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Modulation of Mouse Mammary Tumor Virus Production in the MJY-Alpha Cell Line

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Implantation of the mouse mammary tumor virus (MMTV)-producing mammary tumor cell line MJY-alpha into isogeneic mice elicited both humoral and T-cell responses against MMTV virion antigens. The carcinosarcomas which developed from the implanted cells showed a significant decrease in MMTV synthesis, compared with cells remaining in culture, which was detectable as early as 7 days after implantation and for five transplant generations. Electron microscopic examination of thin sections of the tumors revealed that intracytoplasmic A particles, budding particles, and cell-free MMTV B particles were all affected. However, immunofluorescence assays of tumor sections demonstrated the presence of MMTV viral antigens in the cells. Cell cultures initiated from first-, third-, and fourth-generation tumors were morphologically identical to the original in vitro cell line, although virus production was barely detectable. Analysis of the cultures by electron microscopy revealed a significant increase in MMTV virions after in vitro passage 3. Polypeptide profiles obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of virions purified from these cultures were identical to MMTV. Immunodiffusion demonstrated the cross-reactivity between these virions and MMTV particles obtained from mouse milk. In vitro treatment of MJY-alpha cell cultures with rabbit anti-MMTV antiserum resulted in a reduction of extracellular MMTV virions, as well as alterations in their sodium dodecyl sulfate-polyacrylamide gel electrophoretic polypeptide patterns.

Techniques are now available for the propagation of primary and short-term murine mammary tumor cell cultures releasing mouse mammary tumor virus (MMTV; 13, 15, 17, 21, 23). The levels of MMTV expression and of synthesis of particles in vitro are highly variable; stimulation of virus production in cells with preexisting MMTV expression can be obtained by addition of hydrocortisone or dexamethasone (8, 13, 16-19). However, synthesis of MMTV antigens and virions frequently decreases permanently during in vitro passage, and this has led to difficulties in establishing MMTV-producing cell lines. The reasons for this decline in MMTV replication are unknown, but it demonstrates the fragile nature of this host cell-virus relationship. In this study we present evidence of a transient repression of MMTV production in the mouse mammary tumor cell line MJY-alpha after in vivo transplantation or in vitro treatment with antisera. The data suggest that replication of MMTV virions "modulates" in response to antisera and/or T-cell reactivity against MMTV antigens.

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MATERIALS AND METHODS

Cells. The in vitro MJY-alpha cell line was grown as stationary cultures in T-flasks or petri dishes as previously described (21). After in vivo transplantation, primary cell cultures were initiated by using pools of MJY-alpha cell-induced tumors from transplant generations 1, 3, and 4. Tumors were minced, dissociated with saline A-trypsin-EDTA, filtered, and pelleted as previously described (23). Growth medium for all cultures was RPMI medium 1640 supplemented with 20% fetal calf serum, 10^{-5} M bovine insulin, and antibiotics as previously described (23). Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cultures were routinely checked for bacterial, fungal, and *Mycoplasma* contamination (21); the results were negative.

In vivo transplantation. MJY-alpha cells were implanted subcutaneously in 6- to 10-week-old isogeneic BALB/c/Crgl or BALB/cfC3H/Crgl female mice. Cells were removed from culture supernatants or ves-

sels either with saline A–trypsin–EDTA or by scraping with a rubber policeman, pelleted, suspended in fetal calf serum-free medium, counted, and injected at 10^6 cells per 0.1 ml per site. Tumors were measured every 2 to 3 days along their long and short axes. Tumor size is reported as the product of the two measurements (millimeter squared).

Mice were bled from the tail or retroorbital plexus every 3 to 7 days after implantation. Individual serum samples were obtained by incubation at 37°C for 20 min followed by centrifugation. Sera were stored at 0 or 5°C until tested in two-dimensional, double-diffusion assays for antiviral antibodies or viral antigens.

Mice were sacrificed at 4- to 7-day intervals. Tumor morphology was determined from serial sections stained in hematoxylin and eosin. Samples of tumors from each time interval were also processed for electron microscopic examination. Spleens from mice bearing first in vivo generations of alpha tumors were also obtained for microcytotoxicity tests.

