Induction of Antibodies to the Epstein-Barr Virus Glycoprotein gp85 with a Synthetic Peptide Corresponding to a Sequence in the BXL2 Open Reading Frame

DOUGLAS E. OBA AND LINDSEY M. HUTT-FLETCHER*

Department of Comparative and Experimental Pathology and Department of Pathology, University of Florida, Gainesville, Florida 32610

Received 20 October 1987/Accepted 18 December 1987

Epstein-Barr virus codes for at least three envelope glycoproteins, one of which, gp85, has not yet been mapped to the viral genome. The publication and analysis of the entire Epstein-Barr virus DNA sequence has allowed identification of open reading frames with potential for encoding membrane glycoproteins. To determine whether one of these candidate open reading frames, BXL2, codes for gp85, an antibody was made to a 17-residue peptide derived from positions 518 to 533 of the predicted BXL2 protein. The reactivity of the anti-peptide antibody was then compared with that of the monoclonal antibody F-2-1, which was originally used to define and characterize gp85. Anti-peptide antibody and F-2-1 immunoprecipitated glycosylated molecules with identical electrophoretic mobilities; digestion of the two immunoprecipitated proteins with V8 protease generated comparable peptides; and the anti-peptide antibody reacted in Western immunoblots with the gp85 glycoprotein that had been immunoprecipitated by F-2-1 before transfer to nitrocellulose. In addition, a monospecific rabbit antibody, made against native gp85, reacted with the peptide used for immunization. These results are compatible with the hypothesis that the BXL2 open reading frame codes for gp85.

Epstein-Barr virus (EBV) is a human herpesvirus that causes infectious mononucleosis (14) and is associated with both B-cell neoplasia (8) and nasopharyngeal carcinoma (9). Infectivity of the virus can be neutralized by antibodies to the three major late glycoproteins of 350, 220, and 85 kilodaltons (gp350, gp220, and gp85) that are present on the surface of infected cells and exposed on the outside of enveloped virions (16, 32, 34). This suggests that these molecules play a crucial role in virus infectivity and in development of immunity to virus spread.

EBV replicates poorly in vitro, a property that has impeded study of proteins expressed during the lytic cycle. However, the entire sequence of EBV DNA is known (1), and the gene which codes for both of the two largest glycoproteins, gp350 and gp220, has been identified (4, 17). This has enabled development of strategies for expression and functional analysis of the proteins in procaryotic and eucaryotic cells which have and will continue to expand our understanding of the role of these molecules as virus attachment proteins and mediators of virus penetration (33, 38). A combination of hybrid selection, in vitro translation, and immunoprecipitation was used to identify the DNA fragment that codes for gp350 and gp220. We describe here the use of a synthetic peptide derived from the BXL2 reading frame of EBV DNA to identify the gene coding for the third major membrane glycoprotein, gp85.

MATERIALS AND METHODS

Cells and virus. Two EBV-producing cell lines, the Burkitt lymphoma-derived P3HR1-C113 (15) and the marmoset cell line MCV5 (both gifts of George Miller, Yale University, New Haven, Conn.), were grown at 37°C and diluted at least biweekly in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum, 100 IU of penicillin per ml, and 100 µg of streptomycin per ml.

Virus was harvested by centrifugation from the spent culture medium of these cells 7 days after they had been induced with 30 ng of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) per ml. Media were centrifuged at 4,000 × *g* for 10 min to remove cells, 100 µg of bacitracin per ml was added to the clarified supernatant, and the virus was pelleted by centrifugation at 20,000 × *g* for 90 min. Pellets were suspended in 1/250 the original volume of medium containing 100 µg of bacitracin per ml, reclarified by centrifugation three or four times at 400 × *g*, filtered through a 0.8-µm-pore filter (Millipore Corp., Bedford, Mass.), and stored at -70°C.

