that are essential to their function.\(^23\) Indeed, it has been shown that BHRF1 can protect B lymphocytes from apoptosis in a similar way as the bcl-2 protein.\(^22\) Until recently, BHRF1 has received relatively little attention in lymphoma research, mainly because BHRF1 was primarily identified as a member of the early antigen complex, and it was shown that BHRF1 is dispensable for transformation in vitro.\(^25\) The regulation of BHRF1 expression appears to be complex. High levels of mRNA and the 17-kD protein are detected early after activation of the lytic cycle.\(^26\) However, on Northern blot analysis, differentially spliced BHRF1-containing mRNAs were detected in tightly latent EBV-transformed...
EBV-Associated T-Cell Lymphomas

Six of the nine T-cell lymphomas have been described in detail elsewhere. Three additional cases were obtained from the Department of Pathology, State University of Utrecht, Utrecht, The Netherlands. Sites of origin were nose (all pleomorphic medium and large-cell lymphoma, N = 7), lymph node (unclassifiable, N = 1), and gut (large-cell anaplastic lymphoma, CD30-positive, N = 1). In the nasal T-cell lymphomas, the number of reactive lymphocytes as estimated by morphometry was low, whereas in the latter two cases, the number of reactive lymphocytes was relatively high. EBER 1/2 expression in these cases was not restricted to the malignant cells but was also detected in a number of sporadic, small reactive lymphocytes (15 to 30 EBER 1/2-positive cells per section; Fig 1C and Table 3, cases 1660 and 1624).

EBV-Associated Cases of HD

Cases were classified as nodular sclerosis (N = 3) or as mixed cellularity (N = 2). In one case, the subtype could not be classified because the lymph node was only partially involved. In case 909, many infiltrating B lymphocytes were present. In all cases, the Reed-Sternberg and Hodgkin’s cells were positive using the EBER 1/2 RISH assay, as well as by immunohistochemical staining for LMP1. However, in case 909, sporadic, small EBER 1/2-positive, but LMP1-negative, lymphocytes were also present (10 to 20 EBER 1/2-positive cells per section).

T-Cell Lymphomas With EBER 1/2 Positivity Mainly Restricted to Nonmalignant Cells

In previous studies, we and others have detected several lymphomas in which EBER 1/2-positive staining was mainly restricted to sporadic nonmalignant lymphocytes. We studied two nodal T-cell lymphomas, both Lennert’s lymphomas, harboring 10 to 50 EBER 1/2-positive cells per section. Double-staining with CD3 or CD20 revealed EBER 1/2-positive B cells as well as one or two EBER 1/2-positive T cells per section. However, most EBER 1/2-positive reactive cells expressed no B- or T-cell markers.

Hyperplastic Tonsil With Sporadic EBER 1/2-Positive Lymphocytes

We studied 14 hyperplastic tonsils for the presence of EBER 1/2-positive cells, of which only one harbored enough EBER 1/2-positive cells (ie, more than 10 EBER 1/2-positive cells per section) for detection of EBER-specific transcripts using RT-PCR analysis. In this hyperplastic tonsil, double-staining revealed the presence of EBER 1/2-positive B cells (Fig 1D). EBER 1/2-positive T cells were not observed. Most EBER 1/2-positive cells expressed no B- or T-cell markers.

Cell Lines

The tightly latent EBV-positive Burkitt’s lymphoma cell line RAJI and the EBV-transformed lymphoblastoid cell line KCA were used as positive controls for RT-PCR analysis. The P3HR1-derived HH514C16 cell line served as a negative control for EBNA2 and Y2/HF-spliced BHRF1 transcripts. This EBV strain lacks the EBNA2 open reading frame and the last common EBNA-specific exon due to a deletion spanning the BamHI W fragment (Fig 2). The EBV-negative cell line BJAB was used as a negative control for RT-PCR analysis.

Preparation of Clinical Samples and Cell Lines

For RT-PCR and immunohistochemical analysis, 20 5-μm sections were cut from snap-frozen material. To obtain suitable RNA, 10 sections were homogenized using micropestles (Eppendorf, E.

EBV-Associated B-Cell Lymphomas in Immunocompromised Patients

All five lymphomas consisted of diffuse polymorphous populations of large centroblastic cells with a variable number of CD30-positive blastoid cells, sometimes resembling Reed-Sternberg cells. Within 2 to 12 months, these lymphomas developed in patients who received an allogeneic bone marrow transplantation after having been treated for different malignancies.

