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Viral and Cellular Factors Governing Hamster Cell Infection by Murine and Gibbon Ape Leukemia Viruses

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Hamster cells are resistant to infection by most retroviruses, including Moloney murine leukemia virus (MoMLV) and gibbon ape leukemia viruses (GaLVs). We have constructed MoMLV-GaLV hybrid virions to identify viral and cellular determinants responsible for the inability of GaLV and MoMLV to infect hamster cells. The substitution of MoMLV core components for GaLV core components circumvents the resistance of hamster cells to infection by GaLV, demonstrating that hamster cells have receptors for GaLV but are not efficiently infected by this primate retrovirus because of a postpenetration block. In contrast, hamster cells are apparently resistant to MoMLV infection because although they bear a receptor for MoMLV, the receptor is nonfunctional. Treatment of CHO K1 or BHK 21 hamster cells with the glycosylation inhibitor tunicamycin allows the cells to be infected by MoMLV. The construction of MoMLV-GaLV hybrid virions that can efficiently infect resistant cells has allowed the identification of viral and cellular factors responsible for restricting infection of hamster cells by MoMLV and GaLV.

Both host cell and viral factors regulate the efficient replication of retroviruses in cells, either by influencing viral entry or by affecting viral replication at a postpenetration level (32).

The entry of retroviruses into cells depends on the interaction between the envelope glycoprotein and a specific cell surface receptor. The presence of a functional viral receptor is an absolute requirement for viral infection. Host range restrictions due to the absence of the viral receptor can be overcome by introducing and expressing the appropriate receptor in the resistant cells (1) or by replacing the viral envelope of the restricted virus with one derived from a virus which can infect the cell of interest (26, 33, 34). Cellular resistance to a virus may also be imposed by saturation of the cellular receptors with viral envelope glycoproteins synthesized within a preinfected cell, which then block infection of susceptible cells by the homologous virus or any virus that utilizes the same receptor. This is called superinfection interference (25).

Postpenetration blocks can also result in substantial decreases in the efficiency of infection. Both cellular and viral factors may modulate susceptibility to infection by murine retroviruses at the postpenetration level. The *Fv-1* gene locus in murine cells governs susceptibility to integration of retroviral DNA into a murine host cell genome (11–13). Susceptibility to *Fv-1* restriction has been physically mapped to two adjacent amino acids in the p30 core protein (5, 20, 28). Other factors that regulate viral replication in the later stages of infection are those affecting efficient expression of the integrated viral DNA. Negative regulators in host cells have been proposed to account for the refractory status of stem cells in vivo and of undifferentiated embryonic stem cells in vitro with respect to efficient infection by Moloney murine leukemia virus (MoMLV) (27, 29, 30). The viral determinants responsible for restricting expression in these cells reside in the long terminal repeat and primer-binding sites of MoMLV (6, 7, 14, 21).

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We prepared hybrid gibbon ape leukemia virus (GaLV)-MoMLV virions, containing various proportions of protein and genomic components contributed either by GaLV or MoMLV, to determine what viral component(s) restricts efficient infection of hamster cells by GaLV and murine ecotropic and amphotropic viruses. The 4070A virus is a murine amphotropic virus with a broad host range that includes many types of mouse, cat, dog, guinea pig, rabbit, rat, and human cells. Hamster cells, however, are resistant to 4070A virus (8, 23). GaLV replicates well in a wide variety of cells; however, hamster and mouse cells are resistant to productive infection by GaLVs (31, 33).

Two types of hybrid virions were used to assess the relative contributions of viral genome, core proteins, and envelope proteins to the inability of MoMLV, 4070A, and GaLV to infect hamster cells. One type of hybrid virion, historically referred to as a pseudovirion, contains a genome from one virus and structural proteins from another virus. Such a hybrid virion or pseudovirion is named according to the virus contributing the structural proteins. A second type of hybrid virion, typified by virions produced by the packaging cell lines ΨAM or PA317 (4, 17) and PG13 (18), contains both genome and core components from MoMLV and envelope proteins from a second virus. Thus, PA317 or ΨAM hybrid virions contain an envelope from the murine 4070A virus and PG13 virions contain GaLV envelope proteins.

In addition to resolving the contribution of different viral component(s) to the block of replication of these viruses in hamster cells, we also sought to determine whether a common mechanism of resistance to MoMLV or GaLV infection (such as the absence of expression of the appropriate cellular receptor) is shared by five different hamster cell lines. We examined cell lines derived from three different species of hamster to determine whether different mechanisms that account for the resistance of hamster cells to virus infection by MoMLV, 4070A, and GaLVs exist between hamster species or within cell lines established from the same species.

