VIROLOGY 190, 106–115 (1992)

Expression of the Epstein–Barr Virus Latent Membrane Protein (LMP) in Insect Cells and Detection of Antibodies in Human Sera against This Protein

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Received February 11, 1992; accepted May 11, 1992

Recombinant baculoviruses containing the complete LMP and truncated LMP genes were generated and high levels of the LMP proteins were expressed in *Spadoptera Frugiperda* insect cells. A specific rabbit antiserum directed against the N-terminal part of LMP was obtained by immunizing the rabbits with *Escherichia coli*-expressed trpE-N-terminal part of LMP fusion protein. A total of 127 human sera were studied for their immune response to the recombinant full-length LMP. In immunofluorescence analysis, all sera tested showed no detectable reaction with the recombinant full-length LMP. In immunoprecipitation–immunoblotting analysis, however, sera from patients with nasopharyngeal carcinoma (5/22), patients with Hodgkin's disease (16/27), patients with other diseases exhibiting high EA-IgG titers (3/52), and VCA-IgG-positive healthy individuals (2/26) were shown to contain antibodies against this recombinant LMP. The expressed LMP proteins provided a sufficient and economic source of the proteins for further serological and biological studies.

INTRODUCTION

Epstein–Barr virus (EBV) is an ubiquitous and oncogenic human herpesvirus, which is the causative agent of infectious mononucleosis (IM) and is closely associated with Burkitt's lymphoma (BL) (Lenoir, 1986), polyclonal B-cell lymphomas induced in immunosuppressed patients (Birx *et al.*, 1986), and nasopharyngeal carcinoma (NPC) (de Thé, 1982). Recently, EBV DNA and the expression of latent membrane protein (LMP) have been detected in cells from patients with Hodgkin's disease (HD) (Weiss *et al.*, 1989; Anagnostopoulos *et al.*, 1989; Herbst *et al.*, 1990, 1991a), peripheral T-cell lymphomas (Su *et al.*, 1991), and Ki-1-positive anaplastic large cell lymphomas (Anagnostopoulos *et al.*, 1989; Herbst *et al.*, 1991b). Therefore, EBV has been strongly implicated in the development of human cancers.

LMP is known to be the EBV oncogene which causes hyperplasia and aberrant expression of keratin 6 when expressed in the skin of transgenic mice (Wilson *et al.*, 1990). (LMP is also referred to as LMP-1; a second EBV-encoded membrane protein found in latently infected cells is either named terminal protein, TP, or LMP-2. In the present study, we still use the LMP/TP terminology.) Transfection of an immortalized, nontumorigenic keratinocyte cell line (RHEK-1) with LMP causes a striking morphological transformation: the originally flat, polygonal colonies change to bundles of spindle-shaped cells that form multilayer foci, and cytokeratin expression is down-regulated (Fåhreaus *et al.*, 1990). Furthermore, the expression of LMP in human epithelial cells causes changes of cell surface phenotype which mimick surface changes observed in NPC cells and impairs the cellular response to differentiation signals (Dawson *et al.*, 1990). Two forms of LMP encoded by the BNLF1 open reading frame were reported (Fennewald *et al.*, 1984): full-length LMP mRNA, a latent transcript which is assembled from three exons, and truncated LMP mRNA, a lytic transcript which is composed of the major part of the third exon. Recently, the expression of LMP by using recombinant vaccinia virus was reported (Stewart *et al.*, 1989). The N-terminal part of LMP crossing the cytoplasma membrane appears to be one of the target antigens for the EBV-induced cytotoxic T-cell response (Murray *et al.*, 1988). Furthermore, the deletion of the N-terminal part abolishes the transformation activity of LMP (Wang *et al.*, 1988a). Thus the N-terminal part of LMP seems to play a key role in cell transformation. A specific rabbit antiserum that can distinguish the full-length LMP from the truncated LMP expressed in biopsies of the EBV-associated human cancers would be helpful for the further understanding of the role of LMP.

An immune response of human sera to LMP has been first implied by using the leukocyte migration inhibition (LMI) assay (Szigeti *et al.*, 1984) and by using ELISA, in which short synthetic peptides correspond-

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0042-6822/92 $5.00

ing to LMP amino acid sequences were used as antigens (Modrow and Wolf, 1986). Antibodies against LMP have been detected by using relatively small amounts of LMP expressed in EBV-negative B cells transfected with LMP gene (Rowe *et al.*, 1988). A sufficient and economic source of LMP will be helpful for further immunological and biochemical studies of this protein. Here we describe the expression of high levels of LMP proteins in insect cells, the generation of a specific rabbit antiserum against the N-terminal part of LMP, and the detection of the LMP antibodies in human sera by using the recombinant full-length LMP as antigen. The relatively large amount of the LMP proteins expressed in the insect cells provides an economic and sufficient source of LMP for further serological and biological studies.

