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Glycoprotein 110, the Epstein-Barr Virus Homolog of Herpes Simplex Virus Glycoprotein B, Is Essential for Epstein-Barr Virus Replication In Vivo

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The Epstein-Barr virus (EBV) glycoprotein gp110 has substantial amino acid homology to gB of herpes simplex virus but localizes differently within infected cells and is essentially undetectable in virions. To investigate whether gp110, like gB, is essential for EBV infection, a selectable marker was inserted within the gp110 reading frame, BALF4, and the resulting null mutant EBV strain, B95-110HYG, was recovered in lymphoblastoid cell lines (LCLs). While LCLs infected with the parental virus B95-8 expressed the gp110 protein product following productive cycle induction, neither full-length gp110 nor the predicted gp110 truncation product was detectable in B95-110HYG LCLs. Infectious virus could not be recovered from B95-110HYG LCLs unless gp110 was provided in *trans*. Rescued B95-110HYG virus latently infected and growth transformed primary B lymphocytes. Thus, gp110 is required for the production of transforming virus but not for the maintenance of transformation of primary B lymphocytes by EBV.

Herpesviruses are widely disseminated in nature, with most animal species yielding at least one example (33). The unifying hallmark of the herpesviruses is their ability to establish lifelong latent infections after primary infection. Epstein-Barr virus (EBV), one of eight human herpesviruses, undergoes a primary, productive infection in epithelial tissues, followed by the establishment of latent infection in B lymphocytes (14, 24). It is thought that EBV then reactivates episodically from infected B lymphocytes over the life of the host, leading to periodic production and shedding of virus progeny. Although strategies to establish latency differ greatly among the human herpesviruses, many features of productive replication are conserved among the family members (14, 24). All herpesviruses appear to employ membrane glycoproteins in a variety of important processes during their production, maturation, and transmission, including specific binding to the cell surface, fusion of the viral and plasma membranes during entry, virion assembly, and egress (37, 38). Among the alphaherpesviruses, five glycoproteins which are important either for plasma membrane binding or for entry have been identified. Specific binding to cells is primarily the role of gC, although gB may have an accessory role (11, 12, 18, 36, 40, 45). Delivery of the viral capsid into the cell requires the actions of gB, gD, and the gH/gL complex, all of which are essential for infection (3, 4, 6, 17, 18, 34, 35).

When compared with their homologs among the alphaherpesviruses, the nucleotide and amino acid sequences of the nine defined EBV glycoproteins range from substantially conserved to virtually unrelated (13, 14). For instance, a gH/gL complex homolog (gp85/gp25) is present in EBV-infected cells, but it contains a third glycoprotein, gp42, which has no known relatives in the other human herpesviruses (9, 10, 13, 16, 27, 28). This additional component appears to be important for infection of B lymphocytes but not for infection of epithelial cells (16). Perhaps reflecting its specialized cellular niche, EBV binding to the cell surface utilizes a specific interaction between the type 2 complement receptor CR2 (CD21) and gp350/220, which also has no known herpesvirus homolog (29, 30, 42). Despite having considerable amino acid homology to gB, gp110, unlike gB, is not a major component of the virion, and it is not found in the plasma membranes of infected cells; instead it localizes predominantly to the inner and outer membranes of the nucleus (5, 7, 8, 32). Consistent with such observations, antibodies available against gp110 fail to neutralize EBV infectivity (7). These unique features of gp110 would seem to place it somewhere between a condition in which both sequence and function are conserved, as is seen among other herpesvirus gB molecules, and a condition of nonidentity to gB, as is the case with gp350/220 and gp42. This suggests first that a direct role for gp110 in EBV viral entry is unlikely but, more interestingly, that gp110 activity during EBV infection may have become fundamentally different from that of gB. The possibility that gp110 has an activity unique to EBV infection raises the question of whether this function is dispensable for EBV infection. To begin clarifying the role of gp110, a recombinant EBV bearing a large insertion mutation in gp110 which renders the mutant virus nonviable was created and purified.