MATERIALS AND METHODS

Cell lines and viruses. The cells used in this study include the following: NIH 3T3 murine fibroblasts (ATCC CRL 1658); rat 2 embryo fibroblasts (ATCC CRL 1764); CHO K1 Chinese hamster ovary cells (ATCC CCL 61); HaK Syrian hamster kidney cells (ATCC CCL 15); Don Chinese hamster lung cells (ATCC CCL 16); BHK 21 Syrian hamster kidney cells (provided by Noel Bouck, Northwestern University, Chicago, Ill.), AHL Armenian hamster lung fibroblasts (ATCC CCL 195), EJ human bladder carcinoma cells (provided by S. Aaronson, National Cancer Institute, Bethesda, Md.), HOS human osteosarcoma cells (ATCC CRL 1543), mink lung fibroblasts (ATCC CCL 64), PA317 (ATCC CRL 9078), Ψ2/BAG and ΨAM/BAG (provided by C. Cepko, Harvard Medical School, Boston, Ma. [24]) and PG13 (ATCC CRL 10,683). All cell lines (except CHO K1) were maintained in Dulbecco's modified essential medium (DMEM; Whittaker Bioproducts, Inc., Walkersville, Md.), supplemented with 5% fetal bovine serum (FBS), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 40 mM glutamine. CHO K1 cells were propagated in similarly supplemented alpha MEM (Whittaker Bioproducts, Inc.). Mink lung fibroblasts infected with the 4070A virus were provided by Janet Hartley (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). GaLV SEATO-infected bat lung fibroblasts (CCL 88) and biologically cloned MoMLV was obtained from Electronucleonics, Incorporated (Silver Spring, Md.).

Wild-type virus preparation and RT assays. Wild-type virus obtained from the culture medium of the infected cells was concentrated by centrifugation at 143,000 × *g* for 75 min at 4°C. The viral pellet was resuspended in phosphate-buffered saline and stored in 20-μl aliquots in liquid nitrogen. Serial dilutions of the concentrated virus were used to infect rat 2 fibroblasts. Thirty hours postinfection, a reverse transcriptase (RT) assay was performed on the cellular supernatants and the dilution of virus which generated >10,000 cpm of RT activity per 20 μl of supernatant was used in subsequent experiments.

Hamster or rat 2 cells were seeded in 24-well dishes at 20,000 to 30,000 cells per well and then infected with virus in medium containing 3 μg of Polybrene per ml. Twenty-four hours postinfection, the cells were rinsed and fed with fresh medium. Forty-eight hours postinfection, the cells were seeded at a 1:10 dilution in 24-well dishes. Eight days following infection, the supernatant was assayed for RT activity. Five microliters of a solution containing 50 mM Tris (pH 8.3), 120 mM NaCl, and 1% Triton X-100 was added to 20 μl of supernatant and incubated at room temperature for 10 to 15 min prior to the addition of 25 μl of RT cocktail (50 mM Tris [pH 8.3], 10 mM dithiothreitol, 1.2 mM $MnCl_2$, 10 μg of oligo$(dT)_{12-18}$ per ml, 20 μg of poly(A) per ml, 10 nM [^{3}H]dTTP). Samples were incubated an additional 2 h at 37°C before the reactions were stopped by the addition of 5-μl quantities of 0.25 M EDTA. Samples were filtered onto DEAE paper by using a Titertek cell harvester and were counted in a liquid scintillation counter.

Virion production and infection. Ψ2/BAG and ΨAM/BAG cell lines were used as the source of Ψ2 and ΨAM virions employed in these studies (22). Both the *Escherichia coli lacZ* β-galactosidase and Tn*5* *neo* resistance genes are present in the defective BAG genome transduced by Ψ2/BAG and ΨAM/BAG virions, and viral titers can therefore be determined by counting neomycin-resistant cell clones or by counting blue foci. Transcription of the *neo* gene initiates from an internal simian virus 40 promoter, whereas transcription of the *lacZ* gene initiates from the retroviral promoter contained within the U3 of the long terminal repeat. Helper-free GaLV virions were prepared by infecting the PG13 GaLV packaging cell line (18) with ΨAM virions containing the BAG genome. PG13 virions contain MoMLV genome and core components and GaLV envelope proteins.

Mink cells producing 4070A pseudovirions were prepared by superinfecting 4070A virus-producing mink lung fibroblasts with PG13 virions containing the BAG genome. Mink cell clones expressing pseudovirions were selected in medium containing 800 μg of G418 per ml. GaLV pseudovirions were prepared in an analogous manner, that is, by infecting mink fibroblasts producing GaLV SEATO wild-type virus with ΨAM/BAG virions and selecting for mink cells expressing the BAG genome with medium containing 800 μg of G418 per ml.

