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Fast and High-Affinity Binding of B-Lymphotropic Papovavirus to Human B-Lymphoma Cell Lines

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Binding of B-lymphotropic papovavirus (LPV) to host cells differing in susceptibility to viral infection was determined by a newly established, direct, nonradioactive virus binding assay, which allows quantitative description of the binding characteristics by receptor saturation and Scatchard analysis. LPV binding to the highly susceptible human B-lymphoma cell line BJA-B K88 is specific, saturable, and noncooperative. Binding occurs very fast, with an association rate constant (k_1) of 6.7×10^7 M^{-1} s^{-1}, and is of high affinity, with a dissociation constant (K_d) of 2.9×10^{-12} M; and the virus-receptor complex is stable, with a half life of 70 min. The binding affinities of receptors on four other highly, moderately, or weakly susceptible human B-lymphoma cell lines were similar, with up to twofold variation around a mean K_d value of 3×10^{-12} M, suggesting the presence of the same LPV receptor on all of these cell lines. This view is further supported by the finding that in all cases a terminal sialic acid is necessary for LPV binding. Tunicamycin has been shown to drastically induce LPV susceptibility and LPV binding in weakly and moderately susceptible B-lymphoma cell lines (O. T. Keppler, M. Herrmann, M. Oppenländer, W. Meschede, and M. Pawlita, J. Virol. 68:6933–6939, 1994). The hypothesis that the constitutively expressed and tunicamycin-induced LPV receptors are identical is strengthened by our finding that both receptor types displayed the same high affinity. LPV susceptibility of different B-lymphoma cell lines was correlated with receptor number but not with receptor affinity. The numbers of receptors per cell on highly and moderately susceptible cell lines ranged from 2,000 to 400 and were directly proportional to LPV susceptibility. This indicates that the number of high-affinity receptors per cell is a key regulating factor for the LPV host range.

Attachment of virus particles to specific receptors on the plasma membrane is the initial event in the interaction of viruses with their host cells. The presence and functional state of such cellular receptors are one major determinant of the species and tissue tropism of many animal viruses (8, 10, 13, 27). Numbers of receptors per cell and dissociation constants (K_ds) as a parameter for virus-receptor affinity have been determined for many virus-host cell pairs (for a review, see reference 25) (see also Fig. 6 for examples). However, in many cases, the biological relevance of such numbers remains unclear. Interpretation is especially difficult in view of the huge variations between such pairs, e.g., receptor numbers per cell ranging from 9×10^2 for *Autographa californica* nuclear polyhedrosis virus on HZ 1075 insect cells (41) to 1.8×10^6 for Western equine encephalitis virus on mosquito mesenteronal epithelium cells (17) and K_d values ranging from 2.1×10^{-8} M for human enteric adenovirus type 41 receptor on human epidermoid carcinoma HEp-2 cells (43) to 5×10^{-13} M for Venezuelan equine encephalitis virus on mouse macrophage-like BW-J-M cells (18).

The B-lymphotropic papovavirus (LPV) displays a highly restricted host range for human cells in tissue culture with tropism exclusively for a subset of human B-lymphoid cells mostly derived from Burkitt's lymphomas. LPV, originally isolated from African green monkey lymph node cultures (44), is a monkey polyomavirus related to simian virus 40 (SV40) and to mouse polyomavirus. The presence of a constitutively expressed LPV receptor function on the surface of several human hematopoietic cell lines correlates highly with susceptibility to infection, indicating that this cell surface receptor is at least a major determinant restricting the LPV host range and regulating tropism (14). The biochemical nature of the LPV receptor has not yet been clearly identified; however, the data generated thus far indicate a glycoprotein core with most likely an O-linked α-2,6-sialylated carbohydrate as an essential component of the LPV receptor (21).

LPV binding to host cells has been studied previously by a nonradioactive, highly sensitive indirect assay which is based on the determination by specific enzyme-linked immunosorbent assays (ELISA) of the major structural protein VP1 in the supernatant remaining after removal of cell-bound virus. This allowed the measurement of relative virus-binding capacities of different cell lines under conditions of receptor excess. By this assay, it could be estimated that the number of virus-binding sites on susceptible uncloned BJA-B cells must be very low, i.e., in the order of 600 per cell. In the work presented here, we have modified the nonradioactive LPV binding assay so that binding can be measured directly and under conditions of virus excess. This allowed us to determine more precisely the number of virus-binding sites per cell as well as the K_ds for several cell lines and to test whether LPV susceptibility is correlated with one or both of these parameters. In addition, by using data from both receptor excess and virus excess assays, it was possible to describe quantitatively the kinetics of LPV receptor binding by determining the association rate constant (k_1), the dissociation rate constant (k_2), and the half-life ($t_{1/2}$), of the LPV-receptor complex on BJA-B K88 cells.

MATERIALS AND METHODS

Cells. Human cell lines BJA-B (B-lymphoma) (30), the recently isolated BJA-B subclones (21) K88 (highly susceptible for LPV infection) and K20 (weakly susceptible for LPV infection), Namalwa (Burkitt's lymphoma) (33), P3HR-1 (Burkitt's lymphoma) (16), and Raji (Burkitt's lymphoma) (9) were propagated

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as suspension cultures in glass Erlenmeyer flasks with 10% heat-inactivated fetal calf serum–2 mM glutamine–100 U of penicillin per ml–100 μg of streptomycin per ml in a humidified 5% CO_2 atmosphere.