Construction of gp110 mutant EBV recombinants. To determine if gp110 is essential for EBV replication, a marker selection strategy was used to create a recombinant EBV strain carrying a null mutation in gp110. A cassette expressing hygromycin phosphotransferase from the simian virus 40 early promoter (SVHYG) was inserted into the *Nsi*I site of the BALF4 (gp110) reading frame within the cloned *Bam*HI A fragment of B95-8 EBV (1). In the resulting plasmid, pgp110HYG (Fig. 1), the SVHYG insertion causes premature termination of the production of the 854-amino-acid gp110 protein product after 340 amino acids. Because it lacks hydrophobic transmembrane domains, the gp110 truncation product would likely be se-

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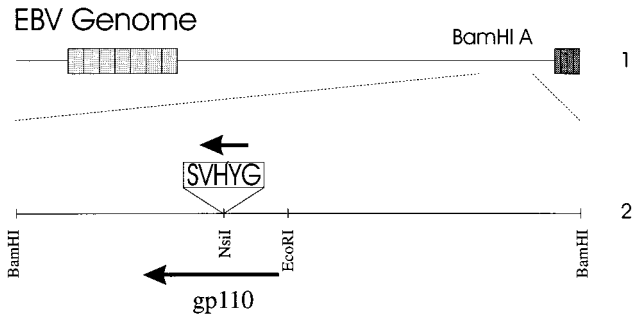

FIG. 1. Schematic representation of the EBV genome. In line 1, the linear form of the EBV genome is shown with the two major repeat areas of the genome (IR1 and TR) indicated as boxes. Line 2 depicts the location of the gp110 reading frame within the *Bam*HI A fragment, the location of the *Nsi*I site into which the selectable marker SVHYG was inserted, and the relative transcriptional orientation of SVHYG.

creted from the infected cell, as are other truncated herpesvirus membrane glycoproteins (2, 39, 44). The mutated *Bam*HI A fragment of pgp110HYG was cloned into the dual-origin-recombination vector pDO (43) and transfected into B95-8 cells, a marmoset cell line latently infected with B95-8 EBV (25, 26). A second plasmid, pSVNaeZ, was cotransfected to express the Zta EBV immediate-early transactivator and induce lytic infection in the B95-8 cells (41). To obtain lymphoblastoid cell lines (LCLs) infected with recombinant EBV gp110 mutants, primary B lymphocytes were infected with cell-free virus prepared from the transfected B95-8 cells, plated in 96-well microtiter dishes, and selected with hygromycin B (50 μg/ml), all as previously described (19, 21, 22). Multiple wells of hygromycin-resistant LCLs were generated from two independent experiments; nine of these were expanded and studied further.

