JOURNAL OF VIROLOGY, Oct. 1979, p. 131-139
0022-538X/79/10-0131/09$02.00/0

Vol. 32, No. 1

Mice with Spontaneous Mammary Tumors Develop Type-Specific Neutralizing and Cytotoxic Antibodies Against the Mouse Mammary Tumor Virus Envelope Protein gp52

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Received for publication 26 March 1979

Sera from C3H mammary tumor-bearing mice contain cytotoxic antibodies for mouse mammary tumor virus (MMTV)-producing cells, based on ^{51}Cr release in a complement-dependent serum cytotoxicity assay. The cytotoxic antibodies could be absorbed by purified C3H MMTV gp52 and C3H MMTV-infected cat cells (C3H [MMTV] CrFK) containing cell surface MMTV gp52. However, purified MMTV p27 and uninfected CrFK cat cells were negative. Absorption of the sera with GR (MMTV) CrFK cells also removed all of the cytotoxicity, whereas absorption with RIII (MMTV) CrFK cells was negative, even though all three infected cat cells contained equivalent amounts of gp52. The same C3H cytotoxic sera also neutralized the focus-forming capacity of a C3H MMTV pseudotype of Kirsten sarcoma virus containing MMTV gp52. In contrast, sera from mammary tumor-bearing GR and RIII mice did not neutralize the pseudotype. Furthermore, neutralization could be achieved only by anti-gp52 but not by anti-gp36, -p27, -p14, or -p10 C3H MMTV sera. The gp52's of C3H, GR, and RIII MMTV could also be distinguished by using a type-specific competition radioimmunoassay employing ^{125}I-gp52 of C3H MMTV and a hyperimmune rabbit anti-C3H MMTV serum. To demonstrate these differences directly, we studied the primary structure of gp52 on the surface of the C3H, GR, and RIII (MMTV) CrFK cells. Two-dimensional tryptic peptide maps of the cell surface lactoperoxidase-catalyzed iodinated gp52's revealed a greater number of peptides common to the gp52's of C3H and GR MMTVs than to RIII MMTV gp52. These results demonstrate that gp52 is a major target antigen for both cytotoxic and neutralizing antibodies, that the cell surface and virion-associated gp52's of C3H, GR, and RIII MMTV contain both group- and type-specific determinants, and that C3H and GR MMTV gp52's are antigenically more related to each other than to RIII MMTV gp52. Furthermore, C3H mammary tumor-bearing mice develop type-specific antibodies capable of recognizing unique gp52 determinants and, therefore, are able to distinguish the gp52 of C3H MMTV from the gp52's of GR and RIII MMTV.

All inbred strains of mice, and probably all feral mice as well, contain the mouse mammary tumor virus (MMTV) in an endogenous form (4, 15). In addition, offspring of mice from strains with high mammary tumor incidence (C3H, GR, and RIII) are also infected with an exogenous MMTV during their suckling stage (D. H. Moore, C. A. Long, A. B. Vaidya, J. B. Sheffield, A. S. Dion, and E. Y. Lasfargues, Adv. Cancer Res., in press). The target tissue for exogenous MMTV is the mammary gland, and infection leads to development of mammary carcinomas in almost all the animals within 1 year. One consequence of the expression of exogenous and possibly endogenous MMTV is the development of an autogenous immune response by the mouse (1, 2, 11). This immune response can be demonstrated by the presence of both cytotoxic and precipitating antibodies for MMTV-infected cells and MMTV, respectively (6). The development of cytotoxic antibodies to MMTV-infected cells represents an important extension of the mouse's humoral response because the development of mammary carcinomas is a result of uncontrolled division of the infected cells. It is equally important to determine whether any of the antibodies capable of binding to MMTV are also able to neutralize the infectivity of the virus.

The major external glycoprotein of MMTV, gp52, has been shown to be the major viral cell surface antigen present on the surface of

MMTV-infected mammary tumor cells (18). Previous studies with murine leukemia virus (MuLV) have shown that the major external virion glycoprotein gp70 exhibits antigenic polymorphism (5, 12, 16, 22). An important question then is whether antigenic polymorphism exists on cell surface and virion-associated gp52's and, furthermore, whether mouse sera contain cytotoxic and neutralizing antibody populations reflecting the various reactivities. Consistent with this possibility are studies demonstrating the presence of both group- and type-specific reactivities associated with the gp52 of the extracellular MMTVs of C3H, GR, and RIII mice by use of a heterologous hyperimmune antiserum (23). In this study, we demonstrate that sera from C3H mammary tumor-bearing mice contain MMTV-specific cytotoxic and neutralizing antibodies. In addition, we show that both cell surface and virion-associated gp52's of C3H, GR, and RIII MMTV contain both group- and type-specific determinants and that C3H and GR MMTV gp52's are antigenically more related to each other than to RIII MMTV gp52. This study also demonstrates that C3H mammary tumor-bearing mice develop antibodies capable of recognizing unique gp52 determinants and are thus able to distinguish the gp52's of C3H MMTV and GR MMTV from the gp52 of RIII MMTV.