MJY-alpha tumors in BALB/c mice were also passaged after 21 to 28 days by transplantation of tumor pieces (1 by 2 mm) subcutaneously with trocars.

Antibody treatment of MJY-alpha cell layers. Normal rabbit serum and rabbit anti-MMTV antiserum against virus from BALB/cfC3H milk were heat inactivated at 60°C for 20 min and filtered using 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.) before use. Antisera were added to regular growth medium at a 2% vol/vol concentration. Confluent, 4- to 6-day-old cell layers were washed three times with RPMI medium 1640 before addition of antiserum-growth medium at 1.3 ml/cm^2. Cell culture supernatants were removed 24 h later and replaced with labeling media containing rabbit antiserum, [^{3}H]glucosamine, and ^{14}C-amino acids as described below. Cultures were chased 24 h later with labeling media containing antiserum. Virions from the label and chase periods were concentrated and purified as described below. Final isopycnic gradients were fractionated into 0.2-ml aliquots and assayed for radioactivity and MMTV antigens by immunodiffusion. Samples of parallel, unlabeled cell layers were also processed on both days for electron microscopy.

Virus. Virions doubly labeled with [^{3}H]glucosamine and ^{14}C-amino acids were obtained from 4- to 8-day-old cell cultures. Hydrocortisone (10^{-6} M) was added to the culture media for 24 h preceding viral harvests. Cells were labeled with [^{3}H]glucosamine (10 μCi/ml) and ^{14}C-amino acids (12.5 μCi/ml) in growth medium containing 10^{-6} M hydrocortisone and 5% fetal calf serum. Virions were harvested every 24 h over a period of 1 to 3 days. Fresh medium was added to cultures after each harvest. Viruses were concentrated and purified by several clarification steps, pelleting, and a series of discontinuous and continuous sucrose gradients (21, 23).

Immunodiffusion assays. Two-dimensional, double-diffusion assays were performed with 0.3% Noble agar and 0.05% agarose in 0.05 M barbital–sodium buffer, pH 8.6, containing 0.035% EDTA as previously described (25). Rabbit anti-BALB/cfC3H milk-derived MMTV was applied to the plate, and readings were made for a period of up to 7 days.

Immunofluorescence. Indirect immunofluorescence was carried out on viable cell culture layers and frozen, cryostat tumor sections as previously described (21). Rabbit antisera against MMTV virions from BALB/cfC3H mouse milk was the specific antisera. Fluorescein-conjugated goat immunoglobulin G or F(ab)'2 anti-rabbit immunoglobulin G was the second reagent.

Microcytotoxicity assay. Spleen cell reactivity of BALB/c and BALB/cfC3H mice implanted with MJY-alpha cells (in vivo generation 1) were assessed by the microcytotoxicity assay procedure as previously described (1, 3). Animals were sacrificed at 3- to 7-day intervals, the spleens were removed, and spleen cells from each donor were tested separately at a ratio of 100 effector cells to 1 target cell. Spleen cells used for controls were obtained from the following animals: (i) BALB/c mice bearing tumors induced by implantation of the FUKU cell line (21); (ii) BALB/c hosts with tumors induced by the D1-DMBA3 cell line (3, 10); (iii) 8- to 12-week-old BALB/c and BALB/cfC3H mice; and (iv) BALB/cfC3H mice bearing spontaneous mammary tumors.

MJY-alpha target cells were obtained from the in vitro cell line and plated in Falcon MicroTest 3034 plates. Cells from the BALB/cfC3H mammary tumor cell lines FUKU (21) and D1-DMBA3 were used as control target cell populations. Target tumor cells were also obtained from spontaneous tumors arising in BALB/cfC3H animals as previously described (2).

The results of these tests are given as the percentage of target cell survival at 48 h after the addition of spleen cells compared with 100% survival in tumor cell controls without added lymphocytes.

Electron microscopy. Serial thin sections of MJY-alpha-induced tumors and MJY-alpha in vitro cell layers were processed for electron microscopy as previously described (21).

