Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5703–5707, August 1986
Medical Sciences

Characterization of two related Epstein–Barr virus-encoded membrane proteins that are differentially expressed in Burkitt lymphoma and *in vitro*-transformed cell lines

(lymphocyte-detected membrane antigen/synthetic oligopeptides/predicted protein structure)

SUSANNE MODROW AND HANS WOLF

Max von Pettenkofer-Institut, Ludwig-Maximilians-Universität, Pettenkoferstrosse 9a, D-8000 Munich 2, Federal Republic of Germany

Communicated by Werner Henle, April 7, 1986

ABSTRACT **Two related but differentially expressed potential membrane proteins of Epstein–Barr virus are encoded by the same reading frame in the *Eco*RI D het region of the viral genome. Potential antigenic sites in the amino acid sequence of these proteins were selected by computer-aided prediction of the secondary structure and two oligopeptides corresponding to regions located in different parts of the proteins were synthesized chemically. Rabbit antisera to these peptides were used for immunoprecipitation of the native viral proteins from Epstein–Barr virus-positive cell lines from various sources. Both predicted membrane proteins could be precipitated from cell lines that had been transformed *in vitro* with EBV or from cell lines derived from infectious mononucleosis patients. In cell lines established from Burkitt lymphoma, only the smaller polypeptide, which lacks 138 amino acids from the amino terminus, could be identified. Using the synthetic peptides as antigens in ELISA, we detected elevated antibody titers in sera from patients with infectious mononucleosis and nasopharyngeal carcinoma.**

Epstein–Barr virus (EBV), a human herpesvirus, causes infectious mononucleosis (1, 2) as a primary disease and remains latent in human B lymphocytes, transforming them into lymphoblastoid cells that proliferate indefinitely upon cultivation *in vitro* (3, 4). EBV has also been found to be associated with African Burkitt lymphoma (5–7) and nasopharyngeal carcinoma (8, 9) and with polyclonal lymphomas in immunosuppressed individuals (10).

At least five regions of the viral genome are transcribed during latency: the small unique (U_S) region is transcribed to give EBER RNAs (11); the internal repeat region with neighboring sequences encodes the nuclear antigen EBNA2 (12); the *Bam*HI fragment-K region encodes EBNA1 (13, 14); a region in the long unique (U_L) region codes for EBNA3 (15); and the region of the *Eco*RI D het fragment at the right end of the viral DNA codes for a potential membrane protein (16–18). Two related membrane proteins were proposed to be expressed from this reading frame, BNLF1 (19), which has promoters with differential activity and a single 3′-terminal end. The shorter of the two proteins would lack 138 amino acids from the amino terminus (ref. 20; Fig. 1). Further, it was predicted that the larger protein would have a highly charged amino terminus followed by a hydrophobic domain, consisting of six transmembrane regions, and a large hydrophilic carboxyl-terminal region. The existence of an EBV-associated membrane protein in latently infected cells was also suggested by studies of cytotoxic T-cell clones (21–24), which recognize an antigen, found only on EBV-infected cells, known as lymphocyte-determined membrane antigen (LYDMA). The biochemical properties of this antigen are not known.

For further characterization of the EBV-encoded membrane antigen in latently infected cells ("latent membrane antigen" or BNLF1-MA), we synthesized oligopeptides corresponding to the amino-terminal part and to the repeat unit that is part of the large hydrophilic carboxyl-terminal domain. (The first one should only be present in the larger protein, whereas the latter is a part of both proteins.) Antisera against the peptides were used for immunoprecipitation of the native proteins from various cell lines.

The peptides were also used as antigens in ELISA assays, in which sera from patients suffering from infectious mononucleosis and the EBV-related nasopharyngeal carcinoma were tested for the presence of the respective antibodies.

MATERIALS AND METHODS

Computer-Predicted Analysis of the Secondary Structure. The secondary structure of the latent membrane protein was predicted by a program, written for a VAX 750 computer, based on suggestions by Cohen *et al.* (25), using the algorithms of Chou and Fasman (26) or Garnier *et al.* (27) to predict secondary structures. These predictions were superimposed with the values of local hydrophilicity determined by the method of Hopp and Woods (28). As alternatives, values for surface probability [modified from Emini *et al.* (29)] or flexibility (30) can be superimposed. The probability of the occurrence of α-helices, β-pleated sheets, random coils, and β-turn regions was evaluated using stringent conditions (31). The parameters for hydrophilicity were averaged over five amino acid residues, with a limit of 0.7. α-Helical structures in a hydrophilic or nonhydrophobic environment are likely candidates for antigenic sites, since they frequently form loop-like structures at the protein surface.