MATERIALS AND METHODS

Cells. The Mm5mt/c_1 cell line was originally derived from C3H mouse mammary tumor cell cultures and was developed into a continuous cell line producing high levels of MMTV (7, 17). The 13D cells are a single-cell clonal isolate of Mm5mt/c_1 cells (18). CrFK feline cells infected with MMTV from C3H, GR, and RIII mice (10) were kindly supplied by J. Schlom. HESM are human embryonic skin and muscle cells in early passage (<passage 10); KNIH cells are NIH Swiss mouse cells nonproductively transformed by Kirsten sarcoma virus (KiSV). All the cells were propagated in Dulbecco-modified Eagle minimal essential medium containing 10% fetal calf serum. The medium for the Mm5mt/c_1, 13D, and infected CrFK cells was supplemented with dexamethasone (10 μg/ml) and insulin (10 μg/ml).

Antisera. Rabbit antisera directed against MMTV and monospecific antisera directed against the MMTV proteins gp52, gp36, p27, p14, and p10 were prepared and characterized as described previously (3). The anti-MMTV serum was highly specific for MMTV and exhibited no detectable cross-reactivity with various MuLV's including ecotropic R-MuLV, xenotropic Balb II virus, the dual tropic mink cell focus virus of AKR mice, and the amphotropic virus (A-MuLV) of feral mice. Mouse sera were obtained from female C3H/HeN, GR/N, and RIII mice bearing spontaneously arising mammary tumors. The sera are pools of four to five mice.

Cell surface iodination. Cell monolayers (90% confluent) were surface labeled by the lactoperoxidase-catalyzed iodination technique as previously described (18, 24). After iodination the cells were washed with growth medium and Earle balanced salt solution, lysed in situ, and clarified before being used for immunoprecipitation.

Radioimmunoprecipitation and gel electrophoresis. Samples of the clarified lysates (150 μl) were incubated for 1 h at 37°C with 50 μl of primary viral antisera and then overnight at 4°C. The immunoprecipitates were collected, washed, and prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10 to 20% gradient slab gels (19, 20). The ^{125}I-labeled proteins were visualized by autoradiography. Purified disrupted MMTV was also iodinated and coelectrophoresed as a marker for gp52.

Tryptic peptide analysis of cell surface ^{125}I-gp52's of C3H, GR, and RIII MMTV-infected CrFK cells. The gel regions containing cell surface ^{125}I-gp52's were excised, and tryptic peptide maps were prepared as previously described (20). Briefly, the individual slices were rehydrated and fixed in a mixture of 50% methanol–10% acetic acid in water. After two changes of methanol–acetic acid–water, the gels were washed twice with 10% methanol and dried with a stream of nitrogen. The gel slices were rehydrated, and the gp52's were digested overnight at 37°C with 200 ng of trypsin (tolyl sulfonyl phenylalanyl chloromethyl ketone-trypsin, Worthington Biochemical Corp.) and 10 μg of myoglobin as carrier; the digestion was repeated once and the digests were pooled. The pooled digests were then removed and lyophilized three times from water. Two-dimensional tryptic fingerprints were prepared on thin-layer cellulose plates. The ^{125}I-containing peptides were detected by autoradiography.

Complement-dependent serum cytotoxicity assay. Rabbit antisera to purified MMTV glycoproteins and nonglycoproteins were tested in a complement-dependent serum cytotoxicity assay based on ^{51}Cr release as previously described (13). ^{51}Cr-labeled Mm5mt/c_1 target cells (10^4) reacted in a microtiter well with 0.05 ml of the heat-inactivated antisera and 0.05 ml of rabbit serum diluted 1:5 as the complement source. This mixture was incubated at 37°C for 5 h; then 0.1 ml of supernatant was removed and the amount of ^{51}Cr release was measured in a Searle gamma counter. The percent cytotoxicity was determined according to the formula given in Table 1, footnote *b*.

The test release was measured with target cells incubated in antisera and complement, the spontaneous release was determined for cells incubated in medium only, and the maximal release was determined by lysing the cells with distilled water and freeze-thawing them three times. The toxicity of the complement was tested in each assay by incubating the target cells in complement without the addition of any antisera. The percent cytotoxicity with complement alone was always less than 10%.

