Identification of the Epstein-Barr Virus gp85 Gene

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Received 16 October 1987/Accepted 20 December 1987

The Epstein-Barr virus glycoprotein gp85 has been mapped to the Epstein-Barr virus DNA open reading frame BXL2 (R. Baer, A. Bankier, M. Biggin, P. Deininger, P. Farrell, T. Gibson, G. Hatfull, G. Hudson, S. Stachwell, C. Sequin, P. Tufnell, and B. Barrell, *Nature* [London] 310:207-211, 1984). A gp85-specific monoclonal antibody reacts with the BXL2 in vitro transcription-translation product. The monoclonal antibody also precipitates an 85-kilodalton protein from rodent cells transfected with the BXL2 open reading frame DNA. In these cells, gp85 localizes to the cytoplasm and nuclear rim rather than to the plasma membrane as in lymphocytes. Northern (RNA) blot hybridization and analysis of a cDNA clone containing BXL2 indicate that gp85 is translated from an unspliced, late, 2.5-kilobase transcript. Similarities between the predicted amino acid sequences of gp85 and herpes simplex virus gH (D. McGeoch and A. Davison, *Nucleic Acids Res.* 14:4281-4292, 1986) are noted.

Epstein-Barr virus (EBV) causes infectious mononucleosis and lymphoproliferative syndromes and has been implicated in the etiology of nasopharyngeal carcinoma (10, 17, 23, 24, 38). The virus is enveloped by a membrane which contains four major virus-specified proteins, gp350, gp220, p140, and gp85 (3, 11-13, 25, 28, 36, 40, 47, 50). These proteins are also present in the plasma membrane of productively infected cells (13, 25, 35, 36, 39, 44, 47-51). A fourth EBV-specified glycoprotein, gp110, resides principally in the nuclear membranes and endoplasmic reticulum of productively infected cells (19).

gp85 is readily detected by immunoprecipitation with EBV-immune human antisera from surface-labeled cells infected with, and induced to replicate, EBV. It possesses both complex and high-mannose asparagine-linked oligosaccharides (14, 43). The nonglycosylated form of gp85 is approximately 70 kilodaltons (kDa) as determined by treatment with tunicamycin or endoglycosidase (14, 43). Monoclonal antibodies to gp85 mediate complement-dependent infected-cell cytolysis and virus neutralization, suggesting that gp85 could be an important target of host immune response to EBV infection (45).

Although the human herpesviruses differ in many properties, regions of their genomes are predicted to encode homologous proteins (1, 5, 7, 9, 18, 33, 37). In fact, the EBV gp110 amino acid sequence has considerable homology to those of herpes simplex virus type 1 (HSV-1) gB (37), HSV-2 gB (5), varicella-zoster virus gpII (29), and human cytomegalovirus gB (7), and EBV gp350 has a short region of homology to HSV-1 gC (2). EBV gp110 is also antigenically related to HSV-1 gB (2, 16). Recently, a low-abundance HSV-1 glycoprotein, gH-1, was identified and mapped within the HSV-1 genome, and its gene sequence was determined (4, 18). HSV-1 gH is homologous to varicella-zoster virus glycoprotein gpIII (30, 33). Both HSV-1 gH and varicella-zoster virus gpIII have weak homology to the EBV BXL2 open reading frame (1, 33). Some features of the predicted protein product of BXL2 are similar to the predicted gH-1 amino acid sequence, including an N-termi-

nal hydrophobic putative signal sequence, a C-terminal potential transmembrane region, and five N-linked glycosylation sites (33).

gH appears to be essential for HSV-1 replication (18, 53). It may also be an important mediator of host immune response to HSV-1 infection. A monoclonal antibody to gH-1 efficiently neutralizes viral infectivity, and addition of the antibody to culture medium during HSV-1 infection inhibits plaque formation (4, 18). Monoclonal antibodies to the homologous VZV gpIII also neutralize virus and inhibit plaque formation (21, 30). Antibody-mediated inhibition of plaque formation suggests a role for gH-1 and gpIII antibody in preventing cell-to-cell virus spread. An HSV-1 gH temperature-sensitive mutant enters cells and replicates normally at the nonpermissive temperature, but releases no infectious virus, further implicating gH-1 in viral egress (18, 53). Finally, gH-1 may be involved in cell fusion since a gH-1 monoclonal antibody prevents syncytium formation in cells infected with syncytial HSV-1 strains (18).

