A Second Epstein-Barr Virus Membrane Protein (LMP2) Is Expressed in Latent Infection and Colocalizes with LMP1

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Received 15 November 1989/Accepted 29 January 1990

Recent cDNA cloning and sequencing of two Epstein-Barr virus (EBV)-specific mRNAs from latently infected cultures revealed that these RNAs are encoded across the fused terminal repeats of the viral genome and that they are likely to encode two nearly identical proteins with the same transmembrane domains. The smaller predicted protein (LMP2B) lacks 119 amino-terminal amino acids found in the larger one (LMP2A). To test whether these proteins are expressed in latently infected lymphocytes, antibodies to the LMP2 proteins were derived by immunizing rabbits with TrpE-LMP2A fusion proteins. Affinity-purified LMP2-specific antibodies recognized 54- and 40-kilodalton proteins, corresponding to LMP2A and LMP2B, in immunoblots of rodent fibroblasts stably transfected with eucaryotic expression plasmids containing either the LMP2A or LMP2B cDNA. Similar-size proteins were also identified in immunoblots of latently infected lymphocytes. LMP2A localized to membranes in cellular fractionation studies. In immunofluorescent studies, LMP2B localized in the plasma membrane of EBV-infected lymphocytes, with the majority of reactivity confined to the region of the LMP1 patch. This reactivity was detected in almost all lymphoblastoid cells latently infected with EBV.

Epstein-Barr virus (EBV), a human herpesvirus, causes infectious mononucleosis in normal adolescents and B-lymphocyte proliferative disease in immune-compromised patients. EBV is also etiologically associated with Burkitt's lymphoma and nasopharyngeal cancer (for reviews, see references 5, 6, 15a, and 23). Normal B lymphocytes, infected in vitro or in vivo, proliferate indefinitely and give rise to lymphoblastoid cell lines which contain the entire EBV genome in an episomal, latent state (20, 22). The EBV genome is 180 kilobase pairs, but only part of the genome encodes mRNA in latent B-lymphocyte infection. Seven virus-encoded proteins are known to be expressed in latent infection. Six of these are the nuclear proteins EBNA-1, EBNA-2, EBNA-3a, EBNA-3b, EBNA-3c, and EBNA-LP (5, 15a, 24, 25). The seventh protein, LMP1, localizes to a small region of the lymphocyte plasma membrane (12, 15a, 19). The proteins expressed in latent infection are being extensively studied since they are likely to be involved with maintaining latent EBV infection or inducing proliferative transformation.

Recent experiments have indicated that there may be other proteins expressed in latent infection. Two polyadenylated cytoplasmic RNAs of 2.3 and 2.0 kilobase pairs are encoded by the left end of the EBV genome in latently infected cells (14, 36). Full-length cDNA clones corresponding to these mRNAs were isolated and sequenced (16, 31), revealing that these RNAs initiate at the right end of the genome and are transcribed across the termini into the left end (Fig. 1). The two mRNA species consist of different 5' exons from the U5 region of the genome followed by eight common exons derived from the U1 region (Fig. 1, line 3). Analysis of the amino acid sequence of the principal open reading frames of these two RNAs revealed that they could each encode nearly identical hydrophobic proteins which differ only in the length of the hydrophilic amino terminus (Fig. 1, line 4). The larger RNA would encode a protein with 119 additional amino acids at its amino terminus. Both proteins would have twelve identical highly hydrophobic domains of at least 17 amino acids which would likely be membrane-spanning domains (Fig. 1, line 4). Assuming a model with an even number of membrane-spanning domains and a cytoplasmic orientation for the amino and carboxy termini, all positively charged amino acids except two would be on the cytoplasmic side of the membrane. The predicted primary amino acid sequences are similar to other membrane-associated proteins in having multiple membrane-spanning domains. No precise homology to previously derived protein sequences in Swiss Protein Data Bank release 11 is evident (Intelligence, Mountain View, Calif.). The predicted molecular masses are 53,011 and 40,383 daltons (Da). In vitro translation of the transcribed cDNAs results in proteins of 54 and 40 kDa which, as expected, are posttranslationally inserted into membranes (31). Since protein size was not affected by microsomal membrane insertion, neither cleavage nor glycosylation probably occurs upon membrane insertion (31). Furthermore, since induction of lytic virus replication did not result in a greater abundance of these RNAs, they are likely to encode latency-associated proteins. These putative latent infection-associated membrane proteins were therefore designated latent membrane proteins 2A and 2B (LMP2A and LMP2B).