Virus preparation and purification. LPV stocks were prepared from LPV (strain P12)-infected BJA-B cells (5). Infection was monitored by indirect immunofluorescence of the viral protein VP1 (14). Virus particles were extracted between days 6 and 9 postinfection by suspending the frozen pellet of washed cells in 1/20 of the original culture volume of hypotonic extraction buffer (14) and sonifying three times for 20 s at level 5 in a Branson sonifier 250. Cell debris was pelleted by centrifugation (10 min, 6,000 × *g*) and reextracted as described above. Extraction supernatants were stored at −20°C. This crude LPV extract could be used for indirect and direct binding assays. However, presumably because of aggregation, at high concentrations the extract gave some background in direct binding assays (see Fig. 1). Therefore, for later experiments, a concentrated, metrizamide gradient-purified LPV stock was prepared. Frozen pellets of LPV-infected BJA-B cells were thawed in 40 ml of extraction buffer (10 mM Tris [pH 7.5], 50 mM NaCl, 0.01 mM $CaCl_2$, 0.01% Triton X-100, 10 μg of aprotinin per ml) (11). LPV particles were extracted by sonication as described above. After centrifugation (20 min, 23,000 × *g*, 4°C), the supernatant was incubated with RNase and DNase I (14) and subsequently sedimentated (2.5 h, 180,000 × *g*, 20°C) through 50% sucrose onto an 80% metrizamide cushion. The recovered virus band was recentrifuged (20 h, 150,000 × *g*, 10°C) after adjustment to 43% metrizamide (34). Gradient fractions were analyzed by silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting (immunoblotting), and LPV VP1-specific ELISA.

LPV VP1-specific ELISA. For detection of LPV VP1, 50 ng of protein A column-purified LPV VP1-specific monoclonal antibody 456-1 was coated in 96-well polystyrene microtiter plates (NUNC, Wiesbaden, Germany) overnight at 4°C. Washing and blocking of residual free protein binding sites as well as the quantification of LPV VP1 by polyclonal rabbit LPV VP-specific antiserum and peroxidase-labeled anti-rabbit immunoglobulin H+L were performed as described previously (14). All samples were analyzed in triplicate. The detection level of this ELISA reached 25 pg of LPV VP1. The absolute protein concentration of the purified LPV preparation used as a standard was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany). To calculate the number of particles from VP1 ELISA determinations, we assumed that all VP1 was present in viral particles and that 360 VP1 molecules with a molecular mass of 40,000 Da formed one particle.

LPV binding assays. LPV binding was determined by nonradioactive virus-binding assays all based on the same principle. Cells in exponential growth phase were washed and incubated with virus at 37°C for 30 min as described previously (14). After sedimentation of cell-virus complexes, either the amount of remaining virus in the supernatant (indirect binding assay) or the amount of virus in the pellet (direct binding assay) was quantified by LPV VP1-specific ELISA.

(i) Receptor excess indirect binding assay. Serially diluted cells were incubated with a constant amount of LPV particles (corresponding to 800 pg of LPV VP1) (21).

(ii) Virus excess indirect binding assay. A constant number of cells (2×10^5/100 μl) was incubated with increasing amounts of LPV particles.

(iii) Virus excess direct binding assay. Saturation kinetic analyses were determined by a direct, nonradioactive binding assay. A constant number of cells (1.2×10^6) in phosphate-buffered saline (PBS) containing 0.2% gelatin was incubated for 30 min at 37°C with increasing amounts of LPV particles in Eppendorf tubes in a final volume of 600 μl. The virus cell suspension was centrifuged at 3,000 × *g* for 10 min through 500 μl of 8% (wt/vol) metrizamide onto a 100 μl of Ficoll-Paque cushion. The cell band was collected with a needle and syringe and resuspended in an equal volume of lysis buffer (10 mM Tris-HCl [pH 7.5], 50 mM NaCl, 0.01 mM $CaCl_2$, 0.01% Triton X-100). Cells were disrupted by sonification, and the amount of LPV VP1 in the resulting extract was quantified by VP1-specific ELISA.

Standard LPV infection assay. To determine the susceptibility of cell lines to LPV infection, 2×10^5 washed cells were resuspended in 1 ml of complete medium containing LPV stock virus (corresponding to 8 ng of VP1, with a multiplicity of infection of approximately 0.5). After incubation for 48 h, the relative frequency of LPV-infected cells was analyzed by indirect immunofluorescence microscopy for LPV VP1 as described previously (14).

Tunicamycin and sialidase treatment. The cells (3.3×10^5/ml) were incubated with 150 ng of the *N* glycosylation inhibitor tunicamycin (Boehringer Mannheim, Mannheim, Germany) per ml for 48 h at 37°C and analyzed for LPV binding as described previously (21). Terminal sialic acids were removed from cell surfaces by incubating 1.5×10^6 washed cells in 100 μl of PBS with 40 mU of *Vibrio cholerae* sialidase (Boehringer Mannheim) for 3 h at 37°C. To remove sialidase or tunicamycin prior to binding assays, the cells were centrifuged and suspended in binding assay buffer.

RESULTS

The characteristics of LPV binding to BJA-B K88 cells, which are a prototype for highly susceptible human B-lymphoma cell lines, were determined in detail with different methods and are summarized in Table 1.

TABLE 1. LPV binding characteristics on highly susceptible BJA-B K88 cells

Characteristic	Value
No. of virus binding sites (receptors) per cell	
Scatchard plot	1,875
Klotz plot	1,850
Affinity (K_d [M])	
Receptor excess binding assay[a]	2.5×10^{-12}
Virus excess binding assay[b]	2.9×10^{-12}
Kinetics	
k_1[c]	6.7×10^7 M^{-1} s^{-1}
k_2[d]	1.7×10^{-4} s^{-1}
$t_{1/2}$ of LPV-receptor complex (min)[e]	70

[a] Determined from the receptor concentration binding 50% of 2.3×10^{-13} M virus particles (Fig. 3).
[b] Determined from the Scatchard plot (Fig. 1C).
[c] Determined from the initial slope of the LPV binding curve (insert in Fig. 3) with a starting receptor concentration of 3.1×10^{-11} M and a starting virus concentration of 1.7×10^{-12} M corresponding to an 18-fold receptor excess. k_1 value is (moles receptors/liter × seconds)$^{-1}$.
[d] $k_2 = K_d \times k_1$.
[e] $t_{1/2} = \ln 2/k_2$.