Generation of KiSV(MMTV) pseudotypes. The KiSV(C3H-MMTV) pseudotype was generated as described elsewhere (18a). Briefly, C3H MMTV-producing 13D cells (10^6) were cocultivated for 17 days with KNIH cells (10^6). The cells were treated with mitomycin C (20 μg/ml) for 1 h and trypsinized. Cells (5

$\times 10^4$) were overlaid onto HESM cells plated 24 h earlier in Eagle modified minimal essential medium with Earle salts (EMEM) containing 2 μg of polybrene per ml. After 2 weeks the cells were transformed, and they were then passaged at least eight additional times. KiSV(C3H-MMTV) pseudotype from the cell-free fluids was pelleted and concentrated in one-twentieth volume of EMEM, filtered to remove any residual cells, and used in a neutralization of focus formation assay on FRE cells.

Neutralization of focus formation. Antisera were heat-inactivated at 56°C for 30 min before use. Stock virus supernatants were diluted to contain 250 foci in 0.5 ml. Then 0.5-ml dilutions of antisera were mixed with 0.5 ml of each virus and incubated for 30 min at 37°C. Virus-antiserum mixtures (0.4 ml) were added to duplicate 60-mm dishes containing indicator cells (5×10^5 cells per dish) that had been seeded 24 h previously in medium containing 2 μg of polybrene per ml. The medium was removed prior to the addition of the virus-antiserum mixture. Incubation was continued for 1 h at 37°C. The dishes were then overlaid with 4 ml of EMEM containing 5% fetal calf serum. Foci appeared between 10 and 14 days, at which time the cells were fixed and stained and the foci were counted.

Purification of MMTV gp52 and competition radioimmunoassay. C3H MMTV gp52 was purified by sequential chromatography on DEAE-cellulose and Sephadex G-100 (1). Purified gp52 was labeled with ^{125}I by the Chloramine-T method (9). Approximately 15 μg of the purified protein was incubated with 10 μg of Chloramine-T and 1 mCi of $Na^{125}I$ in 0.5 M phosphate buffer at pH 7.5 for 1 min. The reaction was terminated by the addition of 20 μg of sodium metabisulfate. The free ^{125}I was separated from ^{125}I bound to protein by sequential Sephadex G-25 and Sephadex G-100 columns, previously equilibrated with radioimmunoassay buffer (10 mM acetate buffer at pH 7.2, 0.15 M NaCl, 0.4% Nonidet P-40, and 0.1% bovine serum albumin).

Competition radioimmunoassays were performed by incubation of 100 μl of sample with limiting dilutions of rabbit anti-C3H MMTV sera for 2 h at 37°C; 100 μl of ^{125}I-labeled protein (10,000 cpm) was added, and incubation was continued for 2 h at 37°C and overnight at 4°C. *Staphylococcus aureus* (Cowan I strain) (14) was added to each tube to facilitate precipitation of immune complexes. Samples were incubated for 15 min at room temperature, immune pellets were collected by centrifugation at 1,500 × *g* for 30 min, and the radioactivity in the pellets was determined.

RESULTS

Demonstration of cytotoxic antibodies in C3H mouse mammary tumor bearer sera directed against MMTV gp52. We have previously shown that sera from mouse strains with high to moderate mammary tumor incidences are specifically cytotoxic for mouse mammary tumor cells expressing MMTV (6). Therefore, it was important to determine whether the cytotoxic antibodies were directed against MMTV-coded protein(s) and to determine which MMTV protein(s) was the target cell surface antigen. To demonstrate that the antibodies were directed against an MMTV protein, antisera from several C3H tumor-bearing mice were pooled and titrated in a complement-dependent serum cytotoxicity assay against Mm5mt/c_1 cells to determine a dilution for subsequent absorption studies. Based on the titration results, a 1:10 dilution of the antisera yielding 50% cytotoxicity was chosen. It should be noted that 100% cytotoxicity was obtained when a high-titered hyperimmune rabbit antiserum directed against MMTV was used. The 1:10 diluted tumor bearer serum pool was then absorbed with increasing amounts of C3H (MMTV) CrFK cells, and the absorbed serum pool was then retested in the complement-dependent serum cytotoxicity assay against Mm5mt/c_1 cells. The results presented in Fig. 1 clearly show that the C3H (MMTV) CrFK cells could completely absorb out the cytotoxicity. The data are plotted as a relative percent cytotoxicity based on the cytotoxicity of the same sera absorbed with various amounts of uninfected cat cells which showed no absorption. Because gp52 is the major MMTV cell surface antigen on the C3H (MMTV) CrFK cells (determined by lactoper-