SDS-PAGE. Virus samples were disrupted for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by boiling for 1 min at 100°C with 1% SDS and 1% β-mercaptoethanol. Electrophoresis was carried out as previously described with 10–20% gradient gels and a discontinuous buffer system (24, 25). Gels were processed for determination of radioactivity as described by Compans (7). Quantitation of the levels of radioactivity associated with MMTV polypeptide peaks was carried out using a generalized curve fit computer program developed by James D. Gerard and adapted for our use by James Allen, Multi-Lab Computer Center, University of Alabama in Birmingham.

Chemicals and isotopes. [^{3}H]glucosamine and ^{14}C-labeled reconstituted protein hydrolysates were obtained from Schwarz/Mann, Orangeburg, N.Y. Components for SDS-PAGE and immunodiffusion were obtained from Bio-Rad Laboratories, Rockville Centre, N.Y.

RESULTS

Cell culture and tumor morphology. The epithelioid morphology of the MJY-alpha mammary tumor cell line has been described previously (21, 22). MJY-alpha cells vary within each passage level from slightly fusiform to polygonal

and back to fusiform with increasing density of the layers. Viable cells are released into the medium from intact monolayers within 24 h after becoming confluent. Hemicysts and other morphological structures usually observed in primary and short-term cultures of murine mammary tumors are not present in these cultures (13, 21).

Implantation of the MJY-alpha cell layers or released cells into isogeneic BALB/c female mice gave rise to carcinosarcomas (21); tumor morphology was unaltered with further transplantation for five generations. Growth rates of the implanted tumors increased slightly with in vivo passage (Fig. 1).

Cell cultures initiated from in vivo tumor passages 1 and 3 were morphologically similar to the in vitro cell line. The cyclic change in cellular shape, as well as the release of viable cells from the monolayers, remained unchanged (Fig. 2). These cultures were passaged in the same manner as the parent cell line by using the released cells.

MMTV virion production. Synthesis of MMTV antigens and extracellular particles by the cell line-induced tumors and newly initiated cell cultures was ascertained by immunofluorescence and by electron microscopic examination of serial thin sections. Immunofluorescent examination of the MJY-alpha cell line revealed that greater than 99% of the cells either adhering to the substrate or released into the culture supernatant were positive for MMTV antigens. The fluorescence was punctate and concentrated on the cell membrane (Fig. 3A; 21). Sections of MJY-alpha-induced tumors were also highly positive by immunofluorescence for MMTV antigens. Figure 3B shows the staining pattern obtained from MJY-alpha tumors after five in vivo passages. Cell layers and released cells from cultures initiated from MJY-alpha tumors were also highly positive by immunofluorescence (Fig. 3C). Approximately 90% of the cells contained MMTV antigens; fluorescent staining was not observed in cells identified as fibroblasts. The staining pattern was punctate and restricted to the cell membrane.

Although the immunofluorescent assays indicated that the synthesis of MMTV antigens remained unchanged during in vivo passage of the cells, electron microscopic examination of serial thin sections demonstrated a significant decrease in MMTV virion production. Nests of intracytoplasmic A particles and numerous budding particles, as well as immature and mature B virions, were routinely observed in MJY-alpha cell cultures (Fig. 4). Pretreatment of these cells with hydrocortisone increased the numbers of particles observed. In contrast, the production

