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Glycosylation-Dependent Inactivation of the Ecotropic Murine Leukemia Virus Receptor

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The ecotropic murine leukemia virus (E-MuLV) receptor expressed on *Mus dunni* tail fibroblast (MDTF) cells is a receptor for all E-MuLVs with the notable exception of Moloney murine leukemia virus (Mo-MuLV). Substitution of isoleucine for valine at position 214 in the third extracellular region (the putative E-MuLV binding site) of the MDTF receptor molecule allows this molecule to function as a Mo-MuLV receptor (M. V. Eiden, K. Farrell, J. Warsowe, L. A. Mahan, and C. A. Wilson, J. Virol. 67:4056–4061, 1993). We have now determined that treating MDTF cells with tunicamycin, an inhibitor of N-linked glycosylation, also renders them susceptible to Mo-MuLV infection. Two potential N-linked glycosylation sites are present in the third extracellular regions of both the NIH 3T3 and MDTF ecotropic receptors. The glycosylation site at position 229 of the MDTF receptor cDNA was eliminated by substituting a threonine codon for the asparagine codon. Mo-MuLV-resistant human HOS cells, expressing this form of the receptor, are susceptible to Mo-MuLV infection. Thus, our studies suggest that without a glycan moiety at position 229, the valine residue at 214 is no longer restrictive for Mo-MuLV infection. BHK-21 and CHO K1 hamster cells also express glycosylation-inactivated forms of the ecotropic receptor. Sequence analysis of these receptors together with our analysis of MDTF receptor function suggests that a single asparagine-linked glycosylation site is responsible for glycosylation inactivation of these receptors.

NIH 3T3 cells express a cell surface protein which functions as a receptor for all members of the ecotropic class of murine leukemia viruses (E-MuLVs). The cDNA for this receptor has been cloned, sequenced, and proposed to encode a glycoprotein containing 14 transmembrane domains (2). The third extracellular region has been shown to be involved in virus binding and entry (1, 4, 16). This domain contains two potential N-linked glycosylation sites. More recently, this receptor has been identified as the widely expressed transporter for basic amino acids (5, 13). The transporter protein is present on the cells of many species, but only the form of the transporter expressed on murine and rat cells can function as the receptor for ecotropic viruses.

Although all ecotropic viruses can use this receptor to enter target cells, some cells that are susceptible to infection by one ecotropic virus remain relatively resistant to infection by another. For example, the *Mus dunni* tail fibroblast (MDTF) cells initially described by Lander and Chattopadhyay are efficiently infected by all ecotropic viruses with the notable exception of Moloney murine leukemia virus (Mo-MuLV) (6). We have now confirmed this observation and extended it to Mo-MuLV-based retroviral vectors (4). MDTF cells can be efficiently infected by the ecotropic Friend murine leukemia virus (F-MuLV) as well as vectors that contain a Mo-MuLV-based genome and core and an F-MuLV envelope. In contrast to NIH 3T3 cells, no evidence of Mo-MuLV infection or spread can be detected in MDTF cells transfected with Mo-MuLV molecular clones. However, replication-competent Mo-MuLV can infect MDTF cells at a high multiplicity of infection, in contrast to human cells, in which resistance is absolute.

MDTF cells that express the NIH 3T3-derived ecotropic receptor after transfection of its cDNA are susceptible to Mo-MuLV-based vectors and replication-competent Mo-MuLV infection. These results suggest that differences in the ecotropic receptor expressed in NIH 3T3 cells and MDTF cells account for their disparate E-MuLV receptor functions (4). We have cloned, sequenced, and functionally characterized the ecotropic receptor cDNA from MDTF cells and compared it with the ecotropic receptor expressed in NIH 3T3 cells. The MDTF ecotropic receptor cDNA is similar to the nonfunctional human ecotropic receptor homolog in that it contains a valine codon at position 214 in the third extracellular loop, whereas the functional NIH 3T3 receptor contains an isoleucine codon at this position (2, 4, 15). Human cells expressing the MDTF ecotropic receptor cDNA are susceptible to F-MuLV but remain resistant to Mo-MuLV and are thus phenotypically indistinguishable from MDTF cells in terms of susceptibility to infection by E-MuLVs. The single amino acid residue difference between the MDTF and NIH 3T3 ecotropic receptors at position 214 accounts for the loss of Mo-MuLV receptor function, since substitution of an isoleucine codon (present in the NIH 3T3 form of the receptor) for the valine codon at position 214 of the MDTF receptor is sufficient to render the MDTF cells susceptible to Mo-MuLV infection (4).