EBV-Associated B-Cell Lymphomas in Patients Without Overt Immunodeficiency

The B-cell lymphomas included two polymorphous centroblastic lymphomas (tonsil, spleen), one diffuse centroblastic lymphoma (cervical lymph node), and one diffuse centroblastic/cleocytic lymphoma (large, peritoneal tumor). These lymphomas showed a diffuse pattern of EBER 1/2-positive neoplastic cells (Fig 1A), comparable with the EBER 1/2 staining pattern observed in the B-cell lymphomas in immunocompromised patients. In the first three cases, a small but significant number (5% to 35%) of the neoplastic cells showed CD30 expression. In the last case, immunohistochemical staining on frozen sections for CD30 and LMP1 was not possible due to poor quality of the material.
Merck Nederland BV, Amsterdam, The Netherlands) in an RNAzol B buffer according to the manufacturer’s protocol (Cinna/Biotech, Houston, TX). Of the EBV-positive cell lines, approximately $5 \times 10^5$ cells were pelleted, and RNA was isolated using the same RNAzol B protocol. RNA was stored in 100% ethanol at −80°C until further processing. The remaining 10 sections were mounted on poly-L-lysine-coated slides, fixed, and used for immunohistochemistry and hematoxylin/eosin (H&E) staining. To confirm that the sections used for RT-PCR contained lymphoma, the first and the last sections of the frozen material used for RT-PCR were H&E stained. Cases were excluded if mRNA appeared to be of poor quality, as determined by the presence of 28s and 18s rRNA bands after gel electrophoresis and by RT-PCR using primers specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a well-described housekeeping gene.

Selection of Oligoprimers and Oligoprobes

RT-PCR analysis was performed using intron flanking primers. The primer sequences and oligoprobes used for analysis of EBNA1, LMP1, BARFO, and H2/HF-spliced BHRF1 transcripts have been published previously. For analysis of EBNA2, ZEBRA, and Y2/HF-spliced BHRF1 transcripts, primers were designated using the PCR plan software (PCgene release 6.7; IntelliGenetics, Mountain View, CA). All primers and oligoprobes are listed in Table 1.

Two primer sets were used to investigate the presence of either latent or lytic BHRF1 transcripts (Fig 2). To detect the H2/HF-spliced BHRF1 transcripts derived from a promoter located on the BamHI H fragment (Hp), activated in lytic infections, a primer combination was used amplifying a sequence of 211 bp, specific for spliced mRNA. In amplifying either DNA or unspliced mRNA, a 650-bp fragment is expected. To detect Y2/HF-spliced transcripts derived from the latent C or W promoter, a 248-bp fragment was amplified using a sense primer (Y2) located on the Y2 exon in combination with the BHRF1-specific H3 antisense primer. PCR products of both spliced transcripts were hybridized with an internal oligoprobe located just upstream of the BHRF1 antisense primer.

To analyze EBNA2 transcription, intron flanking primers located on the Y2 exon (identical to the BHRF1-specific sense primer) and on the 5’ end of the YH exon, which contains the EBNA2 open reading frame (BYRF1), were used (Fig 2). Amplified products were hybridized with an oligoprobe located on the Y3 exon, also used as sense primer for amplification of C or W promoter-derived, EBNA1-specific transcripts.

RT-PCR Analysis

For RT reactions, an amount of RNA equivalent to one 5-µm section (biopsies) and $1 \times 10^5$ cells (cell lines) was used. After centrifugation, pellets were resuspended in 5 µL distilled water. RT reactions were performed in a final volume of 20 µL containing 25 pmol of one to four of each EBV antisense primer specific for the different genes (Table 1), as described previously. To exclude false-positive signals caused by DNA amplification, simultaneous reac-

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**Fig 1.** (A) B-cell lymphoma (case 3796). EBER 1/2 RISH-positive signal is observed in the large majority of neoplastic cells (original magnification, x 600). (B) B-cell lymphoma (case 12707). Strong LMP1-positive signals are detected in large anaplastic cells (original magnification, x 600). (C) Gastrointestinal T-cell lymphoma, large cell anaplastic lymphoma (case 1624). Large EBER 1/2- and LMP1-positive cells. Distant from the neoplastic population, small, EBER 1/2-positive, LMP1-negative cells were observed (inset). Original magnification, x 600. (D) Hyperplastic tonsil (case 20376) with scattered EBER 1/2- and CD20-positive cells (original magnification, x 600).
Fig 2. Schematic presentation of the EBV genome, presenting the different tested BHFR1, EBNA1, and EBNA2 transcripts. Hatched arrows represent the exons containing the different open reading frames. The C or W, F, and BHFR1 specific H promoters are represented by solid arrows. The intron-flanking primers used for amplification by RT-PCR and the internal oligomers used as probe are represented by arrowheads in relation to the specific exons; the direction of the arrowheads correlates with sense or antisense orientation of the oligomer.