The presence of the hygromycin gene in the LCLs was first analyzed by PCR. LCL DNA was prepared and subjected to amplification as previously described (41) with the primers 5′HYG IN (TCGGCACTTTGCATC) and 3′HYG IN (AGC GATCGCATCCAT) (Fig. 2D, line 4). Of the nine LCLs examined, eight exhibited a 207-bp amplification product corresponding to the expected hygromycin-specific fragment (Fig. 2A). A control reaction with pgp110HYG as the template also yielded the 207-bp product. As expected, no amplified product was detected with these primers in LCL2, a B95-8 EBV-transformed lymphoblastoid cell line (19), M12, a murine B-lymphocyte line (15), BJAB, and EBV-negative Burkitt's lymphoma cell line (23), or in reaction mixtures containing primers only (Fig. 2A). The single LCL from which the hygromycin gene could not be amplified, gp110.8, was not further analyzed. To establish that the gp110 reading frame remained disrupted by the SVHYG insertion, primers 3′HYG OUT (TCAGCCAGCAACTCG) and 3′gp110 (TTCTTGACGGT GGCC) were synthesized (Fig. 2D, line 5). These primers amplified the expected 350-bp product from all eight hygromycin-resistant LCLs and the pgp110HYG plasmid control but not from LCL2, M12, BJAB, or reaction mixtures containing primers only. Representative PCR data are shown in Fig. 2B. Similar results were obtained for the 5′ junction of SVHYG and BALF4 (data not shown). Finally, amplifications were done with primers 5′gp110 (TGTGACTGACGAGGG) and 3′gp110 to verify that the wild-type gp110 gene was interrupted (Fig. 2D, line 1). Since these primers could bind to both wild-type and mutant genomes, the sensitivity of competitive amplification was first measured in mixing experiments with plasmid DNA which showed that the gp110 primer pair could amplify a single wild-type copy of gp110 mixed with as many as 500 mutated copies (data not shown). When these primers were then used to amplify LCL DNA, the predicted 235-bp product was detected in wild-type-infected LCLs (LCL2) and in four of the eight hygromycin-positive LCLs (Fig. 2C and data not shown). These four LCLs were therefore either coinfected with wild-type and mutant EBV genomes, or infected with nonhomologous recombinant genomes. By PCR, the remaining four LCLs contained only B95-110HYG EBV. This result indicated that gp110 is clearly not required for continued proliferation of LCLs, as would be presumed of a late replicative gene product (8). Control PCR mixtures with pgp110 HYG, M12 cells, BJAB cells, or primers only did not yield amplification products (Fig. 2C).

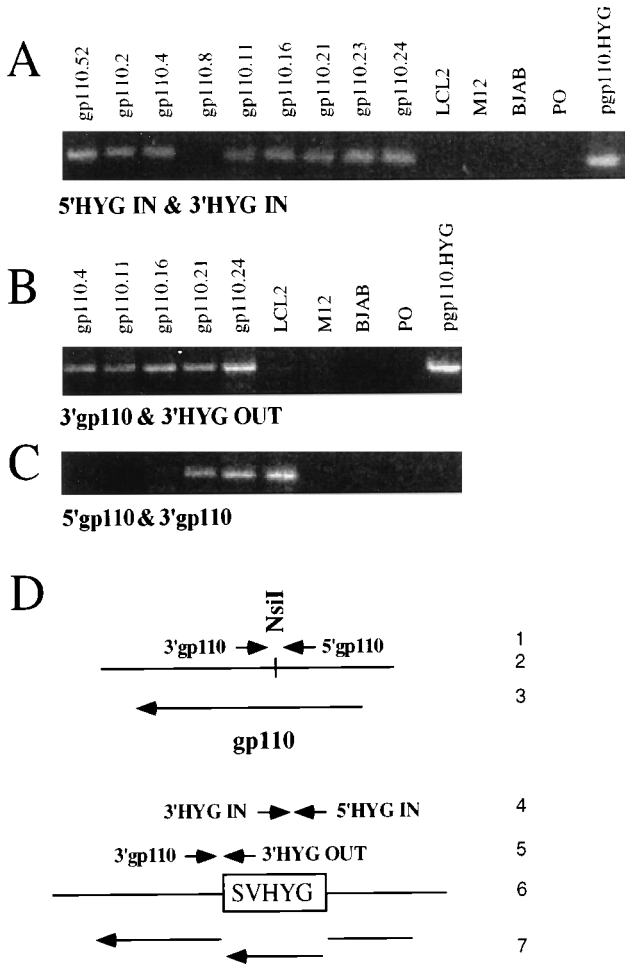

FIG. 2. PCR analysis of the hygromycin-resistant LCLs. (A) Ethidium bromide-stained PCR products obtained from LCL DNA with primers within the hygromycin open reading frame. LCL2, M12, and BJAB lack the hygromycin gene. Lanes PO (primers only) contained a control reaction mixture without template DNA. The plasmid used to introduce the gp110 mutation, pgp110HYG, and all of the gp110-infected LCLs except gp110.8 contain the expected 207-bp amplified product. (B) PCR products obtained from LCL DNA with primers amplifying the 3′ region of the SVHYG cassette and a region of BALF4 3′ to the *Nsi*I site. All of the cell lines positive for the hygromycin cassette from panel A and the pgp110HYG plasmid contain the 350-bp amplified product. (C) PCR products with primers which flank the *Nsi*I site in BALF4. Only LCLs containing wild-type gp110 exhibit the 235-bp amplified product. (D) Schematic representation of the locations of the primers used in the above analyses.