Peptide Synthesis. Two peptides were synthesized (for sequence and location, see Fig. 1*C*) using the Merrifield solid-phase methods (32) with the following modifications. Before and after deprotection of *N-t*-butoxycarbonyl (*N-t*-Boc) amino acids, a series of three washes, with methylene chloride, absolute ethanol, and methylene chloride, respectively, was used; deprotection of *N-t*-Boc amino acids was done with 25% trifluoroacetic acid in methylene chloride. Completeness of deprotection and coupling reactions were monitored by the ninhydrin color test (33). All coupling reactions were carried out using a 4-fold excess of *N-t*-Boc amino acids and a 3-fold excess of dicyclohexylcarbodiimide (Aldrich) according to the amount of the first amino acid. All *N-t*-Boc amino acids were purchased from Sigma; *N-t*-butoxycarbonyl-4-methoxybenzylcysteine-*O*-resin was purchased from Peninsula Laboratories (St. Helens, England).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EBV, Epstein–Barr virus; EBNA, EBV-encoded nuclear antigen; KLH, keyhole limpet hemocyanin.

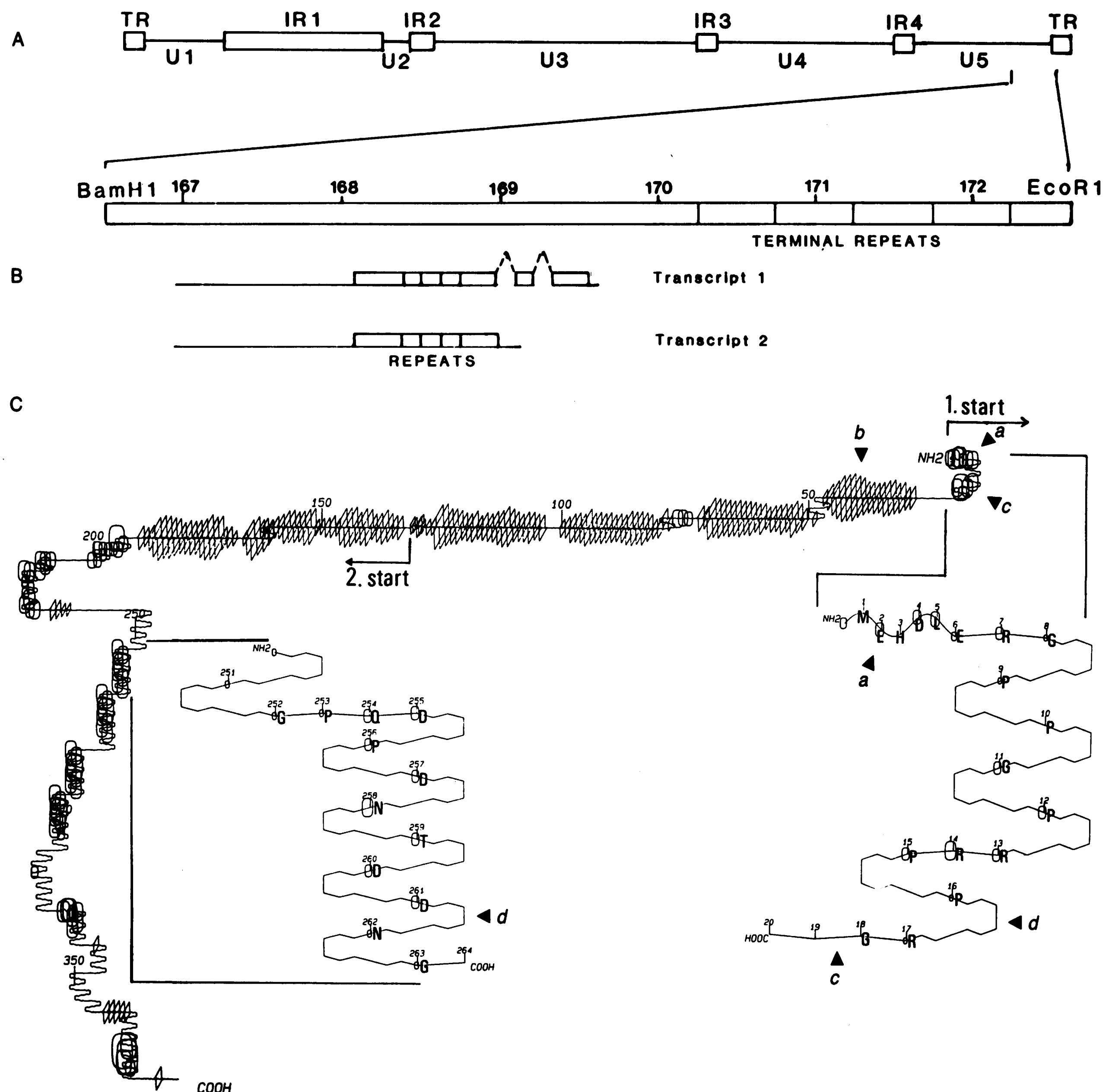

FIG. 1. (*A*) Schematic diagram of the EBV genome indicating terminal repeats (TR), unique regions (U), and internal repeats (IR). (*B*) Organization and transcription of the right-hand end of the U5 region of EBV. Numbering is from the standard sequence, representing thousands of base pairs from the left end of the entire viral genome. Transcripts of this region are represented with boxes showing predicted protein-encoding regions. (*C*) Chou–Fasman prediction of the structure of the proteins encoded by the BNLF1 reading frame. The translation starts of the two predicted gene products and the amino acid sequences (standard one-letter abbreviations) of the peptides synthesized are indicated. Probability of occurrence of α-helices (e.g., arrowheads *a*), β-pleated sheets (arrowhead *b*), random coils (arrowheads *c*), and β-turn regions (arrowheads *d*) was evaluated using stringent conditions. The parameters for hydrophilicity were averaged over five amino acid residues with a limit of 0.7 (hydrophilic regions are shown as ovals, and hydrophobic regions, as diamonds).

The side chains were cleaved from the resin in thioanisole suspension at 0°C with anhydrous hydrogen fluoride (Matheson). After precipitation and extensive washing with ethyl acetate, the peptide was lyophilized, extracted with 1.5% NH_4HCO_3, and purified by molecular sieving (Bio-Gel P-4 column, Bio-Rad).

