Immunological Characterization of a Low Oncogenic Mouse Mammary Tumor Virus from BALB/cNIV Mice

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Antigenic determinants of mouse mammary tumor virus (MuMTV) from the low-mammary-tumor-incidence strain BALB/cNIV were compared by competition radioimmunoassay with those of MuMTV's isolated from several high- and low-mammary-tumor-incidence mouse strains, using rabbit hyperimmune sera against BALB/cNIV MuMTV and against MuMTV from the high-mammary-tumor-incidence strain BALB/cfC3H. Using anti-BALB/cfC3H serum in competition radioimmunoassay, BALB/cNIV MuMTV lacked antigenic determinants present on MuMTV's from the BALB/cfC3H, C3H, and GR strains. With anti-BALB/cNIV serum in competition radioimmunoassay, type-specific antigenicity was detected with BALB/cNIV MuMTV. We found class-specific antigenicities on BALB/cNIV MuMTV that were shared with RIII and C3Hf MuMTV's. BALB/cNIV MuMTV was reportedly derived from the C3Hf strain by infection of BALB/c mouse mammary tissue after transplantation into a (BALB/c × C3Hf)F1 hybrid followed by retransplantation into the BALB/c strain (D. R. Pitelka, K. B. DeOme, and H. A. Bern. *Proc. Am. Assoc. Cancer Res.* 6:51, 1965). However, BALB/cNIV MuMTV contained type-specific antigens not present on C3Hf MuMTV. The possible origin of these determinants is discussed.

Mouse mammary tumor virus (MuMTV) is a causal agent in the appearance of mammary neoplasia in several inbred mouse strains. Variants of MuMTV which differ in their oncogenic potentials have been identified in the C3H/HeN strain: (i) a milk-transmitted variant associated with a high incidence of mammary tumors (90%) occurring within 6 months of age and associated with the appearance of hyperplastic alveolar nodules with high tumor potentials, and (ii) a genetically transmitted variant associated with a lower incidence of tumors (40%) occurring late in life (20 months) and associated with HAN of lower tumor potentials (4, 21). To physically separate these virus variants for biological, immunological, and biochemical comparisons, each variant was transferred to the low-mammary-tumor-incidence BALB/c strain. Transfer of the high oncogenic MuMTV variant to BALB/c was accomplished by foster-nursing BALB/c pups on C3H/Crgl females, resulting in the high-tumor-incidence BALB/cfC3H substrain (21). Transfer of the low oncogenic MuMTV variant from a (BALB/c × C3Hf)F1 mouse to the BALB/c strain was accomplished by mammary gland tissue transplantation and resulted in the

BALB/cNIV substrain (23).

The BALB/cNIV substrain has been the subject of a number of studies (5, 6, 13, 14, 24). BALB/cNIV MuMTV is transmitted via milk and displays a high noduligenic potential, two characteristics of the high oncogenic variant of C3H/HeN, yet it is associated with a relatively low incidence of tumors occurring late in life in BALB/cNIV females (40% at 20 months; 14, 31), a characteristic of the low oncogenic MuMTV variant of C3H/HeN.

Studies of BALB/cNIV MuMTV have left a major unresolved issue: is BALB/cNIV MuMTV identical to the C3Hf MuMTV from which it was apparently derived? To answer this question, we used a competitive radioimmunoassay (RIA) to compare antigenic determinants of BALB/cNIV MuMTV with those of high and low oncogenic MuMTV's from other mouse strains. We found that BALB/cNIV MuMTV contains type-specific antigens that are not shared with the C3Hf MuMTV. Since BALB/cNIV MuMTV was not antigenically identical to C3Hf MuMTV, either BALB/cNIV MuMTV did not arise by a direct transfer of C3Hf MuMTV to the BALB/c strain, or BALB/cNIV and C3Hf MuMTV's have become antigenically distinct during nearly 20 years of inbreeding.

