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Regulation of Susceptibility and Cell Surface Receptor for the B-Lymphotropic Papovavirus by N Glycosylation

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The host range of the B-lymphotropic papovavirus (LPV) in cultured human cells is limited to a few B-lymphoma-derived cell lines. The constitutively expressed cell surface receptor for the virus is a major determinant restricting the LPV host range (G. Haun, O. T. Keppler, C. T. Bock, M. Herrmann, H. Zentgraf, and M. Pawlita, J. Virol. 67:7482–7492, 1993). Here we show that human B-lymphoma cells with low-level susceptibility are rendered highly susceptible to LPV infection by pretreatment with the N glycosylation inhibitor tunicamycin but remain nonsusceptible to infection by the related polyomavirus simian virus 40. Among the selective N glycosylation processing inhibitors, deoxymannojirimycin, but not deoxynojirimycin, swainsonine, or castanospermine, could mimic the effect of tunicamycin. Tunicamycin treatment also induced a drastic enhancement of the cells' LPV-binding capacity, indicating that the induction of LPV susceptibility might be mediated by an increase in the number of functional cell surface receptors and/or by increased receptor affinity. Sialidase sensitivity of the tunicamycin-induced LPV receptor showed that oligosaccharides carrying terminal sialic acids are necessary for binding and are likely to be O linked. The constitutive LPV receptor is also sialic acid dependent, which points to a possible identity with the sialic acid-dependent tunicamycin-induced LPV receptor. We conclude that removal or modification of certain N-linked oligosaccharides in human B-lymphoma cells can enhance expression or functional activity of the sialylated LPV receptor.

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 viruses. Examples are CD4 on T lymphocytes and macrophages for human immunodeficiency virus type 1 (2), the complement C3d receptor CR2 (CD21) on B lymphocytes for Epstein-Barr virus (EBV) (7), the intercellular adhesion molecule 1 (ICAM-1) for the major subgroup of rhinoviruses (10, 24, 26), and aminopeptidase N (CD13) on pig small intestine epithelial cells for the transmissible gastroenteritis virus (3).

The host range of the African green monkey B-lymphotropic papovavirus (LPV), a primate polyomavirus with about equal sequence homology to simian virus 40 (SV40) and mouse polyomavirus (19), is highly restricted in cultured human cells. Productive LPV infection could only be obtained in some Burkitt's lymphoma-derived cell lines and, very inefficiently, in a few EBV-immortalized human B-lymphoblastoid cells (21, 25, 28, 29). The presence of a constitutively expressed LPV receptor function on the surface of several human hematopoietic cell lines correlates with susceptibility to infection, indicating that this cell surface receptor is at least a major determinant restricting the LPV host range (12). Recently, we have isolated subclones of the human B-lymphoma line BJA-B which differ greatly in receptor expression and concomitantly in susceptibility to LPV infection (20). The molecule(s) forming the receptor has not yet been identified. The trypsin and sialidase sensitivity of LPV binding and infection suggests the involvement of proteinaceous and sialylated components in the LPV receptor (12).

Oligosaccharide chains on glycoproteins can play an important role in differentiation and cell recognition. They can be N linked to asparagine residues or O linked to serine and threonine residues. For example, many cells derived from the human hematopoietic system express leukosialin (CD43), a glycoprotein with many O-linked oligosaccharides in which the specific structure of the oligosaccharides is thought to be characteristic for each cell lineage and maturation stage (9, 22). In contrast to being a necessary component in several defined molecular functions, oligosaccharides may also inhibit function by masking sites required for receptor-ligand interactions or by altering the secondary structure of such sites. For example, the removal of terminal sialic acids from serum glycoproteins allows recognition of these asialoglycoproteins by a galactose-specific lectin on hepatocytes, leading to their cellular uptake and degradation (1). Also, cleavage of certain influenza virus hemagglutinins can be inhibited by an oligosaccharide side chain located in the vicinity of the cleavage site between hemagglutinin 1 (HA1) and HA2 (13). More recently, it has been shown that inhibition of N-linked glycosylation or point mutation of a single N glycosylation site of an ecotropic murine leukemia virus receptor resulted in activation of the receptor function (4, 27).

Proteins are N glycosylated by an ordered sequence of catalytic steps in the endoplasmic reticulum and the Golgi apparatus. A variety of N glycosylation inhibitors, which selectively interfere with different steps of biosynthesis and processing of N-linked oligosaccharides and thereby can impair the functions of N-glycosylated proteins, are available as tools to analyze glycoprotein functions and structures (reviewed in

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reference 5). Here we describe that inhibition of cellular glycoprotein N glycosylation by tunicamycin as well as blocking of the formation of complex and hybrid N oligosaccharide chains by deoxymannojirimycin (dManNoj) in human B-lymphoid cell lines enhanced LPV receptor function, conferring increased virus binding and susceptibility to infection. In addition, we demonstrate that both the tunicamycin-induced and the constitutive LPV receptors expressed on LPV-susceptible cell lines are sialic acid dependent, which points to a possible identity of these receptors.