Peptide synthesis and coupling. Peptide V-1, Cys-Ser-Leu-Glu-Arg-Glu-Asp-Arg-Asp-Ala-Trp-His-Leu-Pro-Ala-Tyr-Lys, corresponding to amino acids 518 to 533 of the BXL2 open reading frame of EBV DNA with an additional Cys residue attached to the N terminus, was synthesized by the Protein Chemistry Core Facility of the University of Florida (Gainesville). Peptide B, Cys-Leu-Ile-Asp-Asp-Val-Val-Ser-Leu-Ile-Arg-Gly-Gly-Thr-Arg-Lys, corresponding to residues 399 to 413 of human C9 with an additional N-terminal Cys, was synthesized by Biosearch (San Rafael, Calif.). The coupling reagent *m*-maleimidobenzoyl sulfosuccinimide ester (Pierce Chemical Co., Rockford, Ill.) was used to attach peptide V-1, via the N-terminal cysteine residue, to keyhole limpet hemocyanin according to the procedure of Liu and co-workers (24); coupled peptide was stored at -20°C for use.

Antibody production. Polyclonal antibodies to peptide V-1 (anti-V-1) were made in an adult New Zealand White rabbit. The animal was first inoculated subcutaneously with 400 µg of peptide conjugated to keyhole limpet hemocyanin and emulsified in Freund complete adjuvant; it was reinoculated three times at weekly intervals with the same amount of coupled peptide emulsified in Freund incomplete adjuvant. Immunoglobulin G was purified from preimmune and immune sera by chromatography on protein A-Sepharose

* Corresponding author.

(Sigma Chemical Co., St. Louis, Mo.). Immune sera were further purified by affinity chromatography on peptide V-1 coupled to cyanogen bromide-activated Sepharose 4B (Sigma) according to the manufacturer's instructions. Bound immunoglobulin G was eluted from the Sepharose with 0.1 M acetic acid in 0.1 M NaCl.

Two monoclonal antibodies to gp85 were used. These were F-2-1 (32; hybridoma; a gift of David Thorley-Lawson, Tufts University School of Medicine, Boston, Mass.) and E1D1, which was prepared in our laboratory (2). Antibody E1D1, which immunoprecipitated a glycoprotein with the same mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as gp85, was confirmed to react with the same protein as F-2-1 by the following criteria: preabsorption of lysates with E1D1 removed the protein reactive with F-2-1, and preabsorption with F-2-1 abolished reactivity of E1D1; and V8 protease digestion of the proteins immunoprecipitated by the antibodies produced identical peptide fragments (unpublished data). The E1D1 antibody was used for affinity purification because the monospecific rabbit antibody was made at a time when the antibody F-2-1, but not the hybridoma that produces it, was available to us. Both monoclonal antibodies were purified from hybridoma culture supernatants by chromatography on protein A-Sepharose.

Polyclonal monospecific antibodies to gp85 (R24) were made in an adult New Zealand White rabbit by immunization with gp85 purified from P3HR1-C113 cells by affinity chromatography. Briefly, 10^9 cells were suspended in 600 ml of medium containing 30 ng of TPA per ml. Forty-eight hours later, a sample of 200 ml was pelleted and suspended at a concentration of 10^7 cells per ml in Hanks balanced salt solution, supplemented with nonessential amino acids but with 1/10 normal concentration of methionine and with 2% dialyzed heat-inactivated fetal calf serum, 30 ng of TPA per ml, and 0.5 mCi of [35 S]methionine (specific activity, 1,380 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Twenty-four hours later, all cells were harvested by centrifugation and enriched for those expressing late viral antigens by sedimentation over a discontinuous gradient of Ficoll-Hypaque, as previously described (3). Separated cells were lysed with detergent buffer (0.05 M Tris hydrochloride [pH 7.2], 0.15 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and 100 U of aprotinin per ml), sonicated for 1 min, and centrifuged at $100,000 \times g$ for 1 h. The supernatant was passed five times consecutively through a 3-ml column of Sepharose coupled to antibody E1D1. The column was washed with buffer (0.05 M Tris hydrochloride [pH 7.5], 0.5 M NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 100 U of aprotinin per ml) until the radioactivity in the flowthrough reached background levels. Bound antigen was eluted with 3 M potassium thiocyanate in phosphate-buffered saline (pH 7.2) (PBS), dialyzed extensively to remove thiocyanate, concentrated by dialysis against polyethylene glycol 20,000 (Sigma), and analyzed for purity SDS-PAGE and autoradiography; 85% of the radioactivity was present in a single protein with the molecular weight of gp85. Silver staining of gels was completely negative, indicating the presence of less than 50 pg of protein per ml in the concentrated antigen preparations; detectable amounts of protein were also not obtained if virus instead of TPA-induced cells was used as the starting material, suggesting that gp85 is a minor component of both virus and virus-producing cell membranes. Nevertheless, after immunization once with antigen emulsified in Freund complete adjuvant and four times with antigen

emulsified in Freund incomplete adjuvant, antibody purified from immune serum by chromatography on protein A-Sepharose immunoprecipitated a glycoprotein with the apparent molecular weight of gp85 from virus or virus-producing cells.