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MATERIALS AND METHODS

Virus. Virus was purified from milk of the following mouse strains: BALB/cNIV (Cancer Research Laboratory, Berkeley, Calif.); RIII (Meloy Laboratories, Springfield, Va.); BALB/cfC3H/BiSim (Simonson Laboratories, Gilroy, Calif.); and C3H/Crgl, C3H/CrglfC57BL, and GR/A (Cancer Research Laboratory, Berkeley, Calif.).

Virus was purified from culture fluids from the continuous cell lines GR3A (from a GR/A mammary tumor; 25) and Mm5mt/c1 (from a C3H/Crgl mammary tumor; 22); from primary cultures of mammary tumor cells from BALB/cNIV (Cancer Research Laboratory), BALB/cfC3H/BiSim (Simonson Laboratories), GR/A (Cancer Research Laboratory), and (BALB/c $\times$ GR)F1 (Cancer Research Laboratory) mice; and from CRFK feline kidney cells infected with virus from RIII milk or from the Mm5mt/c1 cell line [(RIII)vp1 and (C3H)vp1, respectively] (provided by D. Howard, Meloy Laboratories). Moloney murine leukemia virus was obtained from Division of Logistics and Resources, National Cancer Institute.

Virus purification. Virus was purified from mouse milk as follows. The milk was skimmed and centrifuged through a two-step density interface gradient (28 and 65% sucrose in TEN buffer [0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris-hydrochloride, pH 7.4]) followed by a linear 15 to 65% sucrose isopycnic zonal gradient in TEN buffer. The light-scattering material at 1.17 g/cm³ was collected and was negative for contaminating type C virus by immunodiffusion assay.

Virus was purified from culture fluids of continuous cell lines or primary cultures as described elsewhere (8, 9, 28) and was negative for type C virus and fetal calf serum by immunodiffusion assay. Virus was stored at -70°C.

Iodination of intact virions with lactoperoxidase or Iodogen. Purified intact MuMTV virions from BALB/cfC3H/BiSim tissue cultures and BALB/cNIV milk were iodinated with ¹²⁵I, using lactoperoxidase (7) or Iodogen (17). After iodination, virus was dialyzed until precipitability by trichloroacetic acid was greater than 90%. Residual unincorporated radioactivity was removed by sucrose density gradient sedimentation. The peak of radioactivity corresponding to the light-scattering material with a density of 1.17 g/cm³ was collected and stored at 4°C. The different iodination techniques did not result in detectable immunological differences.

Preparation of inhibiting antigens. Purified virus was pelleted at 200,000 $\times$ g for 20 min and suspended in TEN buffer. BALB/c lactating mammary gland extract was prepared as follows. Mammary glands from lactating BALB/c females were excised, rinsed, and homogenized in phosphate-buffered saline. The homogenate was centrifuged for 10 min at 10,000 $\times$ g. The supernatant was stored at -20°C. The protein concentration of each antigen was determined by the method of Lowry et al. (16), then diluted in borate-saline buffer (0.126 M sodium borate-0.134 M NaCl (pH 7.4), containing 0.1% bovine serum albumin).

Antisera. Rabbit antiserum against sucrose density gradient-purified virus from BALB/cNIV milk was prepared as described previously (5, 6). Rabbit antiserum against sucrose density gradient-purified virus

from BALB/cfC3H/Crgl primary mammary tumor cell cultures was prepared and characterized as described previously (10). Normal rabbit globulin and goat anti-rabbit globulin were obtained from Antibodies, Inc. (Davis, Calif.).

Absorption of antisera with purified MuMTV virions. MuMTV was purified from BALB/cNIV or BALB/cfC3H milk as described above. Virus (1 mg) was pelleted at 200,000 $\times$ g for 20 min and suspended in 5.3 ml of antiserum (diluted 1:1,000 in borate-saline buffer containing normal rabbit globulin [20 μ g/ml]). The mixture was incubated for 2 h at 37°C and 2 h at 4°C, then centrifuged at 200,000 $\times$ g for 20 min to remove immune complexes and excess virions. The absorbed sera were stored at -20°C.

