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Identification of a Cellular Receptor for Mouse Mammary Tumor Virus and Mapping of Its Gene to Chromosome 16

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Pseudotypes of vesicular stomatitis virus (VSV) containing envelope glycoproteins provided by C3H mammary tumor virus (MTV) instead of the normal VSV G-proteins were prepared and used to assay the presence of an MTV receptor on cells. The assay was specific as demonstrated by competition studies with excess MTV particles and neutralization of the pseudotypes with anti-MTV serum or monoclonal antibodies directed against MTV gp52. The MTV receptor was abundantly present on mouse cells but hardly detectable on nonmurine cells, including the Chinese hamster cell line E36. Somatic cell hybrids between E36 cells and GRS/A spontaneous leukemia cells (GRSL cells) and between E36 and GRS/A primary mammary tumor cells were made. The hybrids retained all Chinese hamster chromosomes but segregated mouse chromosomes. From the analysis of the isoenzymes and chromosomes of the hybrid cell lines we conclude that the gene for the receptor (*MTVR-1*) is located on mouse chromosome 16.

The first step of infection by retroviruses is dependent on the presence of a cellular membrane receptor. The presence or absence of such a receptor often determines whether the virus can infect the cell. For retroviruses this has been clearly documented in the case of murine leukemia virus (MuLV), for which separate receptors exist for ecotropic, xenotropic, amphotropic, and recombinant viruses (3, 6, 12, 28, 31, 32). Other genes, like *Fv-1* on chromosome 4, act later during virus replication and determine the host range of the ecotropic MuLVs (for reviews, see references 19 and 36).

Little is known about the genetic control of the replication of exogenous mammary tumor virus (MTV). The commonly followed approach, mouse cross-breeding experiments, has provided relatively little insight into what host genes are necessary for MTV replication, except for the demonstration of some control by the *H-2* locus (for a review, see reference 2). Other studies have shown that the susceptibility of mouse strains inoculated with different strains of MTV varied greatly (2, 10, 24). It is not clear whether, in this case, receptors play a decisive role in determining the host range of MTV. Studies in the MTV field have been hampered by the lack of an efficient in vitro infection assay. Závada et al. (41) were able to prepare pseudotypes of vesicular stomatitis virus (VSV) containing envelope glycoproteins from MTV. These VSV(MTV) pseudotypes possess several properties of the original MTV such as host range, neutralization, and interference characteristics.

We have used similar pseudotypes to infect somatic cell hybrids between murine and Chinese hamster cells to answer the question of whether there is indeed a specific receptor for MTV. We were able to demonstrate the presence of such a receptor and to map its gene to chromosome 16 of the mouse.

MATERIALS AND METHODS

Viruses and cells. TIB17 and tsO45, two temperature-sensitive mutants of the Indiana strain of VSV, were obtained from S. Schlesinger (Washington University School of Medicine, St. Louis, Mo.). The viruses were cloned twice before stocks were prepared in BHK-21 cells. E36 cells are a Chinese hamster lung fibroblast line lacking hypoxanthine guanine phosphoribosyltransferase (8). GRSL cells are thymic leukemia cells derived from a male GRS/A (GR) mouse (10); they were serially transplanted in ascites form (for the preparation of somatic cell hybrids) or were adapted for growth in vitro (for plaque assays). Primary mammary tumor cells (GR MaTu cells) were derived from a mammary tumor of a GRS/A mouse; they were maintained in minimal essential medium with D-valine medium for one passage to permit preferential growth of epithelial cells (7). GRMT cells, an established line derived from a GR mammary tumor, were a gift from C. Dickson (Imperial Cancer Research Fund, London, England). WP cells were derived from a C3H mammary adenocarcinoma line

established by W. Parks (National Cancer Institute, Bethesda, Md.) All of these mammary tumor cells were grown in the presence of insulin and produced, in the presence of dexamethasone, large amounts of MTV. Primary mouse embryo cells were derived from 10-day-old embryos of BALB/cHeA or GRS/A mice. L929 cells are of C3H/An mouse origin (33), and BHK-21 cells are of Syrian hamster origin (37). The following cells were all obtained from the Naval Supply Center, Oakland, Calif.: CCL64 (mink lung) (9); SIRC (rabbit corneal) (18); CrFK (cat kidney) (4); and AQ, normal mammary gland cells isolated from the NAMRU mouse strain and maintained in culture as a continuous cell line (29).

