Epstein-Barr Virus Recombinants from BC-1 and BC-2 Can Immortalize Human Primary B Lymphocytes with Different Levels of Efficiency and in the Absence of Coinfection by Kaposi's Sarcoma-Associated Herpesvirus

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**Epstein-Barr virus** (EBV) and **Kaposi's sarcoma-associated herpesvirus** (KSHV) are human gammaherpesviruses associated with numerous malignancies. Primary effusion lymphoma or body cavity-based lymphoma is a distinct clinicopathological entity that, in the majority of cases, manifests coinfection with KSHV and EBV. In previous analyses, we have characterized the EBV in the BC-1 and BC-2 cell lines as potential intertypic recombinants of the EBV types 1 and 2. In order to examine the infectious and transforming capacities of KSHV and the intertypic EBV recombinants from the BC-1 and BC-2 cell lines, viral replication was induced in these cell lines and fresh human primary B lymphocytes were infected with progeny virus. The transformed clones were analyzed by PCR and Western blotting. All analyzed clones were infected with the intertypic progeny EBV but had no detectable signal for progeny KSHV. Additionally, primary B lymphocytes incubated with viral supernatant containing KSHV alone showed an unsustained initial proliferation, but prolonged growth or immortalization of these cells in vitro was not observed. We also show that the EBV recombinants from BC-1 were less efficient than the EBV recombinants from BC-2 in the ability to maintain the transformed phenotype of the infected human B lymphocytes. From these findings, we conclude that the BC-1 and BC-2 intertypic EBV recombinants can immortalize human primary B lymphocytes, albeit at different levels of efficiency. However, the KSHV induced from BC-1 and BC-2 alone cannot transform primary B cells, nor can it coinfect EBV-positive B lymphocytes under our experimental conditions with B lymphocytes from EBV-seropositive individuals. These results are distinct from those in one previous report and suggest a possible requirement for other factors to establish coinfecion with both viral agents.
from healthy patients have demonstrated that EBV-1 is most prevalent in these individuals and is the only virus type present in at least 90% of the examined cases (18, 50, 51). However, virus isolation studies of certain T-cell-immunocompromised, human immunodeficiency virus-positive cohorts have shown that EBV-2 exists in much greater proportion in these groups (7, 44, 50, 52, 53). The predominance of a single transforming virus strain, most commonly EBV-1 rather than EBV-2, has been demonstrated in healthy individuals; however, increasing evidence suggests that multiple EBV infections are common within immunocompromised hosts (7, 44, 51, 53). Previous studies have documented the existence of intertypic recombinants of EBV-1 and -2 in both a healthy adult (8) and T-cell-immunocompromised individuals (52).

In earlier studies of BC-1 and BC-2, we have characterized the EBV infecting these cell lines as intertypic recombinants of EBV-1 and -2 (1). The discovery of such intertypic recombinants existing in transformed lymphoblastoid cell lines (LCLs) prompts one to ask whether these intertypic EBV recombinants are responsible for the transformed phenotype and whether they can efficiently transform primary B lymphocytes. Previous work has also shown the ability of these viruses to transform B cells (27). However, the question of whether the KSHV contained in BC-1 and BC-2 can infect and/or immortalize human primary B cells as coinfected or singly infected B lymphocytes is important in understanding the initiation and maintenance of the transformed phenotype. In this work, we have shown that the EBV recombinants in BC-1 and BC-2 can infect and immortalize fresh T-cell-depleted peripheral blood mononuclear cells (PBMC); however, these viruses vary in the efficiency with which they transform B lymphocytes. We have also shown that KSHV from BC-1 and BC-2 alone cannot infect and immortalize human primary B cells. Furthermore, KSHV from BC-1 and BC-2 cannot be established as a coinfection with EBV in immortalized B lymphocytes in our system. Previous work by Haas and colleagues showed immortalization of human primary B lymphocytes by KSHV and coinfection with EBV by using PBMC from EBV-seropositive individuals (24). Further investigations into the ability of KSHV and EBV to coinfect human B lymphocytes will help explain the variations in results from our studies and from previously reported studies.