Competition RIA. Competition RIA was performed with purified, ¹²⁵I-labeled MuMTV virions as labeled antigen as previously described (7), except that borate-saline buffer was substituted for TEN buffer.

RESULTS

Antiserum titrations. The ability of anti-BALB/cNIV MuMTV and anti-BALB/cfC3H MuMTV sera to precipitate purified intact ¹²⁵I-labeled virions was tested by radioimmune precipitation. Figure 1a shows the titration of anti-BALB/cNIV MuMTV serum with ¹²⁵I-labeled BALB/cNIV MuMTV and ¹²⁵I-labeled BALB/cfC3H MuMTV. This antiserum precipitated

FIG. 1. Titrations of anti-BALB/cNIV MuMTV and anti-BALB/cfC3H MuMTV sera with ¹²⁵I-labeled BALB/cNIV MuMTV (●) or ¹²⁵I-labeled BALB/cfC3H MuMTV (○). (a) Anti-BALB/cNIV MuMTV serum; (b) anti-BALB/cfC3H MuMTV serum. RIP, Radioimmune precipitation.

the homologous virus to a maximum precipitation of 97% with a 50% endpoint of 1:250,000, and precipitated ^{125}I -labeled BALB/cfC3H MuMTV to a maximum of 92% with a 50% endpoint of 1:150,000. Normal rabbit globulin precipitated 10% of ^{125}I -labeled BALB/cNIV MuMTV and 1% of ^{125}I -labeled BALB/cfC3H MuMTV.

Figure 1b shows the titration pattern of anti-BALB/cfC3H MuMTV serum with ^{125}I -labeled BALB/cNIV MuMTV and ^{125}I -labeled BALB/cfC3H MuMTV. The antiserum precipitated the homologous ^{125}I -labeled BALB/cfC3H MuMTV up to 94%, with a 50% endpoint of 1:500,000 dilution. The maximum precipitation of ^{125}I -labeled BALB/cNIV MuMTV was 88%, and the 50% endpoint was 1:100,000 dilution.

Competition radioimmune precipitation. To determine whether anti-BALB/cNIV MuMTV and anti-BALB/cfC3H MuMTV sera could distinguish between BALB/cNIV MuMTV and BALB/cfC3H MuMTV, we added increasing amounts of unlabeled MuMTV virions to inhibit the precipitation of ^{125}I -labeled BALB/cNIV or ^{125}I -labeled BALB/cfC3H by their respective antisera. The inhibition was tested at the 50 and 80% endpoint serum dilutions. The serum dilution at the 80% endpoint revealed greater differences in competition between the two viruses than did the 50% endpoint serum dilution. This is in agreement with the observations of others (27). Only the results obtained with the 80% endpoint dilutions of antisera are reported below.

All MuMTV's tested in the competition experiments reported below competed to 100% in heterologous reactions of ^{125}I -labeled BALB/cNIV MuMTV with anti-BALB/cfC3H MuMTV serum and of ^{125}I -labeled BALB/cfC3H MuMTV with anti-BALB/cNIV MuMTV serum (data not shown). Thus, all MuMTV's showed group-specific reactivity, and the incomplete competitions described below were due to a lack of one or more antigenic determinants rather than to inaccurate protein concentration estimations (15). BALB/cNIV and BALB/cfC3H MuMTV's were included as internal controls in each experiment. BALB/c skimmed milk, BALB/c lactating mammary gland extract, Moloney murine leukemia virus, and fetal calf serum did not compete with ^{125}I -labeled BALB/cNIV MuMTV or with ^{125}I -labeled BALB/cfC3H MuMTV in any of the experiments.