Determination of virion titers by β-galactosidase and G418 assays. Target cells were seeded at 50,000 cells per well in a 12-well dish. The next day, target cells were exposed to 2.0 ml of virus in medium adjusted to 3 μg of Polybrene per ml. All virus suspensions were obtained from the filtered supernatant of confluent virus-producing cells. Twenty-four hours postinfection, cells were trypsinized and seeded at 1:2, 1:10, 1:100, and 1:1,000 dilutions into 10-cm-diameter dishes. The next day, cells were fed with medium containing G418. All cells were fed with DMEM containing 5% FBS and the indicated amount (in micrograms per milliliter) of active G418 as follows: all hamster cells and rat 2 fibroblasts, 450; NIH 3T3 cells, 400; EJ and mink cells, 800; HOS cells, 250. Cells were selected for 10 to 15 days after which G418-resistant cell clones were stained with a methanol solution containing 0.75% methylene blue and 0.25% carbol fuchsin (Sigma, St. Louis, Mo.) and quantitated. Relative titers were determined by dividing the actual titer obtained with a particular cell line (e.g., the number of G418-resistant clones per milliliter of virus supernatant) by the titer obtained with rat 2 fibroblasts. Serial dilutions of infected cells prior to selection with G418 allowed differences in the apparent viral titer to be assessed over a 100,000-fold range. To determine virus titers on the basis of β-galactosidase activity, cells exposed to virions were grown to confluence, fixed in a 2% glutaraldehyde–formaldehyde solution, and stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (27). Relative titers were determined by dividing the number of blue foci, obtained with a particular target cell exposed to 1.0 ml of virus supernatant, by the number of blue foci obtained on rat 2 cells infected with 1.0 ml of virus supernatant.

Infection of target cells pretreated with tunicamycin. Target cells were seeded at 50,000 cells per well in DMEM containing 5% FBS with or without 0.15 μg of tunicamycin (Calbiochem Corporation, La Jolla, Calif.) per ml in a 12-well tissue culture dish. Tunicamycin solutions were prepared and stored as described by Heifetz et al. (9). Approximately 18 h later, cells were rinsed three times with fresh medium without tunicamycin and infected with 2 ml of filtered virus suspension. The next day, cells were trypsinized and seeded at 1:2, 1:5, and 1:10 dilutions in a 10-cm-diameter tissue culture dish and selected with G418 as described above.

Construction of hamster and human cells expressing the murine MoMLV receptor cDNA. The pLNS-JET retroviral expression plasmid was constructed by introducing a 2.28-kb *Eco*RI-to-*Bam*HI restriction fragment from the pJET plasmid (J. Cunningham, Harvard Medical School, Boston, Mass.) into the pLNSXe plasmid (19). The pJET-derived

TABLE 1. Susceptibility of hamster cells to infection by GaLV, GaLV pseudovirions, and PG13 virions

Cells	Susceptibility of cells to infection by virus[a]		
	GaLV		PG13 virion[c]
	Wild-type virion[b]	Pseudo-virion[c]	
CHO K1	−	0.001	0.4
Don	−	0.1	1.0
BHK 21	−	<0.0001	0.002
HaK	−	0.0003	0.015
AHL	−	0.0001	0.01
Rat 2	+	1.0	1.0

[a] Wild-type GaLV virions contain GaLV-derived genome and core and envelope proteins. GaLV pseudovirions contain GaLV-derived core and envelope proteins and a MoMLV-based genome. PG13 virions contain MoMLV-derived genome and core components and GaLV envelope proteins.
[b] Susceptibility based on RT assay. Symbols: −, <twofold over background RT activity; +, >200-fold over background RT activity.
[c] Susceptibility based on G418-resistant colony titers, normalized to G418-resistant colony titers on rat 2 cells.

TABLE 2. Susceptibility of hamster cells to infection by MoMLV, 4070A, 4070A pseudovirions, and Ψ2 and ΨAM virions

Cells	Susceptibility of cells to infection by virus				
	Wild-type virion[a]		Ψ2 virion[b]	4070A pseudo-virion[b]	ΨAM virion[b]
	MoMLV	4070A			
CHO K1	−	−	<0.0001	<0.0001	<0.0001
Don	−	−	<0.0001	0.01	0.01
BHK 21	−	−	<0.0001	<0.0001	<0.0001
HaK	−	−	0.01	0.0003	0.015
AHL	−	−	<0.0001	<0.0001	<0.0001
Rat 2	+	+	1.0	1.0	1.0

[a] Susceptibility based on RT assay. Symbols: +, >200-fold over background RT activity; −, <twofold over background RT activity.
[b] Susceptibility based on G418-resistant colony titers, normalized to G418-resistant colony titers on rat 2 cells.

fragment contains the coding region for the ecotropic receptor. CHO K1, BHK 21, and AHL were transfected with 20 μg of pLNS-JET (2). Transfected cells were selected with 450 μg of G418 per ml, and resistant colonies were pooled. PA317 cells were similarly transfected and selected with 400 μg of G418 per ml. Supernatant from the stably transfected PA317 cells was used to infect human EJ bladder carcinoma cells.