To determine whether these two predicted, unusual, hydrophobic membrane proteins are expressed in latent EBV lymphocyte infection, we expressed the common domains of the proteins in Escherichia coli as a TrpE fusion protein, purified the resultant protein, immunized rabbits, and derived antisera which specifically identified these proteins. Using these antisera, we have established that LMP2A and probably LMP2B are expressed in the membranes of latently infected B lymphocytes, where they associate with LMP1.

MATERIALS AND METHODS

Cells and transfections. IB-4, LCL1, and LCL2 are in vitro EBV-transformed, latently infected lymphoblastoid cell lines obtained by infection of cord blood lymphocytes (IB-4 and LCL1) or adult lymphocytes (LCL2) and are not per-
missive for virus replication. Raji and Namalwa are EBV-positive Burkitt's tumor cell lines. B95-8 is an EBV-infected marmoset cell line that is partially permissive for virus replication. BL41 and BJAB are EBV-negative Burkitt's tumor cell lines. All lymphoid cell lines were grown in RPMI 1640 with 10% fetal calf serum and 4 μg of gentamicin per ml.

BALB 3T3 cells used for construction of stable cell lines expressing LMP2A and LMP2B were grown in 10% fetal calf serum and 4 μg of gentamicin per ml. Transfections were done via the calcium phosphate method (8). The cells were selected in the presence of 200 μg of hygromycin B per ml.

**Plasmids and DNA cloning.** The DNA cloning procedures were as previously described (21). The complete cDNA containing the LMP2A and LMP2B coding sequences was as described previously (31). The pATH vector (plasmid amenable to making tryptophan hybrid) used for making LMP2A fusion proteins was as described elsewhere (33). The expression vector pBamHygro was as described previously (41) and kindly provided by A. Korman.

**Induction of TrpE fusion proteins and immunizations.** Bacterial cells were grown and induced, and the insoluble fraction was enriched for the fusion protein essentially as described elsewhere (33). The TrpE-LMP2A fusion protein was recovered in the insoluble protein fraction and electrophoresed on a 7% preparative sodium dodecyl sulfate-polyacrylamide gel. The appropriate band was excised, emulsified in an adjuvant system (Ribi Immunochemo Research, Hamilton, Mont.), and used to immunize two female rabbits. The rabbits received booster shots every 2 to 4 weeks.

**Affinity purification of antibodies.** Purified TrpE-LMP2A fusion proteins and the unfused TrpE protein were purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above. The proteins in the excised bands were then electroeluted from the polyacrylamide gel with an electroelution apparatus (Bio-Rad Laboratories, Richmond, Calif.). The purified proteins (approximately 2 mg) were then coupled to 1-ml Actigel A beads (Sterogene, San Gabriel, Calif.) in 0.1 M phosphate buffer (pH 7.0) in the presence of sodium cyanoborohydride as described by the manufacturer. Coupled beads were then transferred to a small column and washed successively with 1 M NaCl and TBS buffer (20 mM Tris [pH 7.5], 150 mM NaCl), 3 ml of elution medium (Sterogene), and 50 ml of TBS. Immune sera from the immunized rabbits were first applied to the TrpE column and subsequently to the LMP2 fusion protein affinity column. The columns were extensively washed with TBS containing 0.1% bovine serum albumin. Antibodies bound to the affinity matrix were eluted by washing the column with elution medium. The eluate was dialyzed against TBS (pH 7.5)–0.1% sodium azide, divided, and frozen at -80°C.