All purified antibodies were dialyzed extensively against PBS before use.

ELISA. Reactivity of antisera with synthetic peptides was measured in enzyme-linked immunosorbent assays (ELISAs) done in 96-well microtiter plates. A 60- μ g sample of peptide in carbonate-bicarbonate buffer (pH 9.6) was added to each well and dried overnight at 37°C. Plates were then incubated sequentially with 5% skim milk in PBS-Tween 20, dilutions of rabbit or mouse antibody, goat anti-rabbit or anti-mouse antibody conjugated to horseradish peroxidase (Cooper Biomedical Inc., Malvern, Pa.), and the substrate orthophenylenediamine (Sigma). Plates were washed between incubations with 0.05% Tween 20 in 0.85% NaCl. The final enzyme reaction was stopped with 2 N H_2SO_4 , and the color change was measured at 492 nm.

Radiolabeling. Virus protein were labeled extrinsically with ^{125}I (Amersham). Virus was pelleted from 9 ml of concentrated culture supernatant by centrifugation at $100,000 \times g$ for 1 h, suspended in 400 μ l of PBS, and labeled with 0.5 mCi of ^{125}I by use of Iodogen (tetrachlorodiphenylglycouril; Pierce) (11). Labeled virus was then repelleted before solubilization. Cells to be labeled metabolically with [3H]glucosamine (specific activity, 40 Ci/mmol; Amersham) were induced for 2 days with 30 ng of TPA per ml and then suspended in Hanks balanced salt solution supplemented with nonessential amino acids, but with 1/10 the normal concentration of glucose, 2% dialyzed, heat-inactivated fetal bovine serum, 30 ng of TPA per ml, and 100 μ Ci of [3H]glucosamine per ml. Cells were harvested 24 h after addition of label.

Immunoprecipitation and SDS-PAGE. Immunoprecipitation was carried out as previously described (3). Briefly, cells and virus were solubilized in radioimmunoprecipitation buffer containing 0.05 M Tris hydrochloride (pH 7.2), 0.15 M NaCl, 1.0% deoxycholate, 1.0% Triton X-100, 0.1% SDS, 0.1 mM phenylmethylsulfonyl fluoride, and 100 U of aprotinin per ml, sonicated, and centrifuged at $100,000 \times g$ for 1 h. Supernatants were mixed with antibody and protein A-agarose beads (Genzyme Corp., Boston, Mass.). The precipitates were washed, dissociated by boiling in sample buffer containing 2-mercaptoethanol, and analyzed by SDS-PAGE (23) in 9% acrylamide cross-linked with 0.28% N,N' -diallyltartardiamide. Molecular weight markers (Sigma) were electrophoresed in parallel tracks. Gels containing proteins labeled with ^{125}I were stained, destained, dried on filter paper, and placed in contact with XAR film (Eastman Kodak Co., Rochester, N.Y.) at $-70^\circ C$ for fluorography (5). Gels containing proteins labeled with [3H]glucosamine were infused with 2,5-diphenyloxazole before drying.

Western blotting. Immunoprecipitated proteins were electrophoresed in 9% acrylamide and then electrotransferred onto nitrocellulose membranes (0.45- μ m pore size; Schleicher & Schuell, Inc., Keene, N.H.). The transferred sheets were treated for 3 h with blocking buffer (10 mM Tris hydrochloride [pH 7.2], 0.15 M NaCl, 5% skim milk, 0.05% sodium azide) and reacted for 3 h with blocking buffer containing rabbit antibody at 400 μ g/ml. They were then washed five or six times with wash buffer (10 mM Tris hydrochloride [pH 7.2], 0.15 M NaCl, 0.3% Tween 20) for 10 min each, followed by an overnight wash. The washed sheets were reacted with alkaline phosphatase-conjugated

goat anti-rabbit antibodies (Hyclone, Logan, Utah) for 3 h, and the bound anti-rabbit antibodies were detected by reacting with substrate (5-bromo-4-chloro-3-indophosphate and Nitro Blue Tetrazolium; Sigma).