Determination of LPV binding characteristics by Scatchard analysis. Equilibrium binding of LPV to BJA-B K88 cells in virus excess was determined by a direct, nonradioactive assay. Increasing amounts of LPV were incubated with a constant number of BJA-B K88 cells for 30 min at 37°C. It had been previously shown that binding equilibrium is reached under these conditions (14) (see also insert Fig. 3). Cell-bound virus was separated from free virus by low-speed centrifugation through a density step gradient and was quantified in the cell lysate by LPV VP1-specific ELISA. In initial mixing experiments it was established that ELISA sensitivity is not impaired by up to 2×10^6 cells per binding assay sample (data not shown). In addition, in direct binding assays under conditions of receptor excess, in general more than 90% of the LPV added bound to cells and no correction for a nonbinding virus fraction was made in the calculations. In the binding assay with unpurified virus preparations, some unspecific sedimentation of aggregated virus (2 to 3% of total virus added) occurred even in the absence of cells. This background was subtracted from the amount of total sedimented virus in the presence of cells to yield the amount of specifically bound virus (Fig. 1A), although values for numbers of receptor sites and K_ds determined for both total and specific binding differed only marginally (Fig. 1C).

Binding of LPV to BJA-B K88 cells measured by this direct assay was saturable (Fig. 1). Transformation of the saturation binding data by the method described by Klotz (23, 24) results in an S-shaped curve that is symmetric about the inflection point. With increasing concentrations of free virus, the curve asymptotically approaches a plateau at $n = 1{,}850$ virus particles bound per cell which is equal to the total number of binding sites per cell. The inflection point at $n_{1/2} = 950$ virus particles indicates half maximum binding where 50% of the virus binding sites per cell are occupied. Scatchard analysis (Fig. 1C) yields a linear plot, indicating a homogeneous set of noncooperative binding sites.

K_ds were calculated from the slope of the straight line. The K_d for specific LPV receptor binding is 2.9×10^{-12} M, indicating a high-affinity binding. The abscissa intercept of the Scatchard plot yields a result of 1,875 LPV receptors per BJA-B K88 cell, which is in good agreement with the result of

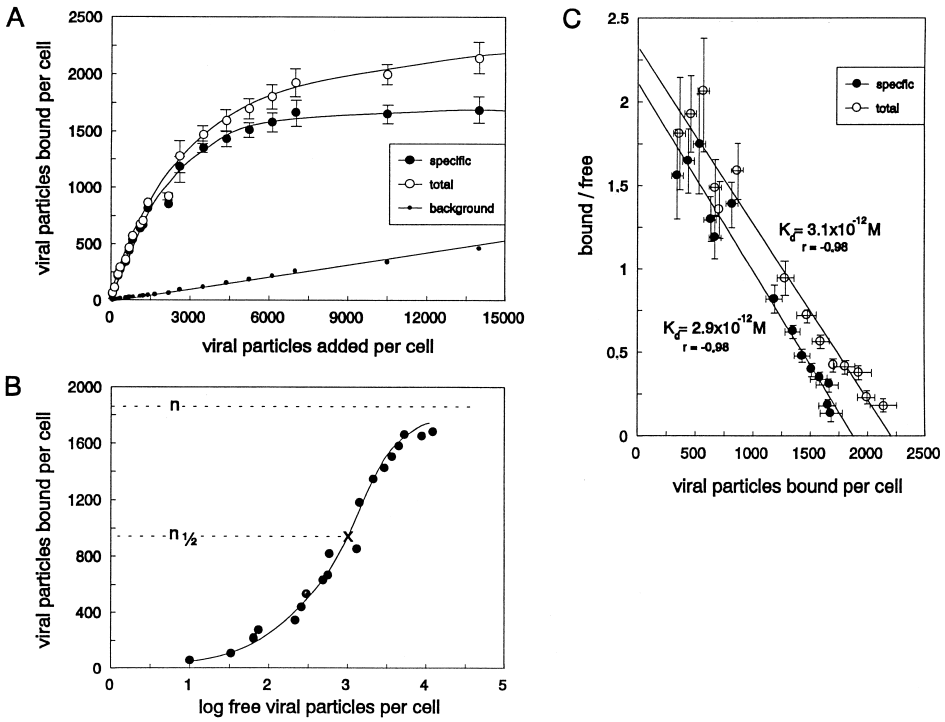

FIG. 1. Binding of LPV in excess to highly susceptible BJA-B K88 cells. A constant number of cells (1.2×10^6) was incubated with various amounts of LPV containing cell extract for 30 min at 37°C. Cell-bound virus was separated from free virus by low-speed centrifugation and was quantified by the LPV VP1-specific ELISA after lysis and hypotonic extraction of the cells (direct binding assay). (A) LPV binding data are presented graphically as the number of viral particles bound per cell versus the number of viral particles added per cell. Specific binding was calculated by subtracting background values, i.e., LPV sedimenting (presumably due to aggregation) in assays without cells, from total values sedimenting together with cells. Each datum point shown is the arithmetic mean ± standard deviation from at least three independent determinations. (B) Data from panel A for specific LPV binding are presented in a semilogarithmic graph (Klotz plot). The inflection point (x) indicates half-maximum binding, and $n_{1/2}$ and n indicate the numbers of virus particles bound at half and complete saturation, respectively. (C) Data from panel A for total and specific LPV binding were transformed according to Scatchard plotting with the ratio of bound to free LPV particles plotted against the number of bound viral particles per cell. The line of best fit was calculated by linear regression (regression coefficient $r = -0.98$, despite the high experimental variation for bound over free values [>1]); K_d was calculated from the slope of the straight line, and the number of LPV receptor sites per cell is equal to its intercept with the abscissa.