MATERIALS AND METHODS

Antibodies and cell cultures. Latent EBNA proteins were analyzed with antibodies derived from a patient serum previously characterized for identification of each of the EBNA proteins (38). Serum samples were adsorbed against the EBV-negative Burkitt’s lymphoma cell line J18B at 4°C for 48 h. Adsorbed serum was then used as a 1:50 dilution in phosphate-buffered saline (PBS) with 1 mM sodium azide. The hybridoma cell lines PE2, A10, and S12 against EBNA2, EBNA3C, and LMP1, respectively, were cultured, and the supernatant from the PE2, A10, and S12 hybridoma cell lines was used as a 1:2 dilution in PBS with 1 mM sodium azide. Antiserum recognizing the EBV lytic antigens was obtained from patient samples which had been previously characterized (40). The serum was typically diluted 1:50 in PBS after absorption with cell lysate from the EBV-negative cell line J18B.

J18B is an EBV-negative cell line obtained from Elliott Kieff. The B95-8 cell line harbors EBV-1, and the J18B cell line is infected with EBV-2 (28, 29). The P3HR-1 cell line is derived from its Jijoye parent and also contains EBV-2; however, the P3HR-1 genome has regions in the EBNA and EBNA genes deleted (34). BC-1, BC-2, and BC-3 were purchased from the American Type Culture Collection. BCBL-1 was obtained from the AIDS Reference and Reagent Program (35). BC-1 and BC-2 are coinfected with EBV and KSHV (20). BC-3 and BCBL-1 are infected with KSHV alone (3, 35). The cell lines were maintained in complete medium, which consisted of RPMI 1640 supplemented with 10% inactivated fetal bovine serum, 2 mM glutamine, and 10 μg of gentamicin per ml. All cell lines were monitored and routinely fed with fresh medium every 3 to 4 days. BC-1, BC-2, BC-3, and BCBL-1 grew best when maintained at a density of at least 400,000 to 500,000 cells per ml.

Primary B lymphocyte infections. Lytic replication of EBV was induced in the BC-1, BC-2, BC-3, BCBL-1, B95-8, and Jijoye cell lines with a combination of tetradecanoyl phorbol acetate (TPA) and sodium butyrate. We have established a cell line harbors EBV-1, and the J18B cell line is infected with EBV-2 (28, 29). The P3HR-1 cell line is derived from its Jijoye parent and also contains EBV-2; however, the P3HR-1 genome has regions in the EBNA and EBNA genes deleted (34). BC-1, BC-2, and BC-3 were purchased from the American Type Culture Collection. BCBL-1 was obtained from the AIDS Reference and Reagent Program (35). BC-1 and BC-2 are coinfected with EBV and KSHV (20). BC-3 and BCBL-1 are infected with KSHV alone (3, 35). The cell lines were maintained in complete medium, which consisted of RPMI 1640 supplemented with 10% inactivated fetal bovine serum, 2 mM glutamine, and 10 μg of gentamicin per ml. All cell lines were monitored and routinely fed with fresh medium every 3 to 4 days. BC-1, BC-2, BC-3, and BCBL-1 grew best when maintained at a density of at least 400,000 to 500,000 cells per ml.

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this restriction 

the prototypic type 2 P3HR-1 genome. This restriction

located at 6,913 bp in the prototypic type 1 B95-8 genome but

endonuclease digestion of PCR-amplified products. The

I (122-kbp) markers have been previously shown to segregate according to

genomic type at each locus (2, 49).