Studies with anti-BALB/cfC3H MuMTV serum. The abilities of BALB/cNIV MuMTV and MuMTV's from several other sources to compete with ^{125}I -labeled BALB/cfC3H for anti-

BALB/cfC3H serum were tested by RIA (Fig. 2). BALB/cfC3H MuMTV from both milk and primary tissue cultures competed completely with identical slopes (Fig. 2a). MuMTV(C3H)vp1 also competed completely, but the competition

FIG. 2. Two experiments using competition RIA with anti-BALB/cfC3H MuMTV serum at 1:40,000 dilution and ^{125}I -labeled BALB/cfC3H MuMTV. Increasing amounts of purified MuMTV's were added as competitors. (a) Experiment 1: MuMTV's which competed well include BALB/cfC3H MuMTV from milk ($\circ$), BALB/cfC3H MuMTV from tissue culture (Δ), and MuMTV(C3H)vp1 ($\bullet$). MuMTV's which competed only partially include BALB/cNIV MuMTV from milk ($\bullet$), RIII MuMTV from milk ($\blacklozenge$), and MuMTV(RIII)vp1 ($\blacksquare$). (b) Experiment 2: MuMTV's which competed well include all C3H- and GR-derived MuMTV's—BALB/cfC3H MuMTV from milk ($\circ$), BALB/cfC3H MuMTV from tissue culture (Δ), GR MuMTV from milk ($\circ$), GR MuMTV from primary tissue culture ($\bullet$), GR3A MuMTV ($\diamond$), (BALB/c $\times$ GR)F1 MuMTV from tissue culture ($\bullet$), Mm5mt/c1 MuMTV ($\square$), and C3H MuMTV from milk (∇). MuMTV's which competed only partially include BALB/cNIV MuMTV from milk ($\bullet$), C3Hf MuMTV from milk ($\blacktriangledown$), and BALB/cNIV MuMTV from primary tissue culture ($\blacktriangle$).

curve was displaced approximately 10-fold from the BALB/cfC3H MuMTV curves. MuMTV from RIII milk and MuMTV(RIII)vp1 competed up to 35% (Fig. 2a), in agreement with previous reports of incomplete competition of RIII MuMTV with C3H MuMTV (3, 18, 26). BALB/cNIV MuMTV resembled RIII MuMTV in competing to only 35% (Fig. 2a).

In a similar experiment all sources of C3H-derived and GR-derived MuMTV's from milk or tissue cultures competed to 100% (Fig. 2b). MuMTV purified from C3Hf milk competed no more than 50%, confirming previous reports of a lack of one or more antigenic determinants on this virus (2, 3, 27). BALB/cNIV MuMTV from milk or from tissue cultures resembled C3Hf MuMTV in competing incompletely (Fig. 2b).

To enhance the apparent antigenic differences between BALB/cfC3H and BALB/cNIV MuMTV's, similar experiments were carried out with the anti-BALB/cfC3H serum after absorption with virus purified from BALB/cNIV milk. The absorption reduced the precipitating ability of the antiserum for 125 I-labeled BALB/cNIV MuMTV to a maximum of 10% above background (Fig. 3a). The absorbed serum, however, still precipitated 125 I-labeled BALB/cfC3H MuMTV to a maximum precipitation of 90% at a 10^{-3} dilution (Fig. 3b). At a dilution of 10^{-4} , the precipitation of BALB/cNIV MuMTV corresponded to background levels. A 10^{-4} dilution was used for the competition radioimmune precipitation reported below.

With the absorbed anti-BALB/cfC3H MuMTV serum, MuMTV from BALB/cfC3H, C3H, and GR sources completely competed with 125 I-labeled BALB/cfC3H MuMTV (Fig. 4a). BALB/cNIV MuMTV, RIII MuMTV, and MuMTV(RIII)vp1 were unable to compete (Fig. 4a). MuMTV's from a variety of other C3H, BALB/cfC3H, and GR sources completely competed (Fig. 4b and c). However, MuMTV's from BALB/cNIV milk and primary tissue cultures (Fig. 4b) and from C3Hf milk (Fig. 4c) were unable to compete using the absorbed serum.

These data indicate that BALB/cNIV MuMTV resembled RIII and C3Hf MuMTV's in lacking antigenic determinants present on MuMTV from BALB/cfC3H, C3H, and GR strains. The antigenic reactivities of the MuMTV's tested with anti-BALB/cfC3H MuMTV serum are summarized in Table 1.