1,850 receptors determined by the method described by Klotz (Fig. 1B). In the following calculations, 1,850 will be used as the number of receptor sites per cell.

The saturation analysis determined by the direct binding assay was compared with that from the previously established indirect binding assay (14), in which the amount of virus remaining in the supernatant after incubation and low-speed centrifugation of cells is quantified by ELISA. The indirect assay could be performed reliably only with less than 2,000 LPV particles added per cell (Fig. 2). With higher virus excess, variation became too large. In the virus concentration range common to both assays, binding data determined by the two different methods did not differ significantly, thus supporting the validity of both determinations.

Determination of kinetic parameters of LPV binding. The Scatchard analysis as used here is based on the assumption that virus-receptor interactions are bimolecular, reversible reactions. It has been shown previously (14) that internalization of LPV particles is much slower than binding and therefore can be neglected in this analysis. This allows description in a first approximation of LPV attachment by the following kinetic equation:

$$R + V \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RV \qquad (1)$$

where R represents the free-receptor concentration, V represents the free-virus concentration, and RV represents the virus-receptor complex concentration. The forward and backward rate constants k_1 and k_2 were designated association rate and dissociation rate constants, respectively. K_d is defined by the following equation, where $R \cdot V$ designates the product of R and V:

$$K_d = \frac{k_2}{k_1} = \frac{R \cdot V}{RV} \qquad (2)$$

An alternative to the method described by Scatchard to determine the K_d has been described for poliovirus by Bibb et al. (4) and was used as follows. According to equation 2, the K_d value is equal to R when 50% of the virus added is bound. Figure 3 shows a receptor excess binding curve for LPV on BJA-B K88 cells analyzed by indirect binding experiments. The broken line indicates the cell concentration at which half of the virus added was bound by the cells. Assuming a total number of 1,850 binding sites per cell, one can deduce a K_d value of 2.5×10^{-12} M. This is in good agreement with the value of 2.9×10^{-12} M as determined by Scatchard analysis from direct binding assay data (Fig. 1C).

On the basis of kinetic equation 1, k_1 can be determined by using the receptor concentration and the initial slope of the line of a time course experiment (Fig. 3 [see legend for details]). Thus, the k_1 value for LPV on BJA-B K88 cells was calculated to be 1.2×10^{-8} ml cell^{-1} min^{-1} or, after transformation with respect to the number of receptor sites, k_1 was calculated to be 6.7×10^7 M^{-1} s^{-1}. From the equation $K_d = k_2/k_1$, k_2 was determined to be 1.7×10^{-4} s^{-1}, yielding a $t_{1/2}$ of 70 min for the LPV-receptor complex.

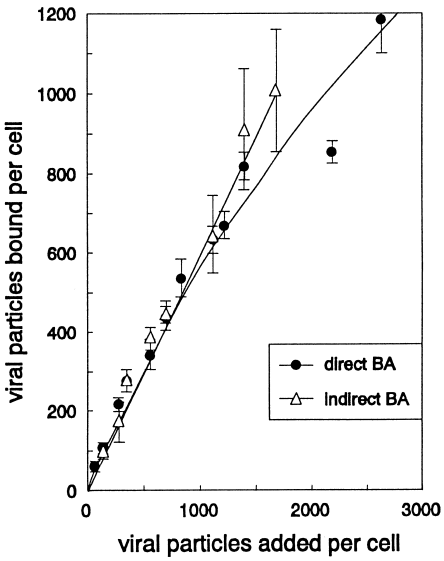

FIG. 2. Comparison of LPV binding in direct and indirect assays (BA). Specific binding to BJA-B K88 cells in direct binding assays was performed as described in the legend to Fig. 1. For indirect binding assays, the amount of free virus remaining in the supernatant after removal of cell-bound virus by low-speed centrifugation was quantified by VP1-specific ELISA. The amount of bound virus was calculated as the difference between free virus incubated without cells and free virus incubated with cells. Each datum point shown is the arithmetic mean ± standard deviation from at least three independent determinations. Under conditions of receptor excess (not more than 1,200 viral particles added per cell), the indirect binding assay shows results nearly identical to those for the direct binding assay.

LPV binding characteristics and susceptibility of B-lymphoma lines. Using the indirect binding assay, we recently established that the LPV binding capacities of different hematopoietic cell lines were positively correlated with susceptibility to LPV infection (14). To determine whether differences in binding capacities are due to differences in the numbers of receptors or affinity or both, several human B-lymphoma lines with different susceptibilities to LPV infection were analyzed by the quantitative methods described above. The susceptibility of these cell lines was determined in standard infection assays in parallel to the binding studies. Two days after infection, which reflects the first round of virus replication, BJA-B K88 cells, P3HR-1 cells, Namalwa cells, and Raji cells showed 8.6, 7.5, 3.8, and 1.8% LPV immunofluorescence-positive cells, (all values are arithmetic means of two independent infections).

The binding saturation curves for P3HR-1 and Namalwa cells are slightly sigmoidal, indicating a possible cooperativity in binding of LPV to the receptor (Fig. 4A). However, replotting the saturation data by the method described by Hill (15) for the first half of the curve yielded Hill coefficients (nH) of less than 1.2, which suggested that there is no significant cooperativity (data not shown). Scatchard analysis yielded about 2,000 LPV binding sites per P3HR-1 cell and about 1,100 binding sites per Namalwa cell and K_ds of 3.0×10^{-12} M for P3HR-1 cells and 6.8×10^{-12} M for Namalwa cells (data not shown). For Raji cells, 440 receptors per cell and a K_d of 5.5×10^{-12} M were determined. However, these numbers deduced from Scatchard analysis are of limited value, since the data points of only the five highest virus concentrations could be fitted on a straight line. Therefore, the binding parameters were also determined with Klotz plots (Fig. 4B) and receptor excess binding assay data (Fig. 4C). For P3HR-1 cells about 2,000 receptors per cell and a K_d of 1.8×10^{-12} M, were found, for Namalwa cells about 1,000 receptors per cell and a K_d of 4.8×10^{-12} M were found, and for Raji cells 360 receptors per cell with a K_d of 1.4×10^{-12} M were found. The corresponding values determined by these two different methods appear to be in good agreement with each other, and the K_d differences seen are assumed to be within the experimental error.