MATERIALS AND METHODS

Cell lines. The human B-lymphoma cell line BJA-B; the Burkitt's lymphoma cell lines Namalwa, Raji, BL60, and Daudi (15); the EBV-immortalized B-lymphoblastoid cell line IARC 277 (16); the T-cell leukemia lines Jurkat and MT-4 (11); the chronic myelogenous leukemia line K562 (17); and the promyelocytic leukemia line HL60 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 lines Vero and TC7 were kept as monolayer cultures in plastic tissue culture bottles in Dulbecco's modified Eagle's medium with the same supplements and incubation conditions. (For other original references on cell lines, see reference 12.)

BJA-B subclones differing in susceptibility to LPV infection were isolated by limiting-dilution culture in 96-well tissue culture plates, and subcultures were subsequently infected with LPV. Cultures of BJA-B subclones which contained less than 0.1% LPV-infectable cells were designated nonsusceptible (K6, K9, K20 and K138) (20).

Viruses. Stocks of LPV and SV40 were prepared from infected BJA-B and TC7 cells, respectively, as described previously (12).

LPV binding and infection. LPV binding was determined by an indirect, nonradioactive virus-binding assay essentially as described previously (12). Pretreated cells were washed, serially diluted in phosphate-buffered saline (PBS) containing 0.2% gelatine, and incubated in 96-well tissue culture plates in a final volume of 200 μl with a constant amount of LPV particles (corresponding to 800 pg of LPV VP1) for 30 min. Analysis of binding kinetics had shown that under these conditions, equilibrium was reached. After low-speed sedimentation of cells (400 × *g*), the amount of unbound LPV VP1 in the supernatant was quantitated by enzyme-linked immunosorbent assay (ELISA) (12).

To determine the susceptibility of cell lines to LPV infection, 10^6 cells were washed and resuspended in 1 ml of medium containing 200 μl of LPV stock virus (corresponding to about one infectious unit per cell). Cells were exposed to LPV at 37°C for 2 h, 2 ml of medium was added, and cells were cultured for 48 to 70 h. The number of LPV-infected cells was determined by indirect immunofluorescence microscopy staining for LPV T and VP antigens as described previously (12). To determine the amount of viral antigen produced in infected cultures, cell pellets were extracted in a hypotonic lysis buffer and LPV VP1 antigen relative to total protein content in the extract was quantitated by ELISA (12, 14).

SV40 infection. SV40 infection in Vero and BJA-B cells was monitored by two methods. Expression of SV40 large T antigen and structural protein VP1 was analyzed by indirect immunofluorescence staining using monoclonal antibodies KT-3 with specificity for the carboxy-terminal undecapeptide of the SV40 large T antigen (18) and SV1-3H9 specific for SV40 VP1 (kind gift of F. Mehnert, Bochum, Germany), respectively. Cells in which viral DNA was replicating were detected by filter in situ hybridization (8) with a molecularly cloned SV40 genome as probe. Briefly, washed cells were resuspended in PBS and filtered onto nitrocellulose with a Makrofold apparatus (Schleicher and Schuell, Dassel, Germany). Cellular DNA was denatured for 5 min in 1.5 M NaCl–0.5 M NaOH and subsequently neutralized in 1.5 M NaCl–0.5 M sodium citrate–0.5 M Tris-HCl (pH 7.5). After being heated for 30 min at 80°C, the nitrocellulose filters were prehybridized and then hybridized overnight at 42°C with a ^{32}P-radiolabelled SV40 DNA probe. Filters were washed three times in SSC buffer (1× SSC is 0.15 M NaCl plus 1.5 mM sodium citrate) containing 0.2% (wt/vol) sodium dodecyl sulfate at 68°C and then analyzed by autoradiography.

For transfection, 2×10^6 washed BJA-B cells were incubated with 5 μg of Hirt-extracted and cesium chloride gradient-purified SV40 DNA in 1 ml of RPMI 1640 containing 500 μg of DEAE-dextran per ml. After 30 min, cells were washed and subsequently cultured for 2 days.

N glycosylation inhibitor treatment. Stocks of two batches of tunicamycin (Boehringer Mannheim) which differed in growth-inhibiting activity were prepared. Batch 1 was used in all experiments except binding assays and had a 7.5-fold-lower specific activity than batch 2. dManNoj, 1-deoxynojirimycin (dNoj), swainsonine, and castanospermine were all obtained from Boehringer Mannheim, dissolved in PBS, and stored at −20°C. For inhibitor pretreatment, cells in the logarithmic growth phase were pelleted and resuspended in medium (3 to 5×10^5 cells per ml) supplemented with either inhibitor or PBS and were cultured in 48-well plastic dishes (NUNC, Wiesbaden, Germany) or Erlenmeyer glass flasks. After 48 h of treatment, cells were counted and cell viability was determined by trypan blue exclusion. Viable cells (10^6) were washed in PBS and analyzed further for virus binding or infection. Only preparations with less than 25% dead cells were used to test the cells' LPV-binding capacity.

Sialidase treatment. Cells (1.5×10^6) were washed in PBS and then exposed to either sialidase (neuraminidase or acylneuraminyl hydrolase; EC 3.2.1.18) from *Vibrio cholerae* (Boehringer Mannheim) (20 mU in 0.1 ml of PBS) or PBS alone. After 2 h at 37°C, cells were washed with precooled PBS at 4°C and kept at this temperature until further testing to prevent endogenous resialylation.