RESULTS

Role of GaLV core components in restricting GaLV infection of hamster cells. We examined five different hamster cell lines for susceptibility to infection by GaLV. These include BHK 21 fibroblast cells and HaK epithelial cells (derived from Syrian hamster neonatal kidney), two Chinese hamster cell lines, CHO K1 and Don (derived from Chinese hamster ovary and lung, respectively), and AHL fibroblast cells (derived from Armenian hamster lung). All of these cells were resistant to infection by wild-type GaLV and demonstrated an increased susceptibility to infection by the PG13 virions compared with the GaLV pseudovirions (Table 1).

GaLV pseudovirions contain GaLV viral proteins and a MoMLV-based defective genome, whereas PG13 virions contain MoMLV core components in addition to a MoMLV genome and GaLV envelope. GaLV pseudovirions did not efficiently infect CHO K1, HaK, BHK 21, and AHL cells. This result indicates that replacing the GaLV genome with a MoMLV-based genome alone is not sufficient to overcome GaLV restriction in these cells. Substitution of both a MoMLV core and MoMLV genome facilitates infection; therefore, core components are responsible for restricting GaLV infection of CHO K1, HaK, BHK 21, and AHL cells. The resistance of these hamster cells to GaLV is thus due to a postpenetration block to infection and not to the absence of a functional GaLV receptor.

Different hamster cell lines demonstrate variable efficiencies of infection by PG13 virions. Susceptibility to infection by PG13 virions was quite variable among the different hamster cells. The HaK and BHK 21 cell lines were established from Syrian hamsters, which are members of the genus *Mesocricetus*. The Chinese and Armenian hamster cell lines used in these studies were established from two different species (*Cricetulus griseus* and *Cricetulus migratorius*, respectively) within the same genus. Chinese hamster cells were the most efficiently infected, while BHK 21 cells were the least efficiently infected (a 1,000-fold lower efficiency of infection of BHK 21 cells compared with Don cells, [Table 1]). Don cells were unique in their ability to be much more efficiently infected by GaLV pseudovirions than other hamster strains were albeit they were 10 times less efficiently infected by GaLV pseudovirions than by PG13 virions.

Hamster cells lack appropriate receptors for MoMLV and 4070A. Wild-type MoMLV and 4070A viruses failed to infect any of the five hamster cell lines employed (Table 2). No G418-resistant clones or β-galactosidase-positive blue foci (data not shown) were observed with CHO K1, BHK 21, and AHL cells following exposure to Ψ2 virions, 4070A pseudovirions, or ΨAM virions. Substitution of the GaLV envelope is sufficient to overcome the resistance of these cells to infection by Ψ2 or ΨAM virions, demonstrating that MoMLV-derived genome and core components function efficiently in hamster cells (Table 1). The viral determinants that restrict the MoMLV and 4070A virions from infecting these hamster cells therefore reside in the envelope.

Hamster cells exhibit variable susceptibility to infection by Ψ2 and ΨAM virions. The only hamster cell line that could be infected with Ψ2 virions was the Syrian hamster cell line, HaK, albeit at a reduced efficiency relative to that of rat 2 fibroblasts (15). The ability to infect HaK with Ψ2 but not wild-type MoMLV may simply reflect differences in the sensitivity between the RT assay and the G418-resistant colony-forming assay.

Cells from the same species of hamster vary in their susceptibility to infection by 4070A pseudovirions and ΨAM virions (Table 2). Don cells are infected 100 times more efficiently than CHO K1, though both are derived from the same species, *C. griseus*. The observed differences in the apparent titer of ΨAM virions on BHK 21 compared with that on HaK demonstrates that variation in susceptibility to infection can exist not only in cells from the same species of hamster (*Mesocricetus auratus*) but even from the same tissue (Table 3). The mechanism for the observed variation in the efficiency of ΨAM infection of different hamster cells is unknown.

Effect of inhibition of glycosylation on infection of hamster

TABLE 3. Summary of infection efficiencies of hamster cells obtained with PG13, Ψ2, and ΨAM virions[a]

Type of virion	Infection efficiency of cells					
	Chinese hamster (*Cricetulus griseus*)		Syrian hamster (*Mesocricetus auratus*)		Armenian hamster (*Cricetulus migratorius*)	Rat 2
	CHO K1	Don	BHK 21	HaK	AHL	
PG13 virions	+++	++++	+	++	++	++++
Ψ2 virions	−	−	−	++	−	++++
ΨAM virions	−	++	−	+++	−	++++

[a] This table summarizes data presented in Tables 1 and 2. One plus sign is equivalent to an increase in titer of 1 order of magnitude.

cells by Ψ2 and ΨAM virions. The resistance to MoMLV observed with the hamster cells could be a consequence of the lack of expression of appropriate MoMLV receptors. It was therefore necessary to determine whether a mRNA for the MoMLV receptor was expressed in hamster cells. All cells examined expressed mRNAs of the appropriate sizes that hybridized to the murine MoMLV receptor cDNA (data not shown), despite the fact that only HaK cells demonstrated even limited susceptibility to infection by MoMLV enveloped virions.

