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Monoclonal Antibodies Identify Individual Determinants on Mouse Mammary Tumor Virus Glycoprotein gp52 with Group, Class, or Type Specificity

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Hybrid cell lines producing monoclonal antibodies against the C3H strain of mouse mammary tumor virus (C3H MMTV) were prepared by the fusion of mouse myeloma cells with the lymphocytes of BALB/c mice that were immunized with C3H MMTV. Approximately 10% of the hybrid cells initially plated after cell fusion produced immunoglobulins that reacted in antibody-binding assays with C3H MMTV; 40 of these cells were cloned, and 6 eventually yielded stable cell lines. High concentrations of monoclonal antibodies (5 to 20 mg/ml) were obtained from serum and ascites fluid of syngeneic mice inoculated with the hybrid cells. All of the monoclonal antibodies were directed against the envelope glycoprotein gp52. Three of the hybrid cell lines produced immunoglobulins of the immunoglobulin M subclass and three produced immunoglobulin G2a. The monoclonal antibodies showed limited charge heterogeneity in light and heavy chains when analyzed by high-resolution, two-dimensional gel electrophoresis. Three serologically distinct specificities were observed when these ascites fluids were tested against different strains of MMTV. The antigenic determinants detected were the following: (i) a type-specific determinant unique to the C3H strain of MMTV; (ii) class-specific determinants shared between C3H and GR MMTVs; and (iii) a group-specific determinant found on C3H, GR, RIII, and the endogenous C3H (C3Hf) MMTVs. Because monoclonal antibodies recognize single antigenic determinants, these results demonstrate for the first time that the three patterns of antigenic reactivity for MMTV are related to individual determinants on the gp52 molecule and also clearly show that one strain of MMTV can be distinguished from other strains.

Antigenic polymorphism for virus-coded proteins and glycoproteins has been evaluated in detail for retroviruses such as murine leukemia virus. Sets of antigenic determinants which are shared by viruses belonging to the same family and others which are unique to a particular isolate have been identified (5, 9, 10, 19, 20, 22, 26, 27). Certain classes of determinants correlate with a particular biological property of the murine leukemia virus on which they are found. Antigenic determinants of p15(E) which are found only in murine leukemia viruses within an ecotropic host range have been identified (19, 26).

For another retrovirus, mouse mammary tumor virus (MMTV), antigenic polymorphism has been shown with heterologous antiserum for the two envelope glycoproteins gp52 and gp36 (4, 16, 24, 28, 30) and the core protein p27 (29). The results of our studies with both naturally occurring antibodies in mammary tumor-bearing mice and hyperimmune rabbit anti-MMTV serum adsorbed with RIII MMTV demonstrated that gp52's from C3H and GR MMTVs share antigenic determinants lacking on the gp52's from both RIII MMTV and C3Hf MMTV, an endogenous MMTV of C3H/HeN mice (17, 24). Although the serological probes used in these studies have been of considerable value, they have so far been unable to clearly distinguish any one MMTV from other MMTVs. This results from the use of a heterogeneous antibody population present in both heterologous and natural antisera and the presence of multiple determinants on MMTV gp52. Furthermore, it has not been shown that the various types of reactivity are each associated with individual antigenic determinants. Greater resolution between individual MMTVs could be achieved through the use of monoclonal antibodies that react with a single antigenic determinant.

We describe the identification of approximately 200 hybrid cell clones producing monoclonal antibodies to C3H MMTV. Forty of these

were cloned, and six eventually yielded stable cell lines. The monoclonal antibodies of these six cell lines were produced in high titers by inoculation of syngeneic mice with the hybridoma cells. All of the monoclonal antibodies were directed against gp52. The following three patterns of reactivity were observed: (i) type specific for C3H MMTV; (ii) class specific for determinants shared between C3H and GR MMTVs; and (iii) group specific for determinants shared between C3H, GR, RIII, and C3Hf MMTVs.

MATERIALS AND METHODS

Viruses and antisera. C3H MMTV was obtained from the Mm5mt/C1 cell line established from a C3H/Crgl mouse mammary tumor (23). Tissue culture-derived GR MMTV was isolated from a GR/N mouse mammary tumor cell line established in our laboratory (3), and antigenic properties of this isolate have been described previously (4). A cloned cell line producing high levels of C3Hf MMTV was derived from a mammary tumor spontaneously occurring in a C3H/HeNf mouse (4). The mouse mammary tumor cell lines were routinely grown at 37°C in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 10 μg of insulin, and 2 μg of dexamethasone per ml. The virus was purified from culture fluids as previously described (8). RIII MMTV, isolated from RIII mouse milk, was supplied by the Office of Resources and Logistics, Biological Carcinogenesis Branch, National Cancer Institute. MMTV concentrations in purified virus stocks were based on group-specific radioimmunoassays for MMTV gp52 as previously described (4). Gross murine leukemia virus, Mason-Pfizer monkey virus, and squirrel monkey retrovirus were all obtained through the Viral Resources Laboratory, Frederick Cancer Research Center.