The structures of the EBV genomes in six representative

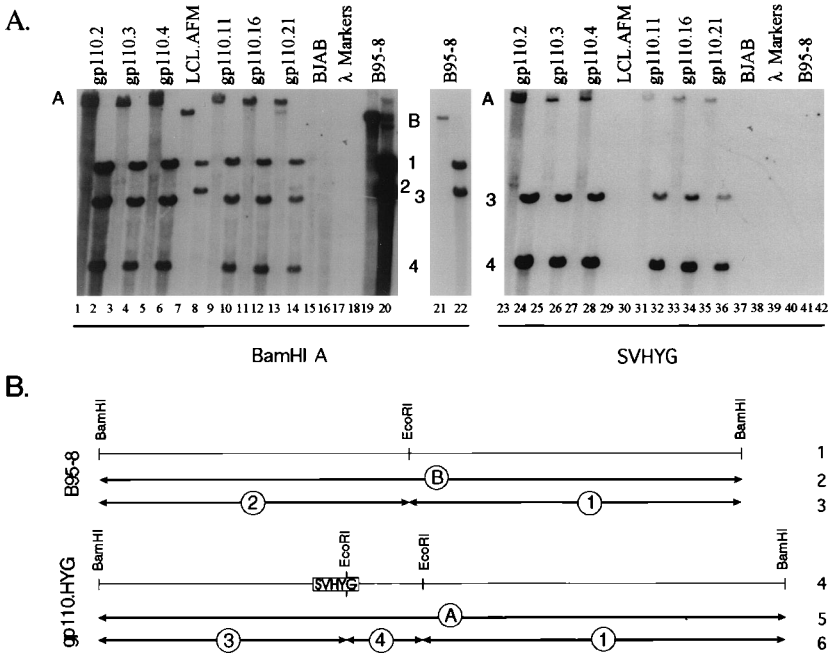

FIG. 3. Southern blot analysis of hygromycin-resistant LCL DNA. DNAs from representative LCLs and control cell lines were digested with *Eco*RI (first lane of each pair) or with *Eco*RI plus *Bam*HI (second lane of each pair), Southern blotted, and first probed with the *Bam*HI A fragment from the B95-8 genome (lanes 1 to 22). The blot was then stripped and reprobed with the SVHYG cassette (lanes 23 to 42). LCL.AFM is a B95-8-transformed LCL, BJAB is an EBV-negative Burkitt's lymphoma cell line, and B95-8 is a marmoset LCL infected with the B95-8 virus. Lanes 17, 18, 39, and 40 contain similarly digested phage lambda marker DNA. The letter and numeral designations between the panels correspond to those in panel B, below. (B) Predicted restriction fragments resulting from *Bam*HI or *Bam*HI-plus-*Eco*RI digestion of the B95-8 genome (lines 1 to 3) and the B95-110HYG genome (lines 4 to 6). Expected fragments from *Bam*HI digests and *Bam*HI-*Eco*RI double digests are designated by letter and number, respectively, for each genome and correspondingly labeled in panel A.