To determine whether hamster cell resistance to MoMLV infection was glycosylation dependent, three hamster cell lines resistant to infection by Ψ2 were analyzed for susceptibility to infection following treatment with an inhibitor of N-linked glycosylation, tunicamycin. The human cell line EJ, which is resistant to infection by Ψ2 but susceptible to infection by ΨAM, was also analyzed. While treatment did not alter the resistance of EJ or AHL cells to Ψ2 infection, resistance to infection was reversed in both BHK 21 and CHO K1 cells by exposing these cells to tunicamycin prior to infection (Fig. 1). EJ cells retained their ability to be infected by ΨAM virions (data not shown), indicating that the inability to overcome EJ cell resistance to Ψ2 infection following treatment with tunicamycin was not attributable to tunicamycin-induced cytopathicity.

Resistance to infection by ΨAM virions was also analyzed after tunicamycin treatment. In contrast to the results obtained with Ψ2 virions, AHL cells were rendered sensitive to ΨAM infection after tunicamycin treatment (Fig. 2), whereas CHO K1 and BHK 21 remained resistant to ΨAM infection (data not shown). Failure to detect any G418-resistant AHL cells following tunicamycin treatment and exposure to Ψ2 virions is probably not attributable to drug toxicity, because AHL cells exposed to ΨAM survived the treatment. Instead, our results suggest that pretreatment of target cells with tunicamycin renders AHL cells selectively permissive to infection by ΨAM, but not Ψ2, virions. Likewise, tunicamycin renders BHK 21 and CHO K1 selectively permissive to Ψ2, but not ΨAM virions.

The apparent titer of the Ψ2 virions on rat 2 cells was 500-fold less than the titer determined on untreated rat 2 cells, presumably reflecting some toxicity of the tunicamycin treatment. Nonetheless, equivalent Ψ2 titers were obtained with tunicamycin-treated rat 2 cells (data not shown) and tunicamycin-treated, MoMLV-resistant BHK 21 and CHO K1 cells (approximately 10^2 G418-resistant CFU/ml [Fig. 1]). These findings demonstrate that inhibition of glycosylation renders functional the existing MoMLV receptors on CHO K1 and BHK 21 cells and receptors for 4070A on AHL cells and thus show the treatment is not acting nonspecifically to allow virus entry.

Expression of a transfected murine MoMLV receptor cDNA in hamster cells confers sensitivity to MoMLV infection. To resolve more precisely how glycosylation renders BHK 21 and CHO K1 cells susceptible to MoMLV infection, the murine MoMLV receptor cDNA was transfected and expressed in these cells. The MoMLV receptor cDNA was transfected into CHO K1, BHK 21, and AHL cells. Susceptibility to infection was determined by exposing these cells to Ψ2 virions and counting X-Gal-positive blue cell foci. Introduction and expression of a functional MoMLV receptor cDNA in CHO K1, BHK 21, and AHL cells overcame the resistance to infection by Ψ2 virions (Fig. 3). Armenian hamster lung fibroblasts are unlike BHK 21 and CHO K1 cells but are similar to human EJ cells, in that these cells express a receptor homolog that cannot functionally substitute for this viral receptor even after pretreatment with tunicamycin (1).

DISCUSSION

Hamster cells express GaLV receptors but are resistant to GaLV at a postpenetration stage of infection. The block to GaLV infection of hamster cells appears to reside in the GaLV core, since substitution of MoMLV core components circumvents the restriction of hamster cells to GaLV infection. This restriction for GaLV in hamster cells is therefore similar to *Fv-1* restriction in murine cells.

The relative efficiency with which MoMLV-GaLV hybrid virions infect different hamster cell lines varies (Table 3). BHK 21 cells are not as efficiently infected by PG13 virions as the two Chinese hamster cell lines, CHO K1 and Don. It is unlikely that the relatively inefficient infection of BHK 21 cells with PG13 reflects a restriction affecting appropriate functioning of the MoMLV genome or core components, because BHK 21 cells expressing the transfected MoMLV receptor are efficiently infected by Ψ2 virions. The observed variations in susceptibility to infection of BHK 21 compared with that of Chinese hamster cells to PG13 infection could be due to subtle alterations in the viral receptors present in the two lines, resulting in inefficient binding of the virus to the receptors expressed on BHK 21 cells, or simply a decreased GaLV receptor density on the surface of these cells.

