JOURNAL OF VIROLOGY, Dec. 1993, p. 7482–7492
0022-538X/93/127482-11$02.00/0

Vol. 67, No. 12

The Cell Surface Receptor Is a Major Determinant Restricting the Host Range of the B-Lymphotropic Papovavirus

GABRIELE HAUN, OLIVER T. KEPPLER, C. THOMAS BOCK, MARKUS HERRMANN, HANSWALTER ZENTGRAF, AND MICHAEL PAWLITA*

Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, D-69120 Heidelberg, Germany

Received 21 June 1993/Accepted 17 September 1993

The B-lymphotropic papovavirus (LPV) productively infects only a subset of human B-lymphoma-derived cell lines while transfection of the viral genome yields infectious viral particles in a much wider variety of human hematopoietic cell lines. We have analyzed the contribution of a putative LPV receptor on the cell surface of B-cell lines in restricting the virus host range. In order to establish a quantitative virus binding assay for LPV, infectious virus particles were highly purified by metrizamide equilibrium density centrifugation and used as immunogens to raise seven mouse monoclonal antibodies specific for LPV VP1. Virus particle binding was quantitated in an indirect, nonradioactive assay with an LPV VP1-specific enzyme-linked immunosorbent assay. Binding of LPV particles to permissive human B-lymphoma cell line BJA-B occured within minutes. Kinetics and capacity of binding were similar at 4 and 37°C. A BJA-B cell was estimated to bind approximately 600 virus particles at conditions under which 50% of the administered virus was bound. The sialidase and trypsin sensitivities of the cellular virus binding moiety show that sialylated and proteinaceous components are necessary components of the LPV receptor on BJA-B cells. Despite a high binding capacity of BJA-B cells for simian virus 40, LPV binding was not significantly affected by a 20-fold excess of simian virus 40 particles, indicating that these related polyomaviruses do not bind to the same receptor on BJA-B cells. Reduction of LPV binding to sialidase-pretreated BJA-B cells was accompanied by a similar reduction of infection, indicating that virus binding may be a limiting factor in the LPV replicative cycle. The two highly LPV-permissive human B-lymphoma cell lines BJA-B and Namalwa displayed high virus binding whereas low and nonpermissive hematopoietic cell lines showed reduced or undetectable virus binding. We conclude that the inability of LPV particles to productively infect the nonpermissive human hematopoietic cell lines analyzed is probably due to the absence or insufficient expression of a functional cell surface receptor.

Viral tropism is the ability of a virus to selectively infect and replicate in cells of distinct species and tissues. Host factors determining viral tropism may operate at various levels of the viral replicative cycle, for example, virus entry or expression of viral genes. Expression of a functional receptor has been demonstrated to be a major determinant for viral tropism in some cases, e.g., CD4 on T lymphocytes and macrophages for human immunodeficiency virus type 1 (13), the intercellular adhesion molecule 1 for the major subgroup of rhinoviruses (21, 45, 49), or aminopeptidase N (CD13) on pig small intestine epithelial cells for the transmissible gastroenteritis virus (14).

The lymphotropic papovavirus (LPV) displays for human cells in tissue culture a highly restricted host range with tropism for a subset of human B-lymphoid cells. LPV is a primate polyomavirus with approximately equal sequence homology to simian virus 40 (SV40) and to mouse polyomavirus (33). LPV was originally isolated from African green monkey lymph node cultures (52) and subsequently passaged in the human B-lymphoma cell line BJA-B (5). The virus can infect and replicate effectively in some human Burkitt's lymphoma-derived cell lines and inefficiently in a few Epstein-Barr virus (EBV)-immortalized B-lymphoblastoid cells but apparently is unable to productively infect other cultured malignant or normal human cells (5, 36, 47, 52, 53). Even closely related EBV-immortalized B-lymphoblastoid cells established from the same patients in parallel with a permissive Burkitt's lymphoma cell line were found to be nonpermissive for LPV infection (36). Despite several efforts, only a single LPV-transformed embryonal hamster cell line has been obtained (48), and attempts to induce tumors in vivo in newborn rodents by LPV inoculation have failed (47, 48, 53). This indicates, in contrast to other polyomaviruses, a low transforming ability of the virus whereas mice carrying a nearly complete LPV genome as transgene readily formed malignant lymphoid and chorioid tumors (7).

Such a narrow host range is not unique among polyomaviruses. Human JC virus is neurotropic, and in tissue culture, this polyomavirus can be propagated almost exclusively in primary human fetal glia cells (24). However, mouse polyomavirus and SV40 display a much wider host range in vitro as well as in vivo, be it for productive infection or for malignant transformation (15, 24).

The LPV genome carries in the noncoding control region a tissue-specific enhancer element which is selectively active in human hematopoietic cell lines (32–34). When the complete viral genome was introduced by transfection, all human hematopoietic cell lines tested expressed both early and late viral genes and produced infectious virus particles. In contrast, nonhematopoietic cell lines (with the single exception of the monkey kidney epithelial cell line Vero) did not express the transfected viral DNA (36). Thus, the expression pattern of transfected LPV DNA coincides with the genetic activity pattern of the transfected LPV enhancer element. However, LPV particles display a very restricted host range within the hematopoietic system, suggesting that an early particle-associated event in the infectious cycle prior to the expression of

* Corresponding author.

early genes from viral DNA in the nucleus cannot take place in nonpermissive cells.

In this report, we describe the purification of infectious LPV particles, the generation of seven monoclonal antibodies with specificity for the main structural protein VP1, and the development of a sensitive enzyme-linked immunosorbent assay (ELISA) to quantitate picogram amounts of LPV VP1. Using these reagents and techniques in an indirect virus binding assay, we show here that of the three early phases of polyomavirus infection, i.e., particle binding to the cellular receptor, particle entry, and uncoating of viral DNA, already the first event does not take place in LPV nonpermissive hematopoietic cells.

MATERIALS AND METHODS

Cells. Human cell lines BJA-B (B lymphoma [31]), Namalwa (Burkitt's lymphoma [37]), Raji (Burkitt's lymphoma [16]), BL60 (Burkitt's lymphoma [28]), IARC 277 (EBV-immortalized B lymphoblastoid [46]), Jurkat (T-cell leukemia [42]), CEM (T-cell leukemia [17]), and HL60 (myeloid leukemia [20]) were propagated as suspension cultures in glass Erlenmeyer flasks with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml in a humidified 5% CO_2 atmosphere. The monkey kidney epithelium cell line TC7 (39), growing as a monolayer, and the mouse plasmacytoma-derived hybrid cell line P3X63Ag8 (26) were kept in plastic tissue culture bottles in Dulbecco's modified Eagle's medium with the same supplements and incubation conditions.

Viruses. To prepare LPV stocks, 2×10^7 BJA-B cells in 100 ml of medium were infected with 2×10^5 infectious units (IU) of LPV (strain P12 [5]); after 2 days, 2×10^7 cells and 300 ml of medium were added; starting at day 4, the culture volume was doubled every 2 days, and the percentage of LPV-infected cells was monitored daily by indirect immunofluorescence (see below). When 40 to 60% of the cells expressed viral protein (VP), usually between days 6 and 9 postinfection, virus particles were extracted by vigorously suspending the frozen pellet of washed cells in 1/20 of the original culture volume of hypotonic extraction buffer (50 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] [pH 7.4], 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2.5 μg of amphotericin B per ml, 200 μg of gentamicin per ml, 200 U of penicillin per ml, 200 μg of streptomycin per ml) and incubating on ice for 30 min. Cell debris was pelleted by centrifugation (10 min, 6,000 × *g*) and reextracted three more times for 5 min each with 1/60 of the original culture volume. Extraction supernatants were pooled and stored at −70°C.

SV40 seed virus was a generous gift from W. Deppert (Hamburg, Germany), and stocks were prepared from infected TC7 cells with massive cytopathic effect by four cycles of freezing and thawing of the washed cells and resuspension of lysed cells in extraction buffer. Pelleted cell debris was reextracted with 0.5% Nonidet P-40 in the extraction buffer after ultrasonication.

Purification of virus particles. LPV and SV40 particles were both purified by metrizamide equilibrium density gradient centrifugation following similar protocols. A total of 100 ml of LPV stock virus was incubated with 10 μg each of RNase and DNase I per ml (both from bovine pancreas; Boehringer Mannheim, Mannheim, Germany) for 30 min at 37°C. Subsequently, particles were precipitated by addition of 50 ml of 30% (wt/vol) polyethylene glycol (PEG) 6000 in extraction buffer and centrifugation (30 min, 12,000 × *g*, 4°C). The PEG precipitate was carefully resuspended in 2 ml of extraction buffer, and low-molecular-weight contaminations were removed by passage through a Bio-Gel A 1.5-m column (Bio-Rad, Munich, Germany). Virus recovered in the exclusion volume (2 ml) was mixed with 2 volumes of 65% (wt/vol) metrizamide in extraction buffer. After adjustment to 42% metrizamide (38), the virus solution was centrifuged (Beckmann Ultracentrifuge XL-70, VTi65 rotor, 40,000 rpm, 10°C, 20 h). Gradient fractions (200 μl) were analyzed by viral infectivity assay, silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot (immunoblot), slot blot hybridization with LPV DNA, and electron microscopy and later also by LPV-VP1 ELISA. Virus-containing fractions were stored at −70°C without further processing. The protein concentration of purified LPV VP1 was estimated from silver-stained SDS-PAGE protein gels with defined amounts of bovine serum albumin run on the same gel as reference.