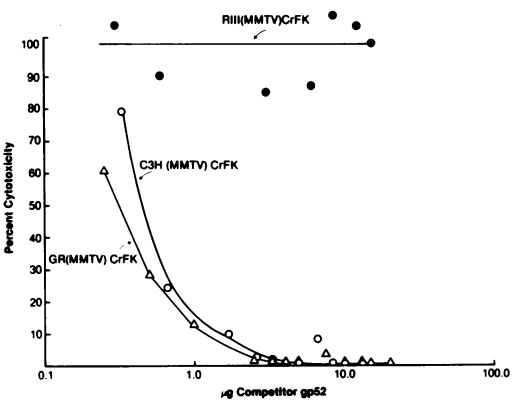

FIG. 1. *Cytotoxicity of sera from C3H mammary tumor-bearing mice absorbed with CrFK cat cells infected with C3H, GR, and RIII MMTV. A pool of sera from C3H tumor bearers was absorbed with increasing amounts of live cells. The absorbed sera were then tested for cytotoxicity against Mm5mt/c_1 cells in the complement-dependent serum cytotoxicity assay as described in Materials and Methods. A sample of each of the cell preparations was used to determine the micrograms of gp52 in a competition radioimmunoassay for gp52. The percent cytotoxicity is plotted relative to the amount of cytotoxicity achieved with equivalent amounts of uninfected CrFK cat cells. Essentially no cytotoxicity was observed with the uninfected cat cells.*

oxidase surface iodination [Fig. 2]), it presumably was the antigen responsible for the absorption of the cytotoxicity.

To further demonstrate that the antibodies in the natural serum responsible for the cytotoxicity were directed against MMTV gp52, the serum was abosrbed with either purified gp52 or the major internal MMTV nonglycoprotein p27 and tested for cytotoxicity against Mm5mt/c_1 cells. The results presented in Table 1 show that gp52 absorbed approximately 40% of the maximum cytotoxicity obtained with unabsorbed serum, whereas p27 was completely negative. The reduction in cytotoxicity by gp52 was probably a minimum value because the purified protein presumably was not in its native three-dimensional configuration. This was substantiated by the fact that gp52 completely absorbed all the cytotoxicity of the hyperimmune rabbit anti-MMTV serum, whereas p27 was negative. Finally, only monospecific anti-gp52 serum was cytotoxic for Mm5mt/c_1 cells, whereas anti-gp36, -p27, and -p10 sera were negative (Table 2). These results all point to gp52 as the major MMTV cell surface target antigen for cytotoxic antibodies.

Recognition of group- and type-specific determinants on cell surface gp52 by sera of C3H mammary tumor-bearing mice. An important question was whether group- and type-specific determinants existed on cell surface gp52 and whether the sera of C3H tumor-bearing mice contained antibodies capable of recognizing these determinants on cell surface gp52's of different strains of MMTV. To demonstrate that the cytotoxic tumor bearer serum possessed group- and type-specific gp52 reactivities, the serum was absorbed with the cat cells infected with MMTV of C3H, GR, and RIII mice. Figure 2 shows the SDS-PAGE profile of lactoperoxidase-catalyzed iodinated MMTV antigens on each of the cat cells immunoprecipitated by anti-MMTV serum. The results show that gp52 is the major MMTV surface antigen on all the infected cat cells. The absorbed serum was then tested in the complement-dependent serum cytotoxicity assay against Mm5mt/c_1 cells. The results presented in Fig. 1 clearly show that both C3H (MMTV) and GR (MMTV) CrFK cells could completely absorb out the cytotoxicity, whereas RIII (MMTV) CrFK cells

TABLE 1. *Absorption of cytotoxic antibodies from sera of C3H mammary tumor-bearing mice*

Sera[a] absorbed with:	Percent cytotoxicity[b]	Percent of control
Unabsorbed	60 ± 0.9[c]	100
gp52	37 ± 2.7	61
p27	65 ± 0.3	108

[a] Pool of sera from C3H mice with mammary tumors. The serum pool was diluted 1:20 and heat inactivated. Amounts of 2 μg of purified gp52 or p27 were added to 50-μl samples of serum, and these mixtures were incubated at 37°C for 60 min and then overnight at 4°C. The absorbed and unabsorbed sera were then tested in a complement-dependent cytotoxicity assay against ^{51}Cr-labeled Mm5mt/c_1 cells.

[b] Percent cytotoxicity = (cpm for test release − cpm for spontaneous release)/(cpm for maximal release − cpm for spontaneous release) × 100. The cytotoxicity of the complement alone was 10%. The maximal release was 78% of the total label incorporated by 10^4 cells pipetted into each test well (2,109 cpm). The spontaneous release was 8% of the total label.

[c] Standard error.

TABLE 2. *Anti-gp52 serum is cytotoxic for Mm5mt/c_1 cells based on ^{51}Cr release*

Antiserum	Percent cytotoxicity[a]	
	1:10	1:20
Anti-gp52 (1:1,600)[b]	69.4 ± 3.3	58.7 ± 1.4
Anti-gp36 (1:1,600)	6.2 ± 0.8	−2.0 ± 0.8
Anti-p27 (1:1,600)	7.7 ± 1.0	2.6 ± 1.5
Anti-p10 (1:3,200)	2.3 ± 1.5	1.8 ± 0.4

[a] Percent cytotoxicity ± standard error at 1:10 and 1:20 dilutions of the individual antisera. Complement cytotoxicity: 2.3 ± 0.8%.