Studies with anti-BALB/cNIV MuMTV serum. To determine whether BALB/cNIV MuMTV contained viral antigens not shared by BALB/cfC3H MuMTV, reciprocal experiments were performed. Using 125 I-labeled BALB/cNIV MuMTV and anti-BALB/cNIV MuMTV serum

FIG. 3. Titration of anti-BALB/cfC3H MuMTV serum after absorption with BALB/cNIV MuMTV from milk. (a) Titration of unabsorbed serum (●) and three preparations of absorbed serum (○) with 125 I-labeled BALB/cNIV MuMTV. (b) Titration of unabsorbed serum (●) and three preparations of absorbed serum (○) with 125 I-labeled BALB/cfC3H MuMTV.

at a 1:30,000 dilution, BALB/cNIV MuMTV completely competed (Fig. 5a). MuMTV from BALB/cfC3H primary tumor cell cultures, MuMTV(C3H)vp1, and MuMTV(RIII)vp1 did not compete more than 10 to 15% (Fig. 5a). However, BALB/cfC3H MuMTV purified from milk competed 70%, and RIII milk MuMTV competed to 90%. In another experiment, MuMTV from BALB/cNIV milk competed to 100%, whereas BALB/cNIV MuMTV from primary tissue culture competed to 35% with 125 I-labeled BALB/cNIV MuMTV (Fig. 5b). Several MuMTV's from primary mammary tumor cell cultures or cell lines competed from 15 to 20% (Fig. 5b). In contrast, all MuMTV's purified from milk competed to between 70 and 80% (Fig. 5b).

Since virions from milks competed more completely than virions from tissue culture sources, milk-derived antigens, in addition to MuMTV polypeptides, appeared to be recognized by the

FIG. 4. Three competition RIA experiments with absorbed anti-BALB/cfC3H MuMTV serum and ¹²⁵I-labeled BALB/cfC3H MuMTV. Increasing amounts of purified MuMTV's and other proteins were added as competitors. (a) Experiment 1: MuMTV's which competed well include BALB/cfC3H MuMTV from milk (○), MuMTV(C3H)vp1 (●), Mm5mt/c1 MuMTV (□), and GR3A MuMTV (◇). MuMTV's which did not compete include BALB/cNIV MuMTV from milk (●), RIII MuMTV from milk (◆), and MuMTV-(RIII)vp1 (■). (b) Experiment 2: MuMTV's which competed well include BALB/cfC3H MuMTV from milk (○), BALB/cfC3H MuMTV from tissue culture (Δ), GR MuMTV from milk (○), GR MuMTV from

anti-BALB/cNIV MuMTV serum. Therefore, we attempted to absorb out antibodies directed against milk and host cell antigens associated with the milk-derived viruses by absorbing with MuMTV purified from the milk of mice with the BALB/c genotype to reveal possible virus-specific differences.

The ability of anti-BALB/cNIV MuMTV serum absorbed with MuMTV purified from BALB/cfC3H milk to precipitate ¹²⁵I-labeled BALB/cfC3H MuMTV and ¹²⁵I-labeled BALB/cNIV MuMTV was compared before and after absorption (Fig. 6). At a 10⁻⁴ serum dilution, only 10% of the ¹²⁵I-labeled BALB/cfC3H MuMTV was precipitated, compared with 90% before absorption (Fig. 6a). In the homologous reaction (Fig. 6b), the titer of the antiserum was reduced approximately 20- to 30-fold after absorption. A 10⁻⁴ dilution of the absorbed serum, at which 50% of the ¹²⁵I-labeled BALB/cNIV was precipitated (Fig. 6b), was selected for the competition radioimmune precipitation experiments described below.

With the absorbed antiserum, MuMTV from BALB/cNIV milk and tissue cultures competed to 100% and 90%, respectively, with ¹²⁵I-labeled BALB/cNIV MuMTV (Fig. 7a). In contrast, BALB/cfC3H and GR MuMTV's from a variety of milk and tissue culture sources competed only 20 to 30% (Fig. 7a). One exception was MuMTV from GR primary tissue culture, which competed to 60% (Fig. 7a). Although the nature of this reactivity might be of interest, it was not pursued further in these studies.