MATERIALS AND METHODS

Cells and virus

The EBV-positive B-cell lines Raji (Pulvertaft, 1965), B95-8 (Miller and Lipman, 1973), and M-ABA (Crawford *et al.*, 1979) and EBV-negative B-cell line BJAB (Klein *et al.*, 1974) were maintained at 37° in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine (200 m*M*), penicillin (40 IU/ml), streptomycin (50 μg/ml), moronal (10 IU/ml), and neomycinsulfate (10 μg/ml) as described (Roeckel and Mueller-Lantzsch, 1985). They were subcultured routinely once a week. 12-*o*-tetradecanoylphorbol-13-acetat (TPA) (20 ng/ml) and *n*-butyrate (4 m*M*) or 5-iodo-2′-deoxyuridine (IDU) (30 μg/ml) were used to induce the cells to express EBV antigens. *Spodoptera frugiperda* (Sf158) insect cells were propagated at 27° as monolayer culture twice a week in TC-100 medium supplemented with 10% fetal calf serum, penicillin (40 IU/ml), streptomycin (50 μg/ml), moronal (10 IU/ml), and neomycinsulfate (10 μg/ml). Wild-type baculovirus AcMNPV was amplified by infection of Sf158 insect cells. Extracellular virions and viral genomic DNA were prepared as described (Summers and Smith, 1987).

cDNA library screening

A cDNA library prepared from M-ABA/CBL cell line was used to screen for LMP cDNA. A probe of 443 bp (bp 169,476–169,033) corresponding to the first and second exons of LMP was labeled with digoxigenin-11-dUTP using a kit (Boehringer-Mannheim) and was used in the screening procedure. Recombinant phages were plated on LB agar plates and transferred to NY 13 N membrane filters (Schleicher & Schuell, Dassel) using standard techniques (Sambrook *et al.*, 1989). The membrane filters were hybridized with the nonradioactive probe and positive clones were isolated and purified.

DNA sequence analysis

LMP cDNA from recombinant phages was cloned into pUC19 and analyzed first with several restriction enzymes to confirm the sequence. DNA sequencing was then carried out by the dideoxy-chain termination method (Chen and Seeburg, 1985), using a sequenase kit (United States Biochemicals) and [alpha-^{35}S]dATP (>1000 Ci/mmol, Amersham) on double-stranded templates.

Construction of a complete LMP gene and the expression vectors

DNA sequence analysis showed that the LMP cDNA contained only the second and third exons and a small part of the first exon including an *Acc*I site, but the majority of the first exon was not present. In order to construct a complete LMP gene, genomic DNA containing the first exon of LMP gene was used. Details of construction of the complete LMP gene and creation of *Bam*HI sites on the LMP gene can be obtained from the authors by request. The complete LMP gene (bp 169,474–168,163) and the truncated LMP gene (bp 168,936–168,163) were cloned in the *Bam*HI site of pAcYM1 (Fig. 1). These recombinant vectors were used to generate recombinant baculoviruses through cotransfection and *in vivo* homologous recombination with wild-type baculovirus (Summers and Smith, 1987). A *Xho*II fragment from the recombinant vector pLMP containing the first and second exons of LMP was cloned in the *Bam*HI site of pATH11 to express the N-terminal part of LMP in *Escherichia coli* cells (Fig. 1).

Rabbit antisera

The production of rabbit antisera against *E. coli*-expressed trpE-LMP C-terminal fusion protein (no. 749) and against a synthetic peptide of seven amino acids corresponding to the LMP C-terminus (no. 492) have been described (Boos *et al.*, 1987). The rabbit antiserum (no. 500) against *E. coli*-expressed trpE-N-terminal part of the LMP fusion protein was generated in the present study.

The expression vector pATH11-E1E2, which contains the N-terminal part of the LMP gene, was used to express a trpE-LMP-N-terminal fusion protein in *E. coli* C600 cells. The fusion protein was predicted to have a molecular mass of about 50 kDa. For unknown reasons, the expressed fusion protein had only a molecular mass of about 40 kDa. To confirm that the fusion protein contained the N-terminal part of LMP, the expression vector was verified by DNA sequencing. This