The hamster cell lines CHO K1 and BHK-21 are resistant to E-MuLVs as a consequence of a glycosylation-dependent receptor-mediated block (14). We have now determined that pretreatment of MDTF cells with tunicamycin, an inhibitor of N-linked glycosylation, renders the MDTF receptor functional as a Mo-MuLV receptor. These data suggest that the ecotropic receptor present on MDTF cells, like the receptors on hamster cells, fails to function as a Mo-MuLV receptor as a result of a glycosylation-dependent process of inactivation. This suggests that N-linked glycans, in the context of specific amino acid residues in the third extracellular region of the ecotropic receptor, contribute to the overall conformation of this loop. Since the human form of the ecotropic receptor is nonfunctional, regardless of its glycosylation status, specific amino acid substitutions in the region of the receptor which has been

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demonstrated to function as the virus binding site within the NIH 3T3 form of the receptor account for its inability to function as an E-MuLV receptor. The hamster and MDTF receptor forms, on the other hand, contain amino acid substitutions which render them nonfunctional in the presence of N-linked glycans.

MATERIALS AND METHODS

Cell lines and viruses. The cells used in this study include NIH 3T3 murine fibroblasts (ATCC CRL 1658), MDTF cells (provided by Janet Hartley, National Institute of Allergy and Infectious Disease, Bethesda, Md.) (6), CHO K1 Chinese hamster ovary cells (ATCC CCL-61), BHK-21 Syrian hamster kidney cells (provided by Noel Bouck, Northwestern University, Chicago, Ill.), Lec 2 hamster cells (ATCC CRL 1736), Lec 8 hamster cells (ATCC CRL 1737), PA317 cells (ATCC CRL 9078), PG13 cells (ATCC CRL 10,683), GP9122 FrBAG cells (4, 8), HOS human osteosarcoma cells (ATCC CRL 1543), CRE BAG cells (ATCC CRL 1858), and CRIP BAG cells (provided by C. Cepko, Harvard Medical School, Boston, Mass.) (3, 10). All cell lines (except CHO K1, Lec 2, and Lec 8) were maintained in Dulbecco's modified essential medium (Whittaker Bioproducts, Inc., Walkersville, Md.) supplemented with 5% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 40 mM glutamine. The CHO K1, Lec 2, and Lec 8 cells were propagated in similarly supplemented alpha minimal essential medium (Whittaker Bioproducts).

Infections of target cells pretreated with tunicamycin. Target cells were seeded at 50,000 cells per well in Dulbecco's modified essential medium–5% fetal bovine serum with or without 0.15 μg of tunicamycin (Calbiochem Corp., La Jolla, Calif.) per ml in a 12-well tissue culture dish as previously described (14). 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, seeded at a 1:10 dilution in a 10-cm-diameter tissue culture dish, and selected with G418 (150 μg/ml for HOS cells and 400 μg/ml for MDTF cells).