Since EBV BXL2 is homologous to gH-1, it may also encode an important glycoprotein. The experiments described here indicate that BXL2 encodes the previously described EBV glycoprotein, gp85.

MATERIALS AND METHODS

Cells and antisera. Lymphoblastoid cell lines were maintained in RPMI 1640 medium supplemented with 10% newborn calf serum. EBV replication was induced in 20 to 30% of the cells by adding phorbol ester (20 ng/ml) and *n*-butyrate (100 µg/ml) to the culture medium for 3 days before harvesting. BHK cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum.

F-2-1 is a murine subclass immunoglobulin G2a monoclonal antibody specific for EBV gp85 which binds protein A. F-2-1 ascites fluid was produced by injecting pristane-treated BALB/c mice intraperitoneally with 10⁶ F-2-1 hybridoma cells. 2L10, an anti-gp350 monoclonal antibody also of the immunoglobulin G2a subclass, was used as a negative control. Another protein A-binding anti-gp350 monoclonal antibody, 72A1, was also used as a control (generously supplied by Andrew Morgan). The EBV-immune human serum was from a patient with chronic active EBV infection who was known to have high-titered antibody to EBV membrane antigens.

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Northern blot analysis and cDNA cloning. RNA for Northern (RNA) blot analysis was purified from induced B95-8 cells by the guanidium thiocyanate-CsCl method (26). Polyadenylated RNAs were isolated by oligo(dT)-cellulose chromatography, denatured in formamide and formaldehyde, and separated by formaldehyde-agarose gel electrophoresis. RNA was transferred to Gene Screen Plus (New England Nuclear Corp.) and hybridized as described elsewhere to ^{32}P -labeled, nick-translated probe. A cDNA library was constructed from the polyadenylated RNA by the method of Gubler and Hoffman (22) with omission of the ligase step. The cDNA was ligated into the *EcoRI* site of lambda gt11, packaged, and plated on *Escherichia coli* Y1090r⁻ (41).

To screen, phage were plated at a density of approximately 3×10^4 per 150-mm plate, grown at 37°C for about 8 h, and transferred to nitrocellulose filters. Phage were lysed, and DNA was denatured by soaking for 2 min in 0.5 M NaOH–1.5 M NaCl. Filters were neutralized in 0.5 M Tris hydrochloride (pH 7.4)–1.5 M NaCl and then rinsed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Filters were dried at room temperature for 30 min and under vacuum at 80°C for 2 h. They were then washed in 50 mM Tris hydrochloride (pH 8.0)–1 M NaCl–1 mM EDTA–0.1% sodium dodecyl sulfate (SDS) for 1 h at 42°C, prehybridized in 50% formamide–6× SSC–5× Denhardt solution–1 mM EDTA–0.1% SDS–100 µg of denatured salmon sperm DNA per ml for 4 h at 42°C, and incubated with ^{32}P -labeled, nick-translated probe (400 ng; 10^8 cpm/µg) for 18 h at 42°C. Filters were washed twice in 1× SSC–0.1% SDS for 15 min at room temperature, followed by washes of 1 h each at 72°C in decreasing concentrations of SSC. Autoradiography was carried out at –80°C. Positive plaques were picked and purified. Phage DNA was isolated, and *EcoRI* fragments were cloned into pUC18 for characterization and sequencing.

