Establishment of a C3Hf Mammary Tumor Cell Line Expressing Endogenous Mouse Mammary Tumor Virus: Antigenic and Genetic Relationships of This Virus with Highly Oncogenic Mouse Mammary Tumor Viruses

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A single-cell clone of C3Hf mammary tumor cells (clone 14) was developed into a continuous cell line expressing high levels of endogenous mouse mammary tumor virus (MMTV) with less than 0.1% murine leukemia virus expression. Comparison of the C3Hf MMTV protein profile on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with that of C3H MMTV revealed that the protein content of the two viruses was quite similar. However, oligonucleotide fingerprints obtained of MMTV 70S RNA revealed that approximately 20% of the large oligonucleotides examined were unique to each virus. The oligonucleotide fingerprint indicated that although the viruses were similar, they differed in their genetic content. The differences in the two viruses extended to immunological differences in the major envelope glycoprotein, gp52. C3Hf MMTV competed only partially in a homologous radioimmunoassay for gp52 of C3H MMTV, whereas C3H MMTV gave complete competition, indicating that gp52 of C3H MMTV contained type-specific determinants not present on gp52 of C3Hf MMTV. Comparison of C3Hf MMTV with highly oncogenic C3H, GR, and RIII MMTVs in a homologous C3H MMTV gp52 assay gave two patterns of reactivity: complete competition by GR and C3H MMTV and incomplete competition by C3Hf and RIII MMTV. Absorption of anti-C3H MMTV serum by either C3Hf MMTV or RIII MMTV removed all antibodies against both viruses but not against GR and C3H MMTVs. These results indicate that C3H and GR MMTVs are more closely related to each other than to RIII and C3Hf MMTVs.

Certain inbred strains of mice, such as C3H/HeN, contain milk-transmitted (exogenous) mouse mammary tumor virus (MMTV) and genetically transmitted (endogenous) MMTV. Foster nursing the C3H suckling on mice of strains of low mammary tumor incidence removes the milk-transmitted virus and reduces the mammary tumor incidence from greater than 95% to approximately 40% (13). Reintroduction of the exogenous MMTV into young mice restores the high mammary tumor incidence (1). The tumors induced by the exogenous MMTV occur early (average 8 months), whereas mammary tumors which arise in foster-nursed C3H (termed C3Hf) mice occur late (14 months of age). DNA-proviral sequences of the exogenous virus are found only in mammary tumors induced by this virus, whereas endogenous MMTV DNA-proviral sequences can be found in all tissues, including late-arising mammary tumors (8, 16, 21). Even though the complete endogenous MMTV can be recovered from late-arising C3Hf mammary tumors, it is still unclear whether the endogenous MMTV is the etiolog-

ical agent for tumor formation in the C3H mouse or whether virus expression was a result of tumor development. Studies to ascertain the tumorigenic properties of C3Hf MMTV have been hampered by the lack of a reproducible source of highly purified virus in which the concentration or infectious dose of MMTV could be controlled. Mouse milk has been used for years as a source of exogenous MMTV; however, milk was not a feasible source of endogenous MMTV due to the low concentration of virus in the milk of the foster-nursed animals. Exogenous MMTV from mammary tumor cells in culture has largely replaced milk-derived MMTV as a source of virus. Tissue culture-derived exogenous MMTV is highly oncogenic (4) and can be obtained reproducibly in large quantities free of contaminating mouse milk proteins. Since production of MMTV from tissue culture has proven successful for the exogenous C3H MMTV, we established cell lines from late-arising tumors of C3Hf MMTV to provide a source of the endogenous MMTV.

In this report we describe the establishment

of a C3Hf cell line producing high levels of MMTV. The protein and nucleic acid composition of this C3Hf-MMTV was similar to the C3H MMTV, although the two viruses were antigenically and genetically distinguishable. Based on a radioimmunoassay (RIA) for the major envelope glycoprotein, gp52, the C3Hf tissue culture-derived MMTV was indistinguishable from the MMTV expressed in C3Hf mammary tumors and milk, indicating that it was the endogenous virus. Comparison of gp52 antigenic determinants of C3Hf MMTV with determinants on gp52 of highly oncogenic C3H, GR, and RIII MMTVs suggested that GR MMTV was more closely related to C3H MMTV than to C3Hf and RIII MMTVs.