Immunization of Rabbits. For the production of antisera, two alternative procedures were used. (*i*) The peptides were coupled to keyhole limpet hemocyanin (KLH) (34) with *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester. Since this coupling reaction requires the presence of a thiol group, cysteine was included as the first amino acid at the carboxyl terminus. (*ii*) The amino acids glycine, glycine, and lysine (Lys-Gly-Gly) were covalently linked to the amino terminus of the peptide, and both amino groups of the terminal lysine were esterified with palmitic acid by following the procedure of solid-phase synthesis (35).

Rabbits were inoculated subcutaneously with a 1-ml emulsion of antigen (200 μg of the palmitoylated peptides or 400 μg of the KLH conjugate) in phosphate-buffered saline (PBS: 130 mM NaCl/2.7 mM KCl/8 mM Na_2HPO_4/1.5 mM KH_2PO_4/0.9 mM $CaCl_2$/0.5 mM $MgCl_2$, pH 7.2) plus complete Freund's adjuvant; booster injections were given at intervals of 3 weeks with incomplete Freund's adjuvant.

ELISA. Either of the two peptides (20 μg per well, 96-well plate, Dynatech) was coupled in 50 μl of 0.2 M sodium carbonate buffer (pH 9.5) overnight. The plates were incubated with gelatin solution (5 mg/ml) for 1 hr and then

were incubated 2 hr with 50 μl of serum diluted in PBS/0.5% Tween 20, followed by incubation with peroxidase-labeled second antibody [rabbit anti-human IgG, Dako (Santa Barbara, CA), or goat anti-human IgA, Teko] diluted 1:500 in PBS/0.5% Tween 20. Staining was done with *o*-phenylenediamine (1 mg/ml in phosphate buffer, pH 6.0) and was stopped after 10 min with 1 M H_2SO_4. The optical density was determined at 486 nm.

Immunoprecipitation. Cells were grown in RPMI 1640 medium with 10% fetal bovine serum and, depending on the experiment, treated with phorbol 12-myristate 13-acetate (40 ng/ml; Sigma) and butyric acid (3 mM) or with phosphonoacetic acid (200 μg/ml) or cycloheximide (50 μg/ml). After incubation with [^{35}S]methionine (20 μCi/ml; 1 Ci = 37 GBq) or [^{14}C]leucine (2 μCi/ml) (Amersham-Buchler, Braunschweig, F.R.G.) for various intervals, cells were washed, suspended in immunoprecipitation buffer [0.5% Nonidet P-40/20 mM Tris Cl, pH 9.0/300 mM NaCl/1 mM $CaCl_2$/0.5 mM $MgCl_2$/2 mM EDTA/10% (vol/vol) glycerol], and lysed by sonication. Lysates were cleared of nonspecifically reacting material by incubation with 10 μl of negative rabbit serum and 3 mg of protein A-Sepharose beads (Pharmacia) per 10^6 cells. The beads with the nonspecific immunocomplexes were removed, the supernatants were incubated with 10 μl of positive serum (preadsorbed with an extract of 10^7 EBV-negative BJAB cells) per 10^6 cells, 3 mg of protein A-Sepharose beads were added. The beads with the bound immunocomplexes were pelleted, washed, suspended in solubilization buffer (2% $NaDodSO_4$/3% sucrose/5% 2-mercaptoethanol/20 mM Tris Cl, pH 7.0, containing bromphenol blue), heated at 100°C, and subjected to electrophoresis as described (36).