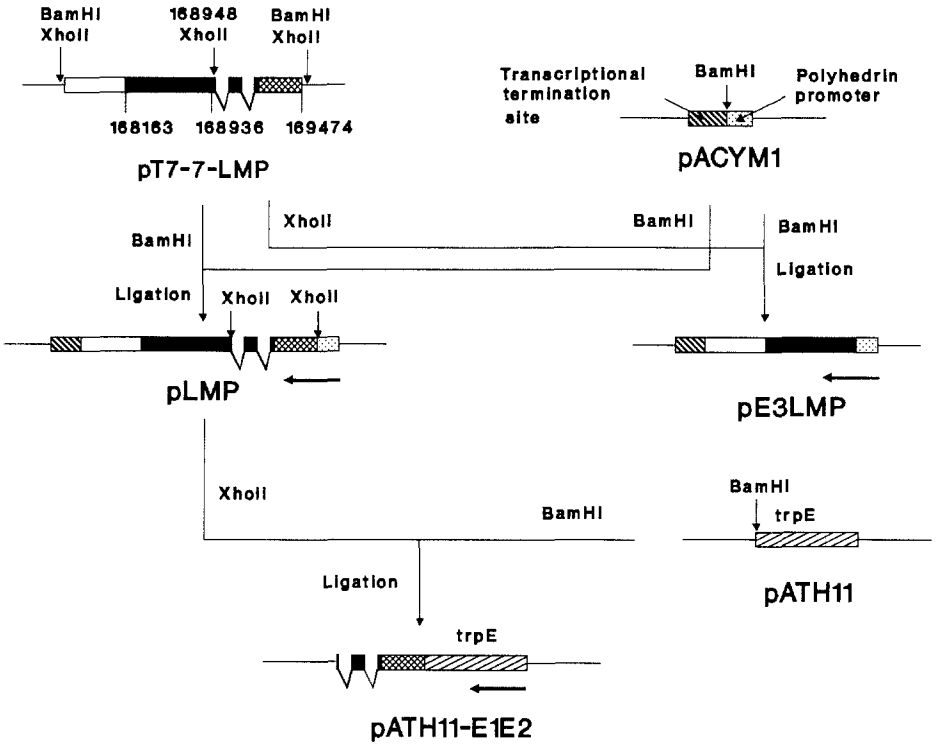

FIG. 1. Strategy of construction of recombinant expression vectors pLMP, pE3LMP, and pATH11-E1E2. The recombinant expression vectors pLMP and pE3LMP were used to generate recombinant baculoviruses and the recombinant expression vector pATH11-E1E2 was used to express the N-terminal part of LMP in *E. coli* cells as described under Materials and Methods.

truncated fusion protein was purified by SDS–PAGE and used for rabbit immunization as described (Chen *et al.*, 1991).

Immunofluorescence

Immunofluorescence was done as described previously (Mueller-Lantzsch *et al.*, 1979).

SDS-polyacrylamid gel electrophoresis

Electrophoresis in SDS–polyacrylamide slab gels was performed in 10 and 15% separating gels or 9.5–20% gradient gels with 5% stacking gel using the discontinuous buffer system (Laemmli, 1970) and proteins were electrophoretically transferred to immobilon membranes (Millipore) or nitrocellulose membranes (Amersham) as described previously (Chen *et al.*, 1991).

Immunoprecipitation–immunoblotting analysis

The anti-LMP peptide-rabbit antiserum (no. 492) was coupled to protein A-Sepharose (Sigma) according to the method described by Schneider *et al.* (1982). The coupled protein A-Sepharose was used to precipitate LMP expressed in insect cells. Briefly, cells were collected, washed with buffer containing 0.15 *M* NaCl, 0.05 *M* Tris–HCl (pH 7.2), and 0.001 *M* EDTA and then lysed in RIPA buffer (0.05 *M* Tris–HCl (pH 7.2), 0.15 *M* NaCl, 0.1% SDS, 1.0% sodium deoxycholate, 1.0% Triton X-100, 0.01% aprotinin, and 0.002 *M* PMSF) through sonication. The lysate was mixed with the antiserum-coupled protein A-Sepharose at 4° for 2 hr and the unbound proteins were washed away with RIPA buffer. For SDS–PAGE, immunoprecipitated LMP was released by boiling the samples for 3 min in gel-loading buffer (0.125 *M* Tris–HCl (pH 6.8), 6% SDS, 10% mercaptopropanediol). The immunoblot was carried out as described (Chen *et al.*, 1991) except that human sera were diluted 1:20 in PBS containing 10% nonfat milk and incubated with LMP for at least 4 hr at room temperature.

Purification of rabbit antibodies

Rabbit antibodies against LMP were purified according to the method described by B. Frech (personal communication). Briefly, Sf158 cell extract was separated on a SDS–polyacrylamid gel and transferred to an immobilon membrane. The membrane strip containing LMP was cut out, blocked with 10% nonfat milk in PBS and incubated with an anti-LMP rabbit antiserum. After a 4-hr incubation, the membrane strip was washed

with PBS and the bound antibodies were eluted first with 100 m*M* glycine (pH 2.5). After washing with 10 m*M* Tris–HCl (pH 9.0), the antibodies were eluted with 100 m*M* triethylamine (pH 12). The eluates were neutralized with 1 *M* Tris–HCl (pH 8.0) and used directly in immunoblot and immunofluorescence analysis.