V8 protease digestion. 125 I-labeled protein was immunoprecipitated by F-2-1 or anti-V-1 and electrophoresed. The dried gel was exposed to XAR film, and that region determined to contain the protein of interest was excised and electroeluted using an Elutrap (Schleicher & Schuell) (20). Samples containing equal amounts of radioactivity were partially digested with *Staphylococcus aureus* V8 protease (Sigma) in 62.5 mM Tris hydrochloride (pH 6.8) for 30 min at 37°C. The reaction was stopped by addition of an equal volume of sample buffer containing 2-mercaptoethanol and boiling for 5 min. Boiled samples were then analyzed by SDS-PAGE in 12.5% acrylamide cross-linked with 0.28% *N,N'*-methylene-bisacrylamide.

Neutralization. Neutralization of virus infectivity by antibody was expressed as the ability to inhibit virus-induced immunoglobulin synthesis by fresh human B cells. Leukocytes were obtained from heparinized human blood by flotation on Ficoll-Hypaque and depleted of T cells by a double cycle of rosetting with 2-aminoethylthioisouronium-treated sheep erythrocytes (28) and centrifugation over 60% Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) as previously described (18). A total of 200,000 cells were incubated with or without virus that had been preincubated with an equal volume of preimmune rabbit antibody and test antibody, adjusted so that the total amount of immunoglobulin remained constant at 100 µg/ml; all antibodies were heated at 56°C for 30 min to inactivate complement. Cells and virus were plated in a total volume of 100 µl in 96-well round-bottomed tissue culture plates. After 6 days in culture, 100 µl of medium was added to each well. On day 12, the immunoglobulin concentrations in the media were measured by a double-sandwich micro-ELISA (35) with appropriate concentrations of rabbit anti-human immunoglobulin, peroxidase-conjugated rabbit anti-human immunoglobulin, and the substrate hydrogen peroxide with 5-aminosalicylic acid. The colorimetric change was measured at 492 nm.

RESULTS

Selection of V-1 peptide sequence. Discriminant analysis of the hypothetical proteins deduced from the EBV DNA sequence (1), by the program of Klein and colleagues (22), indicates that there are at least 33 open reading frames that potentially code for membrane-spanning proteins. The original analysis of the EBV sequence implicated one of these, the BDLF3 open reading frame, as a candidate for the gp85 gene (1). This reading frame is predicted to code for a late integral membrane protein of approximately 24 kilodaltons with nine potential N-linked glycosylation sites. The work of Strnad and colleagues (30), however, indicated that gp85 contains five N-linked oligosaccharide chains and that treatment of the protein with endoglycosidase H reduces its apparent molecular size to around 70 kilodaltons. Thus, of those open reading frames that remain unassigned to known virus proteins, the BXL2 open reading frame (Fig. 1), which encodes a late transcript predicted to have a molecular size of 78 kilodaltons and five potential N-linked glycosylation sites (1), appeared to approximate more closely to the profile of the native molecule. According to the program of Klein et al., the sequence includes three stretches which have a high probability of being membrane-spanning seg-

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1      20      40
MQLLCVFCVLVLLWEVGAASLSEVKLHLDIEGHASHYTIPWTELMKAVPGL
60      80      100
SPEALWREANVTEDLASMLNRYKLIYKTSGLTGIALAEPVDIPAVSEGS
*
120      140
QVDASKVHPGVISGLNSPACMLSAFLEKQLFYIYIGTMLPNTRPHSYVYFQ
160      180      200
LRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLVSLVFGK
220      240
TKDLPDLRGPFPSYPSLTSAQSGDYSLVIVTTFVHYANFHNYPNKLKDMF
260      280      300
SRAVTMTAASYARYVLQKLVLLEMKGCCREPELDTETLTMTFEVSVAFK
320      340
VGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMSYGLER
360      380      400
LAAMLMATVKMEELGHLTTEKQYALRLATVGYPKAGVYSGLIGGATSVL
420      440
LSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLS
460      480      500
TALCSALEIGEVLRLGALGTESGLFSPCYLSLRFDLTRDKLLSMAPQAT
520      540
LDQAAVSNVADGFLGRLSLEREDRDAMHLPAKYCVDRDLKVLMIPLINV
560      580      600
TFIISDREVRSALYEASTTYLSSSLFSPVIMNKCSQGAVAGEPRQIP
620      640
KIQNFTRTQKSCIFCGFALLSYDEKEGLETTYITSQEVQNSILSNYFD
*
660      680      700
FDNLHVHYLLLTNGTVMEIAGLYEERAHVVLAILLYFIAFALGIFLVHK
*
IVMFFL