In vitro translation and immunoprecipitation. To reconstruct BXL2 from the cloned *BamHI* fragments of EBV DNA (8), the 375-base-pair (bp) *HpaII*–*BamHI* fragment *BamHI*-X, which contains the 5' part of BXL2, was ligated to the 1,847-bp *BamHI*–*HindIII* fragment of *BamHI*-T, which contains the 3' part of BXL2 (see Fig. 1; 1). The resulting *HindIII*–*HpaII* fragment, containing all of BXL2, was gel purified and ligated into the *HindIII* site of pTZ19R vector, which contains the T7 RNA polymerase promoter (Pharmacia). After transformation into *E. coli* JM109 cells, the plasmid was isolated, linearized, and transcribed as recommended in capping protocol 2 from Promega Biotec, using T7 RNA polymerase (Pharmacia). The RNA was translated using nuclease-treated rabbit reticulocyte lysate, as described by the manufacturer (Promega Biotec), and [^{35}S]methionine (translation grade; Amersham). Some translations were done in the presence of canine pancreatic microsomal membranes according to the manufacturer's (Amersham) instructions. In vitro translation products were resolved by 7% SDS-polyacrylamide gel electrophoresis.

Immunoprecipitations were done by diluting 10% of the in vitro translation mixture in 0.5 ml of phosphate-buffered saline (PBS) containing 0.5% Nonidet P-40 (NP-40), 1% ovalbumin, 150 µM L-1-chloro-[4-tosylamido]-7-amido-2-heptanone hydrochloride, and 1 mM phenylmethylsulfonyl fluoride (PMSF). Samples were incubated with antiserum overnight at 4°C. Immune complexes were precipitated with protein A-Sepharose CL-4B (Sigma). The protein A-Sepharose was washed five times with PBS containing 0.5% NP-40 and 1 mM PMSF and rinsed once with 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA at 4°C. Samples were boiled

in loading buffer containing SDS and dithiothreitol for SDS-polyacrylamide gel electrophoresis. The gels were stained with Coomassie brilliant blue R-250, destained, and treated with Amplify (Amersham) for 30 min before gel drying. The dried gel was exposed to XAR-5 film at –85°C.

Eucaryotic expression of BXL2. The 2.2-kilobase (kb) fragment containing the BXL2 open reading frame was isolated from *HindIII*–*XbaI*-digested pTZX2, treated with T4 DNA polymerase, and blunt-end ligated to *BamHI*-digested T4 DNA polymerase and calf intestinal alkaline phosphatase-treated pZipNeoSV(X)1. A 20-µg sample of the resultant recombinant plasmid, pZ85 (see Fig. 3A), was transfected into 5×10^6 BHK cells, using CaPO_4 (20), and clones were selected in medium containing G418 at 800 µg/ml. Resistant clones were screened by immunofluorescence and Western blotting (immunoblotting) as described previously (19) using EBV immune serum and by [^{35}S]methionine metabolic labeling and immunoprecipitation.

Immunofluorescence. BXL2-expressing BHK cells were grown on eight-chamber microscope slides (Miles) and acetone fixed for 10 min at –20°C. Fixed cells were incubated at 25°C with PBS containing 20% normal goat serum for 30 min and then with EBV-immune human serum diluted in PBS with 10% normal goat serum. After three washes with PBS, slides were incubated with fluorescein isothiocyanate-conjugated goat anti-human antibody diluted in PBS containing 10% normal goat serum. Slides were viewed with a Zeiss microscope equipped with epifluorescence.

Metabolic labeling and immunoprecipitations. BXL2-expressing BHK cells were incubated for 18 h at 37°C with 50 µCi of [^{35}S]methionine (New England Nuclear Corp.) per ml in methionine-free RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum. Cells were washed at 4°C with PBS–0.2 mM PMSF and lysed in 50 mM Tris hydrochloride (pH 8.0)–150 mM NaCl–5 mM EDTA–1 mM PMSF–0.5% NP-40. Soluble extracts were immunoprecipitated with antibody and protein A-Sepharose CL-4B and then washed three times with 50 mM Tris hydrochloride (pH 7.5)–500 mM NaCl–5 mM EDTA–1% NP-40–5% sucrose and once with 50 mM Tris hydrochloride–150 mM NaCl–1% Triton X-100–1% sodium deoxycholic acid (19). The protein A precipitates were suspended and boiled in sample buffer and subjected to SDS-polyacrylamide electrophoresis as described above.