Quantitation of LPV DNA by slot blot hybridization. Aliquots (0.1 μl) of metrizamide gradient fractions were diluted and denatured in 100 μl of 0.3 M NaOH (room temperature, 10 min), neutralized by addition of 100 μl of 2 M ammonium acetate and incubation at 65°C for 10 min, and subsequently loaded on nitrocellulose filters with a slot blot manifold. DNA was fixed to the filter for 30 min at 80°C, and the blot was subsequently hybridized with a ^{32}P-labeled cloned LPV DNA probe as described previously (18). Twofold serial dilutions of known amounts of LPV DNA on the same filter served as the quantitation standard.

SDS-PAGE, protein silver staining, and Western blot analysis. Proteins were denatured by being boiled for 2 min in 5% SDS and separated in 15% polyacrylamide gels (SDS-PAGE), basically following the procedure of Laemmli (27). Staining with silver nitrate was performed according to the work of Blum et al. (3). For immunological detection, LPV capsid proteins separated by SDS-PAGE were transferred by electroblotting to nitrocellulose filters (2). After being blocked with 10% dry milk in phosphate-buffered saline (PBS), the filters were incubated with polyclonal rabbit anti-LPV serum diluted 1:100 in PBS and then incubated with peroxidase-labeled goat anti-rabbit immunoglobulin heavy and light chains (Ig H+L) serum (Dianova, Hamburg, Germany) and finally stained with diaminobenzidine.

Electron microscopy. Carbon-coated copper grids were glow discharged, and 10-μl aliquots of gradient fractions were allowed to adsorb for 20 s to the coat. Excess liquid was removed, and the specimens were stained for 30 s with 2% aqueous uranyl–acetate and were finally air dried. Specimens were examined in a Zeiss EM10A electron microscope at 80 kV. The magnification indicator was routinely controlled by using a grating replica.

Indirect immunofluorescence microscopy. Suspension cells were washed in PBS and were allowed to settle on glass slides and air dried. Monolayer cells were grown on glass coverslips and washed in PBS. Cells were fixed and permeabilized in acetone at −20°C for 10 min. LPV antigens were detected by a polyclonal LPV T-antigen-specific hamster antitumor serum (18, 48) and an LPV VP-specific rabbit antiserum (see below). Fluorescein isothiocyanate-conjugated goat anti-hamster Ig H+L and rhodamine-conjugated goat anti-rabbit Ig H+L (Dianova) were used as secondary antibodies. SV40-infected cells were stained with an SV40 VP-specific rabbit antiserum (kindly provided by W. Deppert) followed by rhodamine-conjugated goat anti-rabbit Ig H+L.

LPV infectivity titration. Since LPV can be propagated only

in suspension cells, a conventional plaque assay cannot be performed to determine the titer of viral infectivity. Therefore, the number of LPV immunofluorescence-positive cells (T antigen and/or VP antigen positive) detected 48 h after infection (corresponding to about one viral replication cycle) under standardized conditions was used to routinely quantitate infectivity of LPV samples. Exponentially growing BJA-B cells were diluted in medium to 2×10^5 cells per ml and seeded in 1-ml volumes per well in 24-well tissue culture plates (Nunc, Wiesbaden, Germany). LPV-containing samples (50 μl) serially diluted in medium were added and mixed with the cells. After 48 h of incubation, the fraction of LPV-infected cells was determined by double immunofluorescence with LPV T- and VP-specific antibodies. All determinations were performed in triplicate, and only virus dilutions yielding between 0.5 and 5% of infected cells were used for calculations. The concentration of infectious LPV units ($IU_{48\ h}$/ml) was then calculated as the product of the number of infected cells {[(2×10^5) × fraction of LPV-positive] × (1/volume of virus suspension added) × (dilution factor of virus suspension)}. $IU_{48\ h}$ values obtained by this short-term infectivity assay represent approximately one-third of the values obtained by end-point titrations of LPV infectivity (data not shown). Scoring cells at 48 h postinfection by immunofluorescence underestimates the number of total infected cells since weakly stained cells and cells with slow viral replication that may not yet contain enough VP antigen are not included.

Generation of LPV VP-specific polyclonal rabbit antibodies. An LPV VP-specific polyclonal rabbit antiserum was raised by repeated subcutaneous injection of metrizamide gradient-purified nondenatured LPV particles. First, 10^8 $IU_{48\ h}$, corresponding to 5 μg of LPV VP1 in 200 μl of 25% metrizamide–PBS suspended in the same volume of complete Freund's adjuvant was injected; it was followed 4 weeks later by the same amount of antigen in incomplete Freund's adjuvant.

Generation of LPV VP1-specific monoclonal antibodies. BALB/c mice were immunized with metrizamide gradient-purified LPV particles. Per mouse, 5×10^5 $IU_{48\ h}$ of LPV corresponding to 40 ng of LPV VP1 in 200 μl of 5% metrizamide–PBS and suspended in an equal volume of complete Freund's adjuvant was injected first and followed by an intraperitoneal booster injection 4 weeks later with half the amount of antigen suspended in incomplete Freund's adjuvant. Three days later, mouse spleens were isolated and fused with P3X63Ag8 myeloma cells (26). Cell fusion with PEG 1000, seeding and selection in hypoxanthine-aminopterin-thymidine medium with additional unfused mouse spleen cells (5×10^5/ml), subcloning of hybridomas, and production of ascites in pristane-primed (BALB/c × DBA/2)F_1 mice were performed as previously described (35). Hybridomas secreting LPV-specific antibodies were identified by indirect immunofluorescence on LPV-infected BJA-B cells with a fluorescein isothiocyanate-conjugated goat anti-mouse Ig H+L (Dianova). Heavy chain classes of the monoclonal antibodies were identified by ELISA with purified LPV (0.5 μg/ml) as target applied directly to the wells as a coating (see below for ELISA conditions) and a commercially available set of peroxidase-labeled class- and subclass-specific anti-mouse Ig reagents (SBA Clonotyping system III; Southern Biotechnology Associates, Birmingham, United Kingdom).

LPV and SV40 VP1-specific ELISA. For detection of LPV VP1, 100 μl of LPV VP1-specific monoclonal antibody 456-1 either as hybridoma ascites diluted 10^6-fold in 50 mM sodium carbonate, pH 9.6, or later as protein A column-purified antibody at 0.5 μg/ml in the same buffer was applied per well as a coating in 96-well polystyrene microtiterplates (Nunc) overnight at 4°C. PBS containing 0.05% Tween 20 was used throughout as a washing buffer between all incubation steps (1 h, 37°C) and also as a dilution buffer. Residual free protein binding sites were blocked by incubation with 200 μl of 0.2% gelatin per well (Merck, Darmstadt, Germany). Then, incubation with 100 μl of viral antigen dilution per well was followed first by incubation with 100 μl of polyclonal rabbit LPV VP-specific antiserum per well, diluted 1:2,500, and then by incubation with 100 μl of peroxidase-labeled anti-rabbit Ig H+L per well. A total of 100 μl of 100-μg/ml tetramethylbenzidine–0.006% H_2O_2, in 0.1 M sodium acetate, pH 6, per well was used as substrate. After the enzyme reaction was stopped with 50 μl of 1 M H_2SO_4, the A_{450} was determined. All samples were analyzed in triplicate.

The SV40 VP1 ELISA was performed similarly, with the polyclonal rabbit antiserum to SV40 VP diluted $1{:}10^4$ as catching antibody and a tissue culture supernatant, diluted $1{:}10^2$, from an SV40 VP1-specific hybridoma (monoclonal antibody SV1-3H9, kindly provided by F. Mehnert, Bochum, Germany) as the primary detection reagent and peroxidase-conjugated goat antibodies to mouse Ig H+L (Dianova) as the developing reagent. The sensitivity of this assay reached 20 ng of SV40 VP1.

Virus binding assay. Cells (10^6) in exponential-growth phase were washed twice in PBS and incubated with purified virus equivalent to 15 ng of LPV VP1 or 400 ng of SV40 VP1 in 500 μl of PBS at 37°C for 30 min. After the cells were pelleted by low-speed centrifugation (400 × *g*, 5 min), the amount of unbound LPV or SV40 in the supernatant was quantified by VP1-specific ELISA. All samples were analyzed in triplicate.