HI (122-kbp) markers have been previously shown to segregate according to

polymorphisms at these loci. However, our positive

c cavalry different in the amplification products for the type

2 polymorphisms in the EBNA2, -3B, and -3C genes. With

primers specific for these loci, we used PCR analysis to demon- 

strate differences in the amplification products for the type

1 and type 2 alleles. The BC-1 viral genome shows type 2

characteristics at these loci, whereas the BC-2 viral genome

shows type 1 polymorphisms at these markers. Using primers

that amplify from nucleotides 122206 to 122475, we also ana-

lyzed the BC-1 and BC-2 progeny EBV genomes for the pres-

ence of a BamHI restriction site within this amplification frag-

ment. DNA from the B95-8 cell line and the BC-1 and BC-2

parental cell lines has a BamHI restriction site within this PCR

product, while in P3HR-1 DNA this restriction site is absent

(4, 19). The data from these typing experiments suggest that

the EBV infecting the BC-1 and BC-2 progeny cell lines are

the intertypic recombinants derived from the parental cell

lines. Table 1 provides a summary of the PCR analyses of the

progeny cell lines chosen in these series of experiments.

PCR analysis does not detect KSHV DNA in BC-1 and BC-2

progeny cell lines. Using KS330 primers that amplify a distinct

233-bp fragment from the KSHV genome, we performed

PCR analysis for the presence of KSHV in the BC-1 and BC-2

progeny cell lines. We analyzed for the known type 1 and type

2 polymorphisms in the EBNA2, -3B, and -3C genes. With

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progeny cell lines chosen in these series of experiments.
KSHV is not capable of immortalizing T-cell-depleted human primary B lymphocytes in vitro. BC-3 and BCBL-1 are BCBL cell lines that are singly infected with KSHV. In order to examine the infection of primary B lymphocytes by KSHV, we harvested virus after the induction of viral replication with TPA and sodium butyrate. We incubated T-cell-depleted blood B lymphocytes is EBV infected (23). Previous infection studies have shown that the outgrowth of spontaneous LCLs in vitro due to EBV from seropositive individuals is rare (49). After incubation with BC-3 and BCBL-1 supernatants, the cells were plated in 96-microwell plates and observed for evidence of proliferation and immortalization. Table 2 shows the results of three separate experiments. By 2 weeks after plating, initial proliferation and clumping were observed in all wells with a change in pH, as detected by a color change in the medium. After 2 weeks, however, B-cell proliferation did not persist, and no evidence of prolonged growth transformation and immortalization of these cells was observed. Similar initial unsustained proliferation has been seen previously when primary human B lymphocytes were incubated with medium containing TPA and butyrate (E. S. Robertson, unpublished observations). We suggest that the initial proliferation observed in this experiment is not caused by KSHV infection and subsequent mediation of transformation. Rather, we propose that such initial proliferation results from the TPA and butyrate in the media. However, it is possible that KSHV infects primary B cells and triggers proliferation of these cells but this proliferation is not maintained due to the absence of other critical factors.

One important question raised in these experiments was the possibility that the KSHV was not easily induced by TPA and sodium butyrate. Additionally, we wanted to approximate the relative levels of virion particles present in the supernatant. Semiquantitative analysis with KS330 primers (12) for PCR of viral DNA indicated that similar levels of viral DNA were present (Fig. 3B). Moreover, the induction of KSHV was similar for the EBV-positive (BC-1 and BC-2) and EBV-negative (BCBL-1) cell lines. Therefore, KSHV was induced and present at relatively equivalent levels for infection of primary B lymphocytes.