LCL samples were further examined by Southern blot analysis. Duplicate DNA samples for the LCLs and control cell lines were digested with *Bam*HI or *Bam*HI plus *Eco*RI, blotted, and sequentially probed with the *Bam*HI A fragment from B95-8 (Fig. 3B, line 1) and then with SVHYG as previously described (43). To simplify interpretation of the Southern analysis, the expected digestion products are referred to by letters (*Bam*HI fragments) or numbers (double-digest fragments) in the text and in Fig. 3. In *Bam*HI digests of B95-8 and LCL.AFM (a B95-8-transformed LCL), an 11,867-bp fragment (Fig. 3B, line 2) was detected with the *Bam*HI A probe (Fig. 3A, lanes 7, 19, 21). In LCLs infected only with B95-110HYG, this probe detected a 13,617-bp fragment (Fig. 3A, lanes 3, 5, 9, and 11, and B, line 5). In LCL gp110.2 and LCL gp110.21, shown by PCR to contain both SVHYG and wild-type gp110 DNA, both wild-type and mutant-size *Bam*HI fragments were detected with the *Bam*HI A probe (Fig. 3A, lanes 1 and 13). In the *Bam*HI and *Eco*RI double digests, the *Bam*HI A probe detected two fragments of 5,106 and 6,761 bp in LCL.AFM and B95-8 (Fig. 3A, lanes 8, 20, and 22) because of cleavage at an internal *Eco*RI site (Fig. 3B, line 3). In the same digests of LCLs infected only with B95-110HYG, three fragments of 6,761, 4,620, and 2,236 bp were detected with the *Bam*HI A probe (Fig. 3A, lanes 4, 6, 10, and 12), as predicted from the additional *Eco*RI site present in SVHYG (Fig. 3B, line 6). In the coinfected LCLs, both wild-type and mutant fragment patterns were detected (Fig. 3A, lanes 2 and 14). As expected, BJAB DNA did not hybridize with either probe, nor did phage lambda marker DNA that had been added to the LCL samples as a digestion control (Fig. 3A, lanes 15 to 18 and 37 to 40). In Fig. 3A, lanes 21 and 22 are a lighter exposure of lanes 19 and 20 for purposes of reference. Following the first autoradiography, the blot was stripped, verified to be free of residual counts, and reprobed with the SVHYG cassette. The autoradiograph on the right of Fig. 3A shows that the SVHYG probe hybridized to fragment A in *Bam*HI digests and to fragments 3 and 4 in the double digests (lanes 23 to 28 and 31 to 36), also as predicted by the EBV B95-110HYG restriction map (Fig. 3B, line 6). The SVHYG probe failed to hybridize with LCL.AFM, BJAB, phage lambda marker DNA, or B95-8 DNA (Fig. 3A, lanes 29, 30, and 37 to 42). Additional *Eco*RI or *Hin*dIII digests clearly established that the SVHYG cassette was linked to the EBV DNA normally adjacent to the *Bam*HI A fragment, providing final confirmation that the wild-type gp110 allele had been replaced by the gp110HYG allele in B95-110HYG (data not shown).

Analysis of B95-110HYG EBV productive infection. EBV gp110 is expressed late in infection (8); however, since LCLs are generally nonpermissive for EBV lytic infection, the four LCLs infected only with B95-110HYG were initially examined in transfection studies to identify clones in which lytic functions could be studied. Following transfection with pSVNaeZ and treatment with tetradecanoyl phorbol acetate, only one LCL of the four, gp110.16, was induced in sufficient quantities to allow further analysis (data not shown); consequently, only gp110.16 and LCLs derived from it were used for the protein expression and complementation analyses which follow. To facilitate these next experiments, a gp110 expression vector, pSVgp110, was constructed by cloning a 2,830-bp *Tth*111I-to-*Nar*I fragment containing the entire gp110 open reading frame into pSG5 (Stratagene). Protein expression from pSVgp110 was confirmed by immunoprecipitation of labeled cell lysates with a gp110 monoclonal antibody, L2 (Virotech International, Rockville, Md.) (data not shown). In the following series of protein