Trypsin and sialidase treatment of cells. Cells were incubated in 0.1% trypsin–0.1% EDTA in PBS (2×10^6 cells per ml) for 30 min at 37°C. Soybean trypsin inhibitor (Type II-S; Sigma, Deisenhofen, Germany) was added (1 mg of inhibitor per ml), and cells were subsequently washed at 4°C. Binding of 10 ng of VP1 to 10^6 protease-treated cells was measured either directly after treatment or after a recovery phase in complete medium for 2.5 h at 37°C.

For sialidase (neuraminidase, acylneuraminyl hydrolase; EC 3.2.1.18) treatment, BJA-B cells (10^6) were washed in PBS and resuspended in 100 μl of bacterial sialidase solution from either *Arthrobacter ureafaciens*, *Clostridium perfringens* (both from Sigma), *Salmonella typhimurium* (Oxford GlycoSystems, Oxford, United Kingdom), or *Vibrio cholerae* (Boehringer Mannheim) diluted in PBS (0.2 U/ml) or in PBS alone (control) for 2 h at 37°C. Treated cells were washed with precooled PBS, centrifuged (400 × *g*, 10 min, 4°C), and infected with 100 μl of LPV stock virus for 3 h at 4°C (in untreated cells, the infectious dose applied resulted in 15 to 20% infected BJA-B cells 48 h after infection as determined by immunofluorescence microscopy). Infected cells were washed in medium and cultivated for 48 h. Cell pellets were stored at −20°C and extracted in a hypotonic buffer containing 50 mM HEPES (pH 7.4), 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. LPV VP1 antigen in the extract was quantified by LPV VP1-specific ELISA relative to the total protein content of the extract (determined by Bio-Rad protein assay according to manufacturer's instructions).

RESULTS

Purification of infectious LPV particles by metrizamide equilibrium density gradient centrifugation. In order to develop a sensitive LPV VP1 ELISA and a virus particle binding assay, infectious LPV particles had to be purified. Among

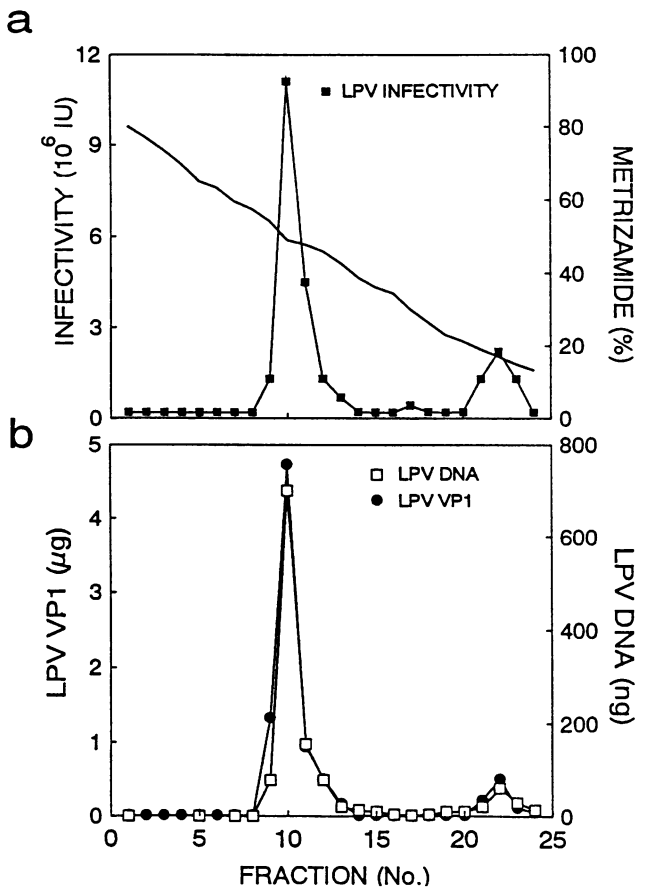

FIG. 1. Purification of LPV particles by metrizamide equilibrium density gradient centrifugation. A total of 24 fractions of 200 to 250 μl were collected and analyzed for infectivity (a) and LPV DNA and LPV VP1 antigen (b). (a) The infectivity of each fraction was titrated on BJA-B cells and is expressed as $IU_{48\ h}$. The density of the fractions is given by the metrizamide concentration as a percent (solid line). (b) Total content of LPV VP1 antigen (open squares) and LPV DNA (closed circles) of each fraction was determined by ELISA and by slot blot hybridization, respectively.

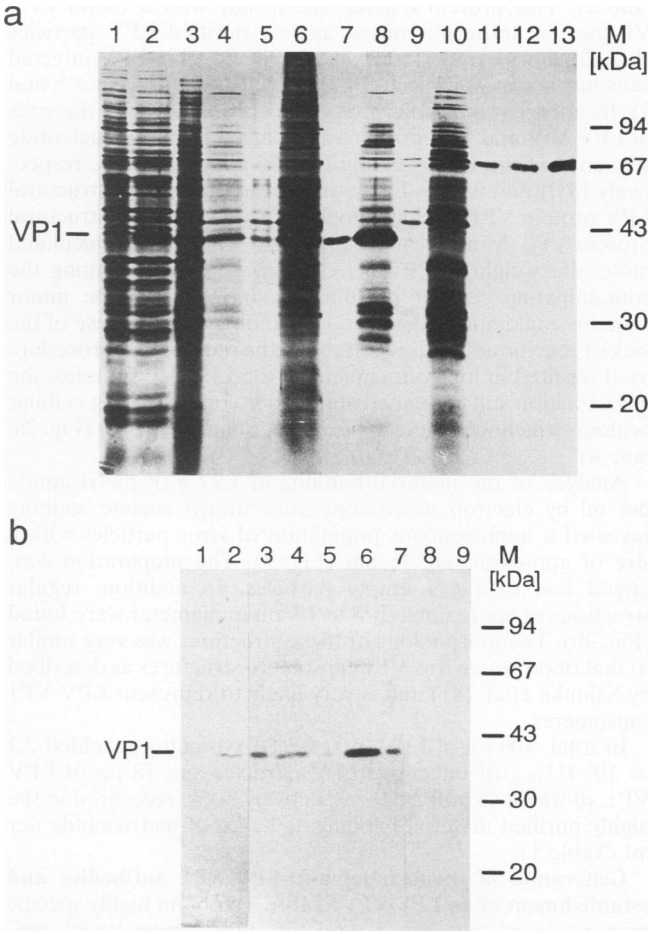

FIG. 2. LPV purification analyzed by SDS-PAGE and Western blot. Aliquots from various steps of the virus purification procedure were run in an SDS–15% polyacrylamide gel and subsequently either silver stained (a) or transferred onto nitrocellulose filters and immunoreacted with rabbit LPV antiserum (b). (a) Crude extract from uninfected (lane 1) and LPV-infected (lane 2) BJA-B cells, PEG precipitate (lane 3), pooled flowthrough fractions after Bio-Gel A 1.5-m column (lane 4), metrizamide gradient (Fig. 1a) fraction no. 22 (lanes 5 and 6) and LPV peak fraction no. 10 (lanes 7 and 8), and fraction no. 10 from control gradient of uninfected cells (lanes 9 and 10). In lanes 6, 8, and 10, 10 times more protein was applied than in lanes 5, 7, and 9, respectively. Bovine serum albumin at 50 (lane 11), 100 (lane 12), and 400 (lane 13) ng was used as a quantitation standard for the VP1 band in lane 7. (b) Crude extracts of uninfected (lane 1) and infected (lane 2) BJA-B cells, of PEG precipitate (lane 3), metrizamide gradient fraction no. 22 (lanes 4 and 5) and fraction no. 10 (lanes 6 and 7), and fraction no. 10 from control gradient of uninfected cells (lanes 8 and 9). In lanes 4, 6, and 8, 10 times more protein was applied than in lanes 5, 7, and 9, respectively.

various permissive B-lymphoma cell lines tested, infected BJA-B cells produced the highest virus titers. LPV particles produced in infected cells were strongly cell associated. Even after prolonged infection with microscopically visible cytopathic effect, less than 10% of viral infectivity was found in the tissue culture supernatant, and the highest yield of infectious virus was observed with hypotonic extraction of cells lysed by freeze-thawing. LPV infectivity was also found to be sensitive to high concentrations of CsCl, the standard density gradient medium used for purification of virus particles (12). Incubation overnight of an LPV suspension in a CsCl solution of 1.35 g/ml in PBS resulted in over 10-fold reduction of infectivity whereas an incubation in an alternative density gradient medium, metrizamide (38) at 1.27 g/ml in PBS, did not change LPV infectivity.