Computer-based protein sequence analysis. Sequence analyses were done on a WYSEpc 286 computer using PC/GENE software from IntelliGenetics, Inc., Mountain View, Calif. The hydrophobic moment plot was generated using the HELIXMEM program based on the method of Eisenberg et al. (15). The hydrophobicity profile was produced by the SOAP program based on the method of Kyte and Doolittle (32). The signal sequence cleavage site was predicted by the PSIGNAL program based on the method of von Heijne (52).

RESULTS

Northern blot and cDNA analysis of BXL2. The EBV BXL2 open reading frame contains 296 bp from EBV *BamHI*-X and 1,821 bp from *BamHI*-T (1). *BamHI*-X and *BamHI*-T have previously been shown by Northern blot analysis to hybridize to a late 2.7-kb message from induced B95-8 cells which encodes a 72-kDa protein (6, 26, 27, 42). To confirm that BXL2 hybridizes to this mRNA, a Northern blot of induced B95-8 RNA was hybridized to the 1,640-bp *BamHI*–*SacI* fragment of *BamHI*-T (Fig. 1A). A single 2.5-kb RNA was detected (Fig. 1C). The difference from the previously reported size is due to the recent

FIG. 1. EBV DNA BXL2 encodes a 2.5-kb RNA. (A) Map of the EBV genome showing the terminal repeat (TR), internal repeat (IR), and unique sequence (U) domains. The region of U4 containing BXL2 is expanded beneath. The EBV DNA *Bam*HI-X and -T *Hpa*II-*Hind*III fragment containing all of BXL2 was cloned into pTZ19R for in vitro transcription. (B) Structure of the BXL2 message as determined by Northern blot and by cloning and analysis of a cDNA clone. Open arrow shows direction of transcription and map position. The EBV genome nucleotide coordinates for the beginning and end of the cDNA clone, for the poly(A) addition site, and for the TATA sequence and the open reading frame are indicated. (C) Northern blot analysis of BXL2. Cytoplasmic polyadenylated RNA from induced B95-8 cells was hybridized to the 1,640-bp *Bam*HI-*Sac*I fragment of *Bam*HI-T. This fragment comprises 77% of BXL2. The positions of the 18S and 28S rRNAs are indicated.

assignment of 1.8 and 4.9 kb as the sizes of the 18S and 28S rRNA markers, respectively.

The fine structure of the BXL2 message was determined by analysis of a cDNA clone derived from infected-cell mRNA. A gt11 cDNA library generated from induced B95-8 polyadenylated RNA was screened with the *Bam*HI-*Sac*I fragment. One positive clone yielded 1,850- and 550-bp *Eco*RI fragments. The combined length is compatible with a full- or near-full-length BXL2 cDNA, and the presence of two fragments reflects the internal *Eco*RI site in BXL2. Three hundred nucleotides from the ends of each *Eco*RI fragment cDNA insert were sequenced. The EBV sequence coordinates for the ends of the mRNA, the poly(A) addition site, and the open reading frame are shown in Fig. 1B. The cDNA begins 65 bp 3' to a TATAAGA sequence shown previously by S1 analysis to be 30 bp 5' to a late mRNA start site (1). A 209-bp, presumably untranslated sequence precedes the first ATG. This ATG is a likely start for initiation of translation since the nucleotide at the -3 position is a purine. However, a C rather than a G at the +1 position makes this ATG somewhat less than an ideal start codon (31). Both the TATA sequence and all but 5 bp of the 5' untranslated sequence of the cDNA are contained in the upstream BXL1 open reading frame. An AATAAA poly(A) addition signal is located 17 bp downstream from the BXL2 stop codon. The poly(A) tract in the cDNA begins 20 bp 3' to the last base of the AATAAA sequence. Digestion of the cDNA clone with *Hae*II, *Rsa*I, and *Ava*II revealed fragments identical in size to those expected from the genomic DNA sequence. Also, in vitro transcription and translation from this cDNA yielded a product which comigrates with the in vitro transcription-translation product from a genomic BXL2 construct (see below and data not shown). These data indicate that the BXL2 cDNA clone is derived from an unspliced mRNA.