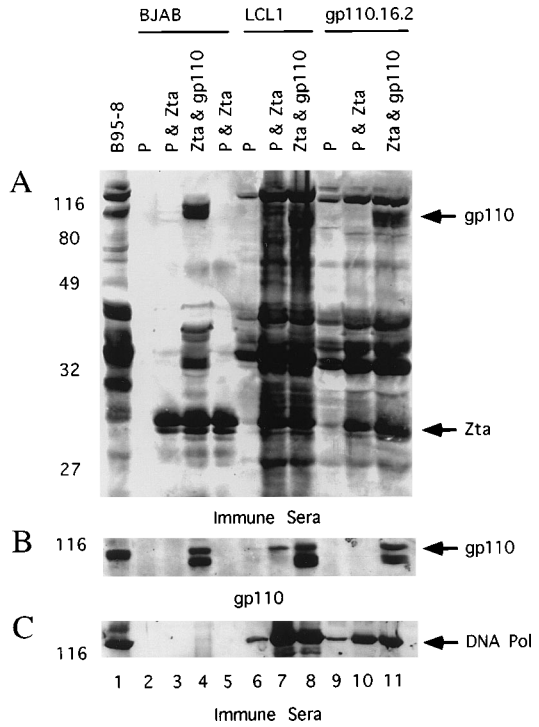

FIG. 4. Lytic-gene and gp110 expression in transfected BJAB cells, wild-type LCLs, and B95-110HYG LCLs. LCL1 is a B95-8-transformed LCL. gp110.16.2 is a secondary-passage B95-110HYG LCL. BJAB, LCL1, and gp110.16.2 cells were transfected with the indicated plasmids, as described in the text, resuspended in media containing 20 ng of tetradecanoyl phorbol acetate, and analyzed after 2 (BJAB) or 4 (LCLs) days. (A) Extracts of approximately 10^6 cells were separated in 10% denaturing polyacrylamide gels, transferred to nitrocellulose, and blotted with an EBV-immune human serum by enhanced chemiluminescence (Amersham). The locations of the gp110, EBV DNA polymerase, and Zta proteins are indicated. (B) gp110 expression determined by immunoprecipitation. gp110 was precipitated from 1% Nonidet P-40 whole-cell lysates with the L2 monoclonal antibody (Virotech), separated in 7% denaturing polyacrylamide gels, and immunoblotted with the EBV-immune human serum in panel A. (C) Induction of the DNA polymerase in the transfected cells. Extracts of approximately 10^6 cells were separated in 5% denaturing polyacrylamide gels, transferred to nitrocellulose, and probed in Western blots (immunoblots) with an EBV-immune human serum by enhanced chemiluminescence. P = pSG5, the parental cDNA expression vector (Stratagene), Zta = pSVNaeZ (41), and gp110 = pSVgp110. The sizes of protein standards are indicated in kilodaltons on the left.

analysis and passage experiments, LCL1, a control B95-8-infected LCL, and B95-110HYG LCLs were transfected pairwise with 12.5 μg each of pSVNaeZ, pSVgp110, or the vector pSG5, as indicated in Fig. 4 and Table 1. pSG5 DNA (25 μg) was transfected in experiments with the vector alone. Prior to all analyses, the cells were cultured for an additional 3 to 5 days in media containing 20 ng of tetradecanoyl phorbol acetate per ml to further induce lytic replication (19, 20).

EBV lytic protein expression in B95-110HYG-infected LCLs. To detect EBV productive antigens, total cellular proteins were immunoblotted with an EBV immune human serum and subjected to enhanced chemiluminescence (Fig. 4A) as previously described (19, 20). Untransfected B95-8 cells reacted strongly with the human serum, whereas no reactivity was observed in EBV-negative BJAB Burkitt's lymphoma cells transfected with the control pSG5 vector (Fig. 4A, lanes 1 and 2). Zta was readily detected in the BJAB cells transfected with pSVNaeZ (Fig. 4A, lanes 3 to 5), and gp110 was detected in BJAB cells transfected with pSVgp110 (Fig. 4A, lane 4). Some possible degradation of gp110 was observed in the pSVgp110-transfected cells (Fig. 4A, lane 4, between the 32- and 49-kDa markers). Following transfection of LCL1 and LCL gp110.16.2 with the pSG5 vector, small amounts of EBV-productive antigens were seen (Fig. 4, lanes 6 and 9), reflecting measurable, but inefficient reactivation of lytic replication by the tetradecanoyl phorbol acetate. Transfection with pSVNaeZ resulted in improved induction of lytic antigens in LCL1 and LCL gp110.16.2, indicating more efficient switching to lytic infection (Fig. 4A, lanes 7 and 10). Nonetheless, the gp110 protein could not be detected in gp110.16.2 (or other similarly inducible B95-110HYG LCLs), even after a prolonged exposure of the film (Fig. 4A, compare lanes 7 and 10), confirming the gp110 null phenotype of B95-110HYG EBV.