Purification of LPV particles by metrizamide gradient equilibrium density centrifugation yielded infectious virus at two different densities with the majority of material banding sharply at about 50% metrizamide, corresponding to a density of 1.27 g/ml (Fig. 1a). A second smaller peak of viral infectivity was present at 1.06 g/ml (18% metrizamide), probably representing virus particles attached to membrane-enriched cell debris. The concentration of viral infectivity in individual gradient fractions coincided with the distribution of LPV DNA, as detected by slot blot hybridization, as well as with the distribution of LPV VP1 antigen, as detected by ELISA (Fig. 1b).

The material banding at a density of 1.27 g/ml (Fig. 1, fraction 10), which contained the majority of infectious virus, consisted mainly of a protein with an apparent molecular mass of 40,000 Da as detected by silver-stained SDS-PAGE (Fig. 2a,

lane 7). This protein reacted specifically with a rabbit LPV VP-specific antiserum raised against purified LPV particles (Fig. 2b, lanes 6 and 7) and was also detected in LPV-infected cells but not in noninfected BJA-B cells (Fig. 2b, lanes 8 and 9). Its apparent molecular mass is in accordance with the sizes of LPV VP1 and VP2 proteins as deduced from the nucleotide sequence of the LPV genome (39,976 and 39,500 Da, respectively [33]) and is thus likely to represent the main structural LPV protein VP1, probably together with the minor structural protein VP2. Minor structural protein VP3, with a calculated molecular weight of 27,513 (33), may be present among the contaminating cellular proteins of similar size. The minor proteins could not be detected immunologically because of the lack of specific antibodies. Although the purification procedure used resulted in high enrichment of the LPV VP proteins, the peak fraction still contained about 20% contaminating cellular proteins which were detectable by overloading the gel (Fig. 2a, lane 8).

Analysis of the material banding at 1.27 g of metrizamide per ml by electron microscopy after uranyl acetate staining revealed a homogeneous population of virus particles with a size of approximately 45 nm (Fig. 3). The preparation contained less than 1% empty particles. In addition, regular structures of approximately 8 to 10 nm in diameter were found (Fig. 3b). The morphology of these structures was very similar to that of polyomavirus VP1 capsomere structures as described by Salunke et al. (41) and is very likely to represent LPV VP1 capsomeres.

In total, 400 ml of LPV-infected BJA-B culture yielded 2.2 $\times$ 10^8 $IU_{48\ h}$ of infectious LPV particles and 58 μg of LPV VP1, of which 18 and 24%, respectively, were recovered in the highly purified material banding at 1.27 g of metrizamide per ml (Table 1).

Generation of monoclonal anti-LPV VP1 antibodies and establishment of an LPV-VP1 ELISA. To obtain highly specific immunological reagents, a series of murine monoclonal antibodies was raised against metrizamide gradient-purified virus by using immunofluorescence with LPV-infected BJA-B cells for screening. Three of the seven monoclonal antibodies analyzed in detail were IgG2a (named 456-1, 464, and 530); the remaining four (479, 520, 542, and 549) were IgG2b. All antibodies neutralized LPV and recognized VP1 epitopes, since they reacted with bacterially expressed VP1 as well as with VP1 produced by a baculovirus expression system (data not shown). However, they did not react with denatured VP1 in standard Western blots.

A sandwich-type ELISA was established to detect and quantitate LPV. Monoclonal antibody 456-1 was used as the catching antibody, and a polyclonal rabbit LPV VP-specific antiserum was used as the detection reagent. With unpurified hybridoma ascites as the source of catching antibody, a minimum of 300 pg of LPV VP1 could be detected. With protein A column-purified antibody, a sensitivity of 20 pg of LPV VP1 was achieved.

LPV particle binding assay. To characterize the interaction of LPV particles with cell surfaces of permissive and nonpermissive human hematopoietic cell lines, an indirect, nonradioactive binding assay was developed. A constant amount of density gradient-purified LPV virions, corresponding to 15 ng of VP1, approximately 10^5 total IU, and about 6 $\times$ 10^8 physical particles (see Discussion for calculation of physical particles), was incubated with increasing numbers of highly LPV-permissive BJA-B cells. After 30 min of incubation, cells were pelleted by low-speed centrifugation, and unbound virus in the supernatant was quantified by LPV VP1 ELISA. Approximately 87% of the viral antigen administered was bound by 2 $\times$ 10^6 BJA-B cells (Fig. 4). The fraction of bound viral antigen did not increase significantly when 5 $\times$ 10^6 BJA-B cells were used in the binding assay. The free VP1 remaining after 60 min in the supernatant of the binding assay in the presence of excess cellular binding sites could not bind to fresh BJA-B cells (data not shown). This indicates that approximately 10% of the viral antigen in this virion preparation apparently represents viral protein reactive in the ELISA but unable to bind to BJA-B cells.

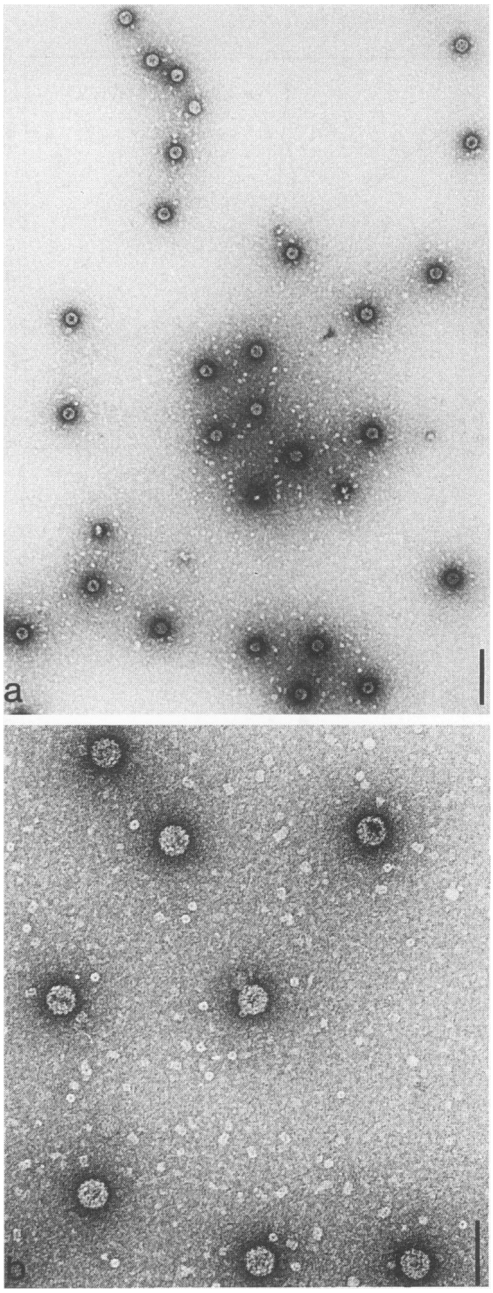

FIG. 3. Electron microscopy of purified LPV particles after metrizamide gradient. An aliquot of gradient fraction no. 10 (Fig. 1a) was negatively stained with uranyl-acetate. Bars indicate 200 nm (in panel a) and 100 nm (in panel b).

Virus binding was clearly dependent on the number of

TABLE 1. Analysis of LPV purification

Purification step	Vol (ml)	Infectivity[a]		LPV VP1 antigen[b]	
		$IU_{48\ h}$	%	μg	%
Crude extract	40	2.2×10^8	100	58	100
DNase-RNase digestion	40	2.2×10^8	100	54	93
PEG precipitation	0.4	1.0×10^8	45	28	48
Bio-Gel A 1.5-m column	4	8.0×10^7	36	18	31
Metrizamide gradient					
Peak 1 (50% metrizamide)	1.5	3.9×10^7	18	14	24
Peak 2 (18% metrizamide)	1.3	1.0×10^7	5	2	3

[a] IU determined by indirect immunofluorescence 48 h postinfection.
[b] Determined by LPV VP1 ELISA.

BJA-B cells present in the binding assay, since with decreasing numbers of BJA-B cells, the fraction of bound virus also decreased. LPV binding was no longer detectable under these assay conditions with 10^5 BJA-B cells (Fig. 4). In all titrations, cells of the LPV nonpermissive human promyelocytic leukemia cell line HL60 were added to obtain a total number of 5×10^6 cells throughout the experiment. Even 5×10^6 HL60 cells displayed no virus binding in the absence of BJA-B cells (Fig. 4; see also Table 4) whereas 2×10^5 BJA-B cells already bound 22% of the 15 ng of VP1 present in the assay. This observation shows that LPV binding to BJA-B is cell specific and that permissive BJA-B cells bind LPV at least 50-fold better than nonpermissive HL60 cells.