LCLs derived from BC-1 and BC-2 expressed essential latent antigens. Using adsorbed, specific EBNA human serum capable of detecting EBNA1, EBNALP, EBNA2, and EBNA3A, -3B, and -3C (40), we analyzed membranes blotted with fractionated cell lysates from BC-1 and BC-2 parents and progeny (Fig. 4). EBNA1 is expressed in all the cell lines analyzed. The intensity of the EBNA1 signal was lower in BC-1 (Fig. 4a) than in BC-2 (Fig. 4b). The notable size variability of the EBNA1 protein from BC-2 can be attributed to different numbers of IR3 repeats located within the EBNA1 coding region. Because EBNA2 was not readily distinguishable in the BC-1 and BC-2 cell lines, we postulated that the EBNA2 band was comigrating with the EBNA1 band. To test this hypothesis, we blotted separately for the EBNA1 and EBNA2 proteins. A human serum that recognizes only the EBV EBNA1 antigen was used to detect EBNA1 expression. Immunoblot analysis indicates that EBNA 1 is expressed in all progeny cell lines (Fig. 5a and b). Numerous breakdown products were seen to be migrating faster on the blot in the BC-2 analysis. EBNA2 expression was determined by blotting with supernatant from a hybridoma cell line specific for detection of the EBNA2 protein (Fig. 5c and d). This analysis clearly indicated that EBNA2 was expressed in both the BC-1 and BC-2 progeny cell lines and was comigrating with the EBNA1 protein. However, as expected, the BC-1 and BC-2 parental cell lines did not express detectable levels of EBNA2 (Fig. 5c and d, lanes 6) (9). Expression of the EBNA3C protein was clearly seen in the BC-2 progeny but was almost undetectable in the BC-1 progeny (Fig. 5e and f). Since the monoclonal antibodies were made to type 1 EBNA3C antigens, the absence of or lowered EBNA3C detection in BC-1 cell lines may represent the type specificity of the antibodies used and not the actual expression patterns (26). Some signals for EBNA3C were seen in the parental lanes in BC-1 and BC-2; however, this is probably due to the

### Table 2. Infection of primary human B lymphocytes by KSHV

<table>
<thead>
<tr>
<th>Expt</th>
<th>Virus</th>
<th>No. of wells positive for B-cell proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 wk</td>
</tr>
<tr>
<td>1</td>
<td>BC-3</td>
<td>0</td>
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<tr>
<td></td>
<td>BCBL-1</td>
<td>0</td>
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<tr>
<td>2</td>
<td>BC-3</td>
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<td>BCBL-1</td>
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<tr>
<td>3</td>
<td>BC-3</td>
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<td></td>
<td>BCBL-1</td>
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* Initial unsustained proliferation observed in all wells with change in pH as detected by color change in medium and clumping of cells. Similar initial proliferation has been seen when primary human B lymphocytes are incubated with medium containing TPA and butyrate, usually used to induce lytic replication (E. S. Robertson, unpublished observations). We suggest that this observation may be a result of TPA and butyrate which were present in the medium during infection.

![Image](http://jvi.asm.org/Downloadedfrom)
nonspecific signals seen in most of the lanes after prolonged exposure. EBNALP can typically be seen as a ladder of bands from 17 to 73 kDa due to the multiple splicing of the *BamHI* W repeats that span the open reading frame. A number of bands are seen in this region in the progeny cell lines, which indicates expression of EBNALP in all progeny lines derived from BC-1 and BC-2 (Fig. 4).

Expression of the essential EBV latent protein LMP1 was analyzed in the progeny cell lines by Western blotting with supernatant from the S12 hybridoma cell line that specifically detects LMP1 (Fig. 6) (25). All progeny LCLs show reasonably high levels of LMP1 expression. A predominant lower band typical of the D1LMP1 protein can be seen in the BC95-8 control lanes. This D1LMP1 signal and other smaller bands were observed in BC-1 progeny. This may indicate relatively high levels of spontaneous lytic replication. Additionally, the BC-1 progeny cell lines express LMP1 at markedly higher levels than do the BC-2 progeny. Moreover, these same LCLs expressed high levels of the D1LMP1 variant. However, this form of LMP1 was not detectable in the analyzed BC-2 progeny LCLs.

Early antigen expression is detected in the BC-1 and BC-2 progeny LCLs. For detection of the EBV early antigens we used a human serum capable of detecting a number of lytic antigens, including BALF2, the single-stranded DNA binding protein, the EA-D (early antigen diffused) complex, and BZLF1 (14, 23). The EA-D antigens range from 43 to 56 kDa. The BALF2 protein and BZLF1 gene product are usually seen in EBV-infected cells undergoing active replication (13, 39). The BC-1 parent expresses EA-D antigens as well as BALF2 (Fig. 7a), whereas the BC-2 parent does not express detectable levels of early antigens (Fig. 7b). Progeny cell lines from both BC-1 and BC-2 appear to be expressing low levels of EA-D antigens. A larger band at approximately 135 kDa indicated expression of the BALF2 single-stranded DNA binding protein in all progeny cell lines (Fig. 7). Only one BC-1 progeny cell line (BC-1.22) was tightly latent, as indicated by undetectable levels of lytic antigen expression (Fig. 7a, lane 5). Another band located at approximately 80 kDa is present in many of the progeny BC-1 and BC-2 cell lines. This band corresponds to the EBNA1 protein that was recognized by the human serum used to detect the early antigens.