BXL2 encodes the 70-kDa precursor to gp85. BXL2 was transcribed in vitro from the EBV DNA BXL2 *Hind*III-to-*Hpa*II fragment or from the cDNA, each of which had been inserted 3' to the T7 promoter in pTZ19R. The RNA was translated in vitro, yielding a 70-kDa protein (Fig. 2B) similar in size to the translation product of *Bam*HI-X hybrid-selected late mRNA (27). When the RNA was translated in the presence of canine pancreatic microsomal membranes, two additional species of 82 and 85 kDa appeared, indicating that BXL2 encodes a membrane protein which undergoes posttranslational glycosylation. The proteins translated with microsomal membranes were immunoprecipitated with EBV-immune human serum, with F-2-1 monoclonal antibody to gp85, or with anti-gp350 monoclonal antibody. The human serum or F-2-1 specifically recognized the 70-kDa precursor protein as well as the 82- and 85-kDa glycosylated proteins, whereas the anti-gp350 monoclonal antibody did not immunoprecipitate any of these proteins (Fig. 2C). Although the F-2-1 monoclonal antibody also specifically precipitated the p70 precursor, the glycosylated proteins were more efficiently precipitated. These data indicate that BXL2 encodes EBV gp85.

Mammalian cell expression. To confirm further that BXL2 encodes gp85, BXL2 was inserted into pZipNeoSV(X)1 (Fig. 3A) and the resultant recombinant plasmid was transfected into BHK cells. G418-resistant clones were isolated. The clones were screened by Western blot and immunofluorescence with EBV-immune human serum. A band of 85 kDa was barely detected in immunoblots of proteins from 3 of 10 clones. Each of the clones was metabolically labeled with [³⁵S]methionine, and an NP-40

FIG. 2. In vitro transcription-translation of BXL2. BXL2 cloned from genomic DNA into pTZ19R, a plasmid containing the T7 RNA polymerase promoter, was transcribed. The in vitro-transcribed RNA was used for in vitro translation. The translation products were separated on a 7% SDS-polyacrylamide gel. (A) Immunoprecipitation of gp85 from surface-iodinated induced B95-8 cells with the F-2-1 monoclonal antibody. (B) In vitro-transcribed BXL2 RNA was translated in vitro in the presence (+) or absence (-) of canine pancreatic microsomal membranes. (C) BXL2 RNA translated in the presence of microsomal membranes was immunoprecipitated with EBV-immune human sera, with anti-gp85 monoclonal ascites fluid (F-2-1), or with anti-gp350 monoclonal ascites fluid (negative control).

FIG. 3. Intracellular localization of BXLF2-encoded protein in transfected fibroblasts. (A) Structure of the recombinant pZipNeoSV(X)1-BXLF2 eucaryotic expression plasmid pZ85. BXLF2 (black box) was cloned into the *Bam*HI site of pZipNeoSV(X)1. Arrow indicates direction of transcription. Open boxes indicate elements of the parent plasmid pZipNeoSV(X)1 from the MuLV long terminal repeats (LTR), the neomycin phosphotransferase gene (NEO), the simian virus 40 origin (SV40), and the pBR322 origin (pBR). The rest of the vector is derived from pBR322 or MuLV. (B) BHK cells were transfected with the plasmid pZ85, and clones were selected using the antibiotic G418. Acetone-fixed cells were incubated with EBV-immune human serum followed by fluorescein isothiocyanate-conjugated goat anti-human antibodies. Nonimmune serum or cells transfected with pZipNeoSV(X)1 alone displayed no fluorescence (data not shown).

extract of each clone was prepared. EBV-immune human serum and the F-2-1 specifically recognized the 85-kDa protein product expressed in BHK cells from the BXLF2-encoding plasmid pZ85, whereas 72A1, an anti-gp350 monoclonal antibody, displayed no specificity for this protein (Fig. 4). No similar size protein was immunoprecipitated from control vector-transfected BHK cells.