A total of 50% of LPV, corresponding to 7.5 ng of LPV VP1, was bound within 30 min when approximately 4×10^5 BJA-B cells were present in the binding assay (Fig. 4). Increasing the binding time to 60 min did not increase the fraction of virus bound (not shown). These data can be used to estimate the LPV binding capacity of BJA-B cells. Assuming that LPV VP1,

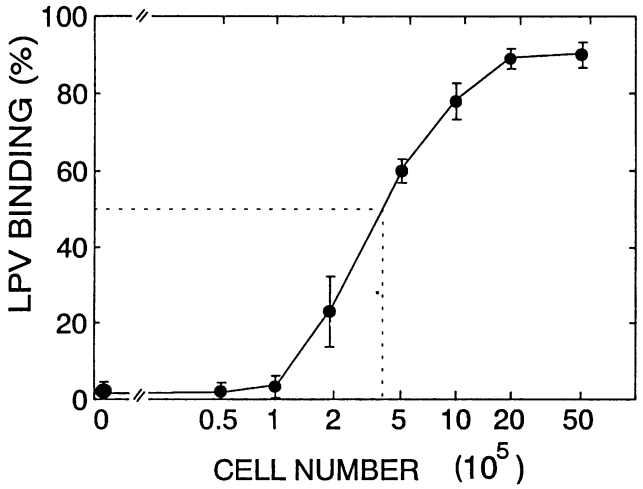

FIG. 4. Titration of BJA-B cells in the LPV binding assay. A constant amount of purified virus was incubated for 30 min with varying numbers of BJA-B cells at 37°C. LPV binding was determined as the percentage of virus bound relative to the total amount of LPV administered (100% = 15 ng of LPV VP1, corresponding to approximately 10^5 total IU and about 6×10^8 physical particles). The total number of cells applied in each determination was adjusted to 5×10^6 with HL60 cells, which do not bind LPV (Table 4). Mean and standard deviation (SD) ($n = 3$) are shown. The broken lines indicate the 50% binding point (4×10^5 BJA-B cells).

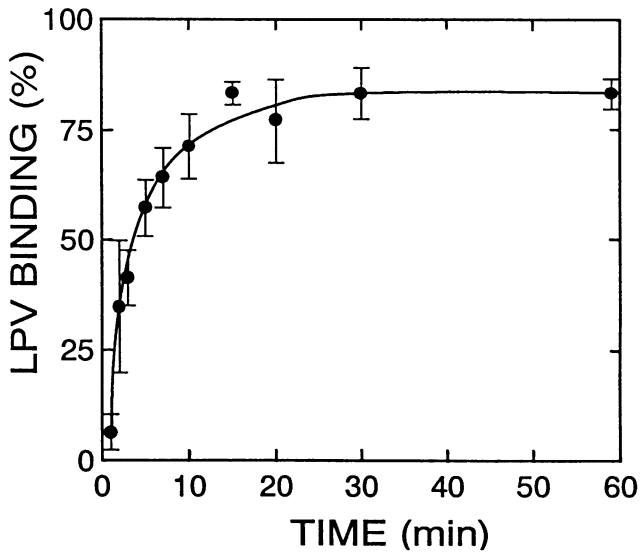

FIG. 5. Kinetics of LPV binding to BJA-B cells. Purified LPV particles (corresponding to 15 ng of LPV VP1) were incubated with BJA-B cells (5×10^6) at 4°C. At the times indicated, LPV binding was determined as the percentage of virus bound relative to the total amount of LPV administered. Mean and SD ($n = 3$) are shown.

like SV40 VP1 (22, 29), constitutes approximately 75% of the total virion protein and that the proteins of an LPV particle, like those of SV40, have a combined molecular weight of 23.6×10^6 (22), one can calculate that the 7.5 ng of LPV VP1 corresponds to 2.6×10^8 virus particles, suggesting that, on average, 650 LPV particles are bound per BJA-B cell. A total of 2×10^5 cells bound 22% of LPV antigen, which corresponds to about 600 virions per cell. Thus, a fourfold excess of free over bound virus at this cell concentration did not increase the number of viruses bound per cell, indicating that the value of 600 to 650 virions bound per cell is close to the mean total binding capacity of a single BJA-B cell.

Kinetics of LPV binding to BJA-B cells. The time course of LPV particle binding was analyzed with 15 ng of LPV VP1 and 5×10^6 BJA-B cells at 4°C. Under these conditions, cellular LPV binding sites were in about sixfold excess over virus particles, allowing binding to occur at its fastest rate. LPV binding took place within minutes, with 50% of the virions bound after 5 min, and was complete after approximately 15 min (Fig. 5). Again, the free VP1 detected after 60 min is assumed to have been unable to bind to BJA-B cells. No significant differences in the virus binding kinetics were apparent when the binding was performed at 37°C (data not shown), making a requirement for an enzymatic or energy-dependent activity in the early phase of virus binding unlikely.

Trypsin and sialidase sensitivity of the cellular LPV binding moiety. The biochemical nature of the virus binding moiety was partly characterized by incubation of BJA-B cells with one protease and one glycosidase (Fig. 6). Trypsin-treated BJA-B cells bound approximately 65% less LPV than did control cells. This indicates that a cell surface protein is an essential component of the LPV binding moiety. After recovery from trypsin treatment in medium for 2.5 h at 37°C, the binding capacity was almost completely restored, which indicates a rapid synthesis and/or cell surface transport of this protein component (Fig. 6a). Sialic acid on the surface of BJA-B cells is required for the attachment of LPV particles since cleavage of terminal sialic acids by sialidase from *V. cholerae*, which

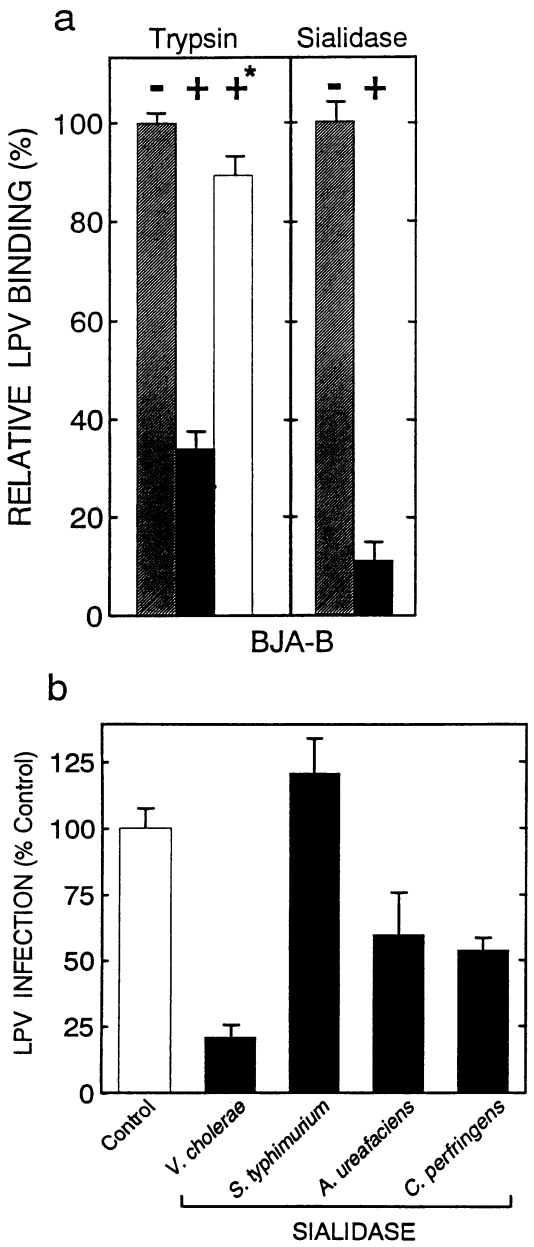

FIG. 6. Trypsin and sialidase sensitivity of LPV binding and infection. (a) BJA-B cells (10^6) were incubated for 30 min in 0.1% trypsin in PBS (+) or in PBS alone (−) at 37°C prior to the LPV binding assay. Reoccurrence of LPV binding was observed on BJA-B cells which had been pretreated with trypsin and had been allowed to recover in conditioned medium for 2.5 h at 37°C (+*). Sialidase sensitivity of LPV binding was examined by incubating cells with *V. cholerae* sialidase (+) or PBS (−; control) for 2 h at 37°C. Values are given as arithmetic means and SDs from triplicate samples. (b) BJA-B cells were pretreated with one of the indicated bacterial sialidases and subsequently infected with LPV for 3 h at 4°C. Washed cells were cultivated for 48 h at 37°C. Cells were extracted, and the relative concentration of LPV VP1, the main structural LPV protein, in the extracted total protein was quantified by ELISA. Values represent arithmetic means from three to six parallel samples, with bars indicating SD, as percentages of values obtained from infected control cells.

cleaves α-2,6- and α-2,3-linked sialic acid residues, resulted in an 88% reduction of the BJA-B cell binding capacity for LPV (Fig. 6a) and a 79% reduction of LPV infection (Fig. 6b) compared with control cells. The lack of effect on LPV infection by treatment with *S. typhimurium* sialidase (Fig. 6b), which has a 260-fold preference for the cleavage of α-2,3-linked sialic acids (25), suggests an involvement of an α-2,6- rather than an α-2,3-linked sialic acid for the LPV receptor moiety.