RESULTS

Selection of Peptide Sequence and Production of the Respective Antisera. The computer-predicted analysis of the secondary structure of proteins combined with the values of hydrophobicity allowed the identification of highly antigenic regions of the EBV latent membrane proteins expressed from the reading frame BNLF1. To study the different expression of the two putative proteins (20), we synthesized peptides corresponding to the sequence of the hydrophilic β-turn region from the amino terminus of the large protein (peptide 1–18) and to the sequence of a hydrophilic repeat unit (peptide 252–263) that is located downstream from the transmembrane region and should be a common antigenic epitope of both forms of the membrane proteins. The sequences of the peptides are indicated in Fig. 1*C*.

Antisera against the peptides were raised in rabbits immunized with dipalmitoyllysylglyrylglycyl or with KLH-peptide conjugate. When tested in ELISA assays, the sera showed titers up to 1:200,000 against the individual peptides.

Screening of Human Sera for Peptide Antibodies. To identify antibodies against oligopeptides derived from the latent membrane antigens, peptides 1–18 and 252–263 were used as antigens in ELISAs to screen sera of healthy, EBV-positive individuals [anti-VCA (viral capsid antigen) IgG titer 1:32 to 1:128, EBNA-positive in immunofluorescence tests]; nasopharyngeal carcinoma patients [anti-VCA IgG titer 1:32 to 1:2056, anti-EA (early antigen) IgA titer 1:16 to 1:128, EBNA-positive]; Burkitt lymphoma patients [anti-VCA IgG titer 1:1280, anti-EA-DR (restricted EA) IgG titer 1:10 to 1:2560, EBNA-positive]; patients with fresh EBV infections (anti-VCA IgG titer 1:18 to 1:64, anti-VCA IgM titer 1:16 to 1:128, anti-EA IgG and IgA titer 1:16 to 1:128, EBNA-negative); and EBV-negative individuals. All sera were used at a dilution of 1:50 in the ELISA, and the optical density was determined at 486 nm. The sera of patients with nasopharyngeal carcinoma or fresh EBV infection reacted very strongly with the amino-terminal epitope peptide 1–18. Those of healthy individuals showed almost no reaction compared to those of EBV-negative persons (Fig. 2); sera of Burkitt lymphoma patients showed a slightly greater reaction. With peptide 252–263 as antigen, the same sera of nasopharyngeal carcinoma, Burkitt lymphoma, and fresh infectious mononucleosis patients showed only slightly greater reactions than did sera of healthy individuals.

Immunoprecipitation of Latent Membrane Proteins from Various Cell Lines. Rabbit antisera raised against both pep-