EGR hybrid clones were prepared by a fusion between GRSL and E36 cells (12). EMT clones were derived from a fusion between primary GR MaTu cells and E36 cells; both hybrid series retained all hamster chromosomes but segregated mouse chromosomes. The fusion procedure and a further description of the hybrids have been given by Hilkens et al. (12, 13).

All cell lines were grown in Dulbecco modified Eagle medium supplemented with 10% newborn calf serum. All hybrids were grown in the same medium supplemented with 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine (HAT). Cell lines were regularly checked for the absence of mycoplasma infection.

Antiserum. Rabbit antiserum was prepared by multiple immunizations with gradient-purified, Triton X-100-disrupted wild-type VSV (strain Indiana). Quantities of 0.1 to 1 mg mixed with Freund adjuvant were injected. The resulting serum gave a 50% neutralization of 100 PFU in a 100,000-fold dilution. Rabbit anti-MTV serum was prepared and absorbed in vivo in BALB/c mice according to Hilgers et al. (11). The serum was used at a final dilution of 1:50 for inactivation of VSV(MTV) pseudotypes and at 1:16 for immunofluorescence. Ascites fluids of monoclonal mouse anti-MTV gp52 sera were obtained from R. Massey (Frederick Cancer Center, Fort Detrick, Md.) (20).

VSV(MTV) pseudotype preparation. VSV(MTV) pseudotypes were prepared by growing VSV in an MTV-producing cell line. Briefly, WP cells, grown in Dulbecco modified Eagle medium supplemented with 10% newborn calf serum and 10 μg of insulin and 0.5 μg of dexamethasone per ml to stimulate MTV production, were infected at 31°C with VSV tlB17 or tsO45 at a multiplicity of infection of 2 PFU/cell. Virus-containing medium was harvested 16 h later, clarified by centrifugation (10 min, 1,000 × *g*) and frozen at −70°C.

VSV(MTV) pseudotype infection assay. Virus stocks were diluted to 2×10^7 PFU/ml and incubated overnight at 40°C in the presence of rabbit anti-VSV serum (1:50 or 1:100 final dilution). The next day, 0.2-ml virus solutions were prepared in phosphate-buffered saline containing 1% heat-inactivated fetal calf serum and 20 μg of Polybrene per ml and adsorbed to confluent monolayers of target cells in 35-mm culture dishes (31°C, 2 h). Plaques were scored after 2 to 3 days at 31°C. Control VSV was grown on the same cell lines or hybrid clones as the pseudotypes, and the test was read only when control VSV was able to grow on the same cell line. All tests were carried out in duplicate and in at least two concentrations of pseudotypes.

GRSL13 cells can only be grown in suspension, and a slightly different assay was used with these cells (13).

Chromosome marker analysis. The presence of the mouse and the Chinese hamster isoenzymes was examined in each hybrid by the difference in electrophoretic mobility of isoenzymes in 12% starch gels; superoxide dismutase and sorbitol dehydrogenase isoenzymes were separated by isoelectric focusing. A total of 22 isoenzymes were tested according to published procedures (25–27, 35, 38, 39): adenine phosphoribosyltransferase (EC 2.4.2.7), peptidases 1, 2, 3, 4, and 7 (α-aminoacylpeptide hydrolases, EC 3.4.11), galactokinase (EC 2.7.1.6), esterase-10 (carboxylesterase, EC 3.1.1.1), glucose-6-phosphate isomerase (glucosephosphate isomerase, EC 5.3.1.9), glutathione reductase [glutathione reductase (NAD(P)H), EC 1.6.4.2], lactate dehydrogenase (EC 1.1.1.27), malate oxidoreductase decarboxylating [malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$), EC 1.1.1.40], mannose phosphate isomerase (EC 5.3.1.8), acid phosphatase (EC 3.1.3.2), purine-nucleoside phosphorylase (EC 2.4.2.1), 6-phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44), phosphoglucomutase (EC 2.7.5.1), triosephosphate isomerase (EC 5.3.1.1), glyoxylase-1 (lactoyl-glutathione lyase, EC 4.4.1.5), glutamate transaminase (aspartate aminotransferase, EC 2.6.1.1), sorbitol dehydrogenase (L-iditol dehydrogenase, EC 1.1.1.14), superoxide dismutase (EC 1.15.1.1). In addition, *H-2* antigens and the *Rev-1* gene on chromosome 5 coding for the MuLV receptor were scored (12).