RESULTS

Tunicamycin enhances LPV susceptibility in BJA-B cells. We have shown previously that the LPV receptor on BJA-B cells is sialic acid dependent (12). To further characterize the oligosaccharide moiety of the receptor, experiments with glycosylation inhibitors were carried out.

The N glycosylation inhibitor tunicamycin blocks the first step in glycoprotein N glycosylation, i.e., the transfer of dolichol phosphate-linked oligosaccharides to the amido group of asparagine residues in the endoplasmic reticulum (5). BJA-B cells were pretreated with 1.2 μM tunicamycin for 48 h and exposed to LPV, and the degree of infection was quantitated 2 days postinfection, which allows only one viral replicative cycle. LPV infection was analyzed by determining the number of LPV capsid antigen-producing cells by indirect immunofluorescence and by ELISA quantitation of the LPV VP1 synthesized in culture. Contrary to expectation, tunicamycin pretreatment increased LPV susceptibility. Greater numbers of LPV-infected cells were found in parental, uncloned

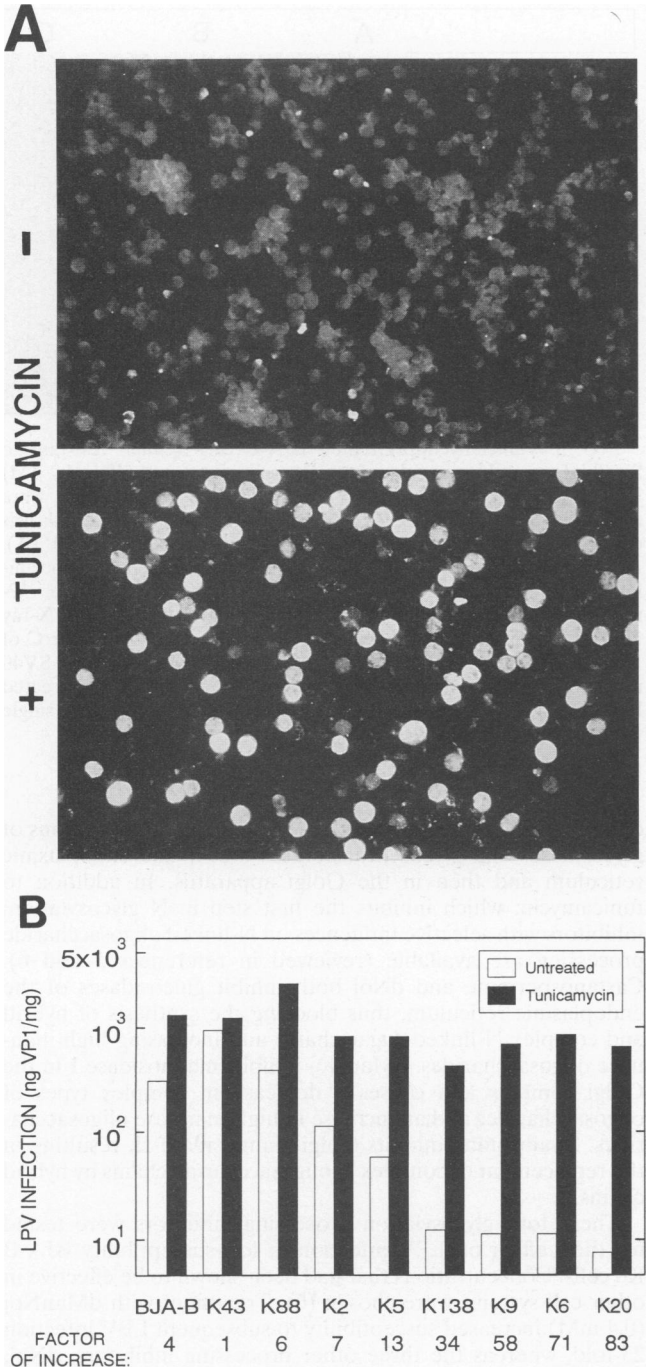


FIG. 1. Tunicamycin pretreatment induces LPV susceptibility in BJA-B cells. Cells were cultured in the absence or presence of 1.2 μM N glycosylation inhibitor tunicamycin for 48 h and were subsequently infected with LPV. Cultivation after LPV infection proceeded for 48 h, which allows one cycle of virus replication. (A) LPV VP-specific immunofluorescence of the low-susceptibility BJA-B subclone K20, showing the drastic tunicamycin-induced increase in the number of LPV-infected cells. +, tunicamycin-treated cells; −, untreated cells. (B) The extent of LPV infection in uncloned parental BJA-B cells and in BJA-B subclones differing in constitutive LPV susceptibility (K2, K5, K6, etc.) is shown as the amounts of viral antigen VP1 produced per milligram of protein in extracts of infected cells. The arithmetic mean from three to six experiments is given; the standard deviation (not shown) ranged from 3 to 23% of the mean. Numbers at the bottom for each subclone show the factor of increase, which was calculated as the ratio of VP1 produced in tunicamycin-treated cultures to VP1 produced in untreated cultures.