Rabbit antisera directed against MMTV (whole virus) and MMTV p27 were prepared and characterized as previously described (2). Goat antisera against purified mouse immunoglobulin M (IgM) and IgG1 were purchased from Meloy Laboratories, Springfield, Va. Rabbit antisera against purified mouse IgG2a, IgG2b, and IgG3 were purchased from Miles Laboratories, Elkhart, Ind. Antisera to mouse immunoglobulins were tested in immunodiffusion assays with purified myeloma proteins (Litton Bionetics Laboratories, Kensington, Md.): IgM was isolated from MOPC 104E, IgG1 was isolated from MOPC 21, IgG2a was isolated from UPC 10, IgG2b was isolated from MOPC 195, and IgG3 was isolated from FLOPC 21. The antisera to mouse immunoglobulin were found to be monospecific and were used in a radial immunodiffusion assay (1) to quantitate immunoglobulin concentrations in sera and ascites fluids.

Mouse immunizations. BALB/c female mice were obtained from the Animal Production Facility, Frederick Cancer Research Center. Two different immunization schedules were used. One group of mice, between 4 and 6 weeks of age, was inoculated subcutaneously with 100 μg of C3H MMTV in complete Freund adjuvant on day 1. The mice were then boosted by intraperitoneal inoculations on days 7 and 14 with 100 μg of C3H MMTV. Lymph nodes and spleens were removed 3 days after the last inoculation. The second group of mice was inoculated with C3H MMTV as described above; however, the mice received an additional intraperitoneal inoculation of 100 μg of C3H MMTV on day 21, and the spleens and lymph nodes were removed 3 days later. The C3H MMTV used for each inoculation was disrupted by freezing and thawing three times with an alcohol-dry ice mixture, followed by thawing at room temperature. The virus was also sonicated between each freeze and thaw.

Preparation of hybrid cell line. The myeloma cell line BALB/c MOPC 21 NSI/1, which produces κ light chain but not γ1 heavy chain (12), was kindly provided by C. Milstein, Molecular Research Council, Cambridge, England. The NSI/1 cells were selected for 8-azaguanine resistance for use in cell fusion procedures. The NSI/1 cell line was routinely grown in RPMI 1640 containing 15% fetal calf serum and supplemented with 1 mM pyruvate, 2 mM glutamine, penicillin, and streptomycin (complete RPMI 1640). NSI/1 cells were subcultured daily for 3 days before use in cell fusions and were seeded at densities not exceeding 10^5 cells per ml. Lymphocytes for the cell fusion were obtained from axillary, inguinal, and mesenteric lymph nodes and from spleens of immunized mice. Spleen and lymph node cell suspensions were prepared by gentle teasing and passage through a fine nylon screen and were pooled together from 10 mice. Lymphocyte cell suspensions were washed three times in serum-free RPMI 1640, and the BALB/c NSI/1 cells were washed once in RPMI 1640.

The hybridization procedure and isolation of hybrid cells used in this study have been described previously (19). Briefly, the two cell populations were mixed at a 4:1 ratio (lymphocytes to NSI/1), and polyethylene glycol 1450 (Eastman Kodak Co., Rochester, N.Y.). (50% polyethylene glycol [wt/vol] in RPMI 1640) was then added dropwise to the cell pellet at a ratio of 1 ml of polyethylene glycol per 1.6×10^8 lymphocytes as described previously (19). After cell fusion with polyethylene glycol, the cell suspension was centrifuged at $200 \times g$ for 5 min, the supernatant was removed, and the cells were gently suspended in complete RPMI 1640 at a final concentration of 10^7 cells per ml. This final cell suspension was then dispensed in 100-μl volumes into the wells of Costar microtiter plates and cultured at 37°C. After 24 h, 100 μl of HAT medium (complete RPMI 1640 supplemented with 1.0×10^{-4} M hypoxanthine, 4.0×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine) was added to each well. Cells were fed every 2 to 3 days as described previously (19).