Chromosome analysis. Mouse chromosomes were identified by trypsin-Giemsa banding followed by centromeric staining with Hoechst dye 33258 (16). Ten or more spreads were examined routinely.

RESULTS

VSV(MTV) pseudotypes. VSV(MTV) pseudotypes were prepared by infecting MTV-producing WP cells with the tlB17 mutant of VSV (40, 41). Since VSV can also form pseudotypes with MuLV, the WP cells were checked for the absence of MuLV antigens by immunofluorescence (11) and found to be negative at the time of infection. VSV infection of the WP cells resulted in a virus mixture with a titer of 1.3×10^8 PFU/ml when plaque titrated on BALB/c or GR embryo cells. A fraction of 10^{-3} of this infectivity was refractive to neutralization with anti-VSV serum but could be neutralized with anti-MTV serum. Control VSV, grown in MTV-negative BHK-21 cells, was neutralized almost 10^6-fold by anti-VSV serum (Fig. 1). It was assumed therefore that a fraction of about 10^{-3} of the VSV particles grown in WP cells consisted of VSV(MTV) pseudotypes containing the MTV envelope glycoprotein (gp52) instead of the normal VSV G protein. When VSV mutant tsO45 was used instead of tlB17, qualitatively similar results were obtained. Because the remaining infectivity, after addition of anti-VSV serum to the virus mixture obtained from VSV-infected WP cells, was due to the VSV(MTV)

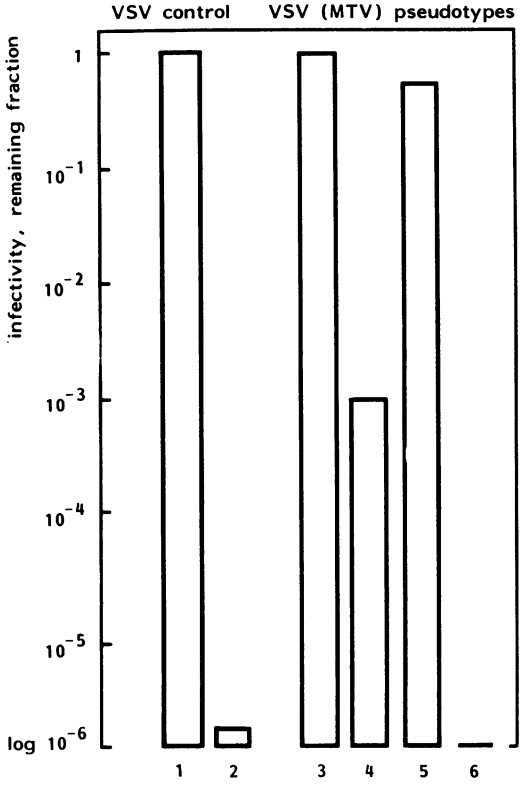

FIG. 1. Neutralization of VSV and VSV(MTV) pseudotypes by anti-VSV and anti-MTV serum. Secondary embryo culture cells of GR mice were infected with VSV tlB17 of a preparation grown on BHK-21 cells and with VSV of the same preparation treated with anti-VSV serum before infection. The relative numbers of counted plaques are indicated in the first and second column. In a parallel experiment, an infection test was carried out with a virus preparation obtained from MTV-producing WP cells infected with VSV. The relative numbers of plaques are indicated. Untreated, column 3; after pretreatment with anti-VSV serum, column 4; with anti-MTV serum, column 5; and with anti-VSV plus anti-MTV, column 6.

pseudotypes, the plaque assays to demonstrate the VSV(MTV) infectivity were carried out with these virus preparations in the presence of anti-VSV serum. The VSV(MTV) pseudotypes infectivity was high on L929 cells (Table 1); therefore, these cells were used in most experiments as control cells rather than the primary mouse embryo cells.

Specificity and further characterization of the VSV(MTV) pseudotypes. Since there was no direct evidence that the cellular receptors used by the VSV(MTV) pseudotypes were the same as the receptors used by MTV, more data were needed to ascertain this point.

GRMT and WP cells were treated with dexamethasone to stimulate the MTV production (23, 30), and after 24 h the medium was harvested and concentrated. The fresh concentrated MTV-containing medium was subsequently used to block the receptors on L929 target cells, before the VSV(MTV) pseudotypes were added to the cells. The results (Table 2) showed that pretreatment of the L929 cells with MTV-containing medium resulted in an 80% reduction of the plating efficiency of the VSV(MTV) pseudotypes, suggesting that the VSV(MTV) pseudotypes use the same receptor as GR and C3H MTV.