[b] Antiserum dilution which precipitated 50% of the radioactivity of iodinated homologous antigen.

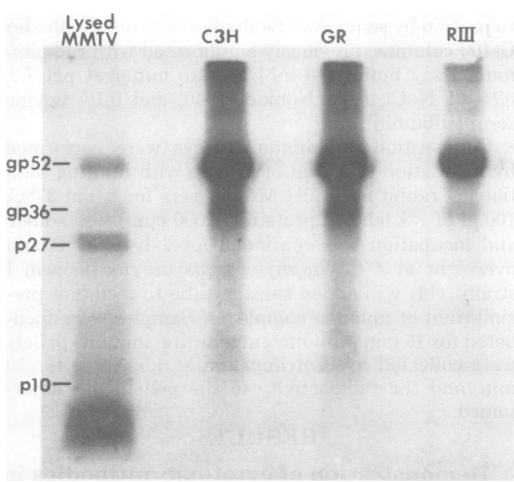

FIG. 2. *Isolation of ^{125}I cell surface-labeled gp52 from C3H, GR, and RIII MMTV-infected CrFK cat cells. Monolayers of each cell line were surface labeled by the lactoperoxidase-catalyzed iodination technique, and ^{125}I-gp52 was isolated by immunoprecipitation and SDS-PAGE as described in Materials and Methods. The labeled proteins were visualized by autoradiography. Iodinated disrupted MMTV was coelectrophoresed as a marker for gp52.*

could not absorb the cytotoxic antibodies. The inability of the RIII cells to remove the cytotoxicity was not due to the lack of surface gp52 because comparable amounts of cell surface gp52 were detected on all the cells by surface iodination and by radioimmunoassay for gp52. Furthermore, all three cell lines were able to absorb all the cytotoxic activity of a heterologous hyperimmune anti-MMTV serum (Fig. 3). These results demonstrate that C3H mice develop type-specific cytotoxic antibodies capable of distinguishing RIII MMTV gp52 from C3H and GR MMTV gp52.

Demonstration of type-specific neutralizing antibodies in the sera of mammary tumor-bearing C3H, GR, and RIII mice. The cytotoxicity data indicated that sera of C3H tumor bearers recognized group- and type-specific determinants on cell surface gp52's of C3H, GR, and RIII MMTV. Although the natural sera in the complement-dependent cytotoxicity assay could distinguish RIII MMTV gp52 from C3H and GR MMTV gp52, they could not distinguish the gp52's of the latter two viruses. It was important to determine whether the natural sera could recognize type-specific determinants on virion-associated gp52's and, furthermore, whether this type specificity extended to the gp52's of GR and C3H MMTV. Therefore, natural sera from C3H, GR, and RIII mammary tumor-bearing mice were tested for their ability to neutralize the focus-forming capacity of a KiSV(C3H-MMTV) pseudotype containing MMTV gp52 as the envelope protein (18a). Tumor bearer sera from these strains of mice have been shown to contain high titers of MMTV precipitating antibodies (6). The target antigen for neutralization of the pseudotype was gp52 because only anti-gp52 serum could neutralize the pseudotype, whereas antisera against the other MMTV proteins gp36, p27, p14, and p10 were negative (Fig. 4A). When the three tumor bearer sera were titrated against the pseudotype, only the C3H tumor bearer serum contained neutralizing antibodies with a 50% endpoint titer of 1:60 (Fig. 4B). The RIII and GR sera possessed no C3H MMTV neutralizing antibodies (Fig. 4B). These results demonstrated that the neutralizing antibodies of tumor bearer mice are highly type specific and capable of distinguishing C3H MMTV from both GR and RIII MMTV.

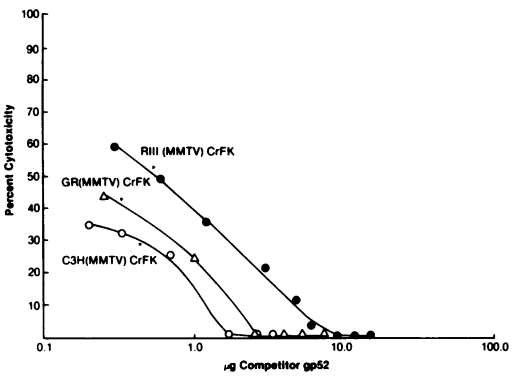

FIG. 3. *Cytotoxicity of hyperimmune rabbit antiserum to MMTV absorbed with CrFK cat cells infected with C3H, GR, and RIII MMTV. The absorbed sera were tested for cytotoxicity against Mm5mt/c$_1$ cells in the complement-dependent serum cytoxicity assay as described in Fig. 1.*