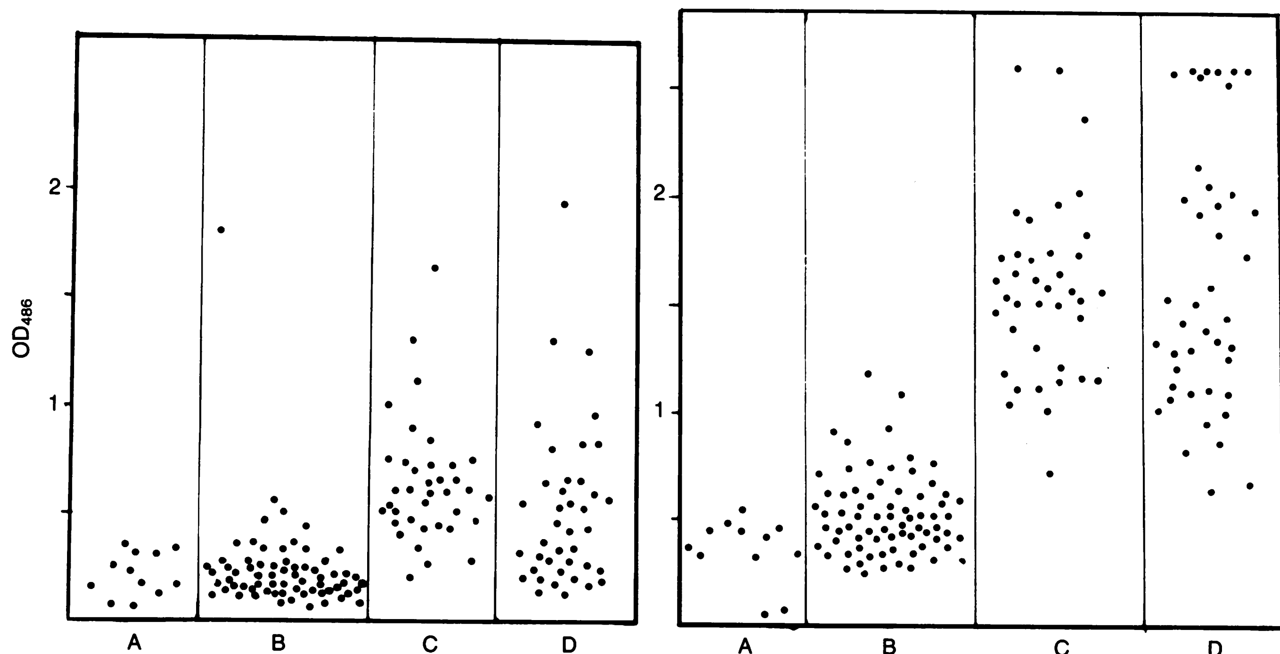

FIG. 2. Distribution of the values for optical density obtained by ELISA using the various groups of human sera. Sera were from EBV-negative individuals (group A), healthy EBV-positive individuals (group B), nasopharyngeal carcinoma patients (group C), or patients with fresh EBV infections (group D). All sera were used at a dilution of 1:50 in phosphate-buffered saline. Peroxidase-conjugated second antibodies were goat anti-human IgA (*Left*) or rabbit anti-human IgG (*Right*). The antigen was underivatized peptide 1–18.

tides were used to precipitate [^{35}S]methionine-labeled polypeptides from the Burkitt lymphoma-derived cell lines Raji (37), Jijoye (38), Namalwa (39), and P3HR1 (40); from the *in vitro*-transformed marmoset cell line B95-8 (41), from the *in vitro*-transformed human cord-blood lines CB176-HK975 (provided by G. Lenoir, International Agency for Research on Cancer, Lyon, France) and J-Aba (42); and from two spontaneous cell lines derived from infectious mononucleosis patients (RB and RS). BJAB (43) and Ramos (44) were used as EBV-negative lines. Antiserum against the amino-terminal peptide 1–18 specifically immunoprecipitated proteins of 60–70 kDa from lysates of *in vitro*-transformed and spontaneous cell lines (Fig. 3*A*). The variation in molecular mass is likely due to variations in the number of repeat units present in the viral genome of the various cell lines (17). No protein that reacted specifically with the antiserum to peptide 1–18 was found in any of the Burkitt lymphoma lines. The result was similar for EBV-negative cell lines.

When the serum directed against peptide 252–263 from the amino acid repeat at the carboxyl-terminal part of the predicted sequence was used for immunoprecipitation (Fig. 3*B*), a protein with the same molecular mass range as described above (60–70 kDa) was identified in the *in vitro*-transformed lines B95-8, CB176HK975, and (data not shown) J-Aba and in the infectious mononucleosis lines RB and (data not shown) RS, together with a second protein of apparent molecular mass ranging from 50 to 60 kDa. Polypeptides of 50–60 kDa were also detected in the Burkitt lymphoma cells Raji, Jijoye, and Namalwa; in P3HR1 we found only a very weak reaction. No protein was identified in the EBV-negative cell lines (Fig. 3 *A* and *B*).

The apparent sizes of the proteins in NaDodSO$_4$/polyacrylamide gels, 60–70 kDa and 50–60 kDa, were different from the predicted values (42 kDa and 28 kDa, respectively). This anomalous behavior of the polypeptides may be due to the high content of aspartic acid and proline and was also reported by other groups (17, 18). Identification of these polypeptides was clearer when [^{14}C]leucine was used for labeling, which is compatible with the presence of only 3 methionine but 34 leucine residues in the sequence.

When cells were treated with phorbol 12-myristate 13-acetate and butyric acid to induce the synthesis of late viral gene products, no polypeptides were identified by immunoprecipitation with the anti-peptide sera (Fig. 3*C*). A similar result was obtained when protein synthesis was blocked by cycloheximide, followed by a labeling period when further RNA synthesis was inhibited by the addition of actinomycin D. When viral DNA synthesis was inhibited by phosphonoacetic acid, the latent membrane proteins were detected in the treated cells. We conclude that the identified proteins belong to a group of viral gene products whose synthesis is blocked by viral DNA synthesis.