Massey and Schochetman made a detailed analysis of the domain of the gp52 molecule of C3H MTV that is involved in the binding to the cellular receptor (21, 22). In the course of their studies they prepared a neutralizing monoclonal antibody against MTV gp52, VII P2G6. Using

TABLE 1. Infectivity of VSV(MTV) pseudotypes on cell lines

Cells	PFU of VSV(MTV) at a virus dilution of:		Permissiveness of cells for VSV(MTV) pseudotypes
	10^{-1}	10^{-2}	
GRSL13	—[a]	224	+
GR embryo cells	—	60	+
BALB/c embryo cells	—	44	+
L929	—	350	+
GRMT	—	45	+
GRMT (grown in the presence of dexamethasone)	2	2	±
WP	—	33	+
AQ 868	91	6	+
E36	0	0	−
CrFK	8	1	±
SIRC	0	0	−
CCL64	3	0	±

[a] —, Too many PFU to count.

TABLE 2. Inhibition of VSV(MTV) pseudotype infection by concentrated MTV-containing culture media[a] of GRMT and WP cells

Pretreatment[b]	PFU of VSV at a 10^{-5} dilution	PFU of VSV(MTV) at a virus dilution of:	
		10^{-1}	10^{-2}
No	33	101	15
Medium GRMT	14	20	3
Medium WP	21	20	0

[a] Concentrated GRMT medium contained 5,000 ng of gp52 per ml and WP medium contained 600 ng/ml as determined in a radioimmunoassay.

[b] The pretreatment was 30 min at 31°C, and the adsorption of the pseudotypes was for 60 min (instead of 120 min).

TABLE 3. Neutralization of VSV(MTV) pseudotypes by anti-MTV gp52 monoclonal antibody VII P2G6

Addition	PFU of VSV at a virus dilution of:		PFU of VSV(MTV) at a dilution of:	
	10^{-5} (control)	10^{-1} (+anti-VSV serum)	10^{-1}	10^{-2}
None	73	0	151	24
VII P2G6	72		1	0

this antibody at a dilution 1:100, we found an almost complete inhibition of the infectivity of the VSV(MTV) pseudotypes on L929 cells, although there was no effect of the monoclonal antibody on the VSV infectivity (Table 3). These results show that gp52 of MTV is involved in the binding of the VSV(MTV) pseudotypes to the cellular receptors.

Host range of the VSV(MTV) pseudotypes. In addition to the GR and BALB/c embryo cells and the L929 cells, several other mouse cell lines and cell lines of nonmurine origin were tested for the presence of the VSV(MTV) receptor. Table 1 shows the results obtained by inoculating the cells with two dilutions of pseudotypes. To eliminate the possibility that negative results with the pseudotypes were due to restriction of VSV replication rather than the lack of a receptor, the cells were always infected with standard VSV as a control. The VSV(MTV) pseudotypes produced plaques on all mouse cells tested, whereas the Chinese hamster line E36 gave no plaques or at most 0.11% of the plaques that the GRSL cells did, indicating that this hamster cell line lacks the MTV receptor. Other nonmurine cells, most of them epithelial cells, had only a few or no MTV receptors.