In a similar experiment with the absorbed serum (Fig. 7b), MuMTV from BALB/cNIV milk was the only virus which competed to 100%. MuMTV from RIII milk competed to 90%, and MuMTV(RIII)vp1 competed to greater than 60%. MuMTV from BALB/cfC3H milk, Mm5mt/c1 cells, and GR3A cells, and MuMTV(C3H)vp1 competed less than 15%.

Absorption of the anti-BALB/cNIV MuMTV serum clearly removed the reactivity we attributed to milk-associated antigens. We have not determined whether these antigens are milk polypeptides or internal MuMTV polypeptides that may have been in milk-derived virus due to

tissue culture (●), and (BALB/c × GR)F1 MuMTV from tissue culture (○). MuMTV's which did not compete include BALB/cNIV MuMTV from milk (●) and BALB/cNIV MuMTV from tissue culture (▲). (c) Experiment 3: MuMTV's which competed well include BALB/cfC3H MuMTV from milk (○), Mm5mt/c1 MuMTV (□), and C3H MuMTV from milk (▽). MuMTV's which did not compete include BALB/cNIV MuMTV from milk (●) and C3Hf MuMTV from milk (▼).

TABLE 1. Maximum competition of MuMTV's with absorbed anti-MuMTV sera^a

Mouse strain	Virus source	Maximum competition (%)	
		Anti-BALB/cfC3H serum	Anti-BALB/cNIV serum
BALB/cfC3H	Milk	85, 87, 91, 96	14, 18, 35
BALB/cfC3H	Primary culture	87, 98	27
C3H	Mm5mt/c1 cell line	89, 92	8, 20
C3H	Milk	83	27
C3H	MuMTV(C3H)vp1	87	15
GR	GR3A cell line	92, 93	14
GR	Primary culture	98	57
GR	Milk	92	34
(BALB/c × GR)F1	Primary culture	96	20
BALB/cNIV	Milk	0, 2, 3, 3	96, 100, 100
BALB/cNIV	Primary culture	8	87
C3Hf	Milk	0	34
RIII	Milk	1, 4	88
RIII	MuMTV(RIII)vp1	3, 4	61

^a Competition RIA using purified MuMTV's (10 µg) with ¹²⁵I-labeled BALB/cfC3H MuMTV and BALB/cNIV MuMTV-absorbed anti-BALB/cfC3H MuMTV serum (1:10,000) or with ¹²⁵I-labeled BALB/cNIV MuMTV and BALB/cfC3H MuMTV-absorbed anti-BALB/cNIV MuMTV serum (1:10,000) was performed as described in text.

FIG. 5. Two competition RIA experiments with anti-BALB/cNIV MuMTV serum at 1:30,000 dilution and ¹²⁵I-labeled BALB/cNIV MuMTV. Increasing amounts of purified MuMTV's were added as com-

petitors. (a) Experiment 1: the best competitors of this unabsorbed antiserum were MuMTV's obtained from milk regardless of strain—BALB/cNIV MuMTV from milk (●), RIII MuMTV from milk (◆), and BALB/cfC3H MuMTV from milk (○). Little or no competition was exhibited by MuMTV's from tissue culture regardless of strain: BALB/cfC3H MuMTV from tissue culture (△), MuMTV(RIII)vp1 (▀), and MuMTV(C3H)vp1 (▴). (b) Experiment 2: the best competitors were milk-derived MuMTV's—BALB/cNIV MuMTV from milk (●), BALB/cfC3H MuMTV from milk (○), C3H MuMTV from milk (▽), C3Hf MuMTV from milk (▼), and GR MuMTV from milk (○). Little or no competition was exhibited by MuMTV's obtained from tissue culture: BALB/cfC3H MuMTV from tissue culture (△), BALB/cNIV MuMTV from tissue culture (▀), GR3A MuMTV (▴), GR MuMTV from tissue culture (▾), (BALB/c × GR)F1 MuMTV from tissue culture (▸), and Mm5mt/c1 MuMTV (□).