DISCUSSION

This work presents data for a differential expression of protein products of the open reading frame BNLF1 (19) in the *Eco*RI fragment-D region of EBV. This reading frame has been shown to code for two viral transcripts (20), the larger of which has been reported to be a latent membrane protein (17, 18), which may be the still-unidentified target antigen of cytotoxic T cells directed against EBV-infected cells. Taking into account computer-predicted analysis of secondary structures and hydrophilicity of amino acids, we synthesized antigenic epitopes from various regions of the protein. Antibodies prepared against the peptides were able to identify the original viral products. It could be shown that these proteins are produced with varying molecular mass according to the number of repeat units present in the cell lines; similar

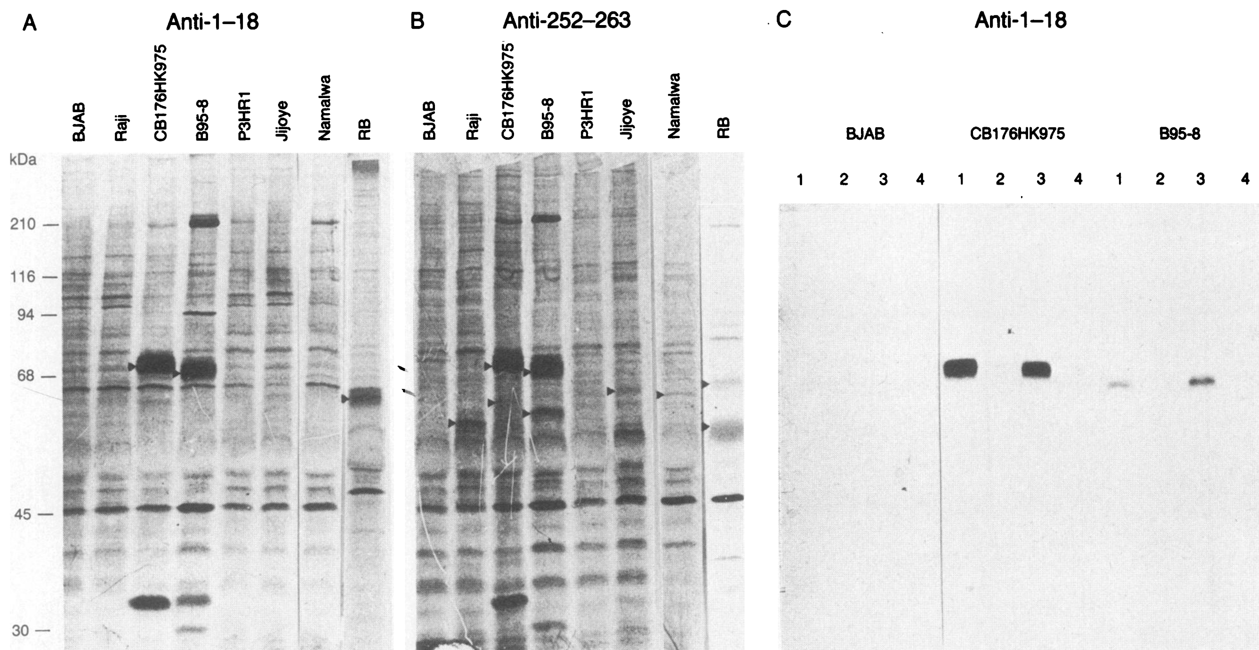

FIG. 3. (*A* and *B*) Immunoprecipitation of lysates from various cell lines (indicated above the lanes). Cells were labeled with [^{14}C]leucine (2 μCi/ml) for 6 hr, washed, suspended in immunoprecipitation buffer, and sonicated. Lysates were incubated first with negative rabbit serum and protein A-Sepharose beads. After the beads with the nonspecific complexes were removed, the labeled extracts were incubated with sera directed against the amino-terminal part of the latent membrane protein (anti-1-18, *A*) or against the internal part (anti-252-263, *B*). After the immunocomplexes were bound to protein A-Sepharose beads, the beads were washed, suspended in electrophoresis buffer, and heated at 100°C, and the labeled proteins were analyzed by NaDodSO$_4$/PAGE followed by autoradiography. Black triangles indicate specifically immunoprecipitated polypeptides discussed in text. High background is due to the very long exposure (2 months) and the labeling with [^{14}C]leucine. (*C*) Immunoprecipitation of proteins from [^{35}S]methionine-labeled cells, using antiserum to peptide 1-18. Lanes 1: cells not treated, labeled for 4 hr. Lanes 2: cells treated with cycloheximide (50 μg/ml) for 6 hr, then labeled in the presence of actinomycin D (2 μg/ml) for 4 hr. Lanes 3: cells treated with phosphonoacetic acid for 24 hr, then labeled for 4 hr. Lanes 4: cells treated with phorbol 12-myristate 13-acetate (40 μg/ml) and butyric acid (3 mM) for 24 hr, then labeled for 4 hr.