Mutagenesis of the MDTF ecotropic receptor cDNA. The ecotropic receptor cDNA encoding a mutant form of the receptor lacking the potential N-linked glycosylation site was made by using a recombinant PCR protocol (7). Four oligonucleotide primers corresponding to specific regions of the NIH 3T3 ecotropic receptor cDNA were designed (2). The J12 sense primer corresponds to bases 176 to 205. The J10 primer is an antisense primer corresponding to nucleotides 1895 to 1924; the two complementary intermediate primers M6 (sense) and M7 (antisense) correspond to bases 851 to 886 and encode a single base change at base 880 resulting in the substitution of a threonine codon for the asparagine codon at this position, thus removing the glycosylation site. In the first round of PCR, two fragments of DNA were generated, using pLNS-mRec DNA as a template: one fragment extending from the J12 primer to the M7 primer, and the other fragment extending from the sense M6 primer to the J10 primer. These reactions were done on an automated thermal minicycler (MJ Research, Watertown, Mass.) (5 cycles of 1 min at 94°C, 2 min at 45°C, and 2 min at 72°C; 20 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C). The PCR products were purified by using Magic PCR Prep columns (Promega, Madison, Wis.) and then annealed at 70°C. The second round of PCR was performed on the annealed product, using the J12 and J10 primers (15 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C). This product was ligated into the TA pCRII cloning vector (Invitrogen, San Diego, Calif.) and sequenced by direct dideoxy sequencing (11) to ensure that the mutation was incorporated and that no unscheduled PCR-induced mutations were present. To construct the pLNS-mut229 retroviral expression plasmid, the *Hpa*I-*Sph*I fragment of this ecotropic receptor mutant (nucleotides 735 to 1792) was used to replace the corresponding fragment of pLNS-dRec.

Retroviral vector infection. CRE BAG and CRIP BAG cell lines were used as the sources of the Mo-MuLV and amphotropic murine leukemia virus (A-MuLV) vectors, respectively, used in these studies. The *Escherichia coli lacZ* β-galactosidase and Tn*5* *neo* genes are present in the BAG genome, and therefore infected cells can be identified by counting neomycin-resistant cell clones or by counting blue foci. The FrBAG vector was produced from GP9122 BAG cells transfected with an F-MuLV envelope expression plasmid as described previously (4). The retroviral vectors pLNS-mRec, pLNS-dRec, and pLNS-Mut 229, packaged in PG13 cells, were used to express the MDTF and NIH 3T3 ecotropic receptor cDNAs in BHK-21 and CHO K1 cells (4, 9, 14). Human HOS cells expressing various ecotropic receptors were established following infection with a PA317-packaged pLNS-mRec, pLNS-dRec, or pLNS-Mut229 genome.

Sequencing of hamster ecotropic receptor cDNAs. An internal segment of the BHK-21 and CHO K1 ecotropic receptor cDNA containing the third extracellular region was isolated by previously described methods (4). Briefly, polyadenylated RNA was isolated from each cell line and was used as template for a reverse transcription reaction primed by random hexadeoxyribonucleotides to make cDNA. Nested oligonucleotide primers were used to amplify the region between amino acid residues 276 and 384. The PCR products were ligated into the TA pCRII vector and sequenced.

RESULTS

The failure of the MDTF ecotropic receptor to function as a Mo-MuLV receptor is glycosylation dependent. CHO K1 and BHK-21 cells are resistant to infection by Mo-MuLV and Mo-MuLV-based retroviral vectors but can be made sensitive to Mo-MuLV infection after tunicamycin treatment (14). We sought to determine whether MDTF cells are resistant to Mo-MuLV infection as a consequence of a similar glycosylation-dependent process. MDTF cells pretreated with tunicamycin were exposed to Mo-MuLV-based recombinant vectors containing the gene encoding neomycin phosphotransferase. Untreated MDTF and human HOS cells and tunicamycin-pretreated HOS cells remained resistant, whereas MDTF cells pretreated with tunicamycin were efficiently infected by Mo-MuLV-based vectors (Fig. 1). We have previously determined that transfection and expression of the NIH 3T3-derived ecotropic receptor cDNA in MDTF cells results in the acquisition of Mo-MuLV susceptibility (4). These data together with the experimental results presented in Fig. 1 suggest that the ecotropic receptor expressed in MDTF cells is inherently different from the NIH 3T3 form of the ecotropic receptor and that the MDTF form of the receptor is inactivated by a glycosylation-dependent process.