RESULTS

High-level expression of full-length and truncated LMP in Sf158 cells

Immunoblot and immunofluorescence were used to study the synthesis of the LMP proteins in the Sf158 cells. The cells were infected with recombinant baculoviruses VpLMP expressing full-length LMP and VpE3LMP expressing truncated LMP. The infected cells were harvested at 0, 12, 16, 20, 24, 36, and 48 hr postinfection (p.i.). After washing with PBS, a small aliquot of the infected cells were fixed with aceton or methanol for immunofluorescence analysis and the remainder was used for immunoblot analysis. The synthesis of recombinant LMP proteins was detected by using polyclonal anti-LMP rabbit antiserum no. 749 (directed against the C-terminal 155 amino acids of LMP). Cells infected with wild-type virus were used as a negative control. The result of an immunoblot analysis is shown in Fig. 2. In the case of VpLMP-infected cells, two immunoreactive bands migrating at 60 and 55 kDa were clearly visible at 20 hr p.i. and increased in amount until 48 hr p.i. as shown in Fig. 2A. We also detected some additional bands migrating at approximately 40 and 50 kDa. These polypeptides together with the 55 kDa polypeptide probably represented the degradation products of the 60 kDa protein. In the case of VpE3LMP-infected cells, two bands migrating at 41 and 38 kDa were visible at 20 hr p.i. and increased in amount until 48 hr p.i. as shown in Fig. 2B. In addition, a larger band migrating at 45 kDa was detected at 36 and 48 hr p.i. Possibly, the truncated 45-kDa LMP protein was degraded very rapidly and was therefore only detected at a high concentration, i.e., at the late time of infection. The results outlined above were confirmed with the anti-peptide rabbit antiserum no. 492 (data not shown). Immunofluorescence analysis also confirmed that the full-length LMP and the truncated LMP expressed in Sf158 cells were recognized by the rabbit antisera nos. 749 and 492 (data not shown).

The immunoblot analysis using purified antibodies from the rabbit antiserum (no. 749) indicated that the Sf158 cells expressed approximately 100 times more LMP than EBV-harboring B cells which provided a sufficient and economic source of LMP for further studies. The full-length LMP expressed in Sf158 cells exhibited the same molecular mass as that expressed in EBV-harboring B-cell lines, which indicated proper modifications of the protein by the insect cells. Although most of the truncated LMP migrated at about 38 and 41 kDa, a small amount of the protein migrated at about 45 kDa, which may also represent the post-translationally modified product (Fig. 3). In addition, the purified antibodies against LMP also crossreacted with a polypeptide of about 67 kDa in extract of M-ABA, B95-8, and Raji cell lines. This protein was also detected in the EBV-negative cell line BJAB (data not shown).

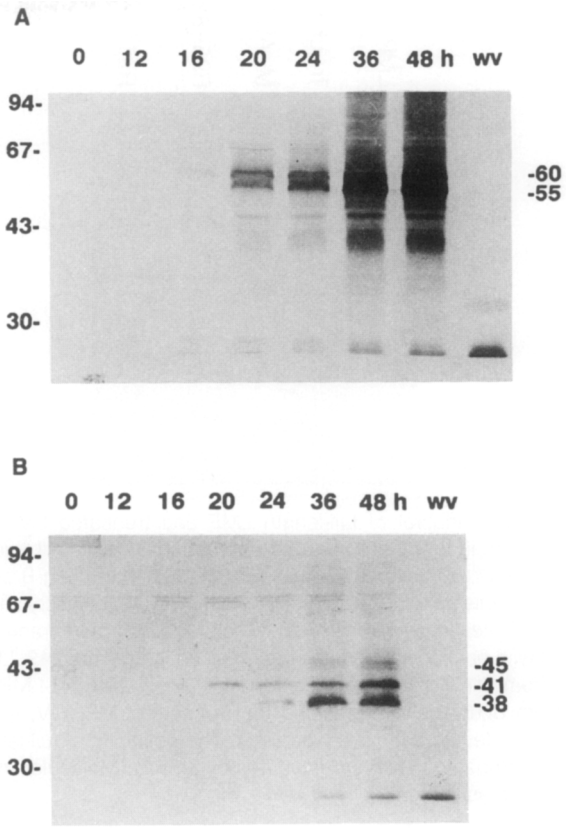

FIG. 2. Expression of full-length LMP (A) and truncated LMP (B) in Sf158 cells. Cells were infected by recombinant baculoviruses and harvested at the indicated time intervals. Cell lysates (3 μl/lane, 3.4×10^7 cells/ml) were separated on a 10% SDS–polyacrylamide slab gel. Proteins were transferred to an immobilon membrane and stained with the rabbit antiserum no. 749 at a dilution of 1:200. h, hour; WV, lysate from cells infected with wild-type viruses. The positions of full-length LMP and the truncated LMP and the molecular mass marker proteins are indicated. The molecular mass markers (Pharmacia) are as follows: phosphorylase b, 94 kDa; bovine serum albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa.

Generation of a specific rabbit antiserum against the N-terminal part of LMP

The aim of this study was to screen sera from patients with EBV-related diseases for the presence of antibodies directed against LMP. As a source of LMP,

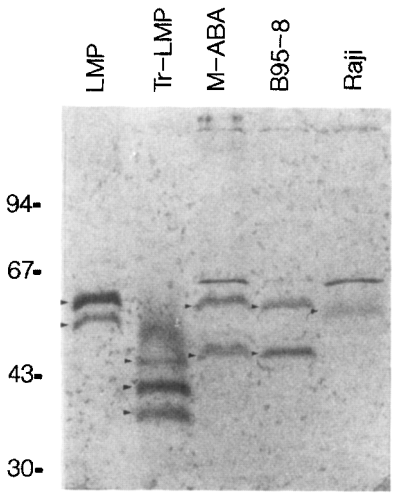

FIG. 3. Immunoblot of full-length LMP and truncated LMP expressed both in Sf158 cells and EBV-harboring B cells. Cell lysates (10 μl/lane; Sf158 cells: 6.8×10^5 cells/ml; EBV-harboring B cells: 7.7×10^7 cells/ml) were separated on a 10% SDS–polyacrylamide slab gel. Proteins were transferred to an immobilon membrane and stained with purified antibodies from rabbit antiserum no. 749. LMP, lysate from Sf158 cells expressing the full-length LMP; Tr-LMP, lysate from Sf158 cells expressing the truncated LMP; WV, lysate from cells infected with wild-type virus. The positions of full-length LMP and truncated LMP are indicated by arrows. Molecular mass markers are as in Fig. 2.