Hamster cells are resistant to infection by MoMLV even though they express a mRNA that is highly related to the MoMLV receptor cDNA isolated from NIH 3T3 cells. There are several mechanisms that could account for the resistance of hamster cells to infection by MoMLV. First, the MoMLV receptor homolog in these cells may be dysfunctional, similar to the MoMLV receptor expressed in human EJ cells (1). Second, the amount of MoMLV receptor actually expressed may be insufficient to allow efficient virus binding or entry

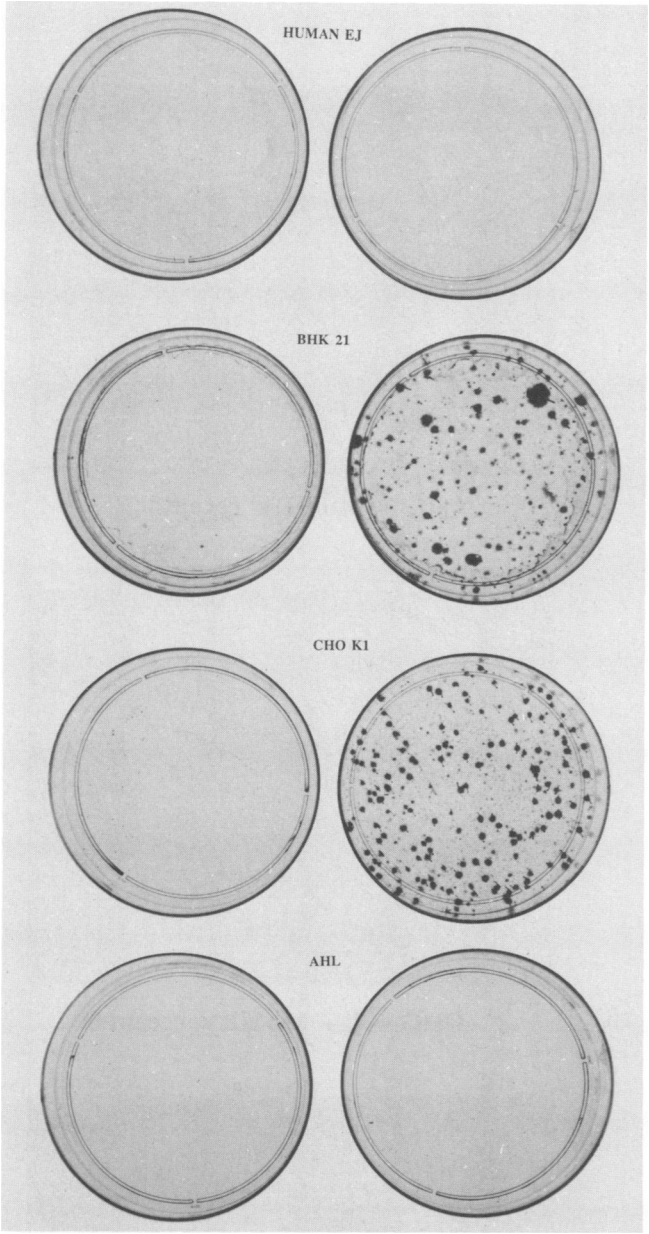


FIG. 1. Analysis of the effect of tunicamycin treatment on target cell susceptibility to Ψ2 infection. Four Ψ2-resistant cell lines, a human cell line (EJ), a Syrian hamster fibroblast cell line (BHK 21), a Chinese hamster ovary cell line (CHO K1), and an Armenian hamster fibroblast cell line (AHL) were assayed for infection by Ψ2 virions following treatment with an inhibitor of glycosylation, tunicamycin. Shown here are cells which were trypsinized and seeded at a 1:5 dilution in a 10-cm-diameter tissue culture dish and selected with G418 after exposure to 2.0 ml of filtered virus. The culture dishes on the left (containing the untreated cells) and the culture dishes on the right (containing tunicamycin-treated cells) depict the presence or absence of G418-resistant clones after exposure to Ψ2 virions. Clones were stained with a methanol solution containing 0.75% methylene blue and 0.25% carbol fuchsin to optimize visualization.

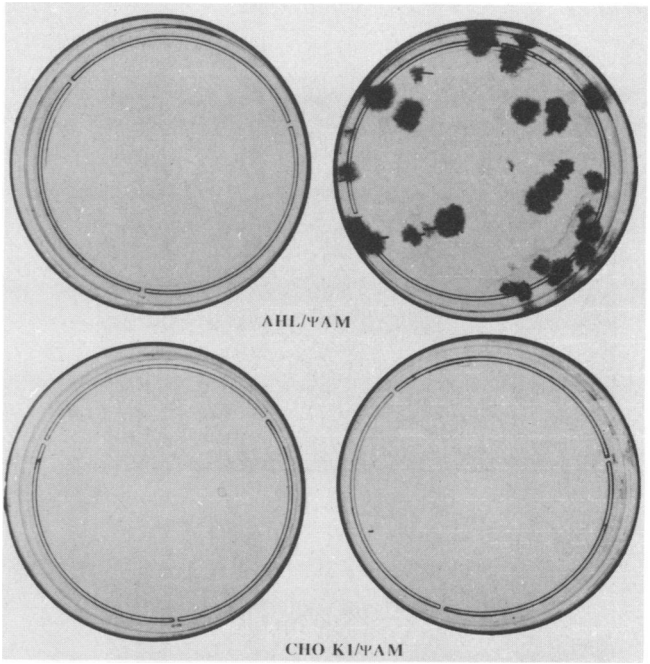
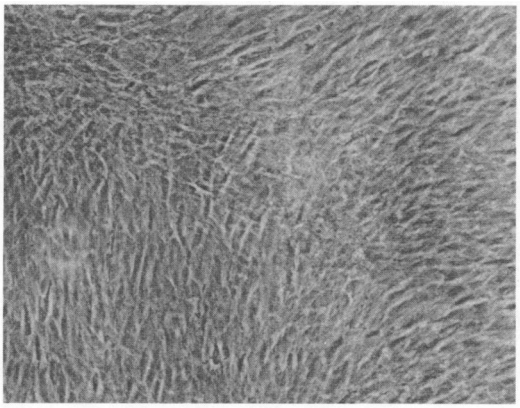