SV40 binding does not interfere with LPV binding to BJA-B cells. SV40 and LPV are both polyomaviruses with 52% protein sequence identity within their VP1 protein (33), the putative viral receptor-binding protein (29). In order to use SV40 as a competitor in the LPV binding assay, SV40 particles were purified from infected monkey kidney epithelial cells (cell line TC7) by metrizamide gradient centrifugation with techniques similar to those for the purification of LPV from BJA-B cells. Binding of SV40 particles to permissive TC7 cells as well as to nonpermissive human cell lines BJA-B (Burkitt's lymphoma) and Jurkat (T-cell leukemia) was determined by applying a type of binding and detection assay similar to that in the case of LPV (Table 2). An equal cell number (10^6) of all three cell lines tested bound approximately 85% of SV40 (equivalent to 400 ng of VP1), which corresponds to about 12,000 virus particles bound per cell. The high SV40 binding capacity appears to be independent of permissivity for viral infection. The high percentage of SV40 bound to all three cell lines probably indicates that the SV40 binding sites were not yet saturated. Therefore, the value of 12,000 SV40 particles bound per cell can only define a minimum for the SV40 binding capacity of BJA-B cells. SV40 was also bound by Jurkat cells to which LPV bound only marginally (see Table 4).

In order to determine whether SV40 binding could interfere with LPV binding, BJA-B cells were preincubated with a 20-fold excess of SV40 particles before LPV particles were added to the binding assay (Table 3). To use the LPV binding assay under its most sensitive conditions (steepest part of the slope in Fig. 4), cell numbers that resulted in approximately 37 to 49% binding of LPV were employed. In the three experiments performed, a slight reduction was seen for LPV binding after preincubation of BJA-B cells with a 20-fold excess of SV40 (Table 3), suggesting that (interpolated from the cell number titration in Fig. 4) up to 22% of the cellular binding sites were no longer occupied by LPV virions.

LPV binding and permissivity in human hematopoietic cell lines are correlated. The importance of the LPV binding capacity as a host range determinant was analyzed by comparing results of binding and permissivity assays on eight human cell lines of hematopoietic origin (Table 4). The B-lymphoma cell lines BJA-B and Namalwa, which are both highly permissive for LPV, displayed the highest capacity to bind LPV

TABLE 2. SV40 binding and infection in cell lines BJA-B, Jurkat, and TC7

Cell line	Origin	% SV40 binding[a] (mean ± SD)	% SV40-infected cells[b]	
			Day 2	Day 8
BJA-B	Burkitt's lymphoma	85 ± 5	<0.1	<0.1
Jurkat	T-cell leukemia	83 ± 2	<0.1	<0.1
TC7	Monkey kidney epithelium	87 ± 4	20	50

[a] Binding of purified SV40 particles (400 ng of VP1) to 10^6 cells was measured after 30 min at 37°C.

[b] The percentage of SV40 T-antigen-positive cells was determined 2 and 8 days postinfection by indirect immunofluorescence.

TABLE 3. SV40 competition assay of LPV binding to BJA-B cells

Expt no.	No. of cells	% LPV binding[a]	
		−SV40	+SV40
1	5×10^5	39	32
2	5×10^5	37	27
3	1×10^6	49	36

[a] BJA-B cells were incubated with purified SV40 particles (300 ng of SV40 VP1) in PBS (+) or in PBS alone (−) for 30 min at 37°C. Subsequently, purified LPV particles were added (15 ng of LPV VP1), and LPV binding was determined.

particles in the LPV binding assay. The binding capacities of the other cell lines tested were greatly reduced. The relative binding capacity of these cell lines was determined by extrapolating (from the graph in Fig. 4) the number of BJA-B cells needed to bind the same amount of LPV as 5×10^6 cells of the other lines (Table 4). Three other lines, i.e., Raji, IARC 277, and Jurkat, revealed a binding of between 20 and 40% of the virus available, corresponding to a binding capacity of 1/14, 1/17, and 1/28, respectively, compared with BJA-B cells. Of Raji cells, up to 1% were infected by LPV. On the other hand, IARC 277 and Jurkat cells as well as three other lines (BL60, CEM, and HL60) with relative binding capacities of less than 1/50 yielded no or only very few LPV antigen-positive cells by immunofluorescence 2 and 8 days after infection.

DISCUSSION

We wanted to study the possible role of a cellular receptor moiety in restricting the LPV host range in cell lines of human hematopoietic origin. To develop the indirect, nonradioactive virus particle binding assay used here, several new reagents and techniques were necessary. These included purified, infectious virus particles and a sensitive LPV VP-specific ELISA.

By establishing the purification protocol for infectious LPV particles, we found that LPV infectivity was highly sensitive to CsCl. This problem could be solved by using metrizamide as an alternative equilibrium density gradient medium. From the 706 ng of LPV DNA and the 4.8 μg of LPV VP1 recovered in the purest LPV particle fraction (Fig. 1b, fraction 10), one can calculate the presence of 1.2×10^{11} or 2×10^{11} physical particles, respectively. This fraction showed an infectivity of 1.1×10^7 $IU_{48\ h}$ (Fig. 1a) in the short-term infectivity assay, corresponding to a total infectivity of approximately 3.3×10^7 infectious particles. Accordingly, a ratio of infectious to physical particles of about 1:3,600 or 1:6,000 can be deduced. A slightly higher ratio of approximately 1:1,000 has been determined for purified mouse polyomavirus (26). The ratio of LPV VP1 to infectivity in the purified particle preparation is similar to that in the crude extract from LPV-infected BJA-B cells, suggesting that no LPV infectivity was lost during the purification.

The virus binding assay applied in this study measures LPV binding indirectly by determining the amount of virus no longer present in the cell supernatant after binding. Alternative direct binding assays using radiolabeled or biotinylated virus preparations have been used, for example, in SV40 or lymphocytic choriomeningitis virus binding studies (4, 8, 10). Binding assays with directly labeled virus particles critically depend on the purity of the virus preparations used for labeling and for competition as unlabeled viruses; contaminations with membrane fragments are conceivable. Binding of labeled components other than virus could occur and would also be inhibited if these components were present in the unlabeled virus preparation used as competitor (4). In the LPV binding assay, high specificity of virus detection is achieved immunologically by LPV VP1 ELISA which employs one monoclonal and one polyclonal antibody preparation. The specificity of this detection system even allows the use of unpurified cell extracts from LPV-infected cells as a virus source for binding studies (unpublished observation).

LPV binding to BJA-B cells as the prototype of permissive human B-lymphoma cells was studied in detail. With an excess of cellular binding sites over LPV particles administered, binding occurred within minutes (Fig. 5) and did not seem to require energy-dependent or metabolic processes. Polyomavirus and SV40 binding to permissive cells has been reported to occur at similar rates (1, 8, 11, 30). On the other hand, binding assays with reovirus serotypes 1 and 3 have demonstrated that only about 25% of virus was bound to endothelial cells after 60 min, and cells were saturated after 4 to 6 h (50). In previous studies, we observed that the addition of neutralizing antibody to the culture up to 8 h postinfection could still lead to a reduction of LPV infection in BJA-B cells (18). Since particle binding apparently is a fast process, later phases of the entry pathway in which the bound particles are transported across the plasma membrane must be much slower, allowing the physical association of bound virus particles with extracellular neutralizing antibodies for several hours.

Only about 80 to 90% of the purified viral antigen was capable of binding even when cellular binding sites were present in more than a fivefold excess over virus particles and

TABLE 4. LPV binding and infection in various hematopoietic cell lines

Cell line	Origin	% LPV binding[a]	Relative binding capacity[b]	% LPV-infected cells[c]	
				Day 2	Day 8
BJA-B	Burkitt's lymphoma	89 ± 6	1	10	>50
Namalwa	Burkitt's lymphoma	82 ± 12	>1/4	3	>50
Raji	Burkitt's lymphoma	40 ± 12	1/14	<0.1	1
BL60	Burkitt's lymphoma	3 ± 8	<1/50	<0.1	<0.1
IARC 277	EBV-immortalized B lymphoblastoid	36 ± 11	1/17	<0.1	<0.1
Jurkat	T-cell leukemia	21 ± 11	1/28	<0.1	<0.1
CEM	T-cell leukemia	6 ± 12	<1/50	<0.1	<0.1
HL60	Promyelocyte leukemia	4 ± 1	<1/50	<0.1	<0.1

[a] Binding of purified LPV particles (15 ng of LPV VP1) to 5×10^6 cells was determined after 30 min at 4°C. Values given are mean ± SD for 3 to 15 individual experiments.

[b] An approximation of the mean binding capacity relative to BJA-B was derived from the curve in Fig. 4. The fraction of BJA-B cells binding the same amount of LPV is given for each cell line.