we used the baculovirus-expressed protein. To test whether the complete protein was expressed in insect cells, we generated a rabbit antiserum directed against the N-terminus of LMP. The N-terminal part of BNLF1 gene containing the first and the second exons of LMP was cloned in the expression vector pATH11. This N-terminal part of BNLF1 gene was predicted to encode a polypeptide of about 13 kDa. Together with the pATH11-encoded N-terminal part of trpE of about 37 kDa, a fusion protein of about 50 kDa was expected from the recombinant vector pATH11-E1E2. However, the fusion protein obtained migrated at approximately 40 kDa. This indicated that only about 3 kDa of LMP-specific polypeptide was synthesized. We do not know why only a truncated protein was expressed in the bacterial cells. To confirm that the expressed fusion protein contained the N-terminal part of LMP, this recombinant vector was again analyzed by DNA sequencing. No mutation was found in the sequence. This *E. coli*-expressed fusion protein was then purified and used to produce rabbit antiserum as described under Materials and Methods. Immunoblot and immunofluorescence analyses were used to test the specificity of the anti-N-terminal rabbit antiserum. In immunoblot analysis, the anti-N-terminal rabbit antiserum recognized only the full-length LMP in insect cells as predicted (Fig. 4C). An additional polypeptide migrating at approximately 35 kDa (Fig. 4C) probably represented a degradation product of the full-length LMP. This confirmed the notion that the full-length protein was expressed, since the presence of the extreme C-terminus of the baculovirus expressed LMP was confirmed with an anti-C-terminal antiserum (Fig. 4A). Surprisingly, the anti-N-terminal rabbit antiserum showed no detectable reaction with LMP expressed from EBV-harboring B cells in immunoblot analysis (data not shown). In immunofluorescence analysis, however, this anti-N-terminal rabbit antiserum showed positive reaction with the LMP expressed both in Sf158 cells (Fig. 5B) and in EBV-harboring B cells (Fig. 5F). The LMP in the B cells was stained as patches of intensive fluorescence. In order to eliminate a possible cross-reaction of the rabbit antiserum with cellular proteins, the antibodies against LMP were purified from the anti-N-terminal rabbit antiserum and were again used in the immunofluorescence analysis. The LMP in the B cells was again stained as patches of intensive fluorescence (Fig. 5G). These results demonstrated that the anti-N-terminal rabbit antiserum specifically recognized the LMP expressed in the EBV-harboring B cells. In addition, we note that only about 5% of the B cells were stained with the anti-N terminal rabbit antiserum. A control test with the parallel rabbit antiserum (no. 749) showed similar results (data not shown). We do not know why only a

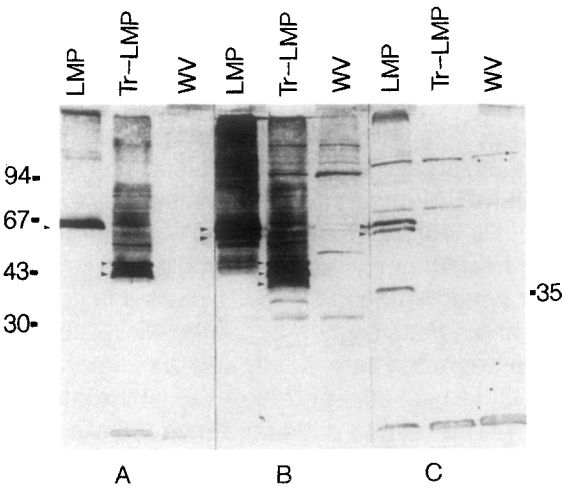

FIG. 4. Immunoblot of full-length LMP and truncated LMP expressed in Sf158 cells. Cell lysates (6 μl/lane, 3.4×10^7 cells/ml) were separated on a 9.5–20% SDS–gradient polyacrylamide slab gel. Proteins were transferred to immobilon membrane and stained with (A) anti-peptide of LMP C-terminus (no. 492), (B) anti-trpE-C-terminal part of LMP (no. 749), and (C) anti-trpE-N-terminal part of LMP (no. 500) rabbit antisera, each at a dilution of 1:200. LMP, lysate from Sf158 cells expressing full-length LMP; Tr-LMP, lysate from Sf158 cells expressing truncated LMP; WV, lysate from Sf158 cells infected with wild-type virus. The positions of full-length and truncated LMP proteins are indicated by arrows. Molecular mass markers are as in Fig. 2.

small percentage of the cells showed a positive reaction.