In addition to similar K_d values, the receptors on these three B-lymphoma lines also share sensitivity to sialidase treatment (Fig. 4D), which is taken as an indication that the LPV receptors on these cells as well as on BJA-B K88 cells carry a terminal sialic acid(s) essential for binding. These findings are consistent with the hypothesis that these receptors are similar or even identical molecules.

Similar affinities of constitutive and tunicamycin-inducible LPV receptors. Weakly LPV-susceptible BJA-B K20 cells can be rendered highly susceptible by pretreatment with the *N* glycosylation inhibitor tunicamycin (21). In standard infection experiments performed in parallel to the binding studies presented here, approximately 0.1% of untreated K20 cells and 8.0% of tunicamycin-pretreated K20 cells were LPV immunofluorescence positive.

Binding saturation curves (Fig. 5A) of untreated BJA-B K20 cells replotted by the method described by Klotz (Fig. 5D) indicated the presence of approximately 180 LPV receptor sites. A K_d of 3.3×10^{-12} M was determined according to the method described by Bibb et al. (4) and by using indirect binding assay data published previously (21) (Fig. 5B). Scat-

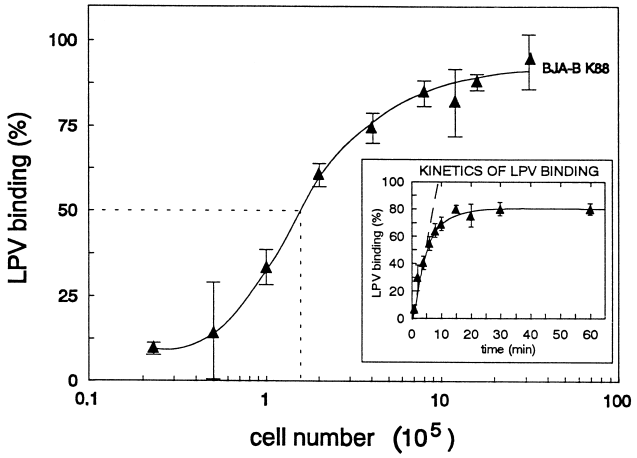

FIG. 3. Characteristics of LPV binding to highly susceptible BJA-B K88 cells determined by indirect binding assay with receptor in excess. A constant amount of LPV (corresponding to 800 pg of LPV VP1) was incubated with various numbers of cells for 30 min at 37°C. Virus binding was determined as described in the legend to Fig. 2. Values are the percentages of virus which have bound and are arithmetic means ± standard deviations from at least five independent determinations. The broken line indicates the number of cells binding 50% of the virus administered. The insert shows association kinetics of LPV binding on BJA-B cells under conditions of high receptor excess. LPV particles were incubated with BJA-B cells at 4°C, and, at the times indicated, LPV binding was determined by the indirect binding assay. The initial slope of the curve used for determination of k_1 is indicated by the broken line. When the concentration of receptor (R) is in large excess over the starting concentration of virus (V_0) (in our experiment, an 18-fold receptor excess was used), the receptor concentration can be assumed to remain constant during the binding assay. In addition, it is assumed that at the beginning of the binding experiment only negligible backward reaction takes place. The reaction rate is the change of the virus concentration (ΔV) over time (Δt), and under these conditions is equal to the association rate. Extrapolation of the initial slope of the binding kinetics curve gave a Δt value of approximately 8 min. This is the time needed to bind 100% of the virus ($\Delta V = V_0$). k_1 can then be calculated from the equation $k_1 = (\text{receptor concentration} \times \Delta t)^{-1}$.