Immunohistochemistry

To detect EBV-specific proteins, antibodies against LMP1 (CS1-4, DAKO and S12, Organon Tecnika, The Netherlands\(^4\)), EBNA2 (pE2, DAKO), and ZEBRA (DAKO) were used. Other antibodies used were anti-CD20 (L26, DAKO), anti-CD3 (DAKO), anti-CD45RO (UCHL-1, DAKO), and anti-CD30 (BerH2, DAKO), and anti-CD45 (LCA, DAKO). A routine ABC immunoperoxidase technique was used.\(^3\)

### Table 1. Sequences of PCR Primers and Oligoprobes

<table>
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<tr>
<th>Transcript</th>
<th>Oligoprobe</th>
<th>Sequence (5'-3')</th>
<th>B95.8 Genomic Coordinates</th>
<th>Amplimer Length (bp)</th>
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<td>EBNA2*</td>
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* The sense primer used for EBNA2 is identical to the BHFR1 Y2 sense primer. EBNA2-specific transcripts were detected with the Y3 primer, also used as sense primer for the detection of EBNA1-specific transcripts.
**Combination RISH and Immunohistochemistry**

After performing the EBER 1/2 RISH protocol, the remaining peroxidase activity was inactivated with 0.3% H2O2/methanol and fixed with 4% paraformaldehyde for 10 minutes, followed by the above-described immunohistochemical detection method.

**RESULTS**

**Cell Lines**

**BHRF1 transcription.** Both latent Y2/HF-spliced and lytic H2/HF-spliced BHRF1 transcripts were clearly detectable in the EBV-transformed cell line KCA. In the RAJI cell line, strong signals were obtained for C or W promoter-derived BHRF1, whereas only a very faint band was observed for H2/HF-spliced BHRF1 transcripts. As expected, the P3HR1-transformed HH514C16 cell line was positive only for the lytic H2/HF-spliced, Hp-derived BHRF1 transcript, because it lacks the last common EBNA Y2 exon (Fig 2). BHRF1-specific transcripts were never observed in the EBV-negative cell line BJAB.

**Transcriptional analysis of EBNA1, EBNA2, BARFO, LMP1, and ZEBRA.** BARFO and LMP1 transcripts were easily detected in all EBV-positive cell lines. C or W promoter-as well as F promoter-derived EBNA1 transcripts were detected in the KCA cell line. This cell line was also clearly positive for EBNA2 and ZEBRA. In the RAJI cell line, EBNA1 was only generated by the C or W promoter, and a strong band was observed for EBNA2, whereas a very faint band was observed for ZEBRA. In the HH514C16 cell line, clear ZEBRA-positive signals were detected in the absence of EBNA2. Again, no positive signals were observed in the BJAB cell line.

**EBV-Associated B-Cell Lymphomas in Immunocompromised Patients**

**BHRF1 transcription.** Transcripts were detected in all five B-cell lymphomas: positive signals for both Y2/HF- and H3/HF-spliced transcripts in three cases and only Y2/HF-spliced transcripts in the two other cases. In patients without overt immune deficiency, BHRF1-specific transcripts were detected in all four EBV-associated B-cell lymphomas. In two cases, Y2/HF-spliced transcripts and in two other cases, H2/HF-spliced transcripts were found.

**Transcriptional analysis of EBNA1, EBNA2, BARFO, LMP1, and ZEBRA.** In these cases, EBNA1-specific transcripts were generated by both the C and W promoter as well as by the F promoter in all cases except one (case 12253). In this case, only Y3/U/K-spliced transcripts were detected. In all cases, strong signals were observed for EBNA2, BARFO, LMP1, and ZEBRA, which is consistent with previous reports.

**EBV-Associated Cases of HD**

**BHRF1 transcription.** In five cases, BHRF1 transcription could not be detected. In these cases, EBER 1/2 expression was only present in large and intermediate-sized neoplastic cells. In the sixth case (Table 3, case 909), a weak Y2/HF-spliced, BHRF1-positive signal was found. In this case, in contrast to the other cases, a very large number of CD20-positive small lymphocytes was present with scattered EBER 1/2-positive, LMP1-negative small lymphocytes. Thus, BHRF1 transcription in HD could again only be detected in the one case harboring EBER 1/2-positive small lymphocytes (Fig 3).
RT-PCR analysis of BHRF1