Relatedness of virion-associated gp52s from C3H, GR, and RIII MMTVs measured by competition radioimmunoassay using a hyperimmune heterologous serum. To further analyze the group- and type-specific antigenic determinants on virion-associated gp52's

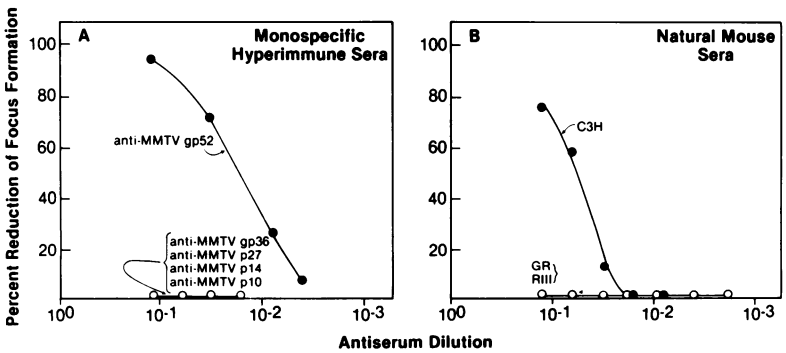

FIG. 4. *Neutralization of the transformation by the KiSV(C3H-MMTV) pseudotype by various antisera. FRE cells were used as indicator cells for transformation, and neutralization was assayed as described in Materials and Methods. The pseudotype stock virus was diluted to yield 100 foci per 60-mm plate. (A) Neutralization with antisera directed against individual MMTV proteins. (B) Neutralization with antisera from C3H, GR, and RIII mammary tumor-bearing mice.*

of C3H, GR, and RIII MMTVs, their immunological cross-reactivity was determined in a competition radioimmunoassay using purified gp52 from C3H MMTV and a rabbit serum directed against C3H-derived MMTV. Lysed C3H, GR, and RIII MMTVs all competed for antibodies binding ^{125}I-gp52 with essentially identical slopes (Fig. 5A). However, only C3H and GR MMTV competed completely, whereas RIII MMTV yielded only 92% competition. This indicated that RIII gp52 lacked antigenic determinants which were shared by C3H and GR gp52. To examine the type-specific differences in greater detail, the antibodies against antigenic determinants common to all three MMTV gp52's were removed by absorption with lysed RIII MMTV. A competition radioimmunoassay was then performed using the absorbed serum. The results clearly show that RIII gp52 is distinct from the gp52 of C3H and GR MMTV since RIII MMTV exhibited relatively little competition compared to the complete competition obtained with C3H and GR MMTV at 10^4 ng of competitor protein. Furthermore, the competition radioimmunoassay using RIII absorbed serum revealed a difference in the affinity of antibody binding to C3H and GR MMTV. This is reflected in the different slopes of the competition curves and is clearly shown in the logit plot of Fig. 5B (see insert). These results agree with the serological analysis of cell surface gp52 and demonstrate that C3H and GR virus gp52's are more closely related to each other than to RIII gp52. The results also demonstrate that C3H and GR gp52's are distinguishable from each other.

Primary structure of cell surface MMTV gp52 by tryptic peptide mapping. The results of immunological analyses with both tumor bearer serum and hyperimmune rabbit anti-MMTV serum demonstrated that the cell surface and virion-associated gp52's of C3H, GR, and RIII MMTV possess both group- and type-specific antigenic determinants. Therefore, to directly characterize the various cell surface gp52's, we analyzed their primary structures. To characterize the primary structure of the exposed portion of the cell surface gp52's of C3H, GR, and RIII MMTV, cells infected with these viruses were surface labeled by the lactoperoxidase technique to iodinate their cell surface gp52. All three MMTVs were propagated in a common cell, the CrFK feline cell, to eliminate possible host modifications of gp52 (e.g., differential glycosylation). Subsequently, the cells were lysed and surface-iodinated gp52 was analyzed by radioimmunoprecipitation with C3H anti-MMTV serum followed by SDS-PAGE. Figure 2 shows the SDS-PAGE profile of the labeled immunoprecipitated gp52's. We have previously shown that cell surface gp52 is sub-