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FIG. 1. Predicted amino acid sequence of protein potentially coded by the BXL2 open reading frame. Solid underlines indicate hydrophobic regions predicted by the program of Klein and colleagues (22) to have a high probability of being membrane spanning regions; *, potential N-glycosylation sites; dashed underline indicates residues synthesized as peptide V-1.

ments: one at the amino terminus, stretching from amino acids 3 to 19, that may represent a signal sequence domain; one at the carboxy terminus, stretching from amino acids 680 to 696, that may anchor the protein in the membrane; and one at amino acids 538 to 554 that might be either a second anchor sequence or, perhaps, resemble the hydrophobic stretches that are found in fusion proteins of ortho- and paramyxoviruses (39). In view of the fact that monoclonal antibody F-2-1 was suspected to inhibit virus replication at a point after virus attachment (19), this internal hydrophobic region at amino acids 538 to 554 was further inducement to examine the possibility that the BXL2 sequence encoded gp85.

Selection of an amino acid sequence within this reading frame for synthesis was made after analysis with the program of Parker and colleagues (27). This program attempts to predict a composite surface profile to be used for selection of synthetic peptides with good potential for inducing antibodies that recognize linear epitopes on the native protein (Fig. 2); residues with composite profile values of greater than 50% are those considered to be most likely to include surface-accessible residues. Of the seven stretches of the BXL2 sequence that had surface profile values of greater than 90%, the sequence corresponding to amino acids 518 to 528, which is adjacent to the internal hydrophobic sequence, was first chosen for synthesis. Since the likely secondary structure of the sequence 530-533 was predicted by the algorithm of Garnier and colleagues (12) to be a turn,

FIG. 2. Composite surface profile for deduced amino acid sequence of potential BXL2-encoded protein as predicted by the program of Parker and colleagues (27). Dashed horizontal line indicates residues synthesized as peptide V-1.

peptide V-1 was extended to include amino acids 518 to 533. A cysteine residue was added to the N terminus of the peptide to facilitate coupling to carrier protein. The V-1 sequence is not duplicated in EBV DNA.

Recognition of peptide V-1 in ELISA. Successful immunization of the rabbit with peptide V-1 was first determined by the ability of the immunoglobulin purified from preimmune and immune sera to react with peptide V-1 and not with an unrelated peptide B, derived from the human C9 sequence. Anti-V1 antibody was further purified by affinity chromatography on immobilized peptide. Its reactivity with peptide V-1 (Fig. 3A) and peptide B (Fig. 3B) was then compared with that of two monoclonal antibodies, F-2-1 and E1D1, that are known to react with gp85, and with a rabbit antibody prepared against affinity-purified gp85. Neither of the two monoclonal antibodies reacted with either peptide. However, the monospecific rabbit antibody, R24, reacted weakly but specifically with peptide V-1; no such reactivity was seen with identical concentrations of immunoglobulin purified from preimmune sera from the same rabbit.