BC-1 and BC-2 intertypic EBV recombinants transform primary B cells with various efficiencies. After induction of EBV lytic replication in the BC-1 and BC-2 parental cell lines, T-cell-depleted PBMC were infected with filtered virus supernatant. The infected B lymphocytes were then plated out in a 96-well plate and screened for outgrowth of transformed B lymphocytes. Outgrowth was designated positive if visible clumping of transformed cells and notable color changes in the medium were observed. The experiment was performed three times for verification of the results, and the data are listed in Table 3. In each experiment, BC-2 progeny virus transformed B cells and led to macroscopic outgrowth at a much higher rate than the virus induced from the BC-1, B95-8, and Jijoye cell lines. In experiment 1, for example, BC-2 gave 14 wells positive for B-cell outgrowth at week 1. BC-1 and Jijoye gave no positive wells at week 1, and B95-8 gave only 8 wells positive for B-cell outgrowth. By week 4 of experiment 1, BC-1 showed 62 wells positive for B-cell outgrowth and BC-2 had 76 wells with visible outgrowth of transformed B cells. At week 4, B95-8 and Jijoye had 68 and 71 positive wells, respectively. The number of wells demonstrating visible B-cell transformation continued to increase for all cell lines until week 8, with the total number of positive wells for each cell line in experiment 1 increasing by 23 to 35%. By week 24, a striking difference in prolonged outgrowth was observed. As expected, the total number of positive wells had decreased due to the eventual death of cells that were not truly immortalized. However, BC-1 showed a much more drastic decline in total number of positive wells than BC-2, B95-8, and Jijoye. By week 24, 71% of the BC-1 wells that were once positive for outgrowth of B cells had lost the transformed phenotype and failed to maintain outgrowth. BC-2 demonstrated the greatest number of stable transformants, with only a 25% reduction in the total number of positive wells as measured from week 8. B95-8 and Jijoye underwent reductions of 41 and 55%, respectively. By week 48, only two wells from BC-1 remained positive for outgrowth. BC-2, on the other hand, did not lose any positive wells over the last 24-week period. B95-8 and Jijoye demonstrated 20 and 10% reductions in cells with the transformed phenotype, respectively. The data from experiments 2 and 3 corroborate the data from experiment 1, as seen in Table 3.

To verify that the experiments were controlled for levels of virion particles, we analyzed the virus supernatant for EBV...
produced upon induction of BC-1 and BC-2 by TPA and sodium butyrate. We used the same volume of virus supernatant to maintain a similar exposure to the TPA and butyrate present in the virus supernatant. Similar to the cases of the B95-8 and Jijoye cell lines, EBV was readily induced in BC-1 and BC-2. Moreover, BC-2, which has a more potent transforming potential, had less virus present in the supernatant (Fig. 2A). Hence, while one could postulate that the observed results in Table 3 could be attributed to relatively higher levels of competent virus in BC-2, Fig. 2A indicates that this was not the case.

To determine if these results may also be due to the type of EBNA2 and EBNA3 loci in BC-1, we compared BC-1 to Jijoye, the prototypic type 2 virus used in our experiment. Although the amount of LCLs obtained from infections with Jijoye was lower than the number derived from the type 1, B95-8 virus, it was substantially higher than that seen for BC-1 infections. Hence, the lower transforming potential of BC-1 EBV cannot be attributed solely to its type 2 EBNA2 and EBNA3 genomic loci.