**Polyacrylamide gel electrophoresis and immunoblots.** Solu-

![Diagram](https://via.placeholder.com/150)
bilization of proteins and electrophoresis in denaturing polyacrylamide gels were essentially done as described previously (11) for each gel except as indicated below in materials and methods. In this case, standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis was conducted with a 7% polyacrylamide gel. After electrophoresis, proteins were either visualized with Coomassie brilliant blue or transferred to nitrocellulose paper and visualized with Ponceau S staining. Either equal amounts of total protein or equal numbers of cells were loaded as indicated in each figure legend. The nitrocellulose blot was blocked with 2.5% milk-phosphate-buffered saline (PBS)-TWEEN 20, and 125I-labeled protein A was used for immunostaining.

**Plasma membrane enrichment.** Membranes enriched for plasma membranes were prepared essentially as described by Kalomiris and Bourguignon (15). Cells used for plasma membrane enrichment were collected and washed once in PBS by centrifugation at 500 × g. The washed cells were suspended in HEM buffer (20 mM HEPES [N-2-hydroxyethyl]piperazine-N'-2-ethanesulfonic acid; pH 7.4), 1 mM EDTA, 1 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 10 μg of aprotinin per ml, 10 μg of leupeptin per ml, and 5 μg of pepstatin per ml) and lyzed with a tight-fitting Dounce homogenizer. Nuclei and unlysed cells were removed by centrifugation (750 × g) for 5 min at 4°C (nuclear fraction). Crude membranes were collected from the supernatant by centrifugation at 43,000 × g for 20 min. The supernatant (cytoplasmic fraction) was removed, and the crude membrane pellet was suspended in HEM buffer containing 10% sucrose (wt/wt) (crude membrane fraction). The membranes were layered over a step gradient of 20, 30, and 40% sucrose (wt/wt). After centrifugation at 100,000 × g for 90 min, the membranes collected from the 20 to 30% interface were enriched for plasma membranes by 20- to 24-fold as determined by its 5'-nucleotidase activity. This plasma membrane-enriched fraction was washed in PBS and stored in aliquots at −85°C in PBS.

**Immunofluorescence.** Cells on slides or lymphocytes in suspension were washed with PBS, dried on slides rapidly at 37°C, fixed in acetone at −20°C for 5 to 10 min, washed with PBS with 20% goat serum, incubated with affinity-purified LMP2 antibodies diluted either 1:10 or 1:50 in 20% goat serum and PBS for 2 h at 25°C, washed with PBS, incubated with fluorescein isothiocyanate-goat anti-rabbit immunoglobulin (Jackson Laboratory, Bar Harbor, Maine), diluted 1:1,000 in 20% goat serum for 30 min at 25°C, and then washed with PBS. For double staining, the monoclonal antibodies CS1 through CS4 directed against LMP1 were included at a dilution of 1:200 with the affinity-purified LMP2 antibodies diluted 1:10. Slides were washed with PBS, incubated for 30 min at 25°C with Texas red–goat anti-mouse immunoglobulin (Jackson Laboratory) diluted 1:1,000 and fluorescein isothiocyanate-goat F(ab')2 anti-rabbit immunoglobulin diluted 1:1,000 in 20% goat serum, and then washed with PBS.

**RESULTS**

**Construction of inducible TrpE-LMP2A fusion proteins.**

Two LMP2A fusion proteins were constructed by using the pATH protein expression vectors (33), which fuse the protein of interest to the TrpE protein carboxy terminus. Fusion proteins of 72 kDa (pFPZ or pFP6) or 87 kDa (pFP7) were obtained by fusing LMP2A codons 19 to 311 or 19 to the termination codon from the LMP2A cDNA to the TrpE carboxy-terminal codons in pATH2. E. coli cells containing the TrpE expression vector, the 72-kDa expression plasmid, or the 87-kDa expression plasmid were tested for their production of the expected protein under induction of the Trp operon with indoleacrylic acid. Cells were grown in the presence or absence of 10 μg of indoleacrylic acid per ml, and total cell extracts were subjected to gel electrophoresis (Fig. 2). Large amounts of the 72-kDa protein were induced by indoleacrylic acid. Variable results were obtained with the 87-kDa fusion protein; some of the protein was degraded (Fig. 2). Preparative amounts of the two fusion proteins were used to immunize two rabbits.