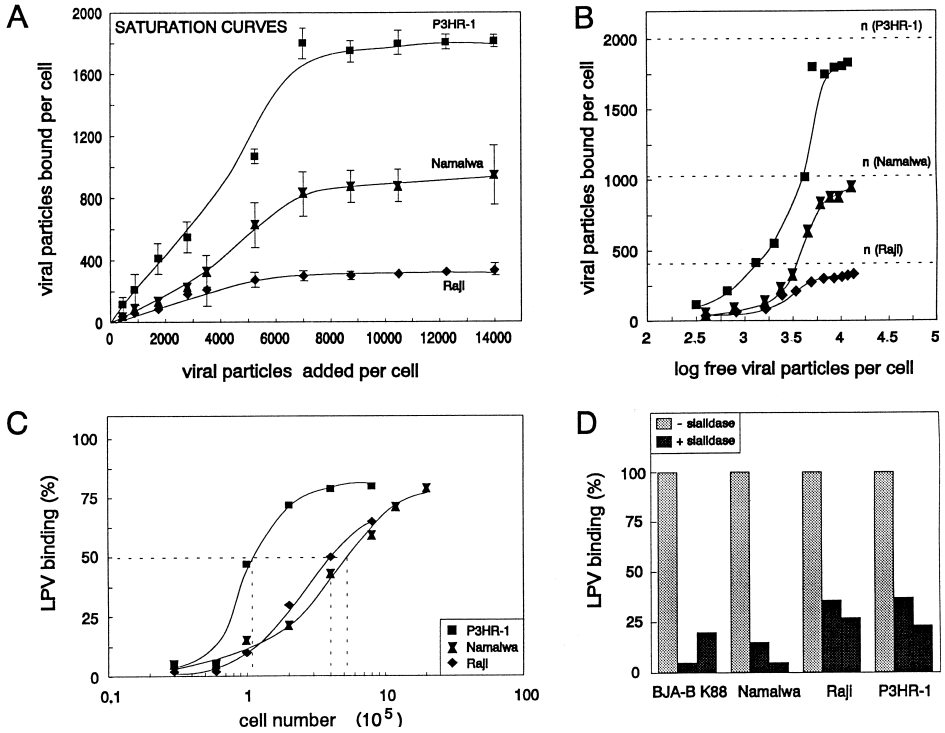

FIG. 4. Sialic acid-dependent LPV binding to human B-lymphoma-derived cell lines with different LPV susceptibilities. (A) Direct binding assaying under conditions of virus excess was performed with purified virus particles and was otherwise as described in the legend to Fig. 1A. Each datum point shown is the arithmetic mean ± standard deviation for at least three independent determinations. (B) Binding data from panel A transformed in a semilogarithmic graph (Klotz plot). The three broken lines indicate the total numbers of binding sites per cell (n) for the respective cell lines. (C) Indirect binding assay under conditions of receptor excess performed as described in the legend to Fig. 3. Values given are percentages of virus bound and are data from two independent determinations. The broken lines indicate the numbers of cells binding 50% of the virus administered. (D) To test the sialic acid dependence of LPV binding, washed cells were incubated in PBS with or without *V. cholerae* sialidase. Sialidase was removed by centrifugation of the cells, and direct binding was assayed with 40 ng of LPV VP1. Binding of sialidase-treated cells relative to binding of untreated cells (100%) is shown from two independent experiments.

chard analysis could not be fitted on a straight line. Tunicamycin-pretreated K20 cells showed a binding saturation graph (Fig. 5A) similar to that for highly susceptible K88 cells, and Scatchard analysis resulted in 1,650 LPV receptor sites and a K_d value of 3.1×10^{-12} M (Fig. 5C). These values are nearly identical to those determined for highly susceptible BJA-B K88 cells and further support the hypothesis of identity of tunicamycin-induced and constitutively expressed LPV receptors on BJA-B subclones.

DISCUSSION

The presence of a functional cell surface receptor for LPV has been recognized as one or perhaps even the most important factor determining the highly restricted host range and tropism of this virus (14, 21, 22). We have modified the nonradioactive binding assay used previously and describe here the binding kinetics and the interaction between LPV and the receptor for highly and weakly susceptible cells in quantitative terms.

As a prototype for a highly susceptible human B-lymphoma cell line, BJA-B K88 cells were studied most extensively. The number of virus binding sites (receptors) per cell was determined from binding assay data with virus excess by using two independent mathematical procedures, i.e., the method of Scatchard (35) and data transformation as described by Klotz (23, 24). The assumption of a monovalent ligand-receptor interaction is inherent in all mathematical procedures used here as well as in all previous studies of other virus-receptor interactions. This may very well be a simplified view of the complex interaction of a repetitive virus surface structure with probably multiple cell surface molecules forming a virus binding site. The term affinity is conveniently used and is also used here to describe this interaction, although avidity probably would be a more appropriate term. Overall, the simplifying mathematical models applied here allow a first approximation of the binding parameters.

With 1,875 and 1,850 receptors per cell, respectively, both methods gave very similar results. The K_d was derived independently from virus excess assays together with data transformation according to the method described by Scatchard and from receptor excess assays with data analysis as described by Bibb et al. (4). The K_d values of 2.9×10^{-12} M and 2.5×10^{-12} M resulting from these two assays are in remarkably good agreement. Thus, the figures determined for the number of LPV receptors on a BJA-B K88 cell as well as for the K_d of the virus-receptor interaction appear to be fairly well substantiated.

Receptor numbers and affinities have been determined for several animal viruses (Fig. 6 gives examples and references). Receptor numbers vary over 3 orders of magnitude, from as little as 3,000 poliovirus receptors on HeLa cells (26) to as much as 1.8×10^6 receptors for Western equine encephalitis virus on mosquito mesenteronal epithelium (17). K_ds vary over 5 orders of magnitude (Fig. 6). Compared with these data, the binding of LPV to susceptible human B-lymphoma cells is characterized by high receptor affinity and low receptor number. The K_d of 2.9×10^{-12} M is among the highest described