Extensive death of the parental myeloma cells and lymphocytes was observed during week 1 of culture in HAT medium. Microtiter wells containing cells producing anti-MMTV antibodies were identified by assaying the medium from each well in a solid-phase radioimmunoassay. The cells in these wells were then transferred at a low cell density to new plates to minimize overgrowth by cells not producing antibodies. A 200-μl portion of a cell suspension, containing hybrid cells at a concentration of 25 cells per ml and thymocytes, as "feeder cells," at a concentration of 4

$\times 10^6$ cells per ml, was plated into each well of a new microtiter plate. This resulted in a final seeding of five hybrid cells per well. With this procedure, 80 to 100% of the wells yielded viable hybrids within 2 weeks. Hybrid cells which continued to produce antibodies were again cloned in the thymocyte feeder cells, but at cell densities of one hybrid cell per three wells. It was sometimes necessary to repeat this second cloning step to ensure that more than 90% of the cells were producing antibodies to MMTV.

IPA assay for antiviral antibodies. Antiviral antibodies were detected by a binding assay with ^{125}I-labeled protein A (IPA) from *Staphylococcus aureus* (7, 19). Viruses were adjusted to a concentration of 10 μg/ml. The amount of MMTV in the original stock was based on MMTV gp52 radioimmunoassay, and the protein concentration for MPMV and squirrel monkey retrovirus was determined by the method of Lowry et al. (15). Briefly, 0.5 μg of virus in 50 μl of phosphate-buffered saline (pH 7.2) was adsorbed to the individual wells of a microtiter plate by incubation overnight at 37°C. On the next morning, the wells of the plate were blocked from further nonspecific protein adsorption by a 2-h incubation with 125 μl of 5% bovine serum albumin in phosphate-buffered saline, pH 7.2. The IPA assay was usually performed in two steps. First, 50 μl of fluid containing antibody was incubated in each of the virus-adsorbed wells for 45 min at 37°C. Nonbound immunoglobulins were then removed from the wells by washing three times with phosphate-buffered saline containing 1% bovine serum albumin. Second, 10^5 cpm of IPA in 50 μl of phosphate-buffered saline was added to each of the virus-adsorbed wells for 45 min at 37°C. The residual nonbound IPA was then removed from the wells by washing with phosphate-buffered saline, and the immune reactions were detected by 24- to 48-h autoradiography of the IPA-treated microtiter plates on Kodak NS-2T film.

Because IPA binds most efficiently to IgG2's, an additional step in the assay was introduced for the detection of other immunoglobulins. An intermediate incubation was performed with a rabbit antiserum that was prepared against murine IgM. This intermediate incubation (between steps 1 and 2) was performed for 1 h at 37°C with 50 μl of 1:1,000-diluted rabbit antiserum; nonbound rabbit antibodies were removed from the wells by washing with phosphate-buffered saline containing 1% bovine serum albumin, and the assay was continued by the addition of IPA as described above.

Radioimmune precipitation and gel electrophoresis. Approximately 1×10^6 to 2×10^6 cpm of lysed, ^{125}I-labeled MMTV in 100 μl of RIP buffer (0.01 M Tris-hydrochloride [pH 7.4], 0.01 M NaCl, 0.001 M EDTA) was incubated for 2 h at 37°C and then overnight at 4°C with 100 μl of ascites fluid or serum, diluted to a concentration of 10 μg of monoclonal antibody per ml. When IgM monoclonal antibodies were tested, rabbit anti-mouse IgM serum was added on the next morning, and the mixture was incubated for an additional 2 h at 37°C. Fifty microliters of *S. aureus* was then added, and incubation was continued for 15 min at room temperature. The immune precipitates were collected, resuspended in 1 ml of RIP buffer, and centrifuged through a sucrose cushion consisting of 5% sucrose and 0.5% sodium deoxycholate. The pellets were then prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10 to 20% gradient slab gels as previously described (25). The ^{125}I-labeled proteins were visualized by autoradiography. Disrupted radiolabeled MMTV was coelectrophoresed as a marker for gp52.

Competition radioimmunoassay. The competition radioimmunoassay was performed with ^{125}I-labeled C3H MMTV as the antigen. The virus was purified and iodinated as previously described (1). The dilution of each monoclonal antibody which gave 50% binding of ^{125}I-labeled C3H MMTV was determined first. Radiolabeled antigen, 20,000 cpm in 100 μl of RIP buffer, was incubated with 100 μl of diluted monoclonal antibodies for 2 h at 37°C and overnight at 4°C. Immune complexes were precipitated with *S. aureus*, and pellets were collected at 1,500 × *g* for 30 min. When IgM monoclonal antibodies were tested, rabbit anti-mouse IgM serum was added, and the mixture was incubated for 2 h at 37°C before precipitation with *S. aureus*. The pellets were washed with RIP buffer, and the radioactivity remaining in each pellet was determined with a Searle gamma counter. Dilutions which gave 50% binding of ^{125}I-labeled C3H MMTV were then incubated with 100 μl of various dilutions of potential competing antigen for 2 h at 37°C. ^{125}I-labeled C3H MMTV was then added, and incubation was continued for 2 h at 37°C and overnight at 4°C. Precipitation of the immune complexes with *S. aureus* and determination of radioactivity in the pellets were the same as described above. The data were normalized to 100% binding based on the counts per minute precipitated when the diluted monoclonal antibody was incubated with ^{125}I-labeled C3H MMTV in the absence of competing antigen.