Reactivity of anti-V-1 in Western blots with gp85. The weak reactivity of R24 with peptide V-1 suggested that this sequence might be present in gp85. We have not found the gp85 protein to be detectable in Western blots of electrophoresed lysates of virus, presumably because of its lack of abundance. However, reactivity with rabbit antibody has been observed if the protein is enriched by immunoprecipitation with F-2-1 before transfer. We therefore immunoprecipitated gp85 from P3HR1-C113 virus with F-2-1, electrophoresed and transferred it to nitrocellulose filters, and examined whether anti-V-1 or immunoglobulin purified from preimmune serum taken from the same rabbit reacted with the transferred protein. In our hands, the protein immunoprecipitated by antibody F-2-1, although known as gp85, has an apparent molecular weight of approximately 80,000 in 9% acrylamide cross-linked with diallyltartardiamide; anti-V-1

FIG. 3. Reactivity in ELISA of monoclonal antibodies F-2-1 and E1D1, monospecific rabbit anti-gp85 (R24), affinity-purified anti-V-1, and preimmune rabbit antibody. (A) Reactivity with peptide V-1; (B) reactivity with peptide B. Symbols: □, F-2-1; ○, E1D1; ●, anti-gp85; ■, anti-V-1; ▲, preimmune rabbit antibody purified from rabbit R24.

antibody, but not preimmune antibody, reacted with a transferred protein of this molecular weight (Fig. 4).

Protein immunoprecipitated by anti-V-1. Immunoprecipitation of 125 I-labeled virus or TPA-induced P3HR1-C113 cells labeled with [3 H]glucosamine also indicated that anti-V-1 recognized a glycoprotein with a mobility in gels similar to that of gp85 immunoprecipitated with either F-2-1 or the monospecific rabbit antibody R24 (Fig. 5). The probable identity of proteins immunoprecipitated by F-2-1 and anti-V-1 was further examined by comparing V8 protease digestion patterns of the two. Virus was labeled with 125 I, immunoprecipitated, and electrophoresed, and those regions of gel containing the immunoprecipitated protein were excised and electroeluted. Eluted proteins were digested with various amounts of V8 protease, and the digestion products were analyzed by SDS-PAGE (Fig. 6). Despite our attempt to digest equal amounts of protein as determined by the amount of radioactivity in each eluted sample, it appeared that more of the F-2-1 than the anti-V-1 protein was available

FIG. 4. Reactivity of preimmune antibody (lane 1) and anti-V-1 antibody (lane 2) with gp85 immunoprecipitated by monoclonal antibody F-2-1 from P3HR1-C113 cell-grown virus, separated by electrophoresis in 9% acrylamide, and blotted onto nitrocellulose. The arrow on the right indicates the band recognized by anti-V-1.

FIG. 5. SDS-PAGE in 9% acrylamide of proteins immunoprecipitated by F-2-1 (lanes 1 and 5), by anti-V-1 (lanes 2 and 6), by preimmune rabbit antibody (lanes 3 and 7), and by monospecific rabbit anti-gp85 (lane 4) from TPA-induced P3HR1-C113 cells metabolically labeled with [3 H]glucosamine (lanes 1, 2, and 3) and from virus labeled extrinsically with 125 I (lanes 4, 5, 6, and 7).

for digestion. Comparison of the digestion patterns nevertheless indicated that the two proteins were identical.

Effect of anti-V-1 on virus infectivity. Antibody F-2-1 inhibits infectivity of EBV in the absence of complement, whereas antibody E1D1 has no neutralizing effect. A comparison of the effects of F-2-1 and anti-V-1 on the ability of EBV to induce immunoglobulin synthesis by normal human lymphocytes indicated that anti-V-1 had only minimal neutralizing ability (Table 1). This observation was not unexpected since anti-V-1 failed to react with virus-producing cells in surface immunofluorescence assays and could not be visualized by fluorescein-conjugated anti-mouse antibody if added to virus before or after attachment of virus to receptor-positive Raji cells (data not shown).

DISCUSSION

Glycoprotein gp85 was first identified as a component of the EBV membrane antigen complex by immunoprecipitation of extrinsically labeled virus and cells with human and rabbit antisera (10, 29, 31). Strnad and co-workers further prepared two monoclonal antibodies, F-2-1 and G-3-1, that were used to characterize the molecule as a late protein, containing at least five N-linked oligosaccharide chains (30). Availability of the EBV DNA sequence, together with a prediction of likely coding regions within the genome (1), then allowed us to identify an open reading frame, BXLF2, as a likely candidate gene for gp85. The data presented in this paper indicate that antibodies made to a synthetic peptide derived from the BXLF2 open reading frame recognize the same glycoprotein as monoclonal antibody F-2-1 and are thus consistent with the hypothesis that BXLF2 encodes gp85. The use of synthetic peptides derived from known gene sequences is an indirect method for protein mapping, but is nevertheless a powerful alternative approach to gene expression that has been extremely useful for isolation and characterization of gene products (36). The as yet imprecise ability to predict antigenic epitopes from primary amino acid sequences is compensated for by the widespread cross-reactivity between proteins and linear peptides.