Transcriptional analysis of EBNA1, EBNA2, BARFO, LMP1, and ZEBRA. EBNA1 transcription was only analyzed in the BHRF1-positive case of HD (Table 3, case 909). Both Y3/U/K- and Q/U/K-spliced EBNA1 transcripts were found. Others have shown that in cases of HD, EBNA1 is usually generated by the F promoter. The explanation for the presence of C or W promoter-derived transcripts (Y3/U/K-spliced EBNA1 and Y2/HF-spliced BHRF1) might be that the Q/U/K-spliced EBNA1 transcripts are derived from the neoplastic cells, whereas C or W promoter-derived EBNA1 and BHRF1 transcripts are derived from the small, EBER 1/2-positive lymphocytes. EBNA2 transcripts were never detected, whereas transcription of BARFO and LMP1 was always found. In one case (Table 2, case 1787), a faint ZEBRA-positive signal was observed.

T-Cell Lymphomas With EBER 1/2 Positivity Mainly Restricted to Nonmalignant Cells

BHRF1 transcription. In these two lymphomas (Table 3, cases 722 and 1484), EBER 1/2-positive staining was detected in 10 to 50 mainly nonmalignant lymphocytes. Both Y2/HF-spliced and H2/HF-spliced BHRF1 transcripts were detected, indicating that BHRF1 was generated by the C or W promoter as well as by the H promoter.

Transcriptional analysis of EBNA2, BARFO, LMP1, and ZEBRA. Although the C or W promoter was activated, as shown by the presence of Y2/HF-spliced transcripts, no EBNA2-specific transcripts were found. ZEBRA- and BARFO-specific transcripts were found in both cases. LMP1 transcription could not be detected.

Hyperplastic Tonsil With Sporadic EBER 1/2-Positive Lymphocytes

BHRF1 transcription. In the hyperplastic tonsil (Table 3, case 20376), approximately 50 EBER 1/2-positive lymphocytes were found scattered in follicle centers as well as in extrafollicular areas. Again, both Y2/HF-spliced and H2/HF-spliced BHRF1 transcripts were detected, indicating that BHRF1 was generated by the C or W promoter as well as by the H promoter (Fig 3).
BHFR1 Expression in EBV-Associated Lymphomas

Table 2. EBV Transcription in Lymphomas With Only EBER 1/2-Positive Neoplastic Cells

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<tr>
<th>Immunocompromised Patients</th>
<th>Diffuse EBER 1/2-Positive B-cell lymphomas</th>
<th>Diffuse EBER 1/2-Positive Nasal T-cell lymphomas</th>
<th>Cases of HD with EBER 1/2-Positive Hodgkin and Reed/Sternberg cells</th>
<th>EBV-Positive Cell Lines</th>
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<tr>
<td>Case No.</td>
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Abbreviations: IB/LC, immunoblast-rich, large-cell lymphoma; CB, centroblastic lymphoma; CC, centrocytic lymphoma; Pleiom m/l, Pleiomorphic medium- and large-cell lymphoma; NS, nodular sclerosing; MC, mixed cellularity; LCL, EBV-transformed lymphoblastoid cell line; ND, not determined; Polyen, polymorphous.

* Cases also immunohistochemically positive for EBNA2, ZEBRA, and LMP1, respectively.

Immunohistochemical analysis of LMP1, EBNA2, and ZEBRA. In all B-cell lymphomas except one (case 92-1959), in patients with or without overt immune deficiency, LMP1 positivity could be demonstrated using both LMP1-specific antibodies in a restricted number of large blastoid cells (Fig 1B). In the T-cell lymphomas, LMP1 expression was detected in only three cases (Table 2, case 305 and Table 3, cases 1660 and 1624). Moreover, although LMP1 was detected at the mRNA level, LMP1 was not detected at the protein level in the hyperplastic tonsil with sporadic EBER 1/2-positive lymphocytes. Immunohistochemical analysis of EBNA2 confirmed the expression of EBNA2 exclusively in the B-cell lymphomas arising in immunocompromised patients. Although ZEBRA-specific transcripts were detected in patients with or without overt immune deficiency, immunohistochemical detection of ZEBRA was only positive in the five B-cell lymphomas arising in immunocompromised patients. Discrepancies between RT-PCR and immunohistochemistry probably reflect differences in expression levels.

**DISCUSSION**

The strong functional homology between bcl-2 and BHFR126 and the involvement of bcl-2 in a number of lymphomas prompted us to determine whether transcripts containing the BHFR1 open reading frame were present in EBV-associated lymphomas, ie, lymphomas where the large majority of neoplastic cells harbor EBV as shown by the EBER 1/2 RISH assay.