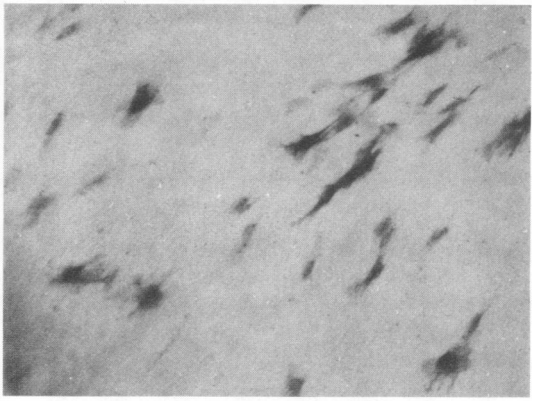
FIG. 2. Analysis of the effect of tunicamycin treatment on the hamster cells CHO K1 and AHL susceptibility to ΨAM infection. Depicted above are untreated cells (plates on the left) and cells treated overnight with tunicamycin (plates on the right) exposed to ΨAM virions and seeded at a 1:5 dilution prior to selection in culture medium containing G418.

into cells. Third, resistance to MoMLV infection may be due to a viral interference-like mechanism whereby hamster cells express a functional MoMLV receptor that is competitively blocked by endogenous ecotropic gp70s. Fourth, the MoMLV receptor expressed by these cells may be rendered nonfunctional as a result of posttranslational modifications of the protein.

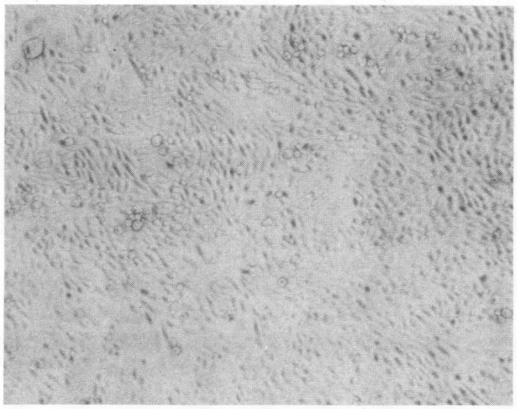
Our finding that CHO K1 and BHK 21 cells were made susceptible to MoMLV infection following treatment with tunicamycin, an inhibitor of N-linked glycosylation, suggests that the refractory status of these cells cannot be attributed to a primary receptor protein product that is either grossly dysfunctional or insufficiently expressed. Tunicamycin treatment could reverse resistance of CHO K1 and BHK 21 cells to MoMLV infection directly by removing oligosaccharides that are N-glycosidically attached to the receptor protein and block virus binding or indirectly by preventing binding of a competing glycoprotein to the MoMLV receptor. Neither viral glycoprotein interference nor a hamster cell-specific posttranslational inactivation of an otherwise conserved (i.e., murinelike) MoMLV receptor can explain the tunicamycin-sensitive block to MoMLV infection of hamster cells, since transfection and expression of the murine MoMLV receptor protein facilitated MoMLV entry into BHK 21 and CHO K1 hamster cells in the absence of inhibitors of glycosylation. Thus, glycosylation inactivation of the endogenous hamster MoMLV receptor is the most likely mechanism for the block to MoMLV infection of the hamster cells and must reflect differences in the primary structure of the hamster and mouse receptor proteins. Comparison of the nucleotide sequence of the MoMLV receptor mRNA homolog expressed in CHO K1 or BHK 21 cells to the murine MoMLV receptor cDNA may resolve nucleotide differences in the receptor-coding region that account for alterations in glycosylation. If so, then glycosylation sites that are present in the hamster MoMLV receptor cDNA and