Immune response of human sera to the recombinant LMP

Immunofluorescence and immunoblot were used to study the immune response of human sera to the recombinant LMP. Cells infected with the recombinant baculoviruses VpLMP for 24 or 48 hr were used for the immunofluorescence studies. Cells infected for 48 hr were used for immunoprecipitation–immunoblotting. In order to confirm that the recombinant LMP contained the same antigenic determinants as that of LMP expressed in EBV-harboring B-cells, three human sera (RA39, PG, and Plomp) that contain antibodies against LMP expressed in EBV-harboring B-cells (Rowe *et al.*, 1988) were used as positive control. In addition, an anti-LMP rabbit antiserum was also used in each test as a positive control. In immunofluorescence analysis, all human sera including the three known positive control sera showed no detectable reaction with the recombinant LMP (data not shown). In immunoprecipitation–immunoblotting analysis, however, sera from patients with HD, from patients with NPC, from patients with other diseases exhibiting high EA titers (EA-IgG > 1:64), and from EBV-positive healthy individuals were found to contain antibodies against the recombinant LMP, whereas sera from EBV-negative healthy individuals did not show any reaction (Table 1). In particular, we found that 16 of 27 sera from patients with HD had antibodies against the LMP protein. A representative result of an immunoprecipitation-immunoblotting analysis is shown in Fig. 6.

DISCUSSION

Recombinant baculoviruses containing the complete and the truncated forms of LMP (BNLF1) were generated and high levels of the recombinant LMP proteins were expressed in the Sf158 cells.

The complete LMP gene is composed of three exons which are predicted to encode a polypeptide of 42,005 Da whereas the truncated LMP is composed of the majority of the third exon which is predicted to encode a polypeptide of 28,851 Da (Baer *et al.*, 1984). From the previous studies of other authors on 20 EBV-harboring lymphoblastoid cell lines, the full-length LMP has a molecular mass of 57–66 kDa (Rowe *et al.*, 1987). The truncated LMP has a molecular mass of 49 kDa (Boos *et al.*, 1987) or 55 kDa (Rowe *et al.*, 1987). This difference between the calculated and the apparent molecular mass deduced from the mobility on SDS–gel of the proteins expressed in the cells is due to the post-translational modifications such as phosphorylation and glycosylation. In the present study, the full-length LMP expressed in Sf158 cells was about 60 kDa, the same as that expressed in M-ABA and B95-8 cell lines (Fig. 3). This result indicated that the full-length LMP expressed in Sf158 cells was also properly modified. Most of the truncated LMP expressed in Sf158 cells migrated at 41 and 38 kDa, whereas only a small amount of the truncated LMP migrated at 45 kDa (Figs. 2 and 3). This truncated 45-kDa polypeptide, although slightly smaller than that expressed in M-ABA and B95-8 cell lines, may also represent the post-translationally modified product.

LMP is known to be toxic to the cells when expressed at high levels and the toxicity is alleviated by deletion of the NH_2 terminus (Hammerschmidt *et al.*, 1989). We have compared the effects of the full-length LMP and the truncated LMP on the growth of the Sf158 insect cells by infection of the cells with the recombinant viruses for 0, 12, 16, 20, 24, 36, and 48 hr. No significant difference between the effects of the full-length LMP and the truncated LMP on the growth of the cells was observed (data not shown). This result may indicate that the NH_2 terminus of LMP was not toxic to the insect cells, although it is toxic to the mammalian cells when expressed at high levels.

Polyclonal rabbit antisera and monoclonal antibodies directed against the C-terminal part of LMP have been reported (Boos *et al.*, 1987; Baichwal and Sugden, 1987; Mann *et al.*, 1985; Rowe *et al.*, 1987). Two reports also described the generation of rabbit antisera against a synthetic peptide corresponding to the N-terminus of LMP (Modrow and Wolf, 1986; Moothy and Thorley-Lawson, 1990). However, no rabbit antiserum against the bacterially synthesized N-terminal part of LMP has been reported. We have cloned the first and second exons of LMP in the *E. coli* expression vector pATH11 and generated a rabbit antiserum against the N-terminal part of LMP by immunizing the rabbits with the *E. coli* trpE-LMP N-terminal fusion protein. Although the expressed N-terminal part of LMP migrated faster than predicted, this fusion protein could contain approximately 30 amino acid residues from the N-terminal part of LMP. In immunoblot analysis, this anti-N rabbit antiserum recognized the full-length LMP expressed in the insect cells (Fig. 4C). However, it showed no detectable reaction with the LMP expressed in EBV-harboring B cells in immunoblot analysis (data not shown). In immunofluorescence analysis, this anti-N rabbit antiserum specifically recognized the full-length LMP expressed both in EBV-harboring B cells and in VpLMP-infected insect cells (Fig. 5). The LMP in the B cells was stained as patches of intensive fluorescence, which was in agreement with the observations of other authors (Liebowitz *et al.*, 1986). This