Two-dimensional gel electrophoresis. Isoelectric focusing was performed in a gel system that was adapted for isotachophoresis (21). Gels were formed in glass tubes (130 by 2.5 mm) with a mixture of 9 M urea, 2% Nonidet P-40, 2% ampholine (pH 3.5 to 10), 4% acrylamide, and 0.11% bisacrylamide. Immune precipitates were solubilized in 30 μl of sample buffer containing 9.5 M urea, 2% Nonidet P-40, 2% ampholine (pH 3.5 to 10), and 5% 2-mercaptoethanol at 37°C for 30 min. Electrophoresis was performed for 3,000 V-h with the anode at the top of the gel.

After isotachophoresis, the isoelectric focusing gels were extruded from the tubes for electrophoresis in the second dimension by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Slab gels of 10% acrylamide, with a 5% acrylamide stacking gel, were prepared as described previously (13). The isoelectric focusing tube gel was held on the top of the sodium dodecyl sulfate slab gel with 1% agarose containing 2.3% sodium dodecyl sulfate, 10% glycerol, 5% 2-mercaptoethanol, and 0.1% bromophenol blue in 62.5 mM Tris-hydrochloride, pH 6.8. Electrophoresis was performed at 20 mA until the bromophenol blue reached the bottom of the gel. Autoradiography was performed by a fluorographic procedure (6).

RESULTS

Isolation of hybrid cell lines producing

anti-MMTV antibodies. Hybridizations were performed between BALB/c NSI/1 myeloma cells and the lymphocytes of BALB/c mice that were immunized with C3H MMTV. A flow diagram for the isolation of antibody-producing hybrid cells is shown in Fig. 1. Approximately 2,000 wells in microtiter plates were seeded with cells from two separate hybridizations. Within 10 days after the fusion, colonies of hybrid cells were detected in each of the wells. At 15 days after fusion, the culture fluid from each of the wells was individually tested in a direct and indirect IPA assay for antibodies against C3H MMTV. Antiviral antibodies in the culture fluids of each well varied considerably from one well to another in their intensity of reaction in the IPA assay. Approximately 200 out of the original 2,000 wells contained cells producing antibodies to C3H MMTV. There was no difference in the percentage of wells positive for antibodies to C3H MMTV when the two groups of mice immunized for different periods of time were compared.

Because previous studies demonstrated that immunoglobulin synthesis was unstable in the majority of hybrid cells (19), efforts were made to prevent the overgrowth of hybrids producing immunoglobulins to MMTV (Ig^+) by non-immunoglobulin-producing (Ig^-) hybrids. For this purpose, cells from 40 positive wells were diluted with thymus feeder cells and seeded into 96-well microtiter plates at a concentration of five hybrid cells per well. This process of low-density passage, termed "minicloning," enabled the rescue of Ig^+ hybrids from the original Ig^+ wells. The number of Ig^+ miniclones obtained from each of the original Ig^+ wells varied considerably. In some instances, fewer than 5% of the miniclones from an original well were Ig^+, whereas in others more than 95% of the miniclones were Ig^+. The miniclone plate, shown in Fig. 1 for day 43, demonstrates that 20 out of 96 wells seeded with cells from well G-6 in the initial plate contained Ig^+ cells. Of the original 40 Ig^+ wells selected for minicloning, 19 remained Ig^+, whereas the others were uniformly negative in the IPA assay.