Two potential sites for N-linked glycosylation are present in the third extracellular domain of both the NIH 3T3 and MDTF receptor molecules, at positions 223 and 229 (Fig. 2). Site-specific mutagenesis was used to selectively eliminate the site at position 229 in the MDTF receptor cDNA, where a threonine codon was substituted for the asparagine codon. A retroviral vector expressing this mutant cDNA, pLNS-Mut 229, was introduced into HOS, CHO K1, and BHK-21 cells, which

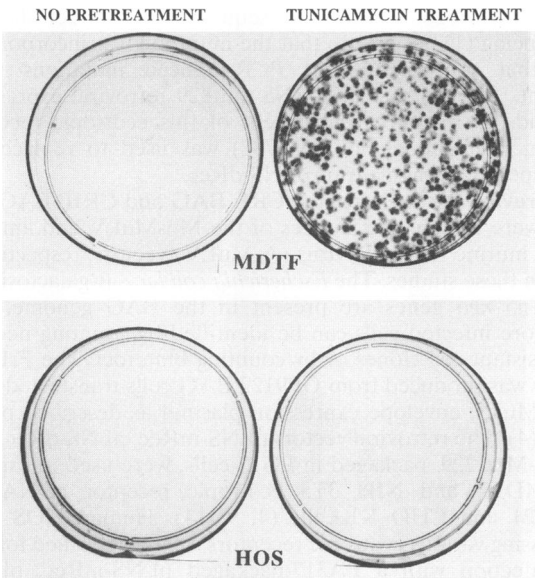

FIG. 1. Analysis of the effect of tunicamycin treatment on MDTF and HOS cell susceptibility to Mo-MuLV (CRE BAG) infection. Cells were trypsinized and seeded at a 1:10 dilution in a 10-cm-diameter tissue culture dish and selected with G418 after exposure to 2.0 ml of filtered virus. The culture dishes on the left (containing the untreated cells) and the culture dishes on the right (containing tunicamycin-treated cells) demonstrate the presence or absence of G418-resistant clones after exposure to CRE BAG virions. Colonies were stained with a methanol solution containing 0.75% methylene blue and 0.25% carbol fuchsin to optimize visualization.

were then tested for susceptibility to E-MuLV infection. Cells expressing this form of the ecotropic receptor were demonstrated to be susceptible to infection by both Mo-MuLV and F-MuLV (Table 1). Therefore, removal of this potential N-linked glycosylation site at position 229 renders the MDTF receptor functional as a Mo-MuLV receptor without compromising F-MuLV receptor function.

Analysis of the effect of N-linked glycans on the function of hamster cell ecotropic receptors. To further characterize the nature of this glycosylation-mediated block to Mo-MuLV infection, we compared the relative susceptibilities of CHO K1 cells and CHO K1 mutant cells exhibiting altered glycosylation of their surface glycoproteins to Mo-MuLV and F-MuLV infection. The Lec 2 and Lec 8 hamster cell lines were independently obtained by selection of CHO K1 cells for resistance to cytotoxic plant lectins and have been identified as mutants with unique primary defects in glycosylation at a stage subsequent to the early block in the N-linked glycosylation pathway effected by tunicamycin (12). It has been determined that the defects in Lec 2 and Lec 8 cells result from a dramatic reduction in CMP-sialic acid (Lec 2) or UDP-galactose (Lec 8) transport into the Golgi compartment (12). CHO K1 cells, unlike MDTF cells, are resistant to infection by both F-MuLV and Mo-MuLV (Table 1). The Lec 2 and Lec 8 glycosylation mutants are susceptible to infection by both F-MuLV and Mo-MuLV (Table 2). These results suggest that the block to E-MuLV infection of CHO K1 cells can be circumvented in CHO K1 mutants containing altered cell surface carbohydrate moieties.