MATERIALS AND METHODS

Cells and virus. MMTV was obtained from mammary tumor cell lines and milk. The virus was purified from the culture fluid as previously described (12). C3H-derived MMTV was from the Mmam/c₁ line established by Owens and Hackett (18), and C3Hf-derived MMTV was obtained from clone (Cl) 14 of a C3H/HeNf mammary tumor cell line described below. GR MMTV was from a GR/N mammary tumor cell line previously established in our laboratory, and RIII virus was obtained from RIII mouse milk. The C3Hf mammary tumor cell line was established from an adenocarcinoma of a 16-month-old multiparous C3H/HeNf NIH Swiss female. The tumor was aseptically removed, sliced into small pieces (1 to 5 mm³), and incubated at 4°C overnight in a tube containing Dulbecco-modified Eagle medium plus 10% fetal calf serum, insulin (10 µg/ml), tylocine (60 µg/ml), penicillin (100 U/ml), and streptomycin (100 µg/ml). The medium was replenished, and the tumor pieces were agitated by gentle pipetting. After the large tumor pieces settled to the bottom of the tube, the medium containing dislodged cells and small tumor fragments was transferred to a 75-cm² tissue culture flask and incubated at 37°C in 5% CO₂. Cells which grew were predominantly epithelial in appearance with few fibroblastic-like cells. Fibroblastic cells detach from the tissue culture flask in the presence of trypsin-EDTA more rapidly than epithelial cells and were therefore removed by incubating trypsin-EDTA on the monolayer until 50% of the cells were detached. The detached cells were discarded and fresh medium was added. After 4 to 6 weeks of this selective trypsinization, islands of epithelial cells were the predominant cell type with no appreciable growth of fibroblasts. At confluency, the cells were transferred by trypsinization and subsequently subcultured at 7-day intervals at a 1:4 split ratio.

Cells from C3Hf mammary tumor cell lines were found to be free of mycoplasma by bioassay (R. Del Guidice, Frederick Cancer Research Center). Karyotypic examination as well as analysis of the glucose-6-phosphate isomerase isozymes confirmed the cells to be of mouse origin (C. S. Stulberg, Child Research Center of Michigan, Detroit; and W. A. Nelson-Rees, University of California, Berkeley). The cells were near tetraploid with a mean chromosome count of 78.

Cells were tumorigenic when inoculated into C3H/HeN, C3H/HeNf, and nude mice. The C3Hf cells did not grow when inoculated into GR/N and BALB/c mice (Arthur and Massey, unpublished data).

Cell cloning. Cells were removed from flasks with trypsin-EDTA and diluted to 10 cells per ml of complete Dulbecco-modified Eagle medium, and 100 µl was placed in each of 96 wells of a Costar 96-well cloning dish. These dishes were incubated at 37°C in 5% CO₂. At 7 days postplanting, the wells were examined microscopically and scored for single colonies. Wells were refed at 7-day intervals, and when the cells were 60 to 80% confluent, they were transferred to a 30-cm² flask and subsequently to a 75-cm² flask. At this stage cells were incubated in completed Dulbecco-modified Eagle medium plus 10⁻⁶ M dexamethasone, and MMTV in the spent medium was monitored for reverse transcriptase activity and MMTV gp52. Cloning efficiency ranged between 10 and 20%.

Polyacrylamide gel electrophoresis. Electrophoretic separation of MMTV polypeptides was performed by procedures previously described (2). Protein was determined by the procedure of Lowry et al. (14) with bovine serum albumin as a standard.