**Affinity purification of LMP2-specific antibodies.**

Western blots (immunoblots) with sera from the two immunized rabbits failed to detect LMP2A- or LMP2B-sized proteins in latently infected lymphocytes, although the sera did react strongly with the purified fusion protein. Purified TrpE or the 87-kDa TrpE-LMP2 fusion protein was used to construct affinity columns to enrich for the LMP2-specific antibodies in the rabbit sera. Antibodies reactive with TrpE were selectively adsorbed to the TrpE column. Antibodies reactive with LMP2 were then selectively absorbed and eluted from the column containing the TrpE-LMP2 fusion protein.

The specificity of the affinity-purified LMP2 antibody was tested by measuring its reactivity with bacterial extracts containing the induced 72- or 87-kDa fusion proteins or induced TrpE protein. The LMP2 affinity-purified sera reacted only with the 72- or 87-kDa fusion proteins and not with TrpE or bacterial proteins (data not shown).

**Detection of LMP2A or LMP2B in BALB 3T3 cell lines transfected with LMP2A or LMP2B open reading frames in eucaryotic expression vectors.** LMP2 expression vectors were constructed by cloning the LMP2A or LMP2B cDNA fragments (31) into pBamHygro (41). The expression plasmid pBamHygro has the murine leukemia virus long terminal repeat for expression of heterologous cDNAs and the simian virus 40 promoter-driven hygromycin B-inactivating enzyme cDNA to enable selection in hygromycin B. Figure 3 is a Western blot of BALB 3T3 transfectant protein extracts developed with LMP2 affinity-purified antibody. The anti-

![FIG. 2. Induction of fusion proteins from plasmid-containing cells. Approximately equal numbers of cells induced (+) or uninduced (−) were solubilized and electrophoresed in sodium dodecyl sulfate-polyacrylamide gels and then stained with Coomassie blue as described in Materials and Methods. Molecular weights (in thousands) of marker proteins are indicated on the left. Arrowheads indicate the induced proteins of interest. PFPZ and pFP6 are different clones of the 72-kDa fusion protein, pFP7 is the 87-kDa fusion protein, and pATH2 is the TrpE vector alone.]
body reacted with a 54-kDa protein in LMP2A transfec- 
tants (Fig. 3, lanes BA4, BA13, and BA14). Variable reactivity was also seen with 108- or 40-kDa proteins. The antibody weakly reacted with a 40-kDa protein in the LMP2B transfec-
tants (BB7 and BB30). The LMP2 antibody did not detect 
similar LMP2-sized proteins in BALB 3T3 lines transfected 
with vector alone (BV9 and BV31). The 40-kDa protein in 
LMP2A transfec- 
tants could be LMP2B, since the LMP2B translational initiation codon is downstream of the LMP2A initiation codon in the LMP2A cDNA. The 108-kDa protein in the LMP2A 
 transfec- 
tants is probably a LMP2A dimer, since it and larger immunoreactive proteins were variably present depending on the conditions used for sample prepara-
tion. These sizes agree with the predicted molecular 
weights for LMP2A and LMP2B, indicating that LMP2A or 
LMP2B do not undergo extensive posttranslational modifi-
cation (glycosylation or cleavage) in transfected mouse 
fibroblasts.