A number of the murine cell lines tested

TABLE 4. VSV(MTV) pseudotype plaque formation on EGR and EMT hybrids

Hybrid clone	PFU of VSV(MTV) at a dilution of[a]:		MTV receptors	Hybrid clone	PFU of VSV(MTV) at a dilution of[a]:		MTV receptors
	10^{-1}	10^{-2}			10^{-1}	10^{-2}	
EMT 3	15	2	+	EMT 25	52	7	+
EMT 3A	76	10	+	EMT 31	14	2	+
EMT 3B	144	19	+	EMT 32A	77	4	+
EMT 3B1	91	6	+	EMT 37A	26	1	+
EMT 3B2	47	7	+	EMT 40	44	6	+
EMT 3B3	0	0	−	EMT 44	56	7	+
EMT 3B4	50		+	EGR 2	2	0	−
EMT 3C	117	18	+	EGR 5	3	0	−
EMT 3C2	86	4	+	EGR 6	>100	55	+
EMT 3C4	2	0	−	EGR 6B	>100	18	+
EMT 3C6	>100	11	+	EGR 6E	0	0	−
EMT 3C6A	>100	38	+	EGR 7/1	1	0	−
EMT 3C7	70	10	+	EGR 7/2	2	1	−
EMT 3E	>100	31	+	EGR 7A	2	0	−
EMT 3F	102	14	+	EGR 11	1	0	−
EMT 6/0	101	6	+	EGR 11A	0	0	−
EMT 6/2	19	2	+	EGR 13	1	0	−
EMT 6A	196	31	+	EGR 15	3	1	−
EMT 6C	58	10	+	EGR 16	1	0	−
EMT 6E	89	10	+	EGR 17B	25	3	+
EMT 8	31	4	+	EGR 17B5	7	0	−
EMT 10	>100	31	+	EGR 17B6	0	0	−
EMT 13	0	0	−	EGR 23	3	0	−
EMT 13A	>100	33	+	EGR 23D	6	1	−
EMT 13B	54	10	+	EGR 25A1	1	0	−
EMT 13D1	0	0	−	EGR 25E	>100	25	+
EMT 13D2	0	0	−	EGR 25K1	1	0	−
EMT 13D3	27	4	+	EGR 25K2	0	0	−
EMT 13E	46	2	+	EGR 26	1	0	−
EMT 18	25	3	+	EGR 30	>100	40	+
EMT 18A	117	13	+	EGR 30B	0	0	−
EMT 18C	84	6	+	EGR 30K	>100	26	+
EMT 18F	56	5	+				

[a] The assays were performed in duplicate and were scored positive when 10^{-1} dilution gave more than 10 plaques, which is 25 times the mean control value with neutralized VSV.

(GRSL, WP, GRMT) produce MTV that might interfere with the binding of VSV(MTV) pseudotypes to these cells as has been described earlier for MuLV (5). This seemed not to be the case as long as the cells were grown in the absence of the MTV inducer dexamethasone. If GRMT cells were grown, however, in the presence of this synthetic hormone, plaque formation by VSV(MTV) pseudotypes was restricted almost completely (Table 1). Dexamethasone had no effect on the plaque formation on cells producing no MTV.

Cell hybrids. We were interested in determining the gene(s) affecting the presence of MTV receptors on mouse cells. Therefore, somatic cell hybrids between VSV(MTV) receptive cells (GRSL or GR MaTu) and E36 cells lacking receptors for VSV(MTV) were prepared. E36 cells lack hypoxanthine guanine phosphoribosyltransferase activity and therefore cannot grow in HAT-containing medium. Since the mouse parental cells are wild type in this respect, the hybrid cells containing the mouse hypoxanthine guanine phophoribosyltransferase enzyme will survive in HAT medium. The GRSL cells normally grow as an ascites tumor and not in vitro without an adaption procedure; moreover, they do not attach so that they could easily be removed from the adhering cells. Primary GR MaTu cells have a limited life span in vitro and could be easily distinguished from the hybrid clones.

Independent clones of both types were isolated and expanded. Isoenzyme tests and karyological analysis showed that the clones were true hybrids, segregating mouse chromosomes while retaining the complete E36 genome. In addition, subclones of several primary clones were selected and characterized. The difference in chromosome segregation was remarkable: the EGR hybrids (GRSL × E36) were very unstable and segregated the mouse chromosomes very rapidly upon culturing. Subclones usually had only a few mouse chromosomes left. In contrast, the EMT hybrids (GR MaTu × E36) kept very stable karyotypes; subclones had lost only a few mouse chromosomes.

Although the parental mouse cells of both types of hybrids express MTV antigens which, if present at a high level, could interfere with the receptor test after fusion, no MTV antigens could be detected in the hybrid cells by immunofluorescence or radioimmunoassay (13).

Segregation of the VSV(MTV) receptor correlation with mouse chromosome 16. Independent primary hybrid clones and subclones were tested for the expression of the MTV receptor by the VSV(MTV) pseudotypes assay. Most, but not all, EGR hybrids were negative for the receptor in contrast to the majority of the EMT hybrids which were positive (Table 4). In addition, all hybrids were tested for 24 chromosome markers distributed among 16 chromosomes (Table 5).