Competitive RIAs. The major envelope glycoprotein of MMTV (gp52) was purified by a combination of lectin chromatography (lens culinaris coupled to agarose polyacrylic hydrazide) and molecular sieving (Sephacryl S-200) as previously described (3). The purified gp52 was iodinated with ¹²⁵I and used as the radiolabeled antigen in a competition RIA as previously described (3). Antisera used in the assays were prepared in rabbits by multiple intradermal inoculations of purified C3H or C3Hf MMTV in complete Freund adjuvant followed by inoculation of each MMTV in incomplete Freund adjuvant at 3-week intervals. Animals were bled before each inoculation, and sera were stored at -70°C until used. Antiserum prepared against C3H-derived MMTV was absorbed with C3H, C3Hf, GR, or RIII MMTV by incubating 2 mg of virus with 10 ml of serum at a 1:100 dilution for 2 h at 37°C and overnight at 4°C. Immune complexes were removed by centrifugation at 100,000 × g for 2 h.

RNA-directed DNA polymerase assay. RNA-directed DNA polymerase assays were performed by determining [³H]dGTP incorporation by detergent-disrupted MMTV as previously described with the synthetic template, oligo(dG)·poly(rC) (12).

Isolation of MMTV 70S RNA. Approximately 6 mg of sucrose gradient-purified MMTV was pelleted and resuspended in 2 ml of TNE buffer (0.5 M Tris-hydrochloride [pH 7.8], 0.1 M NaCl, and 0.001 M EDTA). Protein was digested by addition of proteinase K at a final concentration of 50 µg/ml and incubation for 10 min at room temperature. Sodium dodecyl sulfate was added to a final concentration of 1%, and incubation was continued for 15 min. The 70S RNA was purified by centrifugation in a 15 to 30% sucrose gradient for 3 h at 40,000 rpm with a Beckman SW41 rotor. Fractions were monitored by UV absorption at 260 and 280 nm to locate the 70S RNA, which was then pooled and precipitated by addition of 0.1 volume of 2 M sodium acetate (pH 5.0) and 2.5 volumes of cold ethanol.

Separation of oligonucleotides produced by digestion of MMTV RNA with ribonuclease T₁.

MMTV 70S RNA was digested, and the 5'-phosphate residues were removed by incubating 1 to 5 μ g of RNA with 2 U of RNase T₁ and 0.5 U of alkaline phosphatase in 0.1 M Tris-hydrochloride, pH 7.6, at 37°C for 45 min. The digestion mixture (20 μ l) was twice extracted with 100 μ l of phenol-chloroform (1:1), and the aqueous phase was subsequently extracted twice with diethyl ether. The 5' ends of the oligonucleotides were then labeled with ³²P as described by Maxam and Gilbert (15). The ³²P-labeled oligonucleotides were precipitated with ethanol and separated by two-dimensional gel electrophoresis by the procedure of DeWachter and Fiers (10). The first dimension was run in a 10% acrylamide gel at pH 3.4 and 6 M urea, and the second dimension was run in 21.8% acrylamide at pH 8.0. Radiolabeled oligonucleotides were located by autoradiography with Kodak X-OMAT X-ray film.

RESULTS

MMTV expression in C3Hf cell lines. Mammary tumors were established in culture, and cells with epithelial morphology were selected as described in Materials and Methods. MMTV expression was determined by analyzing virus pellets from spent medium for reverse transcriptase activity and MMTV antigen. Approximately 30 ml of Dulbecco-modified Eagle medium plus 10⁻⁵ M dexamethasone was added to cultures at 80% confluency (approximately 5 × 10⁶ cells) and incubated for 48 h at 37°C. Medium was removed and clarified, and the virus was pelleted by centrifugation at 100,000 × g for 1 h. The virus pellets were resuspended in TNE buffer, and this virus suspension was monitored for reverse transcriptase and MMTV gp52. All values were normalized to 1 ml of virus suspension to allow comparison of antigen and enzymatic activity. Low levels of MMTV (13 pmol of dGTP incorporation per ml per h and 129 ng of gp52 per ml) were detected by both assays in virus pellets from C3Hf cells (Table 1). All C3Hf primary cultures which were established had low MMTV expression. Treatment with dexamethasone, progesterone, insulin, hydrocortisone, and combinations of hormones did not increase virus yields. However, approximately 10% of cultures established by single-cell cloning of C3Hf cells released high levels of MMTV after treatment with dexamethasone at 10⁻⁵ M. The results of the reverse transcriptase and gp52 assays on virus pellets prepared from these clones are given in Table 1. C3Hf Cl 14 yielded virus in comparable levels to the Mm5mt/c₁ mammary tumor cell line. In addition, MMTV of Cl 14 incorporated [³H]dGTP greater than nine times more efficiently in the presence of Mg²⁺ than Mn²⁺. Reverse transcriptase with optimal activity in the presence of Mg²⁺ is indicative of MMTV rather than murine leukemia virus (MuLV). Virus pellets from 12 separate experiments were examined for MuLV p30 by