Intracellular location of gp85. Indirect immunofluorescence of acetone-fixed BXLF2-expressing BHK cells, using EBV-immune human serum (Fig. 3B), revealed that the expressed protein predominantly localized to the cytoplasm, diffusely and in discrete patches, and to the nuclear rim. No plasma membrane fluorescence was observed. Additionally, in an erythrocyte assay on the gp85-expressing clones using EBV-immune serum, the erythrocytes did not aggregate, suggesting that there is very little or no gp85 expressed on the surface of these cells.

DISCUSSION

Several previous lines of evidence made it likely that BXLF2 would encode gp85. First, in vitro translation of hybrid-selected RNA from productively EBV-infected cells, using DNA encompassing the *Bam*HI-X/T region of EBV, resulted in a protein similar in size to the nonglycosylated gp85 precursor (6, 27, 42). BXLF2 is the only significant open reading frame in the EBV DNA *Bam*HI-X/T region, and it would be predicted to translate a protein similar in size to the in vitro translation product (1). Second, the predicted amino acid sequence of BXLF2 includes five potential N-linked glycosylation sites and is homologous to that of the essential HSV-1 glycoprotein gH (33).

The experiments described here provide the first direct proof that the EBV BXLF2 open reading frame encodes the EBV envelope glycoprotein previously designated gp85. An anti-gp85 monoclonal antibody, F-2-1 (45), was a key link in the two experimental approaches we employed to establish

that BXLF2 encodes gp85. First, in vitro transcription-translation of genomic BXLF2 or of a BXLF2-homologous cDNA clone from EBV-infected cells yielded a protein of 70 kDa in the absence of dog pancreas microsomal membranes. In the presence of microsomal membranes, a larger, presumably glycosylated, protein of 85 kDa was produced, identical in size to gp85 expressed in lymphocytes. Both the 70- and 85-kDa proteins were specifically immunoprecipitated by

FIG. 4. Specific immunoprecipitation of gp85 from BXLF2-transfected cells. BXLF2-expressing BHK cells were metabolically labeled with [35 S]methionine (50 μ Ci/ml) for 16 h. Cells were lysed and immunoprecipitated by 72A1, a monoclonal antibody to the EBV-encoded gp350/220, or by F-2-1, a monoclonal antibody to the EBV-encoded gp85. ■, gp85. Positions of the molecular weight standards (myosin, beta-galactosidase, phosphorylase b, bovine serum albumin, and ovalbumin) are indicated on the left.

EBV-immune human sera and F-2-1. Second, rodent cells transfected with BXLF2 under the control of a retrovirus promoter expressed an 85-kDa protein which was also specifically immunoprecipitated by the F-2-1 monoclonal antibody.

gp85 is in the plasma membranes of cells in late stages of EBV replication (13, 35, 36, 44, 47, 49). As judged by the intensity of the signal upon immunoprecipitation from surface-iodinated EBV-infected cells, using monoclonal antibodies against gp350 or gp85, gp85 appears almost as abundant as gp350. However, the 33 tyrosines predicted to be in gp85 from the BXLF2 sequence provide an unusually large number of potential substrates for iodination and may lead to the overestimation of its abundance. Further, the extensive glycosylation of gp350 may result in some of the tyrosines not being exposed to iodination. We raise this issue because we attempted to purify gp85 by using monoclonal affinity chromatography from cells induced to replicate EBV and were astounded at the poor protein yield despite the efficiency of the affinity column in recovering iodinated gp85. We therefore suggest that gp85 is not nearly as abundant as gp350 in EBV-infected cells.