To further confirm the absence of gp110 expression in the B95-110HYG LCLs, immunoprecipitations with the L2 monoclonal antibody were performed with the wild-type and B95-110HYG LCLs. The immunoprecipitates were immunoblotted with the EBV immune human serum, and enhanced chemiluminescence was performed as shown in Fig. 4A. As expected, gp110 was readily detected in B95-8 cells, in wild-type LCLs induced by Zta, and in wild-type- and B95-110HYG-infected LCLs transfected with pSVgp110 (Fig. 4B, lanes 1, 4, 7, 8, and 11). In the Zta-induced LCLs infected with B95-110HYG virus, gp110 or a gp110 truncation product was never detected, even upon prolonged exposure of the blot (Fig. 4B, lane 10, and data not shown).

To ensure that the gp110-mutant-infected cells were switching from latent to lytic infection, whole-cell lysates of the transfected cells were separated on sodium dodecyl sulfate-polyacrylamide gels and immunoblotted with EBV-immune human sera reactive with EBV antigens. The DNA polymerase, an EBV early antigen, is recognized by this serum. Increased levels of expression of the DNA polymerase were detected in both wild-type and B95-110HYG-infected LCLs when they were transfected with pSVNaeZ (Fig. 4C, lanes 7, 8, 10, and 11), compared with pSG5-transfected LCLs (Fig. 4C, lanes 6 and 9). In conclusion, the expression of gp110 could not be detected in the LCLs infected with recombinant EBV containing mutations in gp110, despite gp110 readily being detected in wild-type-virus-infected LCLs in which lytic replication had been induced in parallel.

Lymphocyte transformation by B95-110HYG. The growth of LCLs infected only with B95-110HYG EBV indicated that gp110 was clearly dispensable for the continued proliferation of EBV-infected, transformed B lymphocytes, as previously shown (32a). However, gp110 could have been required for the production of the mutant-transforming virus and would have been provided by wild-type B95-8 virus present in the transfected B95-8 cells in which the recombinant was generated. To address this issue, virus replication was induced in LCL1 or gp110.16 by cotransfection with pSVNaeZ and pSG5 or pSVgp110, and transforming activity was assessed by exposing primary B lymphocytes to cells or media from the induced cultures as previously described (19, 20, 22). Although LCL1 generally transfected more efficiently than the B95-110HYG LCLs, the addition of pSVgp110 to LCL1 transfections did not consistently enhance the yield of transforming virus as measured by the number of progeny LCLs recovered (Table 1, compare the numbers obtained with P-Zta and Zta-gp110). In contrast, transforming virus was obtained from the B95-110HYG-infected LCLs only when pSVgp110 was cotransfected (Table 1), indicating that gp110 is essential for EBV lytic replication. With pSVgp110 for complementation, B95-110HYG EBV was serially passaged twice from LCL gp110.16 (Table 1). At each passage, the structure and purity of the B95-110HYG genomes were confirmed by PCR, as was the absence of gp110 protein expression (data not shown). The

TABLE 1. Transformants obtained by passage of wild-type and B95-110HYG-infected LCLs[a]