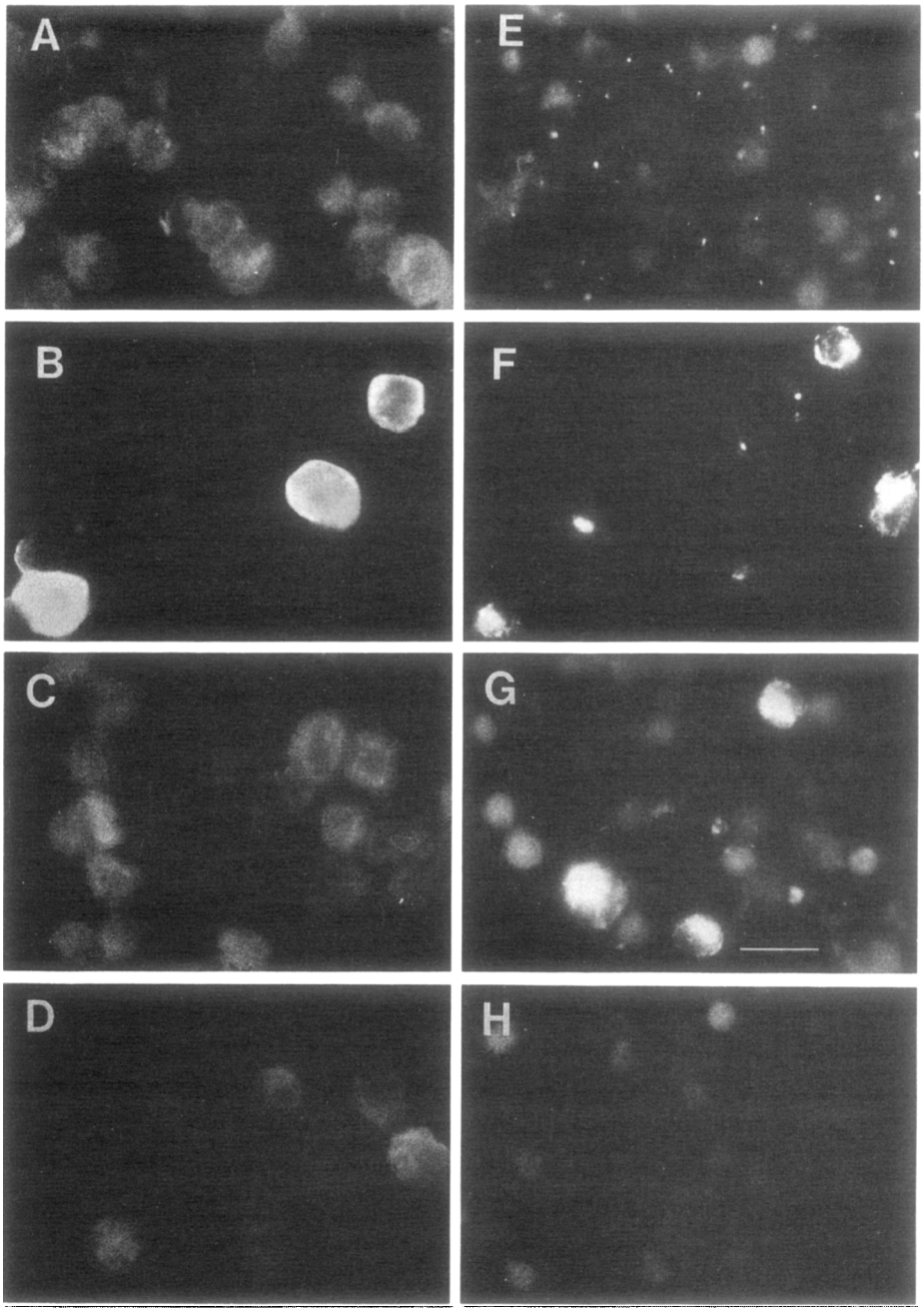

FIG. 5. Indirect immunofluorescence of full-length LMP and truncated LMP expressed in Sf158 cells and Raji cells. Sf158 cells expressing full-length LMP were stained with preimmune rabbit serum no. 500 (A) and anti-N-terminal rabbit antiserum no. 500 (B). Sf158 cells expressing truncated LMP were stained with the anti-N-terminal rabbit antiserum (C). Sf158 cells infected with wild-type virus were stained with the anti-N-terminal rabbit antiserum (D). Raji cells expressing LMPs were stained with the preimmune rabbit serum (E), the anti-N-terminal rabbit antiserum (F), and purified antibodies from the anti-N-terminal rabbit antiserum (G). The EBV-negative BJAB cells were stained with the anti-N-terminal rabbit antiserum (H). The preimmune and anti-N-terminal rabbit sera were used at a dilution of 1:400. Scale bar: 10 μm.

rabbit antiserum may provide an useful tool to probe the full-length LMP expressed in biopsies from patients with EBV associated diseases. It is known that only the full-length LMP has transformation activity (Wang *et al.*, 1988a,b) and is recognized by the cytotoxic T-cells (Murray *et al.*, 1988). Testing the presence of full-length LMP or truncated LMP in biopsies will provide useful evidence to understand the role of LMP in contribution to the development of EBV associated human cancers and to understand the human cellular immune response to LMP.