TABLE 1. Reverse transcriptase and MMTV gp52 in virus pellets from C3Hf mammary tumor cells and clones^a

Cell designation	Reverse transcriptase ^b		MMTV gp52 ^c (ng/ml)
	pmol incorporated	Mg ²⁺ /Mn ²⁺ ratio ^d	
C3H/HeNf line 1	13	0.6	129
C3H/HeNf Cl 1	225	5.9	4292
C3H/HeNf Cl 2	113	4.3	383
C3H/HeNf Cl 3	13	1.3	53
C3H/HeNf Cl 4	146	3.6	441
C3H/HeNf Cl 5	120	2.3	474
C3H/HeNf Cl 6	16	0.9	1,506
C3H/HeNf Cl 7	10	0.5	182
C3H/HeNf Cl 8	151	5.0	378
C3H/HeNf Cl 9	126	5.5	408
C3H/HeNf Cl 10	110	1.6	154
C3H/HeNf Cl 11	109	1.2	626
C3H/HeNf Cl 12	15	0.4	219
C3H/HeNf Cl 13	436	7.0	8,051
C3H/HeNf Cl 14	1,442	9.2	38,550
C3H/HeNf Cl 15	118	6.6	440
Mm5mt/c ₁ cells	2,171	9.6	58,623

^a Virus pellets prepared by centrifugation of spent medium from each cell line treated with 10⁻⁵ M dexamethasone for 48 h were resuspended at 100× and tested for reverse transcriptase activity and MMTV gp52 antigen. All values are normalized to 1 ml of virus suspension.

^b Results are expressed as picomoles of [³H]dGTP incorporated using the synthetic template, oligo(dG)·poly(rC) per milliliter of virus suspension per hour.

^c MMTV gp52 is expressed as nanograms of gp52 per milliliter of virus suspension and was determined with the C3H MMTV gp52 competition RIA.

^d A low Mg²⁺/Mn²⁺ ratio at background RNA-directed DNA polymerase levels is not indicative of MuLV contamination.

competition RIAs and immunodiffusion assays, and less than 0.1% MuLV was detected in any of the pellets. MuLV expression in high-passage cultures has not been ascertained (data not shown).

Polypeptide composition of exogenous and endogenous MMTVs. The protein compositions of C3H and C3Hf MMTV were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Figure 1 shows a Coomassie brilliant blue-stained gel of sucrose gradient-purified C3H- and C3Hf-MMTV. gp52, gp36, p27, p14, and p10 were present in both viruses. Except for the higher relative concentrations of pp20 and p16 in the exogenous MMTV, the protein profiles of the two viruses are essentially identical. MMTV pp20 is a phosphoprotein (20), and p16 has been shown to be a cleavage product of p27 (22).

C3H and C3Hf MMTV gp52's possess group- and type-specific antigenic deter-

minants. With a gp52 RIA, we recently found that tumors from C3H and C3Hf mice were immunologically distinct (3). To determine whether the gp52 antigenic polymorphism extends to MMTV from C3H and C3Hf cells, viruses from both cell lines were examined in competition immunoassays for MMTV gp52. In a homologous assay employing limiting dilutions of anti-C3H MMTV serum and 125 I-labeled gp52 purified from C3H MMTV, both C3Hf and C3H MMTV competed with parallel displacement curves (Fig. 2A). MMTV derived from C3H cells gave complete competition, whereas C3Hf-derived MMTV competed to a maximum of 90%. The lack of complete competition suggests that gp52 of C3Hf MMTV lacked antigenic determinants found on gp52 of C3H MMTV. The incomplete competition by C3Hf MMTV was not due to insufficient concentration of antigen in the C3Hf MMTV preparation. This was demonstrated in a heterologous gp52 RIA with antiserum prepared to C3Hf MMTV and 125 I-labeled gp52 from C3H MMTV where both viruses gave complete competition with identical displacement curves (Fig. 2B). To amplify the type-specific differences, the C3H MMTV antiserum was absorbed with C3Hf MMTV to remove the group-specific antibodies. This absorbed antiserum was then used in a gp52 RIA with gp52 from C3H MMTV as the radiolabeled antigen. As shown in Fig. 2C, MMTV from C3H cells