At 43 days after plating, cells from each of the

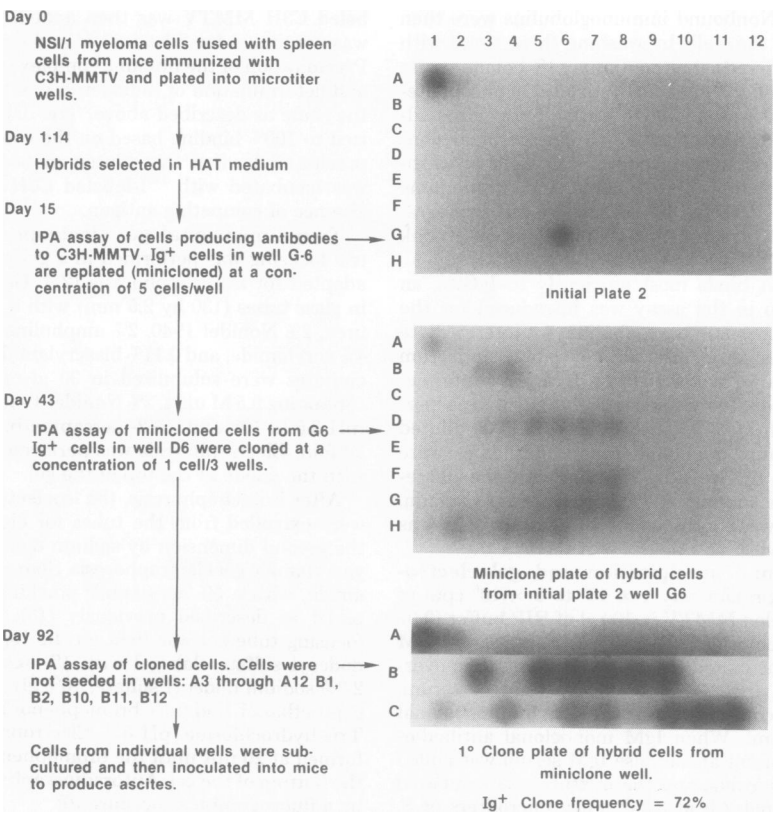

FIG. 1. *Flow diagram for the isolation of hybrid cell lines. See the text for a detailed description. Anti-MMTV serum (1:1,000 dilution) was added to well A-1 of each virus-adsorbed plate as a positive control. On the bottom plate, well A-2 contained anti-MMTV p27 serum (1:1,000 dilution). Direct IPA assays, which do not detect binding of IgM antibodies, are shown.*

19 miniclones still producing antibodies to C3H MMTV were diluted with thymus feeder cells and cloned by limiting dilution in microtiter plates (one cell seeded per three wells). After 10 days, the plates were scored visually, and wells containing two or more colonies were discarded. At 2 to 3 weeks after plating, the clones had reached semiconfluency, and their culture fluids were then tested in the IPA assay for anti-C3H MMTV antibodies. The percentage of these Ig^+ clones derived from each miniclone also varied considerably. Occasionally, fewer than 50% of the clones from a single miniclone were Ig^+, whereas in other instances more than 95% of the clones from a miniclone were Ig^+. Since inactivation of immunoglobulin synthesis was still occurring in some of these clones, hybrids with an Ig^+ clone frequency of less than 90% were again diluted with thymus feeder cells and recloned by limiting dilution (one cell per three wells) in microtiter plates. After 2 weeks, the culture fluids from these twice-cloned cell lines were tested in the IPA assay for anti-C3H MMTV antibodies. In total, six stable clones were isolated from the original wells that produced anti-C3H MMTV antibodies.

Immunoglobulins from the culture fluids of cloned hybrid cell lines were examined by immunodiffusion with antisera that were prepared in rabbits and goats against purified murine myeloma proteins. Three of the hybrid clones produced IgM, and three produced IgG2a (Table 1).

Fine structure of the immunoglobulin products of hybrid cells was determined by high-resolution two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For this purpose, [^{3}H]leucine-labeled immunoglobulins were concentrated by ammonium sulfate precipitation (40% saturation). The immunoglobulins were then isolated by adsorption to *S. aureus* (for the IgG2a) or by immune precipitation with rabbit antisera prepared against IgM, followed by adsorption to *S. aureus*. The immunoglobulin products of each of the cloned hybrid cells exhibited a limited charge heterogeneity by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis, characteristic of monoclonal antibodies (data not shown). Each of the immunoglobulin products contained one or two molecular weight forms of the light chains.

Induction of tumors in mice inoculated with hybrid cell lines. Intraperitoneal inoculation of 10^6 hybrid cells into syngeneic BALB/c mice induced palpable tumors (hybridomas) in more than 90% of the inoculated mice within 2 to 3 weeks. These tumors were accompanied by the production of ascites fluids (0.5 to 3.0 ml per mouse). The immunoglobulin concentration in ascites fluids and sera of hybridoma-bearing mice were determined by a radial immunodiffusion assay. The concentrations of monoclonal antibodies in the serum and ascites fluid of an individual mouse were roughly equivalent, each containing 5 to 50 mg of antibody per ml.