EBV gp85 and HSV-1 gH are in viral envelopes (4, 13, 18, 36, 47, 49), and at least one monoclonal antibody to each neutralizes infection by the virus (4, 18, 45). The envelope location of gp85 and its homology to gH suggest that gp85 could be involved in viral entry or egress. A gH-1 temperature-sensitive mutant allows viral infection and replication, but not release at the nonpermissive temperature (18, 53).

Surprisingly, in view of the location of gp85 in EBV-infected lymphocyte plasma membranes, indirect immunofluorescence of BXLF2-expressing rodent cells using EBV-immune serum suggests that gp85 is localized primarily in the cytoplasmic and perinuclear compartments, with none being detectable in the plasma membrane. The localization to the nuclear membrane could be of significance since herpesviruses acquire their envelopes by budding through the nuclear membranes. The failure to demonstrate gp85 in the plasma membrane of gp85-expressing rodent fibroblasts by using techniques which readily demonstrate the plasma membrane localization of gp350/220 in such cells suggests that gp85 may require association with other EBV or B-lymphocyte proteins for plasma membrane translocation.

The demonstration that BXLF2 encodes gp85 enables us to consider relevant aspects of the primary amino acid sequence predicted from the BXLF2 DNA sequence. First, gp85 has only one region, at the extreme C terminus, of sufficient length and hydrophobicity to be a typical transmembrane anchor sequence (15, 32). This would leave a cytoplasmic tail of only eight amino acids. Similarly, gH-1 is predicted to have a short, 14-amino-acid, cytoplasmic domain (33). Second, gp85 would have a hydrophobic N-terminal putative signal sequence with a predicted cleavage site between residues 17 and 18 (52). Third, gp85 is predicted to be hydrophobic overall, particularly as compared to gp350/220. In addition to the predicted signal and transmembrane sequences, gp85 contains six hydrophobic sequences which have significant helical propensity (18). Two of these

FIG. 5. Sequence analysis of gp85. (A) Hydrophobic moments (μh) of the eight predicted gp85 hydrophobic helices are plotted against their degree of hydrophobicity (H). (B) Hydrophobicity profile of gp85, averaging the hydrophobicity (H) over each 13-amino-acid segment. The numbers mark the predicted hydrophobic helices and correspond to the numbers in the hydrophobic moment plot. Dots mark the N-linked glycosylation sites, and those underlined are conserved in gH-1. The arrows below define the part of the gp85 amino acid sequence which has similarity to gH-1.

have significant hydrophobic moments (Fig. 5, helices 3 and 6), suggesting that they may associate with the surface of the membrane. gp350, the major glycoprotein present in enveloped EBV, has recently been shown to be the ligand for the EBV receptor on B lymphocytes, the CR2 receptor (34, 46). gp350/220 seems to be responsible not only for virus binding to B lymphocytes, but also for virus uptake into the B cell (46). Because gp85 is also present in enveloped EBV and is considerably more hydrophobic than gp350/220, it could play a role in hydrophobic interaction with cell membranes. Monoclonal antibodies to gH-1 can prevent cell fusion in syncytia-forming HSV-1 strains (4, 18). Surface-associated helices may be involved in this process. Notably, gp350 has no analogous sequences. Fourth, although gp85 and gH-1 have only 19% similarity between their sequences, three cysteines and two N-linked glycosylation sites are conserved. These similarities are all in the C-terminal one-third of the molecule, suggesting this region may be more evolutionarily constricted by functional constraints. Our demonstration that BXL2 encodes gp85 makes possible molecular genetic studies of these and other potential functional domains of gp85 as well as analyses of their role in the host response to EBV infection.

ACKNOWLEDGMENTS

Lindsey Hutt-Fletcher and Harvey Rabin provided F-2-1 monoclonal antibody.

T.H., M.G., and J.S. were supported by Public Health Service predoctoral or postdoctoral national research awards HD 07009-13, GM 07218, and CA 07967 from the National Institutes of Health. This research was supported by Public Health Service grant CA 47006 from the National Cancer Institute.

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