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sucrose gradient-purified MMTVs from C3H and C3Hf mammary tumor cell lines. The gel was fixed and then stained with Coomassie brilliant blue.

FIG. 2. Immunological relatedness of the major envelope glycoprotein of MMTV. Detergent-disrupted MMTVs were tested at serial twofold dilutions for the ability to compete with 125 I-labeled gp52 from C3H MMTV for binding limiting amounts of antiserum. The results are normalized for 100% binding in the absence of competing antigen. Competitions were C3Hf MMTV (●) and C3H MMTV (○). Competition immunoassays included: (A) homologous immunoassay in which limiting amounts of anti-C3H MMTV serum was used to precipitate 125 I-labeled gp52; (B) heterologous immunoassay in which anti-C3Hf MMTV serum was used to precipitate 125 I-labeled gp52; (C) homologous absorption immunoassay in which anti-C3H MMTV antiserum which had been extensively absorbed with C3Hf MMTV was used to precipitate 125 I-labeled gp52.

competed to greater than 85%, whereas no competition was detected with C3Hf-derived MMTV. This clearly demonstrates that type-specific antigen determinants on gp52 of C3H MMTV were not present in C3Hf MMTV.

Analysis of 70S RNAs of endogenous and exogenous MMTVs. To determine whether immunological differences were a result of differences in the genetic information of the two viruses and not the result of posttranslational modifications of the gp52, oligonucleotide fingerprint analysis of the genomes of the two viruses was performed. The 70S RNA which was purified from each virus was subjected to ribonuclease T_1 digestion and the resulting oligonucleotides were labeled with ^{32}P and then separated by two-dimensional electrophoresis. Autoradiograms of the large radiolabeled oligonucleotides are given in Fig. 3. The autoradiograms were superimposed to allow identification of common and unique oligonucleotides, and a composite of the autoradiograms is shown in Fig. 4. Of 100 unique oligonucleotides examined, 77 were common to both viruses, indicating that the genomes are similar. However, the 70S RNA of C3Hf MMTV contained 23 oligonucleotides which were not present in C3H MMTV, and C3H MMTV contained 18 oligonucleotides not present in C3Hf MMTV.

Antigenic relationships of C3Hf MMTV gp52 to gp52 of mouse mammary tumors and mouse milk. It has been previously shown that C3Hf mammary tumors and C3Hf milk contain MMTV (19, 26). If the C3Hf MMTV expressed by the C3Hf cell line is the same virus as expressed in C3Hf tumors and milk, it should possess the same group and type antigenic specificities observed between gp52 of C3Hf and C3H MMTV. Therefore, tumor extracts and milk

from various strains of mice were analyzed in the homologous C3H MMTV gp52 assay. As shown in Table 2, C3H MMTV, mammary tumors, and milk give 100% competition, whereas C3Hf MMTV, mammary tumors, and milk compete only 90%. When the anti-C3H MMTV serum that was extensively absorbed with C3Hf mammary tumor extract was used in the gp52 RIA, C3H MMTV and mammary tumors still competed extensively. However, C3Hf MMTV and mammary tumors gave no competition. These results are consistent with the C3Hf MMTV from C3Hf Cl 14 cell line being the same virus as that expressed in C3Hf mammary tumors and milk. As would be expected, the low mammary tumor mouse strains, BALB/c and

FIG. 4. Composite of two-dimensional electrophoretic fingerprint of 70S RNA from C3Hf MMTV and C3H MMTV. Oligonucleotides were generated by RNase T_1 digestion. Those common to both viruses (○), those unique to C3H MMTV (◐), and those unique to C3Hf MMTV (●) are indicated.