FIG. 6. Titration of anti-BALB/cNIV MuMTV serum after absorption with BALB/cfC3H MuMTV from milk. (a) Titration of unabsorbed serum (●) and two preparations of absorbed serum (○) with ^{125}I -labeled BALB/cfC3H MuMTV. (b) Titration of unabsorbed serum (●) and three preparations of absorbed serum (○) with ^{125}I -labeled BALB/cNIV MuMTV.

rine cells, although it should be noted that the reactivity decreased slightly (Fig. 7b). This indicated that the reactivity was related primarily to viral rather than host determinants.

Using the absorbed anti-BALB/cNIV serum, MuMTV's purified from the milks of C3H and C3Hf mice were tested for their ability to compete with ^{125}I -labeled BALB/cNIV (Fig. 7c). Again, MuMTV from BALB/cNIV milk competed completely, whereas MuMTV from BALB/cfC3H milk and from the Mm5mt/c1 cell line competed less than 15%. MuMTV's from C3H and C3Hf milks both competed to 30% (Fig. 7c). This demonstrated that BALB/cNIV MuMTV contained antigenic determinants not shared by C3Hf MuMTV. Further, MuMTV from C3H milk appeared to share some antigenic determinants with BALB/cNIV MuMTV not shared by MuMTV from the C3H tumor cell line Mm5mt/c1. These determinants were not attributable merely to the presence of milk an-

tigens and might be due to the presence of the low oncogenic variant in C3H milk. The antigenic reactivities of the MuMTV's tested with anti-BALB/cNIV MuMTV serum are summarized in Table 1.

DISCUSSION

We analyzed the antigenicity of two viruses isolated in BALB/c mice which presumably originated in C3H mice. One virus, so-called BALB/cfC3H MuMTV, was transferred to the BALB/c mouse by foster-nursing BALB/c pups on C3H mothers, creating the BALB/cfC3H substrain. The MuMTV in this strain is associated with an 87% tumor incidence with a mean latency of 10 months (1). It is also associated with a high incidence of nodules (21) and restriction endonuclease mapping of proviral DNA has indicated that the exogenous MuMTV in BALB/cfC3H mice is the same as that found in C3H mice (11).

The second virus, the nodule-inducing virus, has been more difficult to characterize. It was apparently transferred from C3Hf to BALB/c by transplantation of BALB/c mammary tissue into a (BALB/c $\times$ C3Hf)F1 hybrid mouse followed by retransplantation into a BALB/c mouse (23). After the virus infection was detected in the transplant host, the resulting mouse strain was called BALB/cNIV (14). However, the BALB/cNIV MuMTV differs from the endogenous MuMTV of C3Hf mice in its mode of transmission, via milk rather than gametes, and in its greater noduligenic potential, tumor incidence, and response to lactogenic hormones (14, 23, 31). Since the incidence of tumors in BALB/cNIV is 40% at 20 months, the BALB/cNIV MuMTV is considered a low-tumor-potential virus.

Viral antigens from BALB/cNIV were distinguished from those of BALB/cfC3H by the immunodiffusion assay (6). The present studies confirm and extend those observations by competition radioimmune precipitation, using the same anti-BALB/cNIV MuMTV serum and MuMTV virions from a wide range of sources. These studies further demonstrate that BALB/cNIV MuMTV contains type-specific, as well as group-specific and class-specific, antigens.