[c] The percentage of LPV VP-positive cells in indirect immunofluorescence 2 and 8 days postinfection is given.

the time allowed for binding was increased to 60 min. A fraction of 10 to 20% of the virus preparation may thus consist of free VP1 or particles which have no binding competence but are immunologically detectable by ELISA. However, the large number of noninfectious LPV particles is apparently not defective in binding.

The amount of virus bound clearly depended on the number of cells over 1 order of magnitude (10^5 to 10^6 BJA-B cells, Fig. 4), when the cell number was titrated against a constant amount of virus (15 ng of LPV VP1). These results indicate that one BJA-B cell on average can bind approximately 600 to 650 LPV particles. Single-cell cloning revealed a heterogeneity of BJA-B cells for LPV binding with binding capacities of subclones ranging from 60 to over 1,400 particles per cell (unpublished observation). The LPV binding capacity per BJA-B cell is low compared with the binding capacities of host cells for other viruses. For example, SV40 can occupy about 9×10^4 binding sites per cell on Vero C1008 cells (8). Similarly, 6×10^4 intercellular adhesion molecule 1 molecules serving as receptors for the major group of human rhinoviruses have been detected on HeLa cells by monoclonal antibodies as well as in radioactive virus binding studies (21). The observed low LPV binding capacity of highly permissive cells such as BJA-B may be the reason why our attempts to visualize unlabeled virus binding to the cell surface by indirect immunological detection with fluorescent reagents and FACScan analysis did not yield signals above background (not shown). The absolute number of LPV receptor molecules might be higher than the number of binding sites, assuming that more than one receptor molecule is necessary to build a functional virus binding site.

The LPV binding assay described here can discriminate among high-, low-, and nonbinding cells. Variation of the cell number used in the assay over a wide range to yield binding of between 5 and 80% of administered virus would allow an approximation of the mean LPV binding capacity of any binding cell line. Here we have used the titration data obtained for BJA-B as the standard to determine binding capacities of other cell lines relative to BJA-B. Nonbinding cell lines such as HL60 and BL60 bound less than 1/50 of the virus bound by BJA-B, corresponding to a binding capacity of less than 15 virus particles per cell. We could not only determine the number of binding sites in various cell lines but could also show that cell lines nonpermissive for LPV infection can hardly bind the virus, indicating that they probably lack a functional cell surface receptor.

At present, information on the nature of the LPV receptor on BJA-B cells is rare. The trypsin and sialidase sensitivities of LPV binding indicate the involvement of at least one, probably α-2,6-linked, sialic acid and a membrane protein which appears to have a relatively high recovery rate. Apart from LPV, the requirement of sialic acid for virus attachment to susceptible host cells has also been demonstrated for two other members of the polyomavirus genus, namely, human BK virus (44) and mouse polyomavirus with a sialic acid in α-2,3-linkage (6, 19). The interaction of SV40 with its receptor on Vero cells, however, does not seem to be sialic acid dependent (9). The putative receptor-binding protein VP1 is highly conserved among SV40 and these three other polyomaviruses (22, 33), and yet apparently, these viruses can bind to different classes of receptor structures.

Our experiments demonstrate that the LPV receptor on BJA-B cells is different from the SV40 binding site, since the latter was found to be at least 20 times more abundant and an excess of SV40 particles did not significantly inhibit LPV from binding to its receptor.

Although other steps of the virus entry process besides attachment may also contribute to the resistance of low-binding cell lines to becoming productively infected by LPV, two experimental results presented here indicate that the main mechanism by which the LPV host range in cultured human hematopoietic cells is regulated is the presence of sufficient virus binding sites on permissive cell lines and the lack of sufficient binding sites on nonpermissive cell lines. Firstly, the two highly permissive cell lines BJA-B and Namalwa showed a high virus binding capacity and low or undetectable virus binding correlated with low or absent permissivity in the other hematopoietic cell lines tested. However, the latter are capable of producing complete virus particles upon transfection of LPV DNA (36). Secondly, the drastic reduction in the virus binding capacity of BJA-B cells by sialidase treatment of intact cells was sufficient to strongly reduce the susceptibility to virus infection.

A threshold level of virus binding sites may be necessary to allow productive infection. This could explain why among the three cell lines Raji, IARC 277, and Jurkat, which showed similar low binding capacities, only in Raji were up to 1% of LPV-infected cells detected. Raji could be more heterogeneous than the other two cell lines and could have up to 1% of cells with high LPV binding capacity above threshold. Alternatively, additional mechanisms controlling host range at other levels after attachment, such as penetration, nuclear transport, and uncoating, may be active in these lines.

Burkitt's lymphoma cell lines are heterogeneous in phenotype and have been ordered in four groups according to their tissue culture growth pattern as well as expression of certain cell surface antigens (40). BJA-B, although not classified as a typical Burkitt's lymphoma cell line, displays a pattern of cell surface markers (43) similar to those of group I or I/II Burkitt's lymphoma cell lines. The three other Burkitt's lymphoma lines analyzed here for LPV binding, namely, Namalwa, Raji, and BL60, also display a surface marker pattern (23, 43, 51) compatible with inclusion in group I or I/II. However, these lines differed markedly in permissivity and were concordant in LPV binding. We hypothesize that the receptor function used by LPV to infect human B-lymphoma cells depends on a molecule associated with a subtle differentiation stage of mature B cells represented mostly by Burkitt's lymphoma group I or I/II cells. The fact that not all similarly differentiated Burkitt's lymphoma cell lines are permissive for LPV could be due to small, as-yet-undefined differentiation heterogeneity among the cell lines of these subgroups. LPV receptor function could also depend on a genetic polymorphism in the gene(s) affecting the expression of the LPV receptor molecule(s). Both causes could act either directly on the gene(s) encoding the receptor molecule(s) or indirectly, affecting posttranscriptional modification such as glycosylation or transport of the receptor molecule(s) to the plasma membrane.

ACKNOWLEDGMENTS

We thank H. zur Hausen for continuous support and stimulating discussions and H.-G. Kräusslich and L. Gissmann for critical discussion of the manuscript. We are grateful to W. Deppert and H. Mehnert for gifts of virus stocks and monoclonal antibodies, T. Kanda for the gift of bacterially expressed LPV VP1, and M. Oppenländer and F. Ackermann for expert technical and photographic assistance, respectively.

REFERENCES

1. **Barbanti-Brodano, G., P. Swetly, and H. Koprowski.** 1970. Early events in the infection of permissive cells with simian virus 40: adsorption, penetration, and uncoating. J. Virol. **6**:78–86.
2. **Bjerrum, O. J., and C. Schäfer-Nielsen.** 1986. Buffer systems and