FIG. 3. Two-dimensional electrophoretic fingerprint of digested 70S RNA from C3Hf (A) and C3H (B) MMTV. Oligonucleotides were generated by RNase T_1 digestion of 70S RNAs purified from C3H MMTV and C3Hf MMTV.

NIH Swiss, contained no detectable MMTV gp52 in their milk. Also, no gp52 was found in a dimethylbenzanthracene-induced BALB/c

TABLE 2. Competition by samples from mice of various strains in a C3H MMTV gp52 RIA

Competing antigen	Maximum % competition in RIA employing C3H MMTV antiserum	
	Unabsorbed	Absorbed with C3Hf tumor extract
Virus		
C3H	100	>73 ^a
C3Hf	90	0
Mammary tumors		
C3H	100	>93 ^a
C3Hf	90	0
RIII	92	0
BALB/c (DMBA ^b -induced)	0	0
Milk		
C3H	100	NT
C3Hf	88	NT
BALB/c	0	NT
SWISS	0	NT

^a Competition was not carried to completion. The competition curves were similar to those shown in Fig. 2C and 5C. NT, Not tested.

^b DMBA, Dimethylbenzanthracene.

mammary tumor. Surprisingly, mammary tumor extracts of the high mammary tumor incidence RIII mice competed to the same extent as C3Hf MMTV.

Antigenic relatedness of gp52 of MMTVs from C3H, C3Hf, GR, and RIII mice. The RIA employing ¹²⁵I-labeled gp52 from C3H MMTV and its homologous antiserum, which distinguishes C3H and C3Hf MMTVs, allowed an analysis of the antigenic relatedness of MMTV from different mice. Detergent-disrupted MMTVs from C3H, C3Hf, GR, and RIII mammary tumor cell lines and milk were examined in the homologous gp52 RIA (Fig. 5A). The differences in the extent of competition were sufficiently pronounced to distinguish C3H and GR MMTV from C3Hf and RIII MMTVs. Although the slopes of the competition curves were similar, GR and C3H MMTVs gave complete competition, whereas C3Hf and RIII viruses competed to a maximum of 90 to 92%. This demonstrates that all determinants on C3H MMTV gp52 detected by the assay are also present on GR-N MMTV gp52. In contrast, all four MMTVs competed completely in a heterologous RIA with ¹²⁵I-labeled gp52 from C3H MMTV and limiting dilutions of anti-C3Hf MMTV antiserum (Fig. 5B). This demonstrated that gp52 determinants shared by C3Hf and C3H MMTV were also common to RIII and GR MMTV.

FIG. 5. Immunological relatedness of the major MMTV envelope glycoproteins. Detergent-disrupted MMTVs were tested at serial twofold dilutions for the ability to compete with ¹²⁵I-labeled gp52 from C3H MMTV for binding limiting amounts of antiserum. The results are normalized for 100% binding in the absence of competing antigen. Competitors were C3Hf MMTV (●), C3H MMTV (○), GR MMTV (□), and RIII MMTV (■). Competition immunoassays included: (A) homologous immunoassay in which limiting amounts of anti-C3H MMTV serum were used to precipitate ¹²⁵I-labeled gp52; (B) heterologous immunoassay in which anti-C3Hf MMTV serum was used to precipitate ¹²⁵I-labeled gp52; (C) homologous absorption immunoassay in which anti-C3H MMTV serum which was extensively absorbed with C3Hf MMTV was used to precipitate ¹²⁵I-labeled gp52.