BJA-B cells as well as in a series of BJA-B subclones with large differences in constitutive LPV susceptibility. In low-susceptibility subclone BJA-B K20, for example, the fraction of LPV-infected cells rose from less than 0.1% in untreated cells to approximately 40% in tunicamycin-pretreated cells (Fig. 1A). The increased susceptibility was also reflected in the increased production of viral antigen. In uncloned parental BJA-B cells, virus production rose approximately fourfold from 300 to 1,300 ng of VP1 per mg of extracted protein (Fig. 1B). In low-susceptibility subclones K138, K9, K6, and K20, LPV antigen production was enhanced 34- to 83-fold and reached the level of that in untreated parental BJA-B cells and high-susceptibility subclones K88 and K43. In these high-susceptibility subclones, tunicamycin pretreatment also enhanced LPV antigen production. A maximum was reached in tunicamycin-treated K88 cells, with LPV VP1 making up approximately 0.3% of the total extractable cellular protein.

Tunicamycin-induced enhancement of LPV susceptibility is dose and time dependent. To establish a dose-response curve, cells of the low-susceptibility BJA-B subclone K6 were exposed to various tunicamycin concentrations for 66 h before LPV infection. LPV synthesis increased in a dose-dependent manner from 11 ng of VP1 per mg of total protein in untreated cells and reached a plateau of 1,000 to 1,500 ng of VP1 per mg of total protein with tunicamycin concentrations of 1.2 μM or higher. This level of LPV susceptibility was higher than the level in untreated parental BJA-B cells (Fig. 2a). A fivefold enhancement of LPV infection could already be detected with 0.1 μM tunicamycin. At a tunicamycin concentration of 1.2 μM, about 25% of the K6 cells stained LPV VP positive by indirect immunofluorescence whereas untreated K6 cells displayed only about 1 LPV VP-positive cell per 2,000 total cells (data not shown).

Tunicamycin enhancement of susceptibility became apparent with 1.2 and 2.4 μM tunicamycin after a pretreatment time of 20 h, which corresponds to nearly one cell-doubling cycle. After this period, 10% of the maximal level of LPV infection was reached. The level of infection increased further with pretreatment times of up to 72 h (Fig. 2b).

Cell proliferation was significantly affected at higher tunicamycin concentrations (see Fig. 4b), and, in addition, concentrations above 3 μM were cell toxic. A tunicamycin concentration of 1.2 μM was used in subsequent experiments, since this concentration strongly enhanced LPV infection and inhibited cell proliferation only by a factor of 3.

Tunicamycin-induced susceptibility of BJA-B cells is specific for LPV. To determine if tunicamycin acted in some nonspecific manner to promote virus infection in BJA-B cells, infection by SV40, another polyomavirus with a high degree of sequence homology to LPV (19) but a completely different host range, was analyzed. When BJA-B subclone K6 cells pretreated with tunicamycin (48 h, 1.2 μM), as well as untreated BJA-B cells, were infected for 2 days with SV40 virions, indirect immunofluorescence revealed no signs of expression of early (T antigen) and late (VP1) SV40 genes (data not shown). Also, filter in situ hybridization, which allows the detection of single virus-replicating cells among a large majority of uninfected cells (8), showed no evidence for productive SV40 infection in untreated or tunicamycin-pretreated BJA-B cells, whereas SV40-infected Vero cells were readily detectable (Fig. 3). In control experiments, DEAE-dextran-mediated