Pairwise comparison of the presence or absence of the chromosome markers and the receptor resulted in the percentages of concordancy indicated in Table 5. The highest correlation between the presence of the receptor and a chromosomal marker was found with superoxide dismutase on chromosome 16 (94.4%); there were only three exceptions. All three discordancies were due to hybrid clones expressing the superoxide dismutase marker that could not be infected to a detectable level

TABLE 5. Correlation between the presence of the MTV receptor and chromosome markers in the EGR and EMT hybrid clones

Chromosome marker[a]	Chromosome	Receptor presence/chromosome marker presence (no. of clones)				
		+/+	+/−	−/+	−/−	% Concordancy
PEP3	1	31	6	7	15	77.9
SDH1	2	33	4	14	2	66.0
PGD	4	37	1	18	3	67.7
PGM2	4	35	3	9	12	79.6
PEP7	5	32	6	12	6	67.8
Rev-1	5	12	2	4	1	68.4
TPI	6	29	9	18	4	55.0
GPI	7	38	0	19	3	68.3
LDH1	7	38	0	20	2	66.6
PEP4	7	37	0	19	3	67.6
GR-1	8	25	10	5	13	69.4
APRT	8	25	10	5	13	71.6
MOD-1	9	29	8	8	12	71.9
MPI	9	31	7	11	9	68.9
PEP2	10	29	9	7	14	72.8
GALK	11	3	31	0	13	34.0
ACP1	12	33	4	12	9	72.4
NP1	14	15	23	8	14	48.3
ES10	14	23	15	10	12	58.3
SOD1	16	37	0	3	14	94.4
GLO1	17	37	1	16	0	68.5
H-2	17	6	0	3	0	66.6
PEP1	18	29	9	7	13	72.4
GOT1	19	34	4	7	13	81.0

[a] PEP3, Peptidase 3; SDH1, sorbitol dehydrogenase; PGD, 6-phosphogluconate dehydrogenase (decarboxylating); PGM2, phosphoglucomutase; PEP7, peptidase 7; *Rev-1*, gene on chromosome 5; TPI, triosephosphate isomerase; GPI, glucose-6-phosphate isomerase; LDH1, lactate dehydrogenase; PEP4, peptidase 4; GR-1, glutathione reductase; APRT, adenine phosphoribosyltransferase; MOD-1, malate oxidoreductase decarboxylating; MPI, mannose phosphate isomerase; PEP2, peptidase 2; GALK, galactokinase; ACP1, acid phosphatase; NP1, purine-nucleoside phosphorylase; ES10, esterase-10; SOD1, superoxide dismutase; GLO1, glyoxylase-1; *H-2*, locus on chromosome, 17; PEP1, peptidase 1; GOT1, glutamate transaminase.

with the pseudotypes. This might be explained by the heterogeneity of hybrid clones and the difference in sensitivity for the superoxide dismutase marker, which was still detectable when present in a low percentage of the cells, and the receptor assay. Since all discordancies were in the same direction, this seems the most likely explanation. Alternatively, there could have been chromosome translocations and subsequent loss of one of the translocated chromosomes in these hybrids. Both alternatives were not further investigated since none of the other markers showed comparable high concordancy with the receptor. The next highest correlation (81%) with glutamate transaminase on chromosome 19 has 11 discordancies out of 58 clones tested for both markers. Only low concordancy was found with *MTVR-1* and chromosome 7 and 17 markers, but no discordancies, or in the case of glyoxylase-1 marker, only one of the +/– type. Therefore, additional involvement of these chromosomes for receptor expression cannot be excluded. In addition, a number of clones were karyotyped (Table 6), and the data obtained also strongly support the assignment of the VSV(MTV) receptor to mouse chromosome 16. No discordancies with chromosome 16 were found.