The incomplete competition by RIII MMTV in the homologous assay (Fig. 5A) indicated that the gp52's of C3Hf and RIII MMTVs lacked antigenic determinants present on the gp52's of C3H and GR MMTVs. If the missing determinants were identical, then absorption of C3H MMTV antiserum with C3Hf MMTV would also remove all the antibodies to RIII MMTV gp52. As shown in Fig. 5C, RIII and C3Hf MMTVs gave no competition in the RIA with ^{125}I -labeled gp52 from C3H MMTV and C3H MMTV antiserum absorbed with C3Hf MMTV. However, C3H MMTV and GR MMTV competed greater than 80%. Similar results were obtained with C3H MMTV antiserum extensively absorbed with RIII MMTV (data not shown). Absorption of the antiserum with GR and C3H MMTV removed all precipitating antibodies for ^{125}I -labeled gp52. This demonstrates that gp52's from C3H and GR MMTVs contain unique antigenic determinants lacking on gp52's from both C3Hf and RIII MMTVs.

DISCUSSION

The results presented here describe the establishment of the first C3Hf mammary tumor cell line producing high levels of endogenous MMTV. Comparison of this MMTV with C3H MMTV revealed that the two viruses were similar morphologically (data not shown) and both required Mg^{2+} for optimum reverse transcriptase activity. The polypeptide profile, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was essentially identical for the two viruses. However, the two viruses could be distinguished on the basis of the antigenic properties of their major glycoprotein, gp52. Although group-specific antigenic determinants comprised the major reactivity of the gp52 molecules, the results of the gp52 RIAs clearly demonstrated that C3H MMTV gp52 contained antigenic determinants not found on the gp52 of C3Hf MMTV. These antigenic type-specific differences are not unexpected in view of the observed differences in the oligonucleotide fingerprints of the two viruses reflecting differences in their genetic content.

Immunological type specificity of MMTV from mammary tumors and milk has been reported by Blair (6, 7) and Teramoto et al. (25) by using immunodiffusion and intact virus RIAs, respectively. We have demonstrated type specificity of gp52 for C3H and C3Hf MMTVs and further that the pattern of reactivity extends to C3Hf mammary tumors and C3Hf milk (3; Table 2). This indicated that tissue culture-derived C3Hf MMTV was the same MMTV as that expressed in the milk and mammary tumors of C3Hf mice.

Previous studies of gp52 performed in this laboratory revealed that C3H and GR MMTVs were more closely related to each other than to RIII MMTV (23). This was based on results derived from (i) type-specific RIAs for C3H MMTV gp52, (ii) tryptic peptide maps of cell surface-labeled gp52 of MMTVs grown in a common cell, and (iii) type-specific cytotoxic antibodies in sera of C3H mammary tumor-bearing mice which were directed against cell surface MMTV gp52. Interestingly, results of the gp52 RIA presented here revealed that C3Hf MMTV was more closely related to RIII MMTV than to C3H and GR MMTVs. Since removal of the group-specific antibodies to gp52 by absorption with either C3Hf or RIII MMTV removed activity against both MMTVs, but not against gp52's of C3H and GR MMTVs, it can be concluded that the same type-specific antigenic determinants were missing on the gp52's of C3Hf and RIII MMTVs. This pattern of relatedness, which groups C3H and GR MMTVs together and distinguishes them from C3Hf and RIII MMTVs, was also observed with MMTV neutralizing antibodies from sera of C3H mammary tumor-bearing mice (Massey et al., submitted for publication). It is therefore possible that the type-specific gp52 determinants measured in the RIA are the same determinants as those detected by the neutralizing antibodies of natural sera.

When the degree of oncogenicity of the MMTVs is compared in C3Hf and BALB/c strains of mice, both C3H and GR MMTVs are highly oncogenic, whereas C3Hf and RIII MMTV are considerably less oncogenic (5, 17, 24, 26). This agrees with their grouping based on type-specific antigenic determinants of gp52. Studies of murine leukemia virus indicate that the generation of highly oncogenic viruses correlates with a change in the major envelope glycoprotein, gp70. It has been reported that these changes are due to genetic recombination between ecotropic MuLV and endogenous MuLV sequences coding for gp70 determinants which may confer thymotropic properties to the recombinant virus (9, 11). Further studies will be required to determine whether the acquisition of type-specific differences on gp52's of C3H and GR MMTVs also arose by recombinational events and whether these determinants are involved in enhancing infectivity and subsequently oncogenicity of the MMTVs for mammary glands of various strains of mice.

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