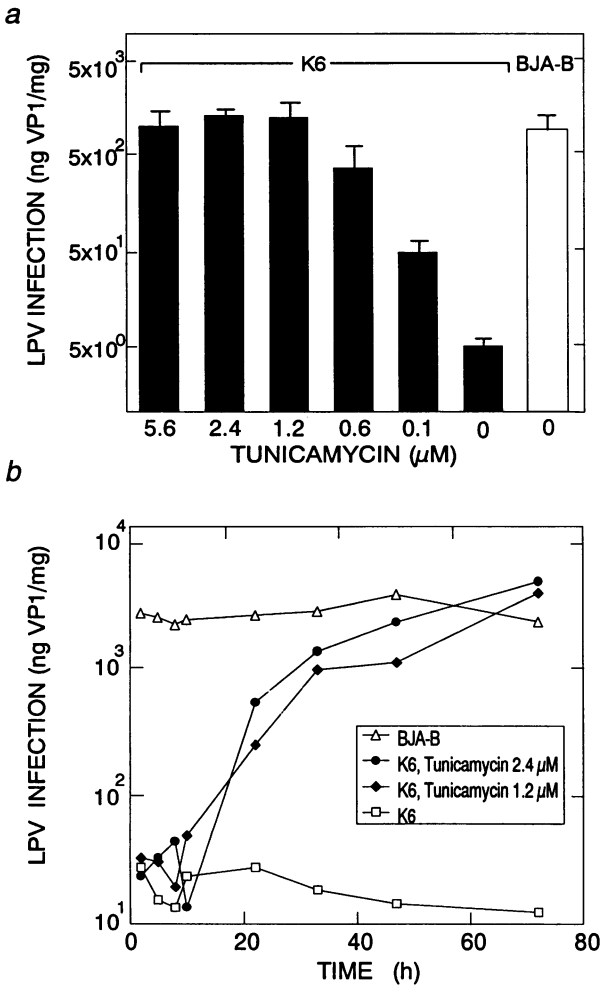


FIG. 2. Dose (a) and time (b) dependence of tunicamycin-induced LPV susceptibility. (a) Low-susceptibility BJA-B subclone K6 cells (filled columns) were exposed to the indicated concentrations of tunicamycin for 66 h, washed with PBS, and infected with LPV for 48 h. The extent of LPV infection was determined by quantitating the amount of LPV VP1 per milligram of extracted cellular protein by LPV VP1 ELISA. Results are given as the arithmetic mean and standard error of the mean (error bars) from triplicates. The extent of infection in untreated BJA-B cells (unshaded column) is shown for comparison. (b) BJA-B cells were left untreated and K6 cells were cultured in the absence or presence of tunicamycin for up to 70 h. At the times indicated, 10^6 cells were removed from the cultures and washed once in PBS, and after infection with LPV they were subsequently cultured for 48 h. The extent of LPV infection was quantitated by LPV VP1 ELISA. Results given are the means from duplicates.

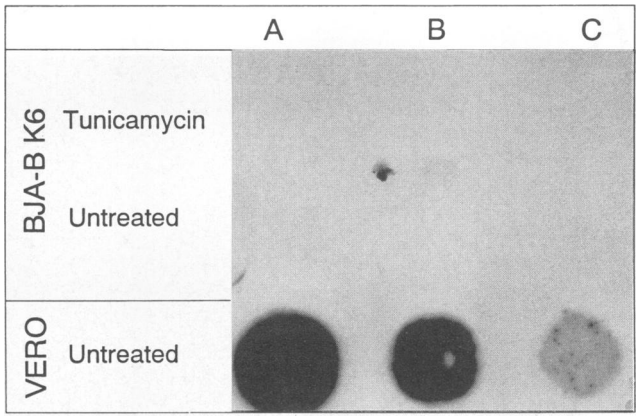


FIG. 3. Tunicamycin-pretreated BJA-B cells remain resistant to SV40 infection. Untreated and tunicamycin-pretreated (48 h, 1.2 μM) BJA-B K6 cells and untreated Vero cells (positive control) were exposed to SV40 virions. SV40 DNA replication in individual cells was determined 2 days postinfection by filter in situ hybridization. 10^4 (A), 10^3 (B), and 10^2 (C) washed cells were sucked onto round nitrocellulose filters and, after denaturation, were hybridized with a ^{32}P-radiolabelled SV40 DNA probe as described previously (8). The X-ray film was exposed overnight by using intensifying screens. Filter C of Vero cells shows that of the 100 cells on the filter, about 30 were SV40 replicating, whereas among 10^4 tunicamycin-pretreated or untreated BJA-B cells infected with the same dose of SV40, not a single virus-replicating cell was detectable.

transfection of purified SV40 DNA into BJA-B cells resulted in about 2% SV40 antigen-positive cells and in the production of progeny SV40 virions infectious for Vero cells but not for BJA-B cells (data not shown). This shows that SV40 virions cannot enter BJA-B cells but that complete SV40 gene expression and replication can take place from transfected SV40 genomes. Thus, tunicamycin treatment of BJA-B cells can induce enhancement of susceptibility to LPV infection but not to infection by the related polyomavirus SV40.

The N glycosylation processing inhibitor dManNoj, but not dNoj, swainsonine, or castanospermine, can also induce LPV susceptibility. After their attachment to proteins, N-linked oligosaccharides are further processed by sequential actions of glycosidases and glycosyltransferases first in the endoplasmic reticulum and then in the Golgi apparatus. In addition to tunicamycin, which inhibits the first step in N glycosylation, inhibitors with selective influences on N-linked oligosaccharide processing are available (reviewed in references 5 and 6). Castanospermine and dNoj both inhibit glucosidases of the endoplasmic reticulum, thus blocking the synthesis of hybrid and complex N-linked sugar chains and increasing high-mannose oligosaccharides. dManNoj inhibits mannosidase I in the Golgi complex and causes a decrease in complex types of oligosaccharides and an increase in high-mannose oligosaccharides. Swainsonine inhibits Golgi mannosidase II, resulting in the replacement of complex N oligosaccharide chains by hybrid chains.

These four glycosylation processing inhibitors were tested for their effect on LPV infection in low-susceptibility BJA-B K6 cells. Concentrations that had been shown to be effective in other cell systems were chosen (6). Treatment with dManNoj (0.4 mM) increased susceptibility to subsequent LPV infection 23-fold, whereas the three other processing inhibitors, dNoj, swainsonine, and castanospermine (4 mM, 20 μM, and 0.5 mM, respectively), had no apparent effect (Fig. 4a). The enhancement of LPV susceptibility by dManNoj to approximately 200 ng of VP1 per mg of protein was not as pronounced as that seen with tunicamycin, with which a level of 3,000 ng of VP1 per mg of protein was reached (Fig. 2a).