FIG. 6. SDS-PAGE, in 12.5% acrylamide cross-linked with methylenebisacrylamide, of 125 I-labeled proteins immunoprecipitated from P3HR1-C113 cell-grown virus by F-2-1 (lanes 1, 3, and 5) and anti-V-1 (lanes 2, 4, and 6), after partial digestion with *S. aureus* V8 protease. Lanes 1 and 2, No enzyme; lanes 3 and 4, 50 μ g of V8 protease; lanes 5 and 6, 100 μ g of V8 protease.

The sequence represented by the V-1 peptide encompassed less than 3% of the BXLF2 reading frame, so the fact that the monospecific rabbit antibody, made against the entire gp85 glycoprotein, reacted only weakly with the peptide was not surprising. The inability of the specific antibody, when titrated into normal rabbit antibody from the same animal, to reduce virus infectivity, except at high concentrations, was also not unexpected in view of our failure to visualize any reactivity with intact virus or virus-infected cells in immunofluorescence assays. This presumably means that, although the composite profile used for selection of peptide V-1 may have correctly predicted the sequence to include residues accessible in partially denatured protein, these residues are not readily accessible in the native protein inserted into the lipid bilayer. The orientation of gp85 in the membrane is not known, and it is possible that the region of the predicted sequence, including V-1, that lies between the hydrophobic termini is internal to the virus envelope. Perhaps a more likely possibility, however, is that the unavailability of the V-1 sequence to antibody is a function of the tertiary folding or assembly of the molecule.

TABLE 1. Comparison of inhibition by monoclonal antibody F-2-1 and rabbit antibody anti-V-1 of the ability of MCV5 cell-grown virus to induce immunoglobulin synthesis by fresh T-depleted human leukocytes

Antibody	Antibody:preimmune rabbit antibody (ng/culture)	Immunoglobulin concn (ng/ml) with:		% Inhibition
		Antibody alone	Antibody + virus	
F2-1	1,000:0	131	198	97
	300:700	125	1,188	85
	100:900	139	4,639	41
	30:970	114	7,858	0
Anti-V-1	1,000:0	60	4,472	43
	300:700	90	6,139	22
	100:900	170	7,612	3
	30:970	123	8,129	0
None	0:1,000	342	7,878	

The fact that anti-V-1 neutralizes virus infectivity very weakly suggests perhaps that at least one epitope included in the V-1 sequence is a neutralization epitope, but that the affinity of the polyclonal anti-V-1 antibody for this epitope is weak. We are in the process of preparing monoclonal antibodies to peptide V-1 in an attempt to isolate one of high affinity with greater neutralizing ability.

Several EBV genes have been shown to have counterparts in other herpesviruses (7, 13, 26), and the BXL2 sequence has been reported to include stretches that have weak homology with genes coding for herpes simplex virus type 1 glycoprotein gH (25) and for glycoprotein III (21) of varicella-zoster virus (26). Preliminary data (N. Balachandran, D. E. Oba, and L. M. Hutt-Fletcher) reveal that the monospecific rabbit anti-gp85 antibody used in this study reacts with gH of herpes simplex virus type 1, indicating that the deduced homology is mirrored by shared antigenicity. Both gpIII and gH are important to the spread of virus in tissue culture (6, 21), and herpes simplex virus gH is essential for efficient egress of virus from infected cells (37). The importance of gp85 to virus infectivity is demonstrated by the fact that antibody to it can neutralize infectivity in a complement-independent manner; antibody F-2-1 does not inhibit virus binding, but does inhibit penetration of virus through the cell membrane (N. Miller and L. M. Hutt-Fletcher, Twelfth International Herpesvirus Workshop, abstr. 109; submitted for publication). The implication of this work is that gp85 is important to virus entry; whether or not it is also, like its alpha herpesvirus counterparts, required for release of EBV from producer cells will be the subject of further study.

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