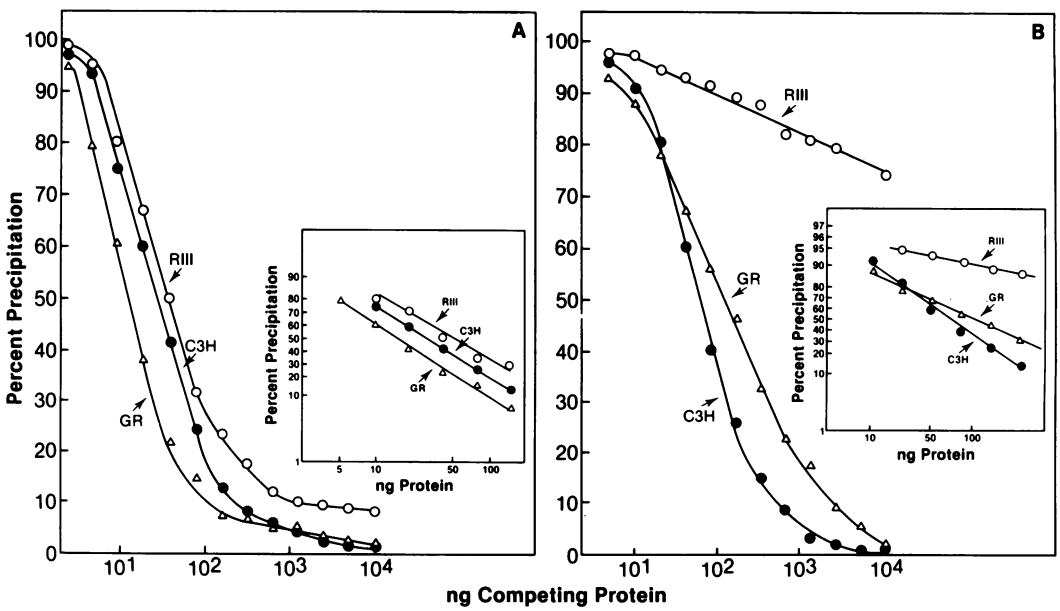

FIG. 5. *Comparison of C3H, GR, and RIII MMTV in a competition radioimmunoassay for MMTV gp52. The competitive radioimmunoassays were performed as described in Materials and Methods. (A) Competition for binding of ^{125}I-gp52 (10^4 cpm) of C3H MMTV by intact C3H, GR, and RIII MMTV using a limiting dilution of anti-C3H MMTV serum. (B) Competition for binding of ^{125}I-gp52 by limiting dilutions of anti-MMTV serum absorbed with RIII-derived MMTV.*

sequently incorporated into extracellular MMTV (18, 19). The immunoprecipitated gp52s were eluted from the slab gels and digested with trypsin. Fingerprints were prepared by chromatography followed by electrophoresis in a second dimension on thin-layer plates. The tryptic peptide maps are presented in Fig. 6, and Fig. 7 is a composite drawing of the representative two-dimensional tryptic peptide maps of Fig. 6. Figures 6 and 7 show 15, 11, and 10 tryptic peptide spots for C3H gp52, GR gp52, and RIII gp52, respectively. A recent evaluation of the amino acid composition of gp52 of C3H MMTV demonstrated that it contains approximately 13 tyrosine residues (25). The number of tyrosine residues agrees with the possibility that most, if not all, of the gp52 tyrosine residues are exposed on the cell surface. Two out of a total of 25 tryptic peptides were common to all three gp52's. These common peptides could contribute to the group-specific antigenic reactivities. There are also peptides that exhibit partial commonality (between C3H and RIII or C3H and GR). Five tryptic peptides were common to both C3H and GR, and two tryptic peptides were common to both C3H and RIII. These partially common peptides would also contribute to the group-specific reactivities, in particular to the MMTVs with the common peptides. The ability to find more peptides common to C3H and GR than to RIII indicates that C3H and GR gp52's are more closely related to each other than to RIII gp52. The data of Fig. 7 also demonstrate a number of peptide spots unique to each gp52 (six for C3H, four for GR, and six for RIII). These unique peptides distinguished the MMTVs from the three mouse strains. More importantly, they presumably contribute to the type-specific antigenic reactivities.

DISCUSSION

This study demonstrates that C3H mammary tumor-bearing mice develop neutralizing antibodies against MMTV and cytotoxic antibodies against MMTV-producing mammary tumor cells. It appears that gp52 was a major MMTV target antigen for both the neutralizing and cytotoxic antibodies. This is based on the following observations: (i) purified MMTV gp52 but not p27 was able to absorb out a significant portion of the cytotoxicity of the C3H mammary tumor-bearing sera; (ii) MMTV-infected cat cells containing cell surface gp52 were able to completely absorb all the cytotoxicity of the same sera, whereas uninfected cat cells were negative; (iii) only anti-gp52 serum was cytotoxic for MMTV-producing cells; and (iv) only anti-gp52 serum was able to neutralize the KiSV(C3H-MMTV) pseudotype.

Equally important is the observation that sera of C3H tumor bearers could distinguish C3H and GR MMTV gp52 from RII MMTV gp52 in a complement-dependent serum cytotoxicity assay based on absorption with cat cells infected with C3H, GR, and RIII MMTV. A potentially more striking observation was that only sera from C3H tumor-bearing mice contained neutralizing antibodies for the KiSV(C3H-MMTV) pseudotype. In contrast, sera from GR and RIII tumor-bearing mice, which have been shown to contain both MMTV precipitating and cytotoxic antibodies (1, 2, 6), contained no neutralizing antibodies for the KiSV(C3H-MMTV) pseudotype. Therefore, the ability of only the C3H tumor bearer sera to neutralize the pseudotype indicated that the neutralizing antibodies were solely directed against type-specific determinants.