We detected either Y2/HF-spliced or H2/HF-spliced BHFR1 transcripts in all B-cell lymphomas in patients with
or without overt immune deficiency. In cases of T-cell lymphomas or HD, BHRF1-specific transcripts were never detected in lymphomas where EBER 1/2 expression was exclusively restricted to the neoplastic cells. However, BHRF1-specific transcripts were detected in two T-cell lymphomas and in one case of HD where EBER 1/2-positive, LMP1-negative, morphologically nonmalignant cells were detected next to EBER 1/2-positive, LMP1-positive neoplastic cells. To be certain in these cases that BHRF1 expression have failed, probably because of the relatively low BHRF1 transcription in these cases was restricted to the expression levels of BHRF1. However, the hypothesis that observed, and by detection of BHRF1 in two T-cell lymphomas, where scattered EBER U2-positive lymphocytes were detected in lymphomas where EBER 1/2 expression was mainly restricted to BHRF1-specific transcripts were detected. Although we cannot exclude the possibility that these transcripts are generated by a yet unidentified promoter, the inclusion of the Y2 exon, characteristic of the EBNA family, strongly suggests that these BHRF1 transcripts are generated by the C or W promoter. In most of these cases, activation of the C or W promoter was confirmed by the detection of Y3/U/K-spliced EBNA1 transcripts. As activation of the C or W promoter is strongly associated with latency, it is likely that Y2/DF-spliced BHRF1 transcripts are generated during latency. This is in agreement with a previous report detecting Y2/DF-spliced BHRF1 in a tightly latent lymphoblastoid cell line. Moreover, the detection of C or W promoter activation exclusively in the B-cell lymphomas and in cases harboring sporadic EBER 1/2-positive lymphocytes is consistent with in vitro data showing that C or W promoter use depends on a B-cell phenotype.

In contrast, either Y2/DF-spliced transcripts or H2/DF-spliced transcripts were detected in the diffuse EBER 1/2-positive B-cell lymphomas in patients without overt immune deficiency. In the three cases harboring only sporadic EBER 1/2-positive cells, both Y2/DF- and H2/DF-spliced BHRF1 transcripts were detected (Table 3, cases 722, 1484, and 20376). Moreover, in all tested cases positive for H2/DF-spliced BHRF1 transcripts, ZEBRA-specific transcripts were also found. As ZEBRA is the first protein to be expressed in lytic cells, lymphoma cells expressing these proteins have obviously entered the virus lytic cycle.

The observed differential promoter use for BHRF1 transcription might implicate an important role for BHRF1 in both the latent and lytic cycles of EBV infection. During latency, even low levels of Y2/DF-spliced BHRF1 transcripts, as observed in vitro, could be sufficient to expand cell survival. During the virus lytic cycle, high levels of BHRF1 expression could be essential to inhibit apoptosis, allowing the generation of a maximal number of virions.

The selective expression of BHRF1 in all EBV-associated
BHRFI EXPRESSION IN EBV-ASSOCIATED LYMPHOMAS

B-cell lymphomas in patients with or without overt immune deficiency could be significant, as its human homologue, bcl-2, also seems primarily involved in the pathogenesis of FCCL. The strong involvement of bcl-2 in the development of FCCL, the presence of BHRFI transcripts in EBV-associated B-cell lymphomas, and the strong functional homology between bcl-2 and BHRFI suggest that both proteins may have identical effects in the pathogenesis of these B-cell lymphomas. The role of bcl-2 in the development of B-cell lymphomas probably involves its ability to prolong cell survival by inhibiting apoptosis, and we hypothesize that BHRFI acts in a similar way in EBV-associated B-cell lymphomas. Moreover, the detection of BHRFI in the B-cell lymphomas might be clinically relevant, because it was shown recently that BHRFI protects against cell death induced by DNA-damaging agents used in cancer therapy.

In conclusion, BHRFI is consistently transcribed in EBV-positive B-cell lymphomas in patients with or without overt immune deficiency. When BHRFI transcription is detected in T-cell lymphomas or in HD, it is probably due to the presence of EBER 1/2-positive, nonmalignant reactive lymphocytes. These data indicate that EBV can be involved in the pathogenesis of lymphomas in different ways. The consistent expression of BHRFI in EBV-associated B-cell lymphomas suggests a pathogenic role for this gene product in these lymphomas in a similar way as the translocated bcl-2 protein. Moreover, in tissues harboring sporadic EBER 1/2-positive lymphocytes, the generation of C or W promoter-derived BHRFI transcripts provides a direct mechanism by which EBV could add to immortalization of latently infected lymphocytes.

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