Detection of LMP2A and LMP2B in latently EBV-infected 
lymphocytes. The affinity-purified LMP2 antibodies were used to probe a Western blot containing proteins from 
latently EBV-infected lymphoblastoid cell lines or control, 
non-EBV-infected B-lymphoma cells. The LMP2 antibodies 
detected LMP2A (54 kDa) in latently infected IB-4, LCL1, 
or LCL2 cells; in partially productive B95-8 cells; or in the 
LMP2A-expressing BALB 3T3 transfec- 
tant, BA5 (Fig. 4). The purified antibodies failed to detect LMP2A in Raji cells (an EBV-positive Burkitt’s tumor cell line). A region in Raji 
cells (Fig. 1, line 2) is deleted for part of the 5’ LMP2A exon 
and the LMP2A promoter region and was not expected to 
make LMP2A (10, 26). Raji cells do have an LMP2B RNA. 
LMP2B was weakly detected in B95-8 and Raji cells. The 
apparent lack of LMP2B in LCL1, LCL2, and IB-4 cells and 
the weak reactivity in B95-8 and Raji cells correlate in part 
with low LMP2B mRNA levels in these cells. In IB-4 and 
B95-8 cells, the ratio of LMP2B mRNA to LMP2A mRNA is 
approximately 1:4 (31). The purified LMP2 antibodies may 
also be weakly reactive with LMP2B protein, since LMP2B 
lacks the 119 amino-terminal LMP2A amino acids. The 
LMP2A-specific amino terminus is hydrophilic and likely 
antigenic. Some LMP2A and LMP2B common epitopes 
could be in the hydrophilic domains between hydrophobic 
domains or in the carboxy terminus. The 12 transmembrane 
domains common to LMP2A and LMP2B are highly hydro-
phobic and not likely to be antigenic. LMP2B could also be 
preferentially degraded or inefficiently translated. The 
purified antibodies failed to react with the BJAB or BL41 (EBV-negative) Burkitt’s tumor cell lines, a BALB 3T3 cell 
line transfected with vector alone (BV31), or the Namalwa 
(EBV-positive) Burkitt’s tumor cell line. Namalwa contains 
a single EBV genome which has integrated into chromo-
somal DNA via the EBV genome terminal repeats and thus 
is not expected to encode LMP2A or LMP2B (22).

LMP2A localization to the cell membrane fraction. Earlier 
experiments had shown that in vitro-translated LMP2A or 
LMP2B posttranslationally insert into membranes (31). To 
verify their likely cytoplasmic membrane localization, mem-
brane fractions were prepared from LCL1 or B95-8 cells. 
LMP2A was enriched in the crude membrane or the plasma 
membrane fractions (Fig. 5). The lack of further enrichment 
of LMP2A in the plasma membrane fraction as opposed to 
the crude membrane fraction could be due to a broad 
distribution of LMP2A in the cytoplasmic membranes or 
to incomplete purification of the plasma membranes. LMP2A 
was not detected in the cytosolic or nuclear fraction (Fig. 5).
LMP2 was surprisingly not evident in the membrane fraction immunoblots despite their enrichment for LMP2A. This could be due to variability in techniques, since LMP2B was barely and irregularly detectable.

**LMP2A and LMP2B localization to BALB 3T3 transfectant plasma membrane and internal cell membranes by immunofluorescence.** Fixed LMP2A- or LMP2B-positive BALB 3T3 transfectants or control cells grown on glass slides were reacted with LMP2 antibody followed by goat anti-rabbit antibody conjugated with fluorescein isothiocyanate. LMP2A transfectants stained strongly with the purified LMP2 antibodies, exhibiting bright, punctate plasma membrane staining and internal cytoplasmic-membrane staining (Fig. 6, panels A, B, and D). Reactivity in the LMP2B transfectants was weaker and more diffuse in nature (Fig. 6, panel C).