results were found by Hennessy *et al.* (17). In addition, we were able to show that this region of the EBV genome is differentially expressed in Burkitt lymphoma cells and EBV-producer cell lines. Burkitt lymphoma cells, in which the viral DNA is present in a latent state and which do not synthesize EBV, do not express the first part of the BNLF1 reading frame, suggesting that only the second proposed promoter (20) is used for the start of transcription. This smaller protein (50–60 kDa) was detected in all EBV-positive cell lines and is a truncated form of the larger one (60–70 kDa), lacking the first hydrophilic domain and four of the proposed transmembrane regions. In precipitations with anti-peptide 1–18 serum, a protein of about 32 kDa was identified in B95-8 and CB176HK1975 cells. Since no protein in the BNLF1 frame could be classed with this product, we assume that the 32-kDa protein either is associated with the 60- to 70-kDa product in the membrane and therefore coprecipitates or is a cellular product with a similar antigenic determinant.

If the latent membrane protein complex described in our experiments is correlated to the lymphocyte-determined membrane antigen (LYDMA) on EBV-producing cells, the target for cytotoxic-T-cell recognition should be located in the first part of the protein, possibly in one of the two turn regions separating transmembrane regions 1 and 2 and transmembrane regions 3 and 4, respectively, which probably are exposed at the surface of the cell, since—due to the lack of a signal peptide—the highly charged amino terminus is not likely to be located on the outside of the cell. After cytotoxic-T-cell recognition and lysis of the LYDMA-positive cells, the amino terminus may be exposed to the immune system of the host. As a consequence, in sera derived from patients with fresh EBV infection or nasopharyngeal carcinoma, we were able to detect elevated antibody titers for the amino-terminal peptide from the latent membrane protein. The fact that some sera from Burkitt lymphoma patients reacted with peptide 1–18 as well is not surprising, since Burkitt lymphoma patients possess, besides the lymphoma cells, peripheral lymphocytes containing EBV genomes without showing the lymphoma genotype or phenotype.

Another possibility is that this protein alone is not sufficient to elicit cytotoxic-T-cell reaction. The highly charged amino terminus and the following hydrophobic transmembrane part may be involved in complex formations and conformational changes with other components of the cell membrane, which then may be the target for the T-cell recognition.

We thank Wolfgang Jilg and Bernhard Dietzschold for helpful discussions. The Burkitt lymphoma patients' sera were a gift of Gilbert Lenoir. This investigation was supported by grants from the Deutsche Forschungsgemeinschaft (SFB217, TP31, and Wo227) and the Bundesministerium für Forschung und Technologie.