It is not surprising that the C3H tumor-bearing mice developed a type-specific response against gp52 of C3H MMTV. Naturally occurring antibodies in mice have been reported to be directed against the type-specific determinants of MuLV gp70 in virus neutralization and cytotoxicity assays. The fact that C3H tumor-bearing mice recognized only a subset of determinants of the virion glycoprotein suggests at least two explanations: (i) the mouse is "tolerant" to

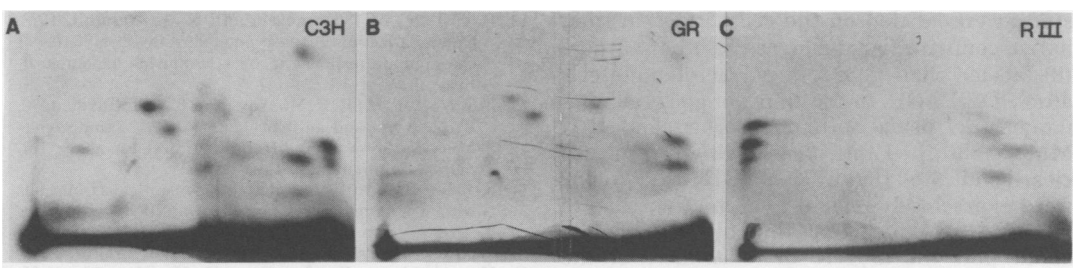

FIG. 6. *Autoradiography of tryptic digest fingerprints of cell surface ^{125}I-gp52 of C3H, GR, and RIII MMTV-infected CrFK cat cells. The gel regions containing ^{125}I-gp52's from Fig. 2 were excised and their tryptic peptide fingerprints were prepared as described in Materials and Methods.*

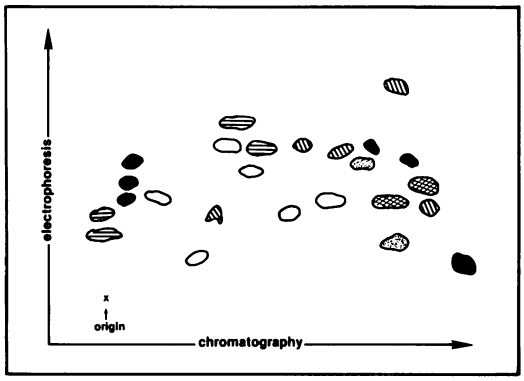

FIG. 7. *Composite of the major peptides spots of the individual gp52's from Fig. 6.* (○) *Unique to C3H;* (●) *unique to RIII;* (⊖) *unique to GR;* (⊛) *common to C3H and RIII;* (⊘) *common to C3H and GR; and* (⊛) *common to C3H, GR, and RIII.*

the group determinants of MMTV gp52 or (ii) the mouse can respond to all of the virion gp52 determinants. However, it is possible that the cytotoxic antibodies directed against the group-specific determinants were completely absorbed in vivo. The latter possibility could arise by absorption of the group-specific antibodies with endogenous MMTV gp52, which is expressed in various organs of the mouse in the absence of virus production (2, 6).

The cytotoxicity results suggested that C3H and GR MMTV gp52 were more closely related to each other than to RIII MMTV gp52. Therefore, the gp52's of C3H, GR, and RIII MMTV were also distinguished by primary structure analysis based on tryptic peptide fingerprinting. The fingerprints revealed a greater number of peptides common to the gp52's of C3H and GR MMTV than to RIII MMTV gp52. This is in disagreement with a previous report indicating that the tryptic peptides of GR and RIII MMTV gp52 are more similar to each other than to C3H MMTV gp52 (8). The reason for the discrepancy is unclear although it may derive from the different iodination methods. The gp52's in this study were labeled on the cell surface in their native configuration, whereas the other authors (8) labeled their gp52's in polyacrylamide gels after SDS-PAGE. In addition, our gp52 radioimmunoassay, using both unabsorbed and RIII MMTV absorbed anti-C3H-MMTV serum, also clearly indicated that C3H and GR MMTV gp52 were more closely related to each other than to RIII MMTV gp52. It is difficult to speculate about how the degrees of relatedness of the different MMTVs arose because of the lack of complete genetic history of the three mouse strains (21).

ACKNOWLEDGMENTS

This work was supported by contract N01-CO-75380 with the National Cancer Institute.

We thank H. O'Connell, H. Rager, G. Clarke, and J. Bruszewski for excellent technical assistance. We also thank J. Olpin for assistance with the tryptic peptide fingerprinting.

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