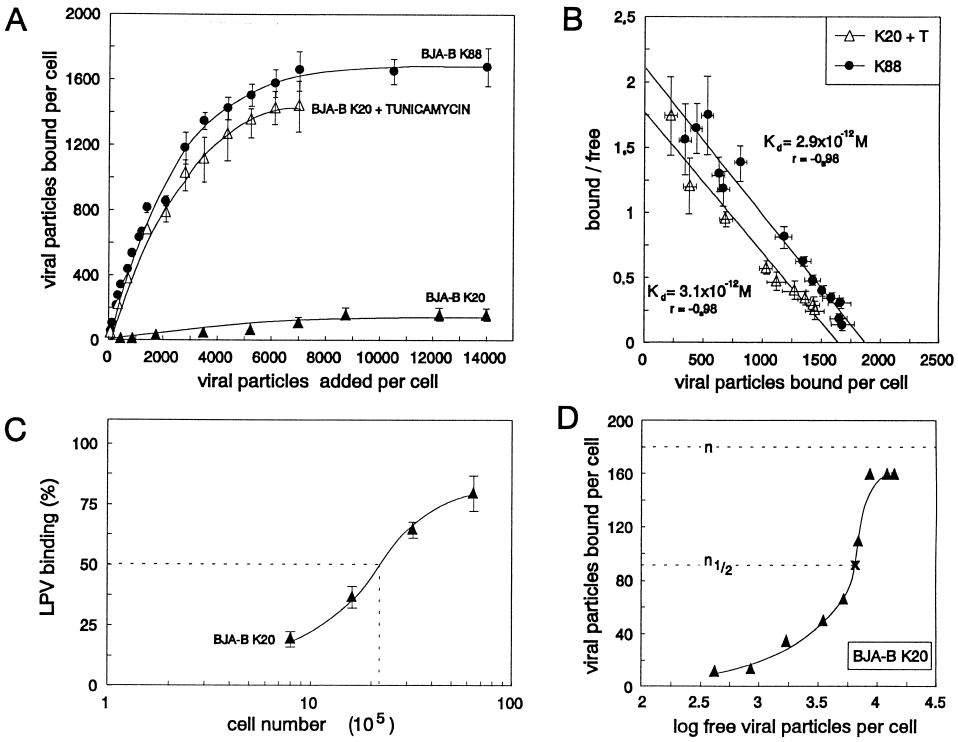

FIG. 5. LPV binding to tunicamycin-induced receptors. Specific binding of LPV to weakly susceptible BJA-B K20 cells and to tunicamycin-pretreated BJA-B K20 cells, which are rendered highly susceptible by this treatment, was determined by direct binding assays with virus in excess as described in the legend to Fig. 1. Binding data of highly susceptible BJA-B K88 cells from Fig. 1 are shown to facilitate comparison. Each datum point is the arithmetic mean ± standard deviation from at least three independent determinations. Data are presented as numbers of viral particles bound per cell plotted against numbers of viral particles added per cell (A) and are transformed into a Scatchard plot (B). The K_d and the number of receptor sites of tunicamycin-pretreated BJA-B K20 cells were determined by the method described by Scatchard. For untreated K20 cells, no straight line could be fitted in a Scatchard plot (not shown). The number of receptors per cell (n = 180, indicated by upper broken line) was determined from a Klotz plot (D) by using the saturation data shown in panel A. (C) LPV receptor excess binding curve as described in the legend to Fig. 3. The broken lines indicate the number of cells (2.2×10^6) binding 50% of the virus administered. The data from panels C and D were used to calculate the K_d of the K20 receptor.

so far for animal virus particles, whereas the receptor number is the lowest for a susceptible cell line.

SV40 is another primate polyomavirus which differs in host range and tropism from LPV and binds to an apparently nonsialylated receptor (7). The affinities of LPV (K_d of 2.9×10^{-12} M) and SV40 (K_d of 3.8×10^{-12} M) (6) for their respective receptors are similar, whereas the SV40 receptor is 45 times more abundant.

The LPV receptors on four human B-lymphoma lines (BJA-B K88, Namalwa, P3HR-1, and Raji) differing in susceptibility to LPV were found to share two characteristics as follows: (i) terminal sialic acid(s) is essential since LPV binding is sensitive to *V. cholerae* sialidase digestion of the host cell surface and (ii) the K_ds of the virus-receptor complexes are similar, with as much as twofold variation around a mean value of 3×10^{-12} M. This indicates that LPV might bind to the same receptor on all four human B-lymphoma lines. In addition, both the residual receptors on weakly susceptible BJA-B subclone K20 and the tunicamycin-induced and sialidase-sensitive K20 receptors have K_ds that are nearly identical to those for the receptors on the highly susceptible BJA-B subclone K88, indicating that these receptors might also be the same molecules.

We have previously shown that the LPV susceptibility of a cell line is positively correlated with its LPV binding capacity (14). The quantitative data now allow further analysis of this correlation with respect to receptor number and affinity. When LPV susceptibilities are plotted against the receptor numbers of the different cell lines tested, a significant linear dependence of these two parameters is evident (Fig. 7A). Susceptibility appears to be directly proportional to receptor number. However, untreated BJA-B K20 cells do not fit in this linear correlation. This may indicate that the residual receptors on these cells are largely nonfunctional. Alternatively, a threshold level of receptors per cell might be necessary for infection, and for LPV and BJA-B cells, between 200 and 300 receptors per cell might be the minimum number to allow infection. In contrast to the number of receptors, the K_ds of the various cell lines appears not to be correlated with susceptibility (Fig. 7B).

In our view, the number of receptors is the most important factor regulating the LPV host range on human B-lymphoma cells. A similar observation was made for *A. californica* nuclear polyhedrosis virus infection of insect cell lines (41). Highly and weakly susceptible cells displayed virus receptors with nearly identical K_ds; however, the numbers of receptors differed by a factor of 15 (see also Fig. 6).

We have been able to determine kinetic parameters for the binding of LPV to its cell surface receptor. To our knowledge, only these data, together with the data published recently for poliovirus and its receptor (4), are available at present to describe the kinetics of animal virus-receptor interactions and to compare them with interactions of other biologically relevant macromolecules. The $t_{1/2}$ of the LPV-receptor complex was found to be about 70 min. This is 15 times longer than the value of 4.5 min for the poliovirus-receptor complex on HeLa cells (4) and indicates a rather stable interaction. Using the