DISCUSSION

The data presented in this study demonstrate that there is a cellular receptor for VSV(MTV) pseudotypes. There is compelling evidence that this is the MTV receptor because (i) the infection of the pseudotypes is inhibited by MTV, and (ii) the binding site for the VSV(MTV) pseudotype receptor is present on the MTV gp52 molecule as was shown by neutralization experiments with a monoclonal antibody directed against gp52. These experiments confirm and extend the data obtained by Schochetman et al. (34), Altrock et al. (1), and Massey and Schochetman (21, 22), who demonstrated cell surface receptors for MTV and the involvement of MTV gp52 in the binding to the receptors by using Kirsten sarcoma virus-MTV pseudotypes and monoclonal antibodies directed against MTV gp52. These authors presented evidence that MTV from C3H and GR use the same receptors, which are different from the ones used by the MTV from C3Hf and RIII mice. Our data confirm that GR-MTV can compete with C3H-MTV for the binding to the receptor, indicating that these viruses use the same receptor. In our experiments, the MTV receptor was hardly detectable on nonmurine cells like cat kidney cells (CrFK), mink lung cells (CCL64), rabbit cornea cells (SIRC), and Chinese hamster fibroblast cells (E36). This is in contrast to the results of Schochetman et al. (34), who found infectivity with Kirsten sarcoma virus-MTV pseudotypes on mink lung, SIRC, and other nonmurine cells. Závada et al. (40) also found infectivity on nonmurine cells. This xenotropic infection of nonmurine cells was preferentially detectable when the MTV-producing donor cells were grown in the absence of dexamethasone. They therefore claimed the existence of dexamethasone-independent xenotropic virus and dexamethasone-stimulated ecotropic MTV. Our pseudotypes were prepared on cells grown in the presence of dexamethasone, and we may preferentially detect the ecotropic MTV receptor and therefore get a low infectivity on nonmurine cells.

The absence of the MTV receptor on CrFK and mink lung cells is interesting since several investigators (14, 15, 17) have isolated host range variants of MTV which apparently adsorb well to CrFK or mink cells. It would be important to know whether these are ecotropic variants that have acquired new binding sites for nonmurine cells, or rather, xenotropic variants already present in the mouse mammary tumor cell (40).

TABLE 6. Chromosome constitution and expression of the VSV(MTV) receptor

Clone	% Cells containing chromosome:																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	*MTVR-1*
EGR-7/1	0	100	0	0	100	0	100	50	0	0	0	0	0	0	70	0	100	0	0	100	–
EGR-30B	85	85	0	0	0	60	60	30	0	0	0	45	10	45	100	0	45	0	30	70	–
EMT-3B	100	100	50	100	100	85	50	70	80	85	0	100	5	0	85	70	85	50	100	100	+
EMT-3B2	90	100	0	90	100	80	70	55	90	0	0	90	25	0	100	55	90	65	80	65	+
EMT-3E	100	100	50	70	100	70	100	70	30	30	0	85	70	85	50	70	100	30	70	80	+
EMT-3F	90	100	0	0	90	50	60	50	50	5	0	35	35	5	75	20	75	5	75	50	+
EMT-3C6A	65	70	65	70	65	10	65	10	65	35	0	70	40	10	70	90	40	50	90	50	+
EMT-6A	100	100	60	70	80	100	60	80	0	100	0	100	70	60	80	80	80	70	30	80	+
EMT-6E	85	100	85	50	100	100	100	85	70	70	0	70	15	100	70	70	100	70	80	100	+
EMT-13D2	80	100	20	80	90	70	60	0	70	80	0	40	60	90	60	0	40	50	10	70	–
EMT-3E	100	100	100	65	90	0	35	55	80	55	0	55	65	100	65	80	45	10	65	90	+
EMT-32A	100	100	0	60	15	40	85	45	0	40	0	60	60	30	85	60	30	75	75	60	+

The lack of infectivity of the VSV(MTV) pseudotypes for nonmurine cells compared to murine cells made it possible to map the gene for the MTV receptor in interspecies somatic cell hybrids. For this purpose, 25 independently derived clones and 40 subclones were tested for the presence of the MTV receptor and 24 chromosome markers located on 16 different chromosomes; several of the hybrid clones were also karyotyped. The isoenzyme data and the karyotyping indicated that the MTV receptor segregates together with chromosome 16. Chromosomes 7 and 17 were specifically retained in these hybrids and were present in all cases when the clone was positive for the MTV receptor. Therefore we cannot exclude that in addition to chromosome 16, chromosomes 7 and 17 are also necessary for expression of the receptor.

We propose *MTVR-1* as the gene symbol for the gene localized on chromosome 16 which is responsible for the expression of the C3H and GR MTV receptor.

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ADDENDUM IN PROOF

Chan et al. (Virology **120**:54–64, 1982) recently reported the observation that monoclonal antibody P2G6 cannot neutralize VSV(C3H MTV) obtained from nonmurine cells. This supports our assumption that only a xenotropic variant of C3H MTV infects nonmurine cells. Our pseudotypes are derived from the ecotropic variant and do not infect CrFK and M1 cells.

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