By expressing different forms of the ecotropic receptor in CHO K1 cells, we demonstrated that their susceptibility to infection by different E-MuLVs could be altered. For example, if the MDTF receptor cDNA is expressed in CHO K1 cells (which are normally resistant to all E-MuLVs), they reconstitute the MDTF phenotype, thus making them susceptible to F-MuLV but not Mo-MuLV (Table 3). Expression of the NIH 3T3 ecotropic receptor cDNA in CHO K1 cells resulted in acquired susceptibility to both Mo-MuLV and F-MuLV infection (Table 1).

In contrast, BHK-21 cells expressing the MDTF ecotropic receptor remain resistant to both Mo-MuLV and F-MuLV (Table 3). However, when BHK-21 cells express the MDTF receptor containing the N-T substitution at position 229, they become susceptible to infection by both F-MuLV and Mo-MuLV (Table 1). Therefore, the position 229 mutation removes both the selective block to F-MuLV function exhibited by the MDTF receptor in some cells and the total block to MDTF ecotropic receptor function exhibited by BHK-21 cells.

Sequence comparison of the third extracellular region of hamster, human, and murine ecotropic receptor cDNAs. To determine the molecular basis for the observed differences in BHK-21, CHO K1, MDTF, human HUT 78, and NIH 3T3 cells regarding susceptibility to E-MuLV infection, we compared the deduced amino acid sequences of the third extracellular regions of these five receptor cDNAs. As shown in Fig. 3A, the NIH 3T3 form of the ecotropic receptor contains 31 amino acid residues (2). The MDTF form contains 32 residues (4), the BHK 21 and CHO K1 forms contain 38 residues, and the human form of the ecotropic receptor contains 39 residues in the third extracellular loop (15). Both CHO K1 and the human receptor homologs contain three additional residues on either side of the first N-linked glycosylation site. The BHK-21 receptor homolog has a similar insertion of six amino acid residues but lacks the first glycosylation site present at position 223 in the other four forms of the receptor (Fig. 3A). A proline residue is present in place of the requisite serine/threonine residue which constitutes the terminal amino acid residue in the Asn-X-Ser/Thr consensus sequence for N-linked glycosylation.

The BHK-21 and CHO K1 receptors are similar to the NIH 3T3 receptor and differ from both the MDTF and human forms of the receptor in that they contain an isoleucine rather than a valine residue at position 214 (Fig. 3A). Finally, both the CHO K1 and BHK-21 receptors lack the additional amino acid residue inserted in the carboxy half of this domain that is present in both the MDTF and human forms of the receptor (positions 236 for MDTF and 242 for human). The MDTF form of the receptor has an additional glycine residue at position 236 (Fig. 3A). All forms of the receptor contain four identical amino acid residues at both of the proposed end regions of this extracellular loop and at least one N-linked glycosylation site. The majority of differences are localized to three positions: position 214, the positions flanking the first of the two N-linked glycosylation sites, and finally at the position downstream of the second N-linked glycosylation site (approximately six amino acids from the C-terminal end of this domain).

DISCUSSION

Classification of three ecotropic receptor subtypes. The ecotropic receptor subtype expressed in NIH 3T3 cells functions as a receptor for all E-MuLVs, such as Rauscher murine leukemia virus, F-MuLV, and Mo-MuLV. MDTF cells, a murine cell line derived from an Asian strain of feral mice, express a distinct subtype of ecotropic receptor that distinguishes between different members of the ecotropic class of viruses, functioning as a receptor for Rauscher murine leuke-