In contrast to tunicamycin, neither dManNoj nor any of the other three glycosylation processing inhibitors significantly affected viability or proliferation of cells during 48 h of treatment (Fig. 4b). To test whether inhibition of cell proliferation in general might contribute to the strong tunicamycin-induced enhancement of LPV susceptibility, K6 cells pretreated with another cell proliferation-inhibiting substance, the protein biosynthesis inhibitor cycloheximide, were infected

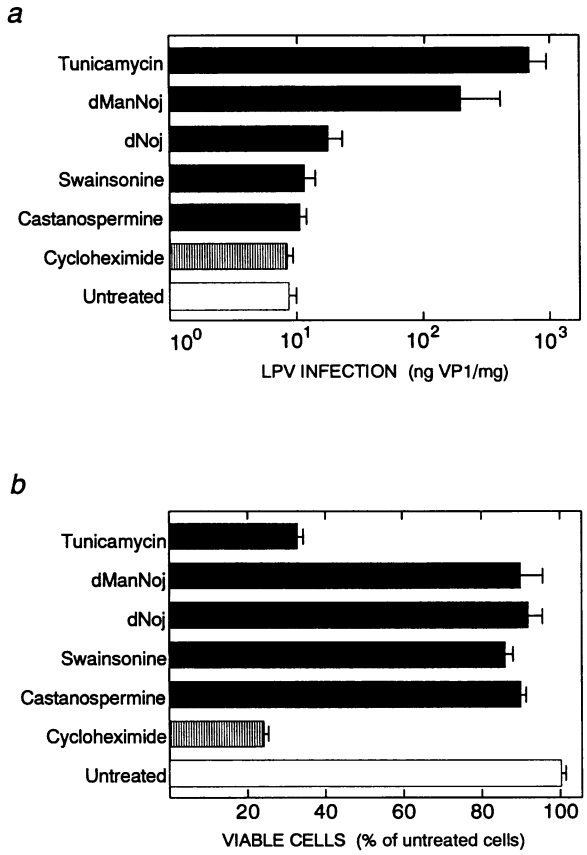
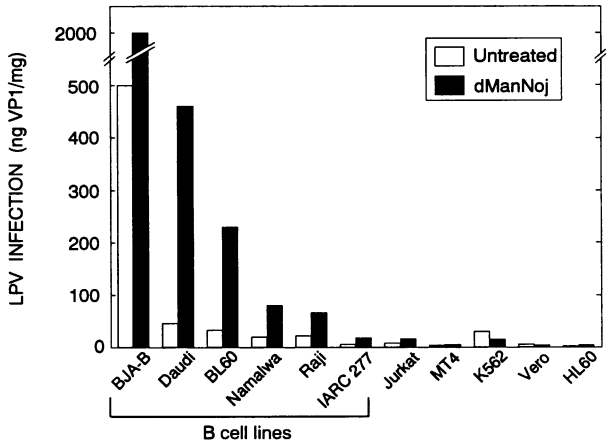


FIG. 4. The effect of various N glycosylation inhibitors and the protein biosynthesis inhibitor cycloheximide on LPV infection (a) and cell proliferation (b). Pretreatment of BJA-B K6 cells with tunicamycin (1.2 μM), dManNoj (0.4 mM), dNoj (4 mM), swainsonine (20 μM), castanospermine (0.5 mM), or cycloheximide (0.4 μM) proceeded for 48 h. (a) LPV infection and quantitation of virus production 69 h postinfection by VP1 ELISA were carried out as described in the legend to Fig. 1. (b) The number of viable cells was determined by trypan blue exclusion. Results for inhibitor-containing cultures are expressed relative to untreated K6 cells. All values given are the arithmetic means and standard deviations (error bars) for three to five independent experiments.



FACTOR OF INCREASE:	4	10	7	4	3	3	2	1	0.5	1	1

FIG. 5. Induction of LPV susceptibility in various cell lines of hematopoietic origin by dManNoj. The indicated cell lines were cultured in the presence or absence of the glycosylation processing inhibitor dManNoj (400 μM, 48 h), washed, and subsequently infected with LPV. The extent of LPV infection in untreated and dManNoj-treated cells was determined by quantitating the amount of LPV VP1 per milligram of extracted cellular protein by LPV VP1 ELISA. Numbers at the bottom for each subclone show the factor of increase which was calculated as the ratio of VP1 produced in tunicamycin-treated cultures to VP1 produced in untreated cultures.

with LPV. Cycloheximide at 0.4 μM reduced cell proliferation and viability to a similar extent as did tunicamycin at 1.2 μM but did not enhance LPV infection (Fig. 3). These data provide evidence that the induction of LPV susceptibility by tunicamycin and dManNoj is caused by a specific effect on protein N glycosylation.