Expt	Expt type	Cell line	No. of clones/96-well plate		
			P[b]	P-Zta	Zta-gp110
1	Cell free	LCL1	45	60	70
		gp110.16	0	0	2
2	Cocultivation	LCL1	0	12	60
		gp110.16.2	0	0	2
3	Cocultivation	LCL1	2	4	2
		gp110.16	0	0	1
4	Cocultivation	LDL1	4	7	4
		gp110.16	0	0	4
5	Cocultivation	LCL1	40	32	60
		gp110.16.2	0	0	2
6	Cell free	LCL1	1	2	3
		gp110.16.2.A	0	0	1
7	Cell free	LCL1	0	4	3
		gp110.16.2	0	0	1
8	Cocultivation	LCL1	1	9	11
		gp110.16.2	0	0	4
9	Cocultivation	LCL1	0	3	4
		gp110.16.2	0	0	2

[a] Cell lines were electroporated with the indicated plasmids, primary human B lymphocytes were infected 3 to 5 days postelectroporation, and wells positive for EBV transformation were counted after 6 weeks. One 96-well plate was used for each transfection. LCL gp110.16.2 was isolated in experiment 1, and LCL gp110.16.2.A was isolated in experiment 5. The presence of the SVHYG insertion in the gp110 mutants was verified as described in the text.

[b] P, pSG5.

B95-110HYG LCLs never yielded an amount of transforming virus comparable to that obtained from LCL1; however, this may simply reflect incomplete complementation by pSVgp110. The growth characteristics of wild-type- and B95-110HYG EBV-infected LCLs were identical during growth in culture, underscoring the dispensability of gp110 for EBV-driven B-cell proliferation.

Conclusions and implications. These experiments demonstrate that gp110 is essential either for EBV entry or assembly but is dispensable for EBV-driven proliferation of latently infected, transformed B lymphocytes. Previous studies have shown that gp110 is not expressed during latency but only during productive infection (7, 8). The apparent lack of gp110 in the virion membrane, its intracellular localization, and the failure of gp110 antibodies to neutralize infectivity have led to the suggestion that gp110 may function during virus assembly and budding (7, 8); our results are consistent with this prediction. Recent work has demonstrated that herpes simplex virus type 1 (HSV-1) gB is also an essential gene, but notably, virions could be produced without complementation in those studies although the virions were noninfectious (3). This result suggests that gB is not required for production, assembly, or release of virus but rather that gB functions exclusively during entry. Little is known about the assembly of herpesvirus virions and their transit from the nucleus to the plasma membrane, but the consistent requirement for gp110 expression observed in our experiments in order to passage B95-110HYG EBV, considered with the observations mentioned above, strongly suggests that gp110 is involved in production or release of infectious EBV. Although this idea has yet to be proved, it predicts that B95-110HYG EBV, unlike HSV-1 gB mutants, should be unable to assemble and/or release virus particles. Current studies are focused on generating sufficient numbers of B95-110HYG-infected cells undergoing lytic replication to study virion assembly and trafficking by electron microscopy.

A comparison of the gp110 and HSV-1 gB nucleotide and protein sequences shows that they have colinear homology and similar structural motifs (5, 7, 8, 31, 32). The mature 110-kDa gp110 species has predominantly endo-H-sensitive, high-level mannose, N-linked oligosaccharides, indicating that gp110 did not traffic through the Golgi apparatus (7). This purely N-linked form is analogous to the immature form of HSV-1 gB but distinct from the mature gB which contains both N- and O-linked sugars (13a, 43a). Thus, despite their relatedness, gp110 and gB seem to have different biological properties. In considering any functional relationship between gp110 and gB, it must be remembered that the modification of existing protein structures to fit new functions is a well-established theme in biology. Keeping this in mind, two interesting and contrasting hypotheses concerning this relationship can be posited. Either gp110 has evolved a different function from that of its gB homologs in the alphaherpesviruses or, alternatively, it has relocated the same biological activity, for example, membrane fusion, to another part of the herpesvirus infectious cycle where it is needed. Since other herpesviruses presumably would need to solve the same problem which gp110 solves for EBV, the latter hypothesis predicts that an HSV-1 gene other than gB which could complement the B95-110HYG defect might exist.

R.E.H. and A.M. contributed equally to this study.

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