Immune precipitation of MMTV proteins by monoclonal antibodies. Monoclonal antibodies in ascites fluids were examined in radioimmune precipitation assays with disrupted ^{125}I-labeled C3H MMTV (Fig. 2). *S. aureus* was used to directly precipitate the immune complexes containing IgG2a, whereas a secondary system was used for the IgM immune complexes. The labeled proteins in the immune precipitates were then analyzed on 10 to 20% slab gels. As shown in Fig. 2, the four monoclonal antibodies shown all reacted with the MMTV envelope glycoprotein gp52. The two remaining mono-

TABLE 1. *Summary of the properties of monoclonal antibodies to C3H MMTV*

Hybrid cell designation	Haplotype	Immunoglobulin class and subclass	MMTV protein recognized	Virus specificity[a]						
				MMTV				G-MuLV	SMRV	MPMV
				C3H	GR	RIII	C3Hf			
Type specific, VII P2G6	*H-2^d*	IgG2a	gp52	+	−	−	−	−	−	−
Class specific										
VI P5D8	*H-2^d*	IgM	gp52	+	+	−	−	−	−	−
VI P5E11	*H-2^d*	IgM	gp52	+	+	−	−	−	−	−
VI P3C5	*H-2^d*	IgG2a	gp52	+	+	−	−	−	−	−
VII P1F1	*H-2^d*	IgG2a	gp52	+	+	−	−	−	−	−
Group specific, VI P1B4	*H-2^d*	IgM	gp52	+	+	+	+	−	−	−

[a] Determined by IPA assays and competition radioimmunoassays. +, Binding of monoclonal antibody to virus or competition by this virus; −, no binding or competition. The titers for the positive ascites fluid ranged from 10^{-4} to 10^{-6}. The titers of the negative ascites fluids were $<10^{-3}$. G-MuLV, Gross murine leukemia virus; SMRV, squirrel monkey retrovirus; MPMV, Mason-Pfizer monkey virus.

clonal antibodies (data not shown) also reacted with gp52. Less than 5% of the radiolabeled gp52 was precipitated by each monoclonal antibody preparation, presumably due to alterations in the three-dimensional structure of purified antigen. Also shown are results with polyvalent sera from BALB/c mice inoculated with C3H MMTV which precipitated gp52 and gp36.

Reactions of monoclonal anti-MMTV antibodies with different MMTVs and other retroviruses. Monoclonal antibodies produced by the six hybrid cell lines were tested in an IPA assay against a variety of retroviruses. Three patterns of reactivity were observed. A representative example for each pattern is shown in Fig. 3 along with the pattern for a polyvalent anti-MMTV serum. The specificity for each monoclonal antibody is shown in Table 1. The patterns can be summarized as follows: (i) type specific for C3H MMTV (represented by monoclonal antibody VII P2G6); (ii) class specific, detecting antigenic determinants shared between C3H and GR MMTVs (represented by VI P3C5, VI P5D8, VI P5E11, and VII P1F1); (iii) group specific, detecting antigenic determinants shared by C3H, GR, C3Hf, and RIII MMTVs (represented by VI P1B4). (The polyvalent anti-MMTV serum was also group specific and recognized all of the MMTVs.)

The patterns of reactivity were confirmed by testing the specificity of each monoclonal antibody in a competition radioimmunoassay with ^{125}I-labeled whole virus. Figure 4 shows the results obtained with the monoclonal antibodies VII P2G6 and VI P1B4. By the use of dilutions of antibody that gave 50% precipitation (1×10^{-5} for VII P2G6 and 5×10^{-5} for VI P1B4), competition assays were then performed. As shown in Fig. 4A for VII P2G6, only C3H MMTV was able to compete completely, yielding 20% competition at 10^3 ng of competitor virus. In contrast, GR, RIII, and C3Hf MMTVs yielded no competition, even at 10^4 ng of competitor virus. In Fig. 4B for VI P1B4, all four MMTVs (C3H, GR, C3Hf, and RIII) competed completely, with 20% competition at 300 ng of competitor virus.

DISCUSSION

This study describes the isolation of six stable hybrid cell lines producing monoclonal antibodies that react with the major MMTV envelope glycoprotein gp52. It is not surprising that all of the monoclonal antibodies are directed to gp52, in view of our observation that sera from mice immunized with C3H MMTV contain antibodies to gp52 and gp36, but not to p27, pp20, p14, and p10. The antigenic reactivities for the monoclonal antibodies fall into three categories: (1) group specific, recognizing all four MMTVs; (ii) class specific, distinguishing C3H and GR MMTVs from RIII and C3Hf MMTVs; and (iii) type specific, recognizing only C3H MMTV.