**LMP2 expression in all latently EBV-infected cells.** A variety of EBV-positive or -negative lymphoblasts were tested for their reactivity with the purified LMP2 antibodies by immunofluorescence microscopy. The LMP2-specific antibodies reacted with small, discrete dots on the surfaces of EBV-infected lymphocytes, including LCL1 (Fig. 6, panel E), LCL2 (Fig. 6, panel F), IB-4 (Fig. 6, panel H), B95-8 (Fig. 6, panel K), W91 (data not shown), or Akata (data not shown) cell lines. Some reactivity in most cells localized to a patch at the cell periphery, reminiscent of LMP1 staining (12, 18, 19). In EBV-infected cell lines containing intact LMP2A- and LMP2B-coding domains, almost all cells were LMP2 antibody positive. The fraction of LMP2-positive cells was similar among cell lines (such as LCL1 or LCL2) which were almost entirely latently infected, as it was among cell lines (such as B95-8) which contained a small fraction of cells permissive for virus replication. No reactivity was observed with the EBV-negative Burkitt’s cell lines BJAB or BL41 (data not shown) or with the EBV-positive cell lines Namalwa or Raji (data not shown). No reactivity was observed with antibodies eluted from the TrpE affinity column (data not shown).

The positive reactivity observed in the immunofluorescence studies with the purified antibodies was likely a consequence of reactivity with LMP2A and not LMP2B. In proof, Raji cells which lacked LMP2A had only weakly detectable LMP2B in Western blots and were negative in immunofluorescence (data not shown).

**LMP2 colocalization with LMP1.** Since LMP2 partially localized to the cell periphery in a patch similar to an LMP1 patch, double immunofluorescence was performed with LMP1-specific mouse monoclonal antibody and LMP2-specific rabbit polyclonal antibody. Goat anti-rabbit antibody-conjugated fluorescein isothiocyanate and goat anti-mouse antibody-conjugated Texas red were used to detect the primary antibodies. LMP2 patches colocalized with LMP1 patches in IB-4 cells (Fig. 6, panels G, H, or I), B95-8 cells (Fig. 6, panels J, K, or L), or recently transformed cord blood lymphocytes (data not shown). More than 80% of the B95-8 cells exhibited LMP1 and LMP2 colocalization. Either the remaining cells lacked staining for LMP2 or LMP1, or localized staining was not evident for LMP2 or LMP1. LMP2 staining was frequently only partially confined to LMP1 patches, with spots of LMP2 fluorescence evident through other cell cytoplasmic and plasma membranes. When either primary antibody was omitted, no spillover in fluorescence into the other channel was evident.

**DISCUSSION**

These experiments demonstrate that EBV encodes a second membrane protein in latently infected, growth-transformed lymphocytes. These results were not entirely unexpected because of the previous findings of two unexplained RNAs in latently infected lymphoblastoid cell lines (14, 36), the results of subsequent cDNA cloning and sequencing (16, 31), the results of in vitro transcription and translation of the cDNAs (31), and the posttranslational membrane insertion of the resultant protein (31). However, the documentation of LMP2 expression in almost all cells in latently infected lymphoblastoid cell lines is a necessary step in the delineation of the association of LMP2 with latent infection, since RNAs have also been detected in latently infected cells which are abundant, early, virus replication cycle-associated RNAs expressed in a small fraction of cells in an otherwise latently infected cell culture. In fact, early RNAs have been detected in latently infected lymphoblastoid cell lines without concomitant protein expression (J. Sample, C. Alfieri, and E. Kief, unpublished observations).

LMP2A and LMP2B probably complete the repertoire of EBV proteins expressed in latently infected, growth-transformed lymphocytes. This brings to eight (or nine, if LMP2B
is considered to differ from LMP2A) the number of EBV proteins which are likely to play a role in maintaining latent infection or cell growth transformation.