1. Henle, G., Henle, W. & Diehl, V. (1968) *Proc. Natl. Acad. Sci. USA* **59**, 94–101.
2. Henle, W. & Henle, G. (1972) in *Oncogenesis and Herpesviruses*, eds. Biggs, P. M., deThe, G. & Payne, L. N. (International Agency for Research on Cancer Scientific Publications, Lyon, France), pp. 269–274.
3. Henle, W., Diehl, V., Kohn, G., zur Hausen, H. & Henle, G. (1967) *Science* **157**, 1064–1065.
4. Gerber, P., Whang-Peng, J. & Monroe, J. H. (1969) *Proc. Natl. Acad. Sci. USA* **63**, 740–747.
5. Levy, J. A. & Henle, G. (1966) *J. Bacteriol.* **92**, 275–276.
6. Henle, G., Henle, W., Clifford, P., Diehl, V., Kafuko, G. W., Kirya, B. G., Klein, G., Morrow, R. H., Munube, G. M. R., Pike, P., Tukei, P. M. & Ziegler, I. L. (1969) *J. Natl. Cancer Inst.* **43**, 1147–1157.
7. deThe, G., Geser, A., Day, N. E., Tukei, P. M., Williams, E. H., Beri, D. P., Smith, P. G., Dean, A. G., Bornkamm, G. W., Feorino, P. & Henle, W. (1979) *Nature (London)* **274**, 756–761.
8. Henle, W., Henle, G., Zajac, B., Pearson, G., Waubke, R. & Scriba, M. (1970) *Science* **169**, 188–190.
9. Wolf, H., zur Hausen, H. & Becker, V. (1973) *Nature (London) New Biol.* **138**, 245–247.
10. Bar, R., DeLar, C. L., Clausen, K. P., Hurtubise, P., Henle, W. & Hewetson, J. F. (1974) *N. Engl. J. Med.* **290**, 363–367.
11. Lerner, M. R., Andrews, N. C., Miller, G. & Steitz, J. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 805–809.
12. Dambaugh, T., Hennessy, K., Chamnankit, L. & Kieff, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7632–7636.
13. Fischer, D. K., Robert, M. F., Shedd, D., Summers, W. P., Robinson, J. E., Wolak, J., Stefano, J. E. & Miller, G. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 43–47.
14. Hennessy, K. & Kieff, E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5665–5669.
15. Hennessy, K., Fennewald, S. & Kieff, E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5944–5948.
16. Fennewald, S., van Santen, V. & Kieff, E. (1984) *J. Virol.* **51**, 411–419.
17. Hennessy, K., Fennewald, S., Hummel, M., Cole, T. & Kieff, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7207–7211.
18. Mann, K. P., Staunton, D. & Thorley-Lawson, D. A. (1985) *J. Virol.* **55**, 710–720.
19. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrell, P. J., Gibson, T. G., Hatfull, G., Hudson, G. S., Satchwell, C. S., Seguin, C., Tuffnell, P. S. & Barrell, B. G. (1984) *Nature (London)* **310**, 207–211.
20. Hudson, G. S., Farrell, P. J. & Barrell, B. G. (1985) *J. Virol.* **53**, 528–535.
21. Menezes, J., Jondal, M., Leibold, W. & Dorval, G. (1976) *Infect. Immun.* **13**, 303–310.
22. Wallace, L. E., Rickinson, A. B., Rowe, M. & Epstein, M. A. (1982) *Nature (London)* **297**, 413–415.
23. Tanaka, Y., Sugamura, K. & Hinuma, Y. (1982) *J. Immunol.* **118**, 1241–1245.
24. Slovin, S. F., Schooley, R. T. & Thorley-Lawson, D. A. (1983) *J. Immunol.* **130**, 2127–2132.
25. Cohen, G. H., Dietzschold, B., Ponce de Leon, M., Long, D., Golub, E,, Varrichio, A., Pereira, L. & Eisenberg, R. J. (1984) *J. Virol.* **49**, 102–108.
26. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13**, 222–245.
27. Garnier, J., Osgutthorpe, D. J. & Robson, B. (1978) *J. Mol. Biol.* **120**, 97–120.
28. Hopp, T. P. & Woods, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3824–3828.
29. Emini, E. A., Hughes, J. V., Perlow, D. S. & Boger, J. (1985) *J. Virol.* **55**, 836–839.
30. Karplus, P. A. & Schulz, G. E. (1985) *Naturwissenschaften* **72**, 212–213.
31. Chou, P. Y. & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* **47**, 45–148.
32. Steward, J. M. & Young, J. P. (1969) *Solid Phase Peptide Synthesis* (Freeman, San Francisco).
33. Kaiser, E., Colescott, R. L., Bossinger, C. D. & Cook, P. J. (1970) *Anal. Biochem.* **34**, 595–598.
34. Liu, F. T., Zinnecker, M., Hamaoka, T. & Katz, D. H. (1979) *Biochemistry* **15**, 690–697.
35. Hopp, T. P. (1984) *Mol. Immunol.* **21**, 13–16.
36. Modrow, S. & Wolf, H. (1983) *J. Gen. Virol.* **64**, 37–46.
37. Pulvertaft, R. J. V. (1964) *Lancet* **i**, 238–240.
38. Ragona, G., Ernberg, I. & Klein, G. (1980) *Virology* **101**, 553–559.
39. Klein, G. & Dombos, L. (1973) *Int. J. Cancer* **11**, 327–337.
40. Klein, G. (1979) in *The Epstein–Barr Virus*, eds. Epstein, M. A. & Achong, B. (Springer, Berlin), pp. 339–350.
41. Miller, G., Shope, T., Lisco, H., Stitt, D. & Lipman, M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 383–387.
42. Trumper, P. A., Epstein, M. A. & Giovanella, B. C. (1976) *Int. J. Cancer* **17**, 578–587.
43. Klein, G., Lindahl, T., Jondal, M., Leibold, W., Menezes, J., Milsson, K. & Sundstrom, C. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3283–3286.
44. Klein, G., Giovanella, B., Westman, A., Stehlin, J. & Mumford, D. (1975) *Intervirology* **5**, 319–334.