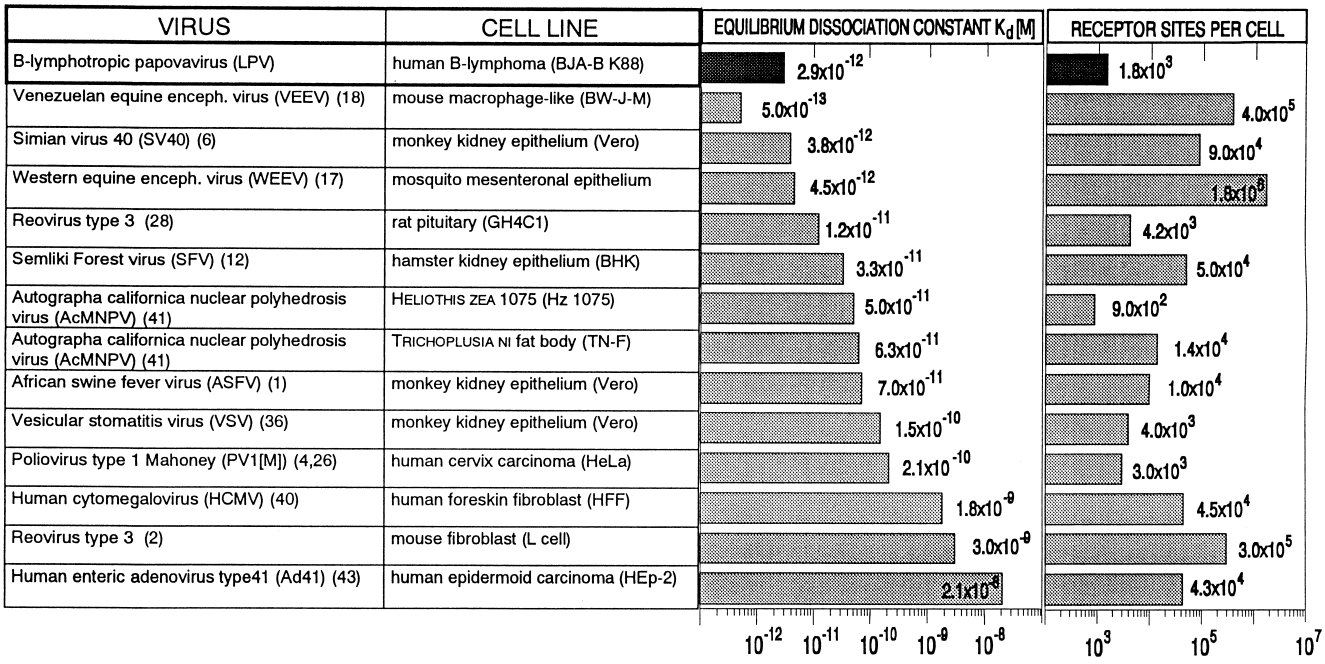

FIG. 6. Binding characteristics of animal virus-receptor interactions. The K_ds and the average numbers of receptor sites per cell determined for LPV binding to BJA-B K88 cells are shown at the top in boldface. The corresponding values published for 13 other virus particle-host cell pairs are given for comparison.

equation $t_{1/2} = \ln 2/k_2$ and published data, we have calculated the $t_{1/2}$s of some other complexes for comparison. The $t_{1/2}$s of hormone-receptor complexes can vary from 11 min for gastrin with its receptor on rat oxyntic gland mucosa cells (37) to nearly 120 h for ovine prolactin and rabbit mammary epithelial cells (39). Three complexes of monoclonal antibodies with their respective antigens, DNA (3), the lipoprotein apoC-I (42), and the differentiation antigen Thy-1 on rat thymocytes (29), all have $t_{1/2}$s of approximately 2 h.

The k_1s of protein molecules to form dimers or larger complexes are typically in the range of 0.5×10^6 to 5×10^6 M^{-1} s^{-1} (31). For example, the association of ^{125}I-Tyr-11-labeled-somatostatin with its receptor on rat pituitary tumor cells occurs with a k_1 of 2.8×10^6 M^{-1} s^{-1} (32); the association of hemoglobin dimers to form tetramers has a k_1 of 4×10^5 to 6×10^5 M^{-1} s^{-1} (19, 20). The k_1s for virus-receptor binding were found to be 6.7×10^7 M^{-1} s^{-1} for LPV and 1.2×10^7 M^{-1} s^{-1} for poliovirus (4). Apparently, the association of a whole virus particle with its cellular receptors occurs considerably faster than most protein oligomerization processes or antibody-antigen reactions. The fast association reaction of viruses could be a consequence of the highly repetitive nature of the receptor binding site on the surface of the particle. For example, the structure of murine polyomavirus complexed with an oligosaccharide receptor fragment shows 360 binding sites per particle (38). These repeated binding sites in the correct and fixed docking orientation probably cover more of the particle surface than a single docking area on a monomeric protein and could result in a faster association rate. In other, more simple words, in whatever orientation the virus meets the cell, at least one of the many docking sites is always looking toward the cell surface receptors.

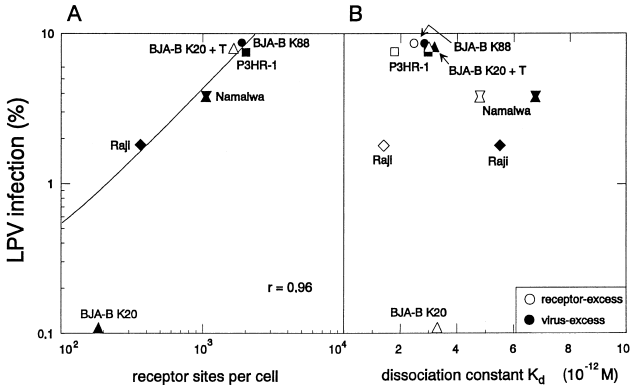

FIG. 7. Correlation of LPV susceptibility and receptor number on human B-lymphoma cell lines. (A) The frequency of LPV-infected cells 2 days after standard infection is given as a parameter of susceptibility; data are arithmetic means of two independent infections. Susceptibility was plotted against the number of LPV receptors per cell determined in virus excess binding assays (see Fig. 1, 4, and 5). A linear regression line ($r = 0.96$) with slope 1 can be fitted for the five LPV-susceptible cell lines, including tunicamycin-pretreated BJA-B K20 cells (BJA-B K20 + T), but not for untreated, weakly susceptible BJA-B K20 cells. (B) The susceptibility data presented in panel A were plotted against the K_ds determined from Scatchard analysis of virus excess assays or from receptor excess assays. No correlation of susceptibility and affinity can be detected.

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