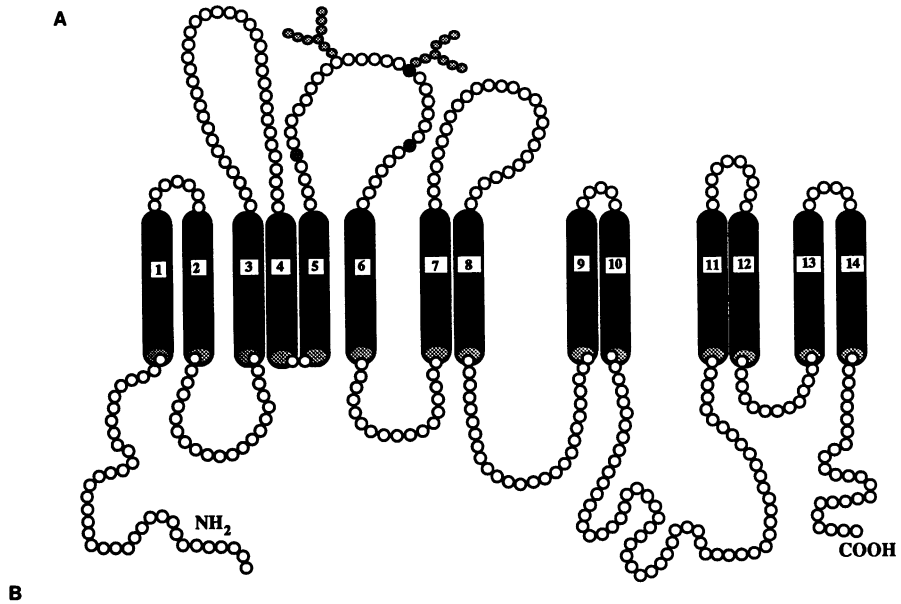

FIG. 2. (A) Schematic representation of the MDTF form of the ecotropic receptor. Rods depict transmembrane regions, and individual circles correspond to amino acid residues in the cytoplasmic and extracellular regions of the receptor. Two potential N-linked glycosylation sites are contained within the third extracellular loop at positions 223 and 229 and are marked by a branched structure in the diagram. The two amino acid changes which distinguish the MDTF receptor from the NIH 3T3 receptor (the substitution of a valine for isoleucine at position 214 and the insertion of an additional glycine at position 236) are represented by shaded circles. (B) Alignment of the amino acid sequences of the third extracellular regions (extending from amino acid residues 210 to 242/243) of the NIH 3T3 (2), MDTF (4), and Mut 229 ecotropic receptors. The glycosylation consensus sequences (NXS or NXT) are underlined. The Mut 229 form of the receptor contains a threonine in place of the asparagine at the corresponding position of the NIH 3T3 and MDTF receptors. This substitution results in the loss of the second potential N-linked glycosylation site at position 229.

mia virus and F-MuLV but not Mo-MuLV. Hamster cells such as CHO K1 and BHK-21 express an ecotropic receptor subtype that is not functional in its native form for any of the E-MuLVs but can be rendered functional by the removal of N-linked carbohydrates (Fig. 3B).

TABLE 1. Susceptibilities of cells expressing the Mut 229 ecotropic receptor to infection by E-MuLVs

Target cells	Focus formation[a]		
	Mo-MuLV	F-MuLV	A-MuLV
BHK-21	0	0	0
BHK-21 + Mut 229	411	367	0
BHK-21 + NIH 3T3 receptor	378	114	0
CHO K1	0	0	0
CHO K1 + Mut 229	289	356	0
CHO K1 + NIH 3T3 receptor	311	75	0
HOS	0	0	362
HOS + Mut 229	426	411	416
HOS + NIH 3T3 receptor	512	101	388

[a] Number of blue foci observed 72 h after exposure to equivalent volumes of medium containing Mo-MuLV, F-MuLV, or A-MuLV enveloped retroviral vectors. Mo-MuLV retroviral vectors were produced from CRE BAG cells and contained the Mo-MuLV-based BAG genome. F-MuLV retroviral vectors were produced from GP9122 BAG cells transfected with an F-MuLV envelope expression plasmid (4). A-MuLV retroviral vectors also contain the Mo-MuLV-based BAG genome. They were produced from CRIP BAG cells and therefore contain the 4070A A-MuLV envelope.