The six nuclear proteins appear to have specific actions in maintaining latent infection or in B-lymphocyte activation. EBNA-1 is an EBV DNA origin (oriP)-specific binding protein necessary for EBV episome maintenance (27, 29, 34, 42, 43). EBNA-1 can also transactivate an origin-associated enhancer (28, 39). Since cell proteins can specifically compete with EBNA-1 for oriP binding, EBNA-1 interaction with oriP may be an example of a broader range of cell EBNA-1-like protein-DNA interactions (40). EBNA-2 is essential to growth transformation (4, 4a, 7, 9, 32) and conveys much of the EBV-type specific differences in growth transformation (4a, 30). EBNA-2 is a specific transactivator of the B-lymphocyte activation markers CD23 and CD21 (38b, 39). EBNA-3C is a specific transactivator of CD21 but not other B-lymphocyte-specific markers (38b). EBNA-3A and EBNA-3B are colinearly homologous to EBNA-3C (13) and may therefore also be cell gene transactivators. EBNA-LP has not had discernible effects in B-lymphoma or rodent fibroblast cell assays (38b, 39).

In contrast to the restricted EBNA effects, LMP1 has broad effects on B-lymphocyte and even rodent fibroblast
growth (37). In NIH 3T3 cells, LMP1 enables cell growth in low serum (37). In BALB 3T3 cells, LMP1 causes anchorage-independent growth (2, 37, 38). In Rat-1 cells, LMP1 causes reduced serum dependence, loss of contact inhibition, anchorage independence, and tumorigenic growth in nude mice (2, 37, 38). LMP1 is also a broad inducer of B-lymphocyte activation and adhesion molecules and of vimentin (3, 38a). LMP1 induction of activation or adhesion molecules or of vimentin usually correlates with an increase in the respective mRNA, indicating that LMP1 acts at a distance to increase mRNA levels, probably by nuclear activation. LMP1 associates with vimentin and other cellular proteins forming a patch in the lymphocyte plasma membrane (18, 19). Vimentin is not normally associated with a plasma membrane patch but is brought to the plasma membrane by LMP1, mimicking its association with cap structures (1, 18, 19). LMP1 patches, however, differ from cap structures in the lack of other associated cytoskeletal elements, such as tubulin or actin. Although vimentin is associated with LMP1 complexes, vimentin is not required for complex formation or B-lymphocyte activation (17). A LMP1 mutant which lacks the amino terminus and first four transmembrane domains does not patch in the plasma membrane or cause lymphocyte activation (38a). Thus, LMP1 appears to be capable of forming patches in the B-lymphocyte plasma membrane without vimentin and causes a constitutive up regulation of lymphocyte adhesion and activation molecule expression.

Our data indicate that LMP2 is also characteristically expressed in latently infected B lymphocytes and, in part, associates with LMP1 in the plasma membrane. LMP2 is therefore likely to also be involved in maintaining proliferative transformation. By associating with LMP1, LMP2 could function as a modulator of LMP1 activity or might use the LMP1 patch as a structure for assembly of LMP2-specific activities. LMP1 and LMP2 have multiple membrane-spanning domains similar to other membrane proteins responsible for ion or small-molecule transport or for the mediation of membrane protein interactions. LMP2, with its many transmembrane domains, most resembles a membrane transport protein. By associating with LMP1, LMP2 could influence LMP1 activity by altering the concentration of an ion or small molecule in the vicinity of LMP1. For example, LMP2 could create a locally high concentration of divalent cations, altering an enzymatic activity, which could modify LMP1 or an LMP1 activity. These hypotheses should be testable in B-lymphoma cell transfection assays.

ACKNOWLEDGMENTS

David Liebowitz, Cheryl Wilson, Jeff and Clare Sample, Michael Kurilla, Caroline Alfieri, Fred Wang, Andy Marchini, and Kevin Hennessy contributed advice and assistance. X-Qian Miao provided excellent technical help. A. Korman kindly provided pBamHygro. This research was supported by Public Health Service grant no. CA47006 from the National Cancer Institute. R.L. is a postdoctoral fellow supported by the Leukemia Society of America. E.K. and R.L. are also partially supported by funds from the Sandoz Corporation and the Baxter Foundation.

LITERATURE CITED