Previous RIA studies of high and low oncogenic MuMTV's from C3H/HeN emphasized the lack of one or more antigenic determinants on the low oncogenic virus (2, 3, 27). They have not presented reciprocal evidence that the low oncogenic virus contains a specific determinant(s) lacking on the highly oncogenic MuMTV. In our experiments, anti-BALB/cNIV

FIG. 7. Three competition RIA experiments with absorbed anti-BALB/cNIV MuMTV serum and 125 I-labeled BALB/cNIV MuMTV. Increasing amounts of purified MuMTV's were added as competitors. (a) Experiment 1: MuMTV's which competed well include BALB/cNIV MuMTV from milk (●) and BALB/cNIV MuMTV from tissue culture (▲). MuMTV's which competed only partially include BALB/cfC3H MuMTV from tissue culture (Δ), GR MuMTV from milk (○), GR MuMTV from tissue culture (●), (BALB/c × GR)F1 MuMTV from tissue culture (●), and BALB/cfC3H MuMTV from milk

serum identified one or more antigenic determinants on 125 I-labeled BALB/cNIV MuMTV, the precipitation of which could not be inhibited by BALB/cfC3H MuMTV or any other competitor. Thus, BALB/cNIV is different from BALB/cfC3H MuMTV and all the other MuMTV's tested. Therefore, BALB/cNIV MuMTV may be said to have type-specific or BALB/cNIV-specific determinants.

RIII MuMTV was more closely related to BALB/cNIV MuMTV than to the high oncogenic C3H or GR viruses. Since the assay used here is directed against the surface antigens, it is probable that the antigenic similarities between RIII and BALB/cNIV MuMTV's reside in the virion glycoproteins. This could be determined by using RIAs employing purified polypeptides. Since RIII MuMTV is considered to be a high oncogenic virus, we conclude that the determinants studied here are not critical in oncogenicity. Thus, although the RIII and BALB/cNIV virions share a class of antigenic determinants, the class specificity does not appear to be related directly to oncogenicity.

Multiple MuMTV proviruses are present in the genomes of laboratory mouse strains, including C3Hf (11, 12, 20). Genetic and biochemical evidence suggests that only one provirus, corresponding to the *Mtv-1* locus, is expressed and is responsible for the late-occurring tumors in C3Hf and a closely related strain DBAf (19, 29, 30). If BALB/cNIV MuMTV were identical to the virus originally transmitted from C3Hf, complete immunological identity would be anticipated. However, MuMTV from C3Hf did not compete completely with BALB/cNIV MuMTV. Incomplete identity might be explained if the MuMTV found in BALB/cNIV had not been simply transferred from C3Hf. Instead, it might represent an induced BALB/c MuMTV, an activated C3Hf MuMTV provirus other than *Mtv-1* or a recombinant or other mutant form. Another possible explanation might be a common origin of these viruses with a divergence of antigenic structure in the last 16 years as a result of mutations of the viral ge-

(○). (b) Experiment 2: MuMTV's which competed well include BALB/cNIV MuMTV from milk (●), BALB/cNIV MuMTV from tissue culture (▲), and RIII MuMTV from milk (◆). MuMTV's which showed little or no competition include BALB/cfC3H MuMTV from milk (○), MuMTV(C3H)vp1 (○), Mm5mt/c1 MuMTV (□), and GR3A MuMTV (◇). (c) Experiment 3: BALB/cNIV MuMTV from milk (●) competed well. BALB/cfC3H MuMTV from milk (○), Mm5mt/c1 MuMTV (□), C3Hf MuMTV from milk (▼), and C3H MuMTV from milk (▽) competed only partially.

nomes or as a result of host modification of the virion carbohydrate structure. Restriction endonuclease maps of DNA copies of RNA purified from milk-derived BALB/cNIV MuMTV suggest that, in some preparations, BALB/cNIV MuMTV represents two viruses, both of which can be distinguished from C3H and C3Hf MuMTV's (J. P. Puma and R. D. Cardiff, unpublished data). Since both viruses can be distinguished from C3Hf MuMTV on the basis of restriction maps, it is possible that both viruses would also be antigenically different from C3Hf MuMTV. The observed lack of complete immunological identity with the C3Hf virus may be due to antigenic differences present on one or both viruses. Whatever the origin of the virus in BALB/cNIV mice, it can clearly be distinguished from viruses expressed in C3Hf and in other strains of mice.

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