These results indicate that the BC-2 intertypic recombinant EBV is a more potent transforming agent than either the B95-8 and Jijoye controls or the BC-1 EBV. In addition to initiating transformation more effectively, BC-2 EBV also maintained the transformed phenotype at significantly higher levels throughout the experiment. Additionally, BC-1 was unable to maintain long-term outgrowth of LCLs as efficiently as BC-2, suggesting possible genomic abnormalities. The fact that BC-1 and BC-2 are intertypic recombinants of EBV-1 and -2 may provide one explanation. However, BC-1 was capable of transforming B lymphocytes with relatively high efficiency. Therefore, we would suggest that there is a greater probability for genomic abnormalities in the BC-1 recombinant.

FIG. 5. Western blot analysis with antibodies specific for EBNA1, -2, and -3. Western blots of BC-1 (a) and BC-2 (b) with human serum specific for EBNA1 recognition are shown. Cross-reactive antibodies to EBNA1 recognized both type 1 and type 2 variants of the protein. All BC-1 and BC-2 parents and progeny express the EBNA1 protein. Western blots with monoclonal antibodies specific for EBNA2 are shown for BC-1 (c) and BC-2 (d). The progeny cell lines appear to be expressing EBNA2 (lanes 1 to 5), but the parent cell lines do not (lane 6). These results confirm that EBNA2 was comigrating with EBNA1 in our experiments. Western blots with monoclonal antibodies specific for recognition of the EBNA3C protein are shown for BC-1 (e) and BC-2 (f), respectively. Since the monoclonal antibodies were made in response to type 1 antigen, apparent expression differences between BC-1 and BC-2 may be attributable to type-specific differences in antigen recognition.

DISCUSSION

The PCR data presented in this work clearly show that the progeny BC-1 and BC-2 EBV are intertypic recombinants that were derived from the parental cell lines. PCR evidence also demonstrates that no coinfection with KSHV occurred in over 500 LCLs analyzed in our experiments. Western blot analyses of the EBV proteins expressed in the progeny cell lines indicate an expression pattern that is consistent with the latent transformed state. Unlike the parental cell lines, which demonstrate a latency II (Lat II) pattern of expression (9), the
progeny cell lines appear to demonstrate a Lat III pattern that is characteristic of transformed LCLs (37). In Lat III, a full pattern of latent gene expression is observed, including expression of the Cp- and Wp-driven set of EBNA transcripts and the BamHI N-derived mRNAs encoding several virus latent membrane proteins (for a review, see reference 32). The fact that the progeny cell lines are expressing the EBNA2 protein, essential for EBV-induced B-cell transformation, and the LMP1 oncogene argues strongly for the ability of the intertypic recombinant viruses to drive cell proliferation.

The discovery of intertypic EBV recombinants in the transformed BC-1 and BC-2 cell lines prompted some crucial questions about the transforming efficiencies of these viruses. Assays to determine the transformation efficiencies of the BC-1 and BC-2 intertypic EBV recombinants indicate that the BC-2 EBV is a more potent transforming virus than the type 1 B95-8 or the type 2 Jijoye. On the other hand, BC-1 EBV was shown to be the least potent of the analyzed transforming viruses. BC-2 EBV was also shown to be most successful in maintaining the transformed phenotype over time, while BC-1 EBV appeared to be least capable of maintaining transformation in cells. B cells transformed by BC-1 EBV showed a significant decrease in the transformed phenotype when observed over a 48-week period following initial infection. It is possible that the recombination events had resulted in aberrant genomes of BC-1 and BC-2 and that the BC-1 genome is rearranged in a manner deleterious to the long-term survival of LCLs in vitro. However, this does not suggest that the BC-1 intertypic EBV has no role in inducing PELs in vivo. It is possible that the BC-1 recombinant virus provides critical functions in patients infected with KSHV to drive cell proliferation in the immunocompromised individuals (20). The BC-1 transformed phenotype may be maintained by the synergistic actions of EBV and KSHV infecting these cells in vivo.