Other human B-lymphoma cell lines are also rendered susceptible to LPV infection by inhibition of N glycosylation. The possible enhancement of LPV susceptibility by N glycosylation inhibition was also tested with a series of other cell lines with no or low-level LPV susceptibility. All the lines used are capable of expressing and replicating transfected LPV DNA (20). dManNoj was chosen since it can effectively induce LPV susceptibility without affecting cell proliferation. In all cell lines outside the B-lymphoid differentiation pathway, i.e., the promyelocytic leukemia line HL60, the chronic myelogenous leukemia line K562, and T-lymphoid leukemia lines Jurkat and MT-4, as well as the monkey kidney epithelium cell line Vero, dManNoj pretreatment could not induce LPV susceptibility more than twofold (Fig. 5). In pretreated EBV-immortalized B-lymphoblastoid IARC 277 cells, again no significant LPV susceptibility was detectable. All these cell lines produced less than 30 ng of LPV VP1 per mg of extracted protein. In contrast, in all four Burkitt's lymphoma cell lines, Raji, Namalwa, BL60, and Daudi, LPV susceptibility was induced and resulted in more than 60 ng of VP1 per mg of protein. In Daudi cells, LPV susceptibility increased 10-fold from 45 ng of VP1 per mg of protein in untreated cells to 460 ng of VP1 per mg of protein after dManNoj treatment. These results show that induction or enhancement of LPV susceptibility by inhibition of N glycosylation processing is not unique to a particular cell line (BJA-B and its subclones) but rather is common among B-lymphoma cell lines and is restricted to cells of this differentiation compartment.

The enhancement of LPV susceptibility by N glycosylation inhibition is mediated by enhancement of LPV binding. Since LPV receptor expression had previously been shown to be a major determinant of LPV host range (12), we analyzed whether the enhancement of LPV susceptibility by tunicamycin could be due to enhanced LPV binding to host cells. Varying numbers of cells were incubated with a constant amount of LPV, virus binding was allowed to occur for 30 min, and after low-speed centrifugation, the amount of free LPV in the supernatant was quantitated by LPV VP1 ELISA. The cell numbers required to bind 50% of the administered virus were taken as the definition of the relative LPV-binding capacities of inhibitor-treated and untreated cells (Fig. 6).

Untreated cells of the low-LPV-susceptibility BJA-B subclone K20 display a very low LPV-binding capacity, with 2×10^6 cells needed to bind 400 pg of LPV VP1 (Fig. 6b). In contrast, 3×10^5 untreated cells of the highly susceptible subclone K88 are sufficient to bind the same amount of virus. This is equivalent to an approximately sevenfold-higher LPV-binding capacity. Tunicamycin pretreatment of K20 and K88

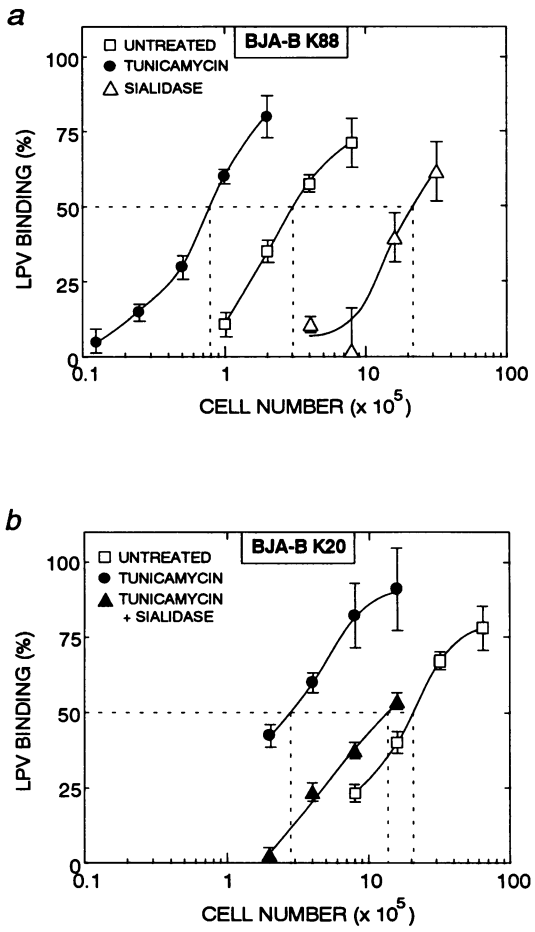


FIG. 6. Induction of sialidase-sensitive LPV binding in low- (a) and high- (b) LPV susceptibility BJA-B clones by tunicamycin. LPV binding was determined by incubating a constant amount of purified LPV particles (corresponding to 800 pg of VP1) with varying numbers of K20 (a) or K88 (b) cells. Cells had been cultivated for 48 h in the presence (closed symbols) or absence (open symbols) of tunicamycin (0.3 μM, batch 2) and were subsequently incubated for 2 h at 37°C with sialidase from *V. cholerae* (△, ▲) or PBS (□, ●). Values given represent the amount of virus bound relative to the total virus offered and are arithmetic means and standard deviations (error bars) from triplicate samples. Broken lines indicate the relative binding capacities of the different cell populations, i.e., the number of cells binding 50% of the virus administered.

cells increased the binding capacities 10-fold and 4-fold, respectively. Therefore, tunicamycin-induced enhancement of LPV susceptibility appears to be mediated at least to a large extent by enhancement of the virus-binding capacity of the cell surface.

The tunicamycin-induced LPV receptor on BJA-B cells is sialic acid dependent and possibly identical with the constitutively expressed LPV receptor. Sialic acid is an essential component of the constitutively expressed receptor (12, 14). We therefore analyzed whether the tunicamyin-induced receptor also requires sialic acid to bind LPV. On BJA-B K20 cells with low-level constitutive LPV binding and susceptibility, the tunicamycin-induced LPV binding was reduced by 85% after enzymatic desialylation with *V. cholerae* sialidase (Fig. 6b). This enzyme also efficiently removes the functionally essential sialic acid from the constitutively expressed LPV receptor on untreated K88 cells (Fig. 6a). Thus, both the constitutive receptor on K88 cells and the tunicamycin-induced K20 receptor contain a functionally essential terminal sialic acid(s). This points to a possible identity of the constitutive and the induced LPV receptors.