transfer parameters for semidry electroblotting with a horizontal apparatus. Electrophoresis **86:**315–327.
3. **Blum, H., H. Beier, and H. J. Gross.** 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8:**93–99.
4. **Borrow, P., and M. B. A. Oldstone.** 1992. Characterization of lymphocytic choriomeningitis virus-binding protein(s): a candidate cellular receptor for the virus. J. Virol. **66:**7270–7281.
5. **Brade, L., W. Vogl, L. Gissman, and H. zur Hausen.** 1981. Propagation of B-lymphotropic papovavirus (LPV) in human B-lymphoma cells and characterization of its DNA. Virology **127:**228–235.
6. **Cahan, L. D., R. Singh, and J. C. Paulson.** 1983. Sialyloligosaccharide receptors of binding variants of polyoma virus. Virology **130:**281–289.
7. **Chen, J., K. Neilson, and T. Van Dyke.** 1989. Lymphotropic papovavirus early region is specifically regulated in transgenic mice and efficiently induces neoplasia. J. Virol. **63:**2204–2214.
8. **Clayson, E. T., and R. W. Compans.** 1988. Entry of simian virus 40 is restricted to apical surfaces of polarized epithelial cells. Mol. Cell. Biol. **8:**3391–3396.
9. **Clayson, E. T., and R. W. Compans.** 1989. Characterization of simian virus 40 receptor moieties on the surface of Vero C1008 cells. J. Virol. **63:**1095–1100.
10. **Clayson, E. T., L. V. Jones Brando, and R. W. Compans.** 1989. Release of simian virus 40 virions from epithelial cells is polarized and occurs without cell lysis. J. Virol. **63:**2278–2288.
11. **Crawford, L. V.** 1962. The adsorption of polyoma virus. Virology **18:**177–181.
12. **Crawford, L. V.** 1969. Purification of polyoma virus, p. 75–81. *In* K. Habel and N. P. Salzmann (ed.), Fundamental techniques in virology. Academic Press, New York.
13. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapman, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312:**763–767.
14. **Delmas, B., J. Gelfi, R. L'Haridon, L. K. Vogel, H. Sjöström, O. Noren, and H. Laude.** 1992. Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. Nature (London) **357:**417–420.
15. **Eddy, B. E.** 1969. Polyoma virus, p. 1–114. *In* S. Gard, C. Hallauer, and K. F. Meyer (ed.), Virology monographs, vol. 7. Springer-Verlag, Vienna.
16. **Epstein, M. A., Y. M. Achong, Y. Barr, B. Zajac, G. Henle, and W. Henle.** 1966. Morphological and virological investigations on cultured Burkitt tumor lymphoblasts (strain Raji). J. Natl. Cancer Inst. **37:**547–559.
17. **Foley, G. E., H. Lazarus, S. Farber, B. G. Uzman, B. A. Boone, and R. E. McCarthy.** 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer **18:**522–529.
18. **Forbes, B., L. Gissmann, and M. Pawlita.** 1988. Detection of individual virus infected cells by filter in situ hybridization. Mol. Cell. Probes **2:**245–253.
19. **Fried, H., L. D. Cahan, and J. C. Paulson.** 1981. Polyoma virus recognizes specific sialyloligosaccharide receptors on host cells. Virology **109:**188–192.
20. **Gallagher, R., S. Collins, J. Trujillo, K. McCredie, M. Ahearn, S. Tsai, R. Metzgar, G. Aulakh, R. Ting, F. Ruscetti, and R. Gallo.** 1979. Characterization of the continuous differentiating myeloid cell line (HL60) from a patient with acute promyelocytic leukemia. Blood **54:**713–733.
21. **Greve, J. M., G. Davis, A. M. Meyer, C. P. Forte, S. C. Yost, C. W. Marlor, M. E. Kamarck, and A. McClelland.** 1989. The major human rhinovirus receptor is ICAM-1. Cell **56:**839–847.
22. **Griffin, B. E.** 1981. Structure and genomic organization of SV40 and polyoma virus, p. 61–124. *In* J. Tooze (ed.), DNA tumor viruses. Molecular biology of tumor viruses, 2nd ed., part 2, revised. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
23. **Guy, K., J. A. Ross, and C. M. Steel.** 1989. Non-coordinate expression of B-cell and myeloid antigens on variant sublines of the Burkitt lymphoma cells Namalwa, p. 200–201. *In* W. Knapp et al. (ed.), Leukocyte typing IV. Oxford University Press, Oxford.
24. **Howley, P. M.** 1980. Molecular biology of SV40 and the human polyomaviruses BK and JCV, p. 489–550. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
25. **Hoyer, L. L., P. Roggentin, R. Schauer, and E. R. Vimr.** 1991. Purification and properties of cloned *Salmonella typhimurium* LT2 sialidase with virus-typical kinetic preference for sialyl α-2,3 linkages. J. Biochem. **110:**462–467.
26. **Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody secreting hybrid cell lines. J. Immunol. **123:**1548–1550.
27. **Laemmli, U. K.** 1974. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
28. **Lenoir, G. M., M. Vuilaume, and C. Bonnardel.** 1985. The use of lymphomatous and lymphoblastoid cell lines in the study of Burkitt's lymphoma, p. 309–318. *In* G. T. O'Conor and C. L. M. Olweny (ed.), Burkitt's lymphoma: a human cancer model. International Agency for Research on Cancer Scientific Publication 60. International Agency for Research on Cancer, Lyon, France.
29. **Liddington, R. C., Y. Yan, J. Moulai, R. Sahli, T. L. Benjamin, and S. C. Harrison.** 1991. Structure of simian virus 40 at 3.8-A resolution. Nature (London) **354:**278–284.
30. **MacKay, R. L., and R. A. Consigli.** 1976. Early events in polyoma virus infection: attachment, penetration and nuclear entry. J. Virol. **19:**620–636.
31. **Menezes, J., W. Leibold, G. Klein, and G. Clements.** 1975. Establishment and characterization of an Epstein-Barr virus (EBV)-negative lymphoblastoid cell line (BJA-B) from an exceptional, EBV-genome negative African Burkitt's lymphoma. Biomedicine **22:**276–284.
32. **Mosthaf, L., M. Pawlita, and P. Gruss.** 1985. A viral enhancer element specifically active in human haematopoietic cells. Nature (London) **315:**597–600.
33. **Pawlita, M., A. Clad, and H. zur Hausen.** 1985. Complete DNA sequence of lymphotropic papovavirus: prototype of a new species of the polyomavirus genus. Virology **143:**196–211.
34. **Pawlita, M., L. Mosthaf, A. Clad, and P. Gruss.** 1984. Genome structure and host range restriction of the lymphotropic papovavirus (LPV): identification of a viral lymphocyte specific enhancer element. Curr. Top. Microbiol. Immunol. **113:**26–30.
35. **Pawlita, M., E. Mushinski, R. J. Feldmann, and M. Potter.** 1981. A monoclonal antibody that defines an idiotype with two subsites in galactan-binding myeloma proteins. J. Exp. Med. **154:**1946–1956.
36. **Pawlita, M., M. Oppenländer, and H. zur Hausen.** Submitted for publication.
37. **Reedman, B. M., and G. Klein.** 1973. Cellular localization of an Epstein-Barr (EBV)-associated complement fixing antigen in producer and non-producer lymphoblastoid cell lines. Int. J. Cancer **11:**499–520.
38. **Rickwood, D., and G. D. Birnie.** 1975. Metrizamide: a new density gradient medium. FEBS Lett. **50:**102–110.
39. **Robb, J. A., and K. Huebner.** 1973. Effect of cell chromosome number on simian virus 40 replication. Exp. Cell Res. **81:**120–126.
40. **Rowe, M., D. T. Rowe, C. D. Gregory, L. S. Young, P. J. Farrell, H. Rupani, and A. B. Rickinson.** 1987. Differences in B cell growth phenotype reflect novel patterns of Epstein-Barr virus latent gene expression in Burkitt's lymphoma cells. EMBO J. **6:**2743–2751.
41. **Salunke, D. M., D. L. D. Caspar, and R. L. Garcea.** 1986. Self-assembly of purified capsid protein VP1. Cell **46:**895–904.
42. **Schneider, U., H. U. Schwenk, and G. Bornkamm.** 1977. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int. J. Cancer **19:**621–626.
43. **Schwarz-Albiez, R., and G. Moldenhauer.** 1989. Immunochemistry and epitope analysis using CD10, CD19, CD20, CD21, CD23, CD24, CD37, CD38, CD39 and CD40 mAb, p. 142–154. *In* W. Knapp et al. (ed.), Leukocyte typing IV. Oxford University Press, Oxford.
44. **Sinibaldi, L., P. Goldoni, V. Pietropaolo, C. Longhi, and N. Orsi.**

1990. Involvement of gangliosides in the interaction between BK virus and Vero cells. Arch. Virol. **113:**291–296.

45. **Staunton, D. E., V. J. Merluzzi, R. Rothlein, R. Barton, S. D. Marlin, and T. A. Springer.** 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell **56:**849–853.
46. **Szajnert, M.-F., S. Simon, G. W. Bornkamm, H. Wajcman, G. M. Lenoir, and J.-C. Kaplan.** 1987. Clustered somatic mutations in and around first exon of non-rearranged c-myc in Burkitt's lymphoma with t(8;22) translocation. Nucleic Acids Res. **15:**4553–4565.
47. **Takemoto, K. K., A. Furuno, K. Kato, and K. Yoshiike.** 1982. Biological and biochemical studies of African green monkey lymphotropic papovavirus. J. Virol. **42:**502–509.
48. **Takemoto, K. K., and T. Kanda.** 1984. Lymphotropic papovavirus transformation of hamster embryo cells. J. Virol. **50:**100–105.
49. **Tomassini, J. E., D. Graham, C. M. DeWitt, D. W. Lineberger, J. A. Rodkey, and R. J. Colonno.** 1989. cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is the intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA **86:**4907–4911.
50. **Verdin, E. M., G. L. King, and E. Maratos-Flier.** 1989. Characterization of a common high-affinity receptor for reovirus serotypes 1 and 3 on endothelial cells. J. Virol. **63:**1318–1325.
51. **Walter, J., P. Möller, G. Moldenhauer, V. Schirrmacher, M. Pawlita, and J. Wolf.** 1992. Local growth of a Burkitt's lymphoma *versus* disseminated invasive growth of the autologous EBV-immortalized lymphoblastoid cells and their somatic cell hybrids in SCID mice. Int. J. Cancer **50:**265–273.
52. **zur Hausen, H., and L. Gissmann.** 1979. Lymphotropic papovavirus isolated from African green monkey and human cells. Med. Microbiol. Immunol. **167:**137–153.
53. **zur Hausen, H., L. Gissmann, A. Mincheva, and J. F. Böcker.** 1980. Characterization of a lymphotropic papovavirus, p. 365–373. *In* M. Essex, G. Todaro, and H. zur Hausen (ed.), Viruses in naturally occuring cancers. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.