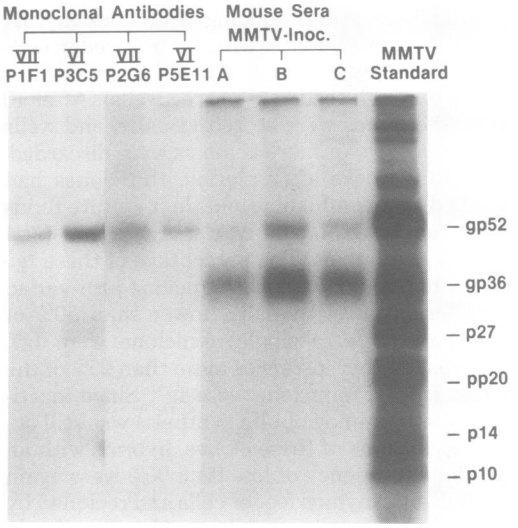

FIG. 2. *Radioimmune precipitation of viral proteins. Disrupted ^{125}I-labeled C3H MMTV was used in immune precipitation assays with individual monoclonal antibodies and sera from BALB/c mice inoculated with C3H MMTV. ^{125}I-labeled MMTV proteins in the immune precipitates were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10 to 20% gradient slab gels. Disrupted ^{125}I-labeled C3H MMTV was coelectrophoresed as a marker (MMTV standard).*

Group-specific determinants have been reported previously for C3H, GR, RIII, and C3Hf MMTVs when natural mouse sera in whole virus-precipitating antibody assays were used (11, 17, 18). Because the natural mouse sera and MMTV gp52 antigen were polyvalent, it was difficult in those studies to determine whether the group specificity resulted from a single determinant common to all four MMTVs or to sets of determinants shared between different groups of MMTVs. The latter possibility could have arisen because the C3H mouse contained both the C3H exogenous MMTV and the C3Hf endogenous MMTV. Since C3H and GR MMTVs contained common determinants missing on C3Hf and RIII MMTVs (4, 17; G. Schochetman, B. Altrock, L. O. Arthur, G. G. Lovinger, and R. Massey, *in Cold Spring Harbor Conferences of Cell Proliferation: Viruses and Naturally Occurring Cancer*, vol. 7, in press) and C3Hf and RIII MMTVs contained common determinants missing on C3H and GR MMTVs (L. O. Arthur and G. Schochetman, submitted for publication), it is possible that the natural mouse sera

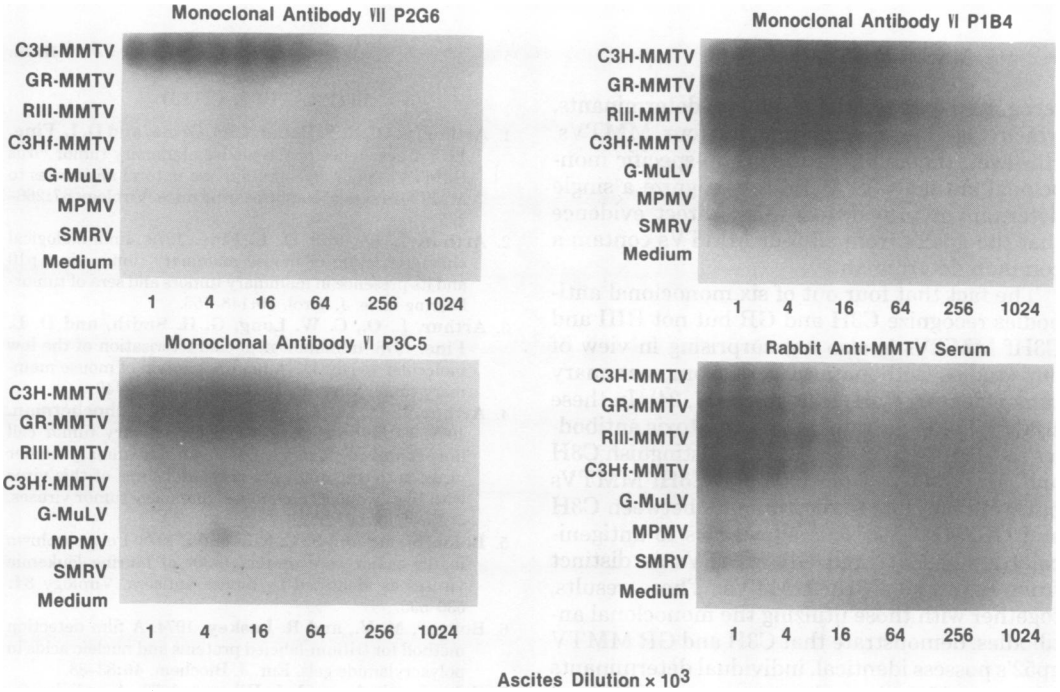

FIG. 3. *Antibody-binding assays with ascites fluids from mice bearing hybridomas. Serial twofold dilutions of ascites fluids (starting at a 1:1,000 dilution) were incubated in the wells of virus-adsorbed microtiter plates (see the text for details). Immune complexes were detected by the addition of IPA and subsequent autoradiography. Prototype antibodies for each of the classes of reaction were: (i) type specific, VII P2G6; (ii) class specific, VI P3C5; and (iii) group specific, VI P1B4. Reactions obtained with the polyvalent rabbit anti-MMTV antibody were also included. Abbreviations: G-MuLV, Gross murine leukemia virus; MPMV, Mason-Pfizer monkey virus; SMRV, squirrel monkey retrovirus.*