Identification of three regions within the third extracellular region that effect E-MuLV binding and entry. Yoshimoto et al. have previously shown that substitution of a single amino acid residue from the nonfunctional human receptor for the corresponding tyrosine residue at position 235 in the NIH 3T3 receptor is sufficient to prevent Mo-MuLV entry (16). Similar results obtained by Albritton et al. suggested that the substitution of at least two amino acid residues, $PGV_{242-244}$, from the human receptor for the corresponding residues in the NIH 3T3 receptor, $YGE_{235-237}$, was required to abrogate Mo-MuLV entry and binding (1). It is important to note that E-MuLVs other than Mo-MuLV were not tested in these

TABLE 2. CHO K1 glycosylation mutants are susceptible to Mo-MuLV and F-MuLV

Target cells	Focus formation[a]	
	Mo-MuLV	F-MuLV
CHO K1	0	0
Lec 2	246	211
Lec 8	311	289

[a] Number of blue foci observed 72 h after exposure to equivalent volumes of medium containing either Mo-MuLV or F-MuLV enveloped retroviral vectors. Mo-MuLV retroviral vectors were produced from CRE BAG cells and contained the Mo-MuLV-based BAG genome. F-MuLV retroviral vectors were produced from GP9122 BAG cells transfected with an F-MuLV envelope expression plasmid (4).

TABLE 3. Susceptibilities of human and hamster cells expressing MDTF receptor cDNA to infection by E-MuLVs

Target cells	Focus formation[a]		
	Mo-MuLV	F-MuLV	A-MuLV
MDTF	2	189	406
NIH 3T3	567	115	489
CHO K1	0	0	0
CHO K1 + MDTF ecotropic receptor	1	114	0
BHK-21	0	0	0
BHK-21 + MDTF ecotropic receptor	0	0	0
HOS	0	0	626
HOS + MDTF ecotropic receptor	0	78	611

[a] Number of blue foci observed 72 h after exposure to equivalent volumes of medium containing either Mo-MuLV or F-MuLV enveloped retroviral vectors. Mo-MuLV retroviral vectors were produced from CRE BAG cells and contained the Mo-MuLV-based BAG genome. F-MuLV retroviral vectors were produced from GP9122 BAG cells transfected with an F-MuLV envelope expression plasmid (4). A-MuLV retroviral vectors also contain the Mo-MuLV-based BAG genome. They were produced from CRIP BAG cells and therefore contained the 4070A A-MuLV envelope.

studies, and it is therefore conceivable that these ecotropic receptors can function as receptors for other E-MuLVs such as F-MuLV. Our findings with the MDTF subtype of the ecotropic receptor further support such a possibility. This receptor subtype allows F-MuLV infection but fails to bind Mo-MuLV as a consequence of the additional glycine residue at position 236 (4). We now show that this receptor does not allow Mo-MuLV entry due the presence of an N-linked glycan at position 229, an indirect inactivation event mediated by the valine-for-isoleucine substitution at position 214. Therefore, the valine residue at position 214 of the MDTF receptor is restrictive for Mo-MuLV only in the presence of adjacent N-linked oligosaccarides.

CHO K1 cells differ from murine cells in that they are resistant to Mo-MuLV and F-MuLV (Table 1). They differ from human cells in that resistance to E-MuLV is glycosylation mediated (14). It is of interest to note that the CHO K1 form of the receptor contains a histidine residue in place of the tyrosine residue found in the NIH 3T3 receptor at position 241. The placement of a tyrosine residue in this position has been suggested to have a critical effect on ecotropic receptor function (1, 16). Our finding that the CHO K1 ecotropic receptor can function as an ecotropic receptor with a histidine at position 241 suggests that a tyrosine amino acid residue at this position is not a critical part of an E-MuLV binding and entry epitope in the absence of asparagine-linked oligosaccarides.