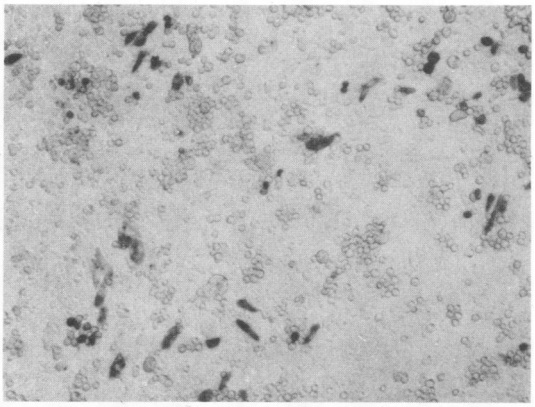
AHL



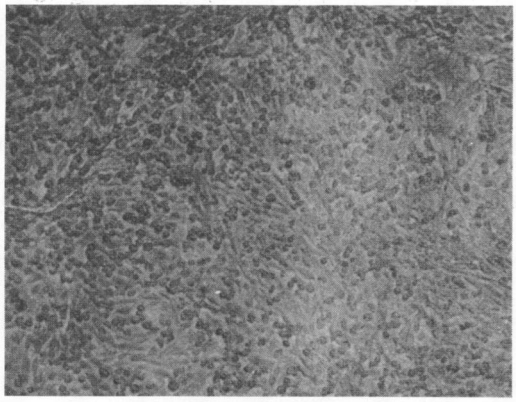
AHL + MoMLV receptor



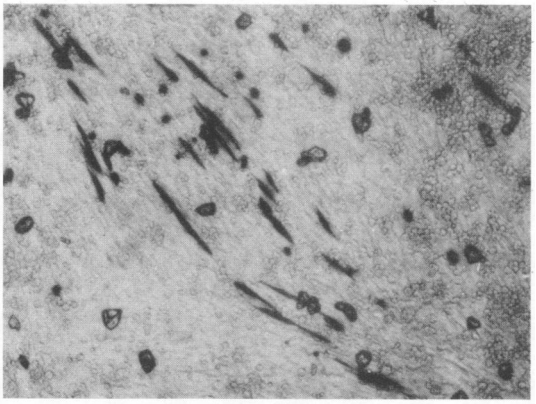
CHO K1



CHO K1 + MoMLV receptor



BHK 21



BHK 21 + MoMLV receptor

FIG. 3. Susceptibility of resistant hamster cell lines to infection with Ψ2 recombinant virions before and after transfection of MoMLV receptor cDNA. A plasmid expressing the murine MoMLV receptor cDNA was introduced into hamster cells via $CaPO_4$-mediated gene transfer (2). A Nikon inverted microscope was used to obtain phase-contrast micrographs of control AHL, CHO K1, and BHK 21 cells and their transfected counterparts that stably expressed MoMLV receptor cDNA after exposure to Ψ2 virions containing the β-galactosidase gene. Three days after exposure to virus, cells were assayed for β-galactosidase activity by using the histochemical procedure described in the text. Magnification, ×40.

not found in the murine MoMLV receptor cDNA are likely to represent virus-binding regions.

No common mechanism of cellular resistance to infection by Ψ2 or ΨAM was discerned for hamster cells derived from the same genus, species, or even from the same hamster tissue (Table 3). The AHL and CHO K1 cell lines, both established from the hamster genus *Cricetulus* differ in that tunicamycin pretreatment renders CHO K1 but not AHL cells permissive for Ψ2 infection and AHL but not CHO K1 cells permissive to ΨAM infection. ΨAM virions can infect some but not all cells derived from *C. griseus* (Chinese hamster). The Chinese hamster ovary cell line CHO K1 is resistant to ΨAM infection, whereas Chinese hamster lung fibroblasts (Don cells) are not. Both BHK 21 and HaK cell lines were established from a Syrian golden hamster kidney, yet these two cell lines demonstrate marked differences in their susceptibility to ΨAM and Ψ2 virions as well as PG13 virions. One factor governing increased susceptibility to infection among hamster cells may be cellular phenotype. BHK 21 cells are fibroblasts, whereas the more readily infectible HaK cells are epithelioid cells. A phenotype-dependent induction of functional viral receptor upon in vitro culture of Syrian hamster kidney cells may explain these results. An analogous observation has been reported for infection of cultured fetal kidney cells by poliovirus. Expression of poliovirus receptor mRNA occurs in kidney cells both in vivo and in vitro (16); however, primate kidney cells do not bind poliovirus in vivo but can be induced to do so after they are trypsinized and grown as a monolayer in vitro (10). It has been proposed that regulation of poliovirus binding could be affected by posttranslational modifications of its receptor induced by culturing kidney tissue in vitro.

The general approach employed here may prove useful in the elucidation of the viral and cellular determinants responsible for the refractory status of many human cells to infection by recombinant virions and in designing recombinant hybrid virions that can successfully mediate transduction and expression of foreign genetic material in these cells. It has been previously reported that some human hematopoietic cell lines are resistant to infection by ΨAM/PA317-derived virions due to postpenetration blocks to infection (3). Attempts to design hybrid virions similar to the ones described in this report that can efficiently infect these cells may prove fruitful.

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