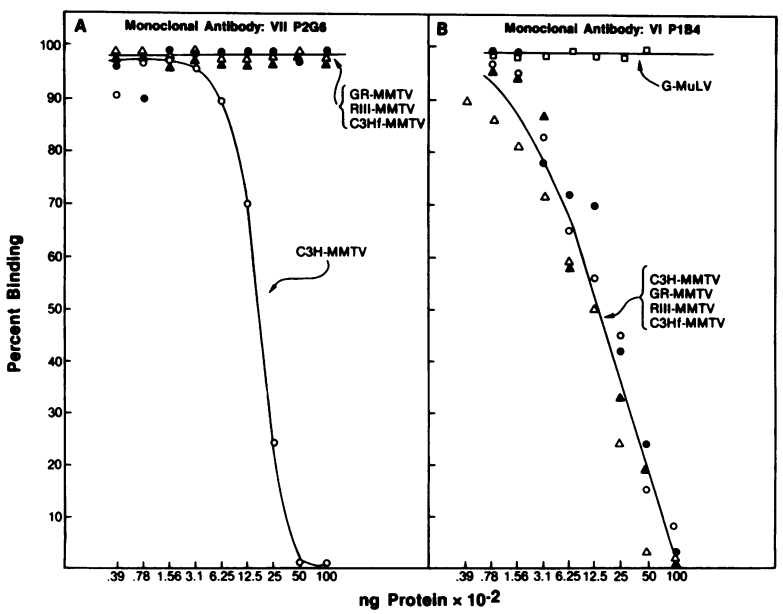

FIG. 4. *Competition radioimmunoassays for determining the specificity of two monoclonal antibodies. Monoclonal antibodies VII P2G6 and VI P1B4 (at 10^{-5} and 5×10^{-5} dilutions, respectively) were used to precipitate ^{125}I-labeled intact C3H MMTV. The specificity of binding was measured by the ability of increasing amounts of C3H, GR, RIII, and C3Hf MMTVs and Gross murine leukemia virus to compete for this binding. Monoclonal antibody VII P2G6 is an IgG_{2a} and VI P1B4 is an IgM which required the addition of rabbit anti-mouse IgM serum before precipitation with S. aureus. Symbols: ○, C3H MMTV; ●, GR MMTV; ▲, RIII MMTV; △, C3Hf MMTV; □, Gross murine leukemia virus.*

recognized two distinct sets of determinants, which together precipitated all four MMTVs. However, the ability of the group-specific monoclonal antibody VI P1B4 to recognize a single determinant provides the first direct evidence that the gp52's from all four MMTVs contain a common determinant.

The fact that four out of six monoclonal antibodies recognize C3H and GR but not RIII and C3Hf MMTVs is also not surprising in view of our studies with natural sera from mammary tumor-bearing C3H/HeN mice (17, 24). In these studies, both neutralizing and cytotoxic antibodies to MMTV gp52 were able to distinguish C3H and GR MMTVs from RIII and C3Hf MMTVs but were not able to discriminate between C3H and GR MMTVs. This allowed us to antigenically group C3H and GR MMTVs as distinct from RIII and C3Hf MMTVs. These results, together with those utilizing the monoclonal antibodies, demonstrate that C3H and GR MMTV gp52's possess identical, individual determinants (class-specific epitopes) not present on C3Hf and RIII MMTVs. The results further indicate that these determinants are immunodominant in BALB/c mice inoculated with C3H MMTV. Class-specific epitopes for MMTV gp52 are analogous to antigenic determinants on MuLV gp70, identified with monoclonal antibodies which are also class specific, i.e., found on some, but not all, types of MuLV (14).

Although studies from this laboratory and others (4, 28) have demonstrated antigenic differences among gp52's of various MMTVs, they have not identified a determinant unique to one strain of MMTV. The isolation of monoclonal antibody VII P2G6 which is capable of reacting with the gp52 of only C3H MMTV represents the first clear demonstration of an MMTV gp52 type-specific determinant and provides the ability to distinguish C3H MMTV from GR, RIII, and C3Hf MMTVs. It also suggests that appropriate immunizations will yield a battery of monoclonal reagents capable of distinguishing each of the different strains of MMTV.

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