The main purpose of this study was to investigate

the possible use of the recombinant LMP in testing the immune response of human sera to this protein. Sera from patients with NPC and freshly infected individuals were reported to show immune response to a synthetic peptide corresponding to the N-terminus of LMP (Modrow and Wolf, 1986). A further study indicated that sera from patients with NPC, BL, RA (rheumatoid arthritis), and EBV-positive healthy individuals contained antibodies against LMP expressed in EBV-harboring B-cells (Rowe *et al.*, 1988). Since EBV-harboring B cells express only small quantities of LMP, were expressed the full-length LMP in large quantities in Sf158 cells and used this recombinant protein to test the immune response of human sera to LMP. Surprisingly, all sera tested in immunofluorescence showed no detectable reaction to the recombinant LMP. Furthermore, it was also difficult to detect the immunological reaction of human sera to LMP in immunoblot by using unpurified LMP. The cross-reaction of human sera to the cellular proteins which migrated at the position of LMP obscured the results (data not shown). Therefore, we employed the immunoprecipitation–immunoblotting technique to analyze the reaction of human sera to LMP. Our results indicated that sera from patients with HD (16/27), from patients with NPC (5/22), from high EA titer patients with other diseases (3/52), and from EBV positive healthy individuals (2/26) contained antibodies against LMP (Table 1 and Fig. 6). At the present time we do not know why the human sera showed negative reaction in immunofluorescence but positive reaction in immunoprecipitation–immunoblotting analysis with the recombinant LMP. One of the explanations might be that the human sera recognized only SDS–denatured but not aceton- or methanol-fixed LMP. Another explanation might be that the antibodies against LMP were very weak and the sensitivity of immunofluorescence was lower than that of immunoprecipitation–immunoblot. It is of interest that antibodies against LMP were detected in a large percentage of sera from HD patients. HD has been associated with EBV infection by the demonstration of elevated antibody titers against EBV (Gutensohn and Cole, 1980; Mueller *et al.*, 1989) and the detection of EBV genome in the Hodgkin and Reed–Sternberg (HRS) cells (Herbst *et al.*, 1990; Weiss *et al.*, 1989). LMP has been reported to be expressed in HRS cells of up to 38% of HD cases (Herbst *et al.*, 1991a). It would be interesting to study the relationship between the expression of LMP in HRS cells and the serum immune response to LMP in a large collection of sera from HD patients.

TABLE 1

TEST OF ANTIBODIES (IgG) IN HUMAN SERA AGAINST LMP THROUGH IMMUNOPRECIPITATION–IMMUNOBLOTTING ANALYSIS

Source of sera	Total no. of sera	No. of positive sera against LMP (1:20)
NPC	22	5 (22.7%)
Hodgkin disease	27	16 (59.3%)
Other diseases exhibiting high EA-IgG titers	52	3 (5.8%)
VCA-IgG-positive healthy individuals	26	2 (7.7%)
VCA-IgG-negative healthy individuals	12	0 (0%)

Note. NPC, nasopharyngeal carcinoma.

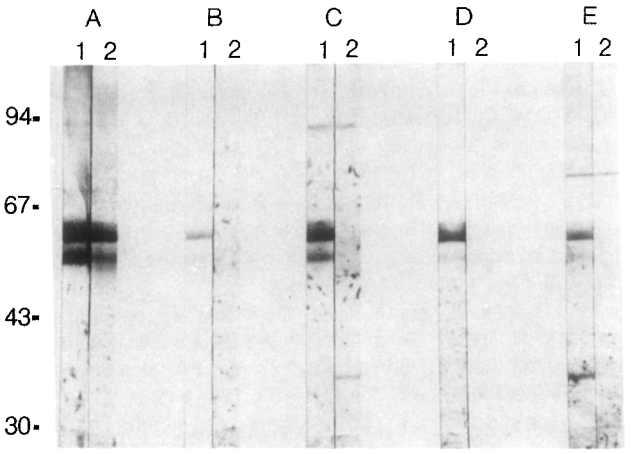

FIG. 6. Immunoprecipitation–immunoblot analysis of full-length LMP expressed in Sf158 cells. LMP was purified through immunoprecipitation and separated on a 10% SDS–polyacrylamide slab gel. The proteins were transferred to an immobilon membrane and stained with a panel of human sera diluted 1:20. The positive control sera (A) included the anti-LMP rabbit antiserum no. 749 (lane 1, diluted 1:800) and the human serum RA39 (lane 2, diluted 1:20). The experimental human sera were from NPC patients (B), HD patients (C), patients with other diseases exhibiting high EA-IgG titers (D), and EBV-positive healthy individuals (E). In the experimental groups, lane 1 shows the positive reaction of the human serum with the full-length LMP and lane 2 shows the undetectable reaction. Molecular mass markers are as in Fig. 2.

ACKNOWLEDGMENT

The authors are very grateful to Michael Pfreundschuh for providing the sera from patients with Hodgkin's disease, to Martin Rowe for providing LMP-positive human sera, and to Dr. M. Sauter for help with the generation of rabbit antisera. This work was supported by Deutsche Krebshilfe (W4/91/Mü 1).

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