Previous studies of human immunodeficiency virus-positive T-cell immunocompromised cohorts have indicated a greater prevalence of type 2 EBV in these individuals as well as an increased frequency of multiple EBV infections (50, 53). The discovery of intertypic EBV recombinants in these patients (52) has suggested that the impaired immune surveillance and higher titers of EBV in immunocompromised individuals may foster the development of novel recombinant strains. The demonstration of the BC-2 intertypic EBV recombinants as distinctly more potent at transforming PBMC in vitro is an important finding that may provide evidence for intertypic recombination as an evolutionary mechanism for the emergence of more potent and efficient transforming strains of EBV. The selective advantage of a virus with enhanced transforming capacity could lead to altered tropisms for host cells and an increase in the virulence of these strains of EBV. Type 1-type 2 EBV chimeras may also exhibit novel mechanisms of immune evasion. Changes in patterns of gene expression or alterations in programs of EBV latency may enable these recombinant variants to elude established immune responses to EBV infection. New combinations of EBV-1 and -2 antigens could help these emerging intertypic strains to elude the established host immune response, which most often involves antibody recognition of the widely prevalent type 1 antigens. Since the recent discovery of KSHV, much study has focused...
on the potential transforming properties of this virus. The virus appears to have much potential to trigger malignancy, including many candidates for transforming genes (for a review, see reference 5). One recent report proposes that KSHV is capable of transforming primary human endothelial cells (15). This is the first report declaring that KSHV can transform any cell type; however, it should be noted that the virus was lost over time in the majority of cells (15). Our data suggest that KSHV alone cannot transform human primary B lymphocytes. One recently published study by Kliche and colleagues posits that KSHV can maintain persistent infection of EBV-positive B lymphocytes and can be serially passaged from KSHV+ EBV− LCLs to fresh PBMC (24). Furthermore, this group presents data from infection of PBMC from EBV+ donors with BCBL-1 supernatants and suggests that KSHV infection can induce LCL outgrowth of primary B lymphocytes. They also state that KSHV+ EBV− immortalized cell lines have been derived from infections of PBMC from EBV-seropositive donors with supernatants from the BCBL-1 cell lines. Based on numerous infection studies, we have no evidence that KSHV can induce immortalization of T-cell-depleted human primary B lymphocytes. We also have no data to suggest that infection of PBMC from EBV-seropositive patients with supernatants from induced BCBL-1 or BC-3 cell lines can produce KSHV+ or KSHV+ EBV+ immortalized cell lines. Since Kliche and colleagues observed that infection of PBMC with BC-1 supernatant did not result in the outgrowth of EBV+ LCLs but instead resulted in the outgrowth of LCLs infected with both EBV and KSHV, they propose that this observation may indicate a selection for infection with both viruses. However, our data suggest that the KSHV from the KSHV+ EBV+ BC-1 and BC-2 cell lines can potentially infect primary B cells but cannot maintain a sustained coinfection with EBV in human primary B lymphocytes, thus indicating no selection for coinfection. The contradiction in the observed results from these two studies may indicate the necessity of important cofactors for KSHV infection that were present in one study and absent in the other. Hence, we propose that important differences in the experimental setup and in the isolation methods used in preparing the PBMC for infection in our experiments and in those of Kliche and colleagues may account for the observed differences.

The study of PEL-derived cell lines continues to produce new insights into their unique biology. Of central importance to this biology is the potential synergistic relationship between KSHV and EBV. Much evidence suggests that cells infected with both viruses may have a growth advantage (24). Our data suggest that coinfection of KSHV with EBV may alter the gene expression patterns of EBV. For example, all progeny cell lines express both LMP1 and EBNA2; however, neither parental cell line expresses EBNA2. While a model remains to be established, differences between EBV expression patterns of KSHV-positive parental and KSHV-negative progeny cell lines may indicate that the functions of some KSHV proteins can supplement or possibly even substitute for the functions of certain EBV proteins. Further study of the BC-1 and BC-2 cell lines will continue to elucidate any potential synergism between these two latently infected herpesviruses.

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