We have shown that the presence of an N-linked carbohydrate moiety at position 229 of the MDTF receptor is not required for the entry of E-MuLVs such as F-MuLV. Indeed, elimination of this site retains F-MuLV receptor function and extends the function of this molecule to serve as a receptor for Mo-MuLV. Comparison of the function of the MDTF ecotropic receptor expressed in BHK-21 cells and CHO K1 cells suggests that cell-specific patterns of N-linked glycosylation can result in altered receptor function. Expression of the MDTF ecotropic receptor cDNA in CHO K1 cells results in reconstitution of the MDTF phenotype in terms of E-MuLV infection; i.e., they are susceptible to F-MuLV but not Mo-MuLV infection (Table 3). BHK-21 cells expressing the MDTF ecotropic receptor cDNA remained resistant to infection by both F-MuLV and Mo-MuLV (Table 3). These collective findings suggest that both N-linked glycosylation patterns potentiated by different ecotropic receptor cDNAs and cell-specific N-linked glycosylation patterns affect the ability of various E-MuLVs to use these ecotropic receptors. Similar cell-specific posttranslational receptor modifications may account for the discrepancies between the previous reports regarding mutational inactivation of the ecotropic receptor by substitution of amino acid residues from the human receptor

A.

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                  214                                   229
NIH 3T3: V K G S I K N W Q L T E K      N F S      C N N N D T N V K Y G  E G G F M
MDTF:    V K G S V K N W Q L T E K      N F S      C N N N D T N V K Y G G E G G F M

CHO K1:  V K G S I K N W Q L T E E D F L N R S S P L C G N N D T N V K H G  E G G F M
BHK 21:  V K G S I K N W Q L K E E D F L N S P S A P L G N N D T N V K Y G  E G G F M

HUT 78:  V K G S V K N W Q L T E E D F G N T S G P L C L N N D T K E G K P G V G G F M
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B.

RECEPTOR SUBTYPE	Mo-MuLV	F-MuLV
1. NIH 3T3	GLYCOSYLATION INDEPENDENT	GLYCOSYLATION INDEPENDENT
2. MDTF	GLYCOSYLATION DEPENDENT	GLYCOSYLATION INDEPENDENT
3. BHK 21 CHO K1	GLYCOSYLATION DEPENDENT	GLYCOSYLATION DEPENDENT

FIG. 3. (A) Comparison of the amino acid residues contained within the third extracellular regions of the ecotropic receptors present in murine NIH 3T3 (2) and MDTF (4), hamster CHO K1 and BHK-21, and human HUT 78 (17) cells. The N-terminal amino acid is at position 210 for all five receptors and extends to positions 241 for NIH 3T3, 242 for MDTF, 246 for the hamster receptors, and 247 for the human form of the receptor. (B) Comparison of the ability of the three receptor subtypes expressed in NIH 3T3, MDTF, and hamster cells to function as either an F-MuLV or Mo-MuLV receptor. The receptors classified as glycosylation independent are present on cells that are susceptible to virus infection in the absence of tunicamycin treatment. Glycosylation-dependent receptors are receptors expressed on cells which, although normally resistant to virus infection, can be rendered susceptible to F-MuLV and/or Mo-MuLV by tunicamycin treatment.

(see above). Albritton et al. (1) conducted their experiments in human 293 cells, whereas Yoshimoto et al. (16) transfected their mutant receptors into CHO K1 cells.

Comparing both the functions and the nucleotide sequences of the BHK-21, CHO K1, NIH 3T3, MDTF, and human ecotropic receptor cDNAs has allowed us to identify three discontinuous epitopes or regions of the ecotropic receptor that facilitate, either directly or indirectly, E-MuLV entry. The two regions that appear directly involved in Mo-MuLV entry lie on opposing ends of this extracellular loop (position 214 and the residues at positions 235 to 237; Fig. 3). Glycosylation appears to block access of the viral particle to this critical region of the receptor, thereby restricting use of the hamster receptor by E-MuLVs and use of the MDTF receptor by Mo-MuLV.

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