DISCUSSION

While attempting to characterize the oligosaccharide(s) necessary for the B-lymphotropic papovavirus receptor on a susceptible human B-lymphoma line, we observed that pretreatment of the cells with the N glycosylation inhibitor tunicamycin enhanced susceptibility to LPV infection. Enhancement of susceptibility was due to an increase in the number of infected cells and was accompanied by an enhancement of the cellular virus-binding capacity. A similar correlation between susceptibility to LPV infection and virus-binding capacity has previously been seen for various human cell lines (12). We have also shown previously that in susceptible BJA-B cells treated with sialidase, the amount of LPV receptor is reduced and limits the infection. The increase in binding and infection induced by tunicamycin pretreatment described here shows that, even in constitutively susceptible BJA-B cells, LPV receptor expression is the limiting factor for viral infection.

For the high-susceptibility BJA-B subclone K88, tunicamycin pretreatment enhanced virus binding fourfold and susceptibility sixfold, suggesting a positive correlation. However, in the low-susceptibility K20 cells, a 10-fold increase in virus-binding capacity was accompanied by an over 100-fold increase in the number of infected cells and an 83-fold increase in virus production, indicating a nonlinear relationship between virus-binding capacity and susceptibility. The overproportional increase in susceptibility in the low-binding-level cells after tunicamycin treatment could be explained by a model in which several virus-binding sites have to cooperate to form a site sufficient for virus uptake. Hence, in cell membranes in which the concentration of binding sites is higher, the formation of a functional uptake site would occur more efficiently, leading to an overproportional increase in infection.

Although the molecular natures of the constitutive LPV receptor and the tunicamycin-induced receptor are still unknown, some information on the oligosaccharide(s) necessary for virus binding and infection is available. Both receptors are sensitive to enzymatic desialylation by *V. cholerae* sialidase. The sialic acid-containing oligosaccharide(s) on the tunicamycin-induced receptor is unlikely to be N linked. The high binding capacity of tunicamycin-treated K88 cells is best explained by an additive effect of constitutive and tunicamycin-induced receptors on these cells, which suggests that the sialic acid on the constitutive receptor is also not N linked. This raises the possibility that the constitutive and the tunicamycin-induced receptors are identical. This interpretation is further supported by the finding that tunicamycin induction of LPV susceptibility appears restricted to B-lymphoma-derived cell lines, i.e., the cell type to which almost all cells with known constitutive LPV susceptibility belong (21, 25, 28, 29). It remains to be determined whether the sialic acid(s) necessary for receptor function is part of the O-linked oligosaccharides on a glycoprotein or is located on membrane glycolipids. The former is favored by the trypsin sensitivity of the constitutive LPV receptor (12).

Besides inhibiting the N glycosylation of nascent proteins in the endoplasmic reticulum, at the concentrations used here, tunicamycin might also affect protein synthesis, as has been described for several experimental systems (reviewed in reference 6). In our experiments, tunicamycin clearly had a potent

effect on cell proliferation and cell viability. However, we conclude that with tunicamycin also, the effect is specifically due to inhibition of correct N glycosylation and not to inhibition of cell proliferation per se, since the N glycosylation processing inhibitor dManNoj displayed a similar effect on LPV susceptibility without inhibition of proliferation and since protein synthesis impairment by cycloheximide had no apparent influence on LPV susceptibility. The failure to detect susceptibility enhancement with three other N glycosylation processing inhibitors may point to the type of N-linked oligosaccharide involved in the susceptibility enhancement. dManNoj inhibits mannosidase I, leading preferentially to the accumulation of high-mannose structures without terminal glucose residues, whereas the other processing inhibitors used result in accumulation of either high-mannose structures with terminal glucose residues (castanospermine and dNoj) or hybrid-type oligosaccharides with terminal *N*-acetyl glucosamine residues (swainsonine).

Several mechanisms may be envisaged to explain the induction of LPV receptor function by interfering with cellular N glycosylation. Firstly, an N-linked oligosaccharide on the receptor protein itself might block the virus-binding site directly or indirectly by inducing a conformational change in the molecule. The masking of functional sites on cell surface molecules by oligosaccharides as a regulatory tool has been suggested (23). One precedent for such a mechanism has recently been described. A receptor for an ecotropic murine leukemia virus is blocked in some cell lines by N glycosylation at a single specific site; removal of the site resulted in activation of receptor function (4, 27). Secondly, the inhibitory oligosaccharide might be located on a membrane glycoprotein spatially closely associated with the receptor and thus may sterically hinder virus binding. Thirdly, the inhibitory oligosaccharide could be on a glycoprotein regulating posttranscriptional modification, transport, or degradation of the LPV receptor. Molecular identification of the LPV receptor should allow definition of which of these N glycosylation-dependent mechanisms is regulating LPV receptor expression.

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