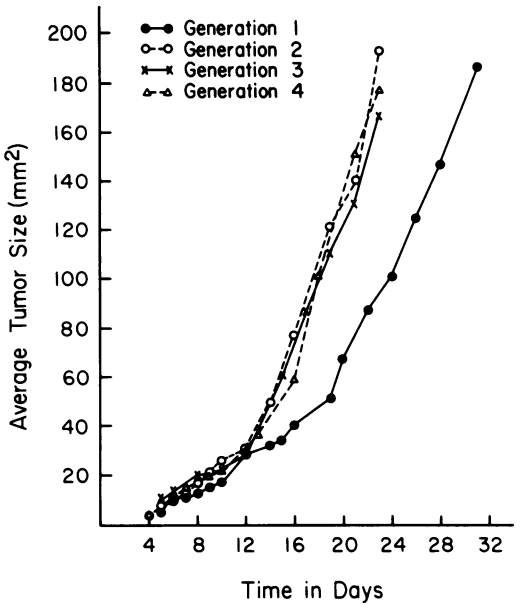

FIG. 1. *Average tumor size of MJY-alpha cells implanted in 6- to 10-week-old BALB/c female mice as a function of time after implantation. The readings are averages obtained from six series of experiments with a total of 34 to 54 animals per tumor generation.*

of MMTV virions was extremely low in MJY-alpha-induced tumors. This decrease was observed as early as 7 days after implantation of MJY-alpha cells. Serial sections of tumors from in vivo passages 1, 3, and 5 were almost devoid of MMTV-associated particles. Occassionally, small numbers of budding and A particles were observed (Fig. 5). Examination of serial sections from over 35 tumors suggested that the number of MMTV-associated particles decreased by greater than 90% compared with the in vitro cell line. This reduction in MMTV virions was also observed in the first two passages of cultures initiated from the MJY-alpha tumors. Only a few scattered A and budding particles were observed (Fig. 6). The number of particles increased by passages 3 and 4 of the cells, although they were never as numerous as in the parent line. The addition of hydrocortisone did not appear to affect the numbers of MMTV-associated particles observed in the cells. C particles associated with murine leukemia virus were not observed in any of the tumors and cell cultures.

Cell culture supernatants were harvested from newly initiated cultures which had been labeled with [^{3}H]glucosamine and ^{14}C-amino acids to determine the presence and identity of extracellular virions. Final sucrose isopycnic gradients were fractionated, and the viral band and/or virus-associated radioactivity having the buoy-

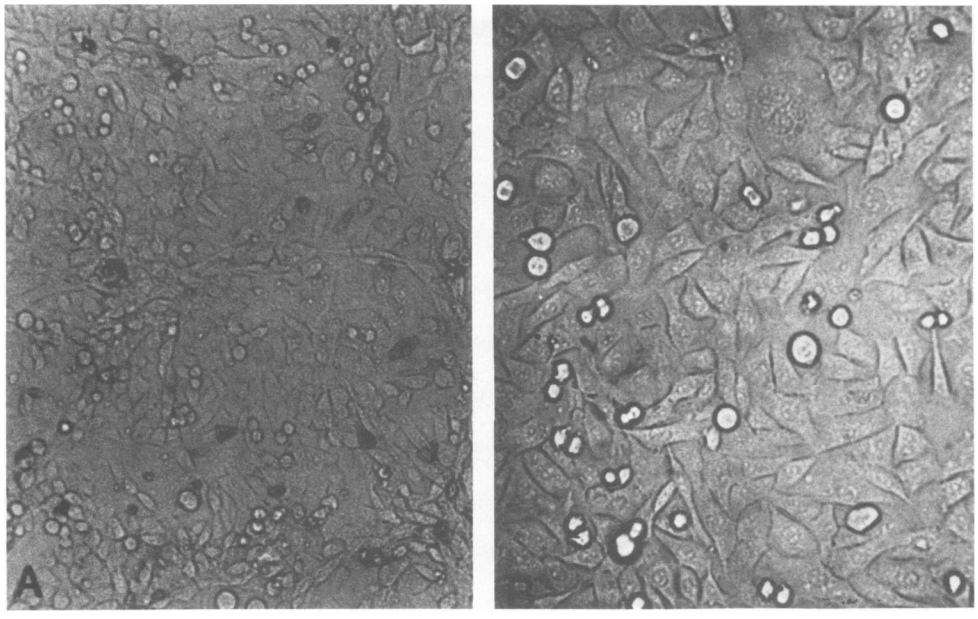

FIG. 2. *MJY-alpha cell cultures in situ initiated from in vivo generation 3 of MJY-alpha tumors in BALB/c females. (A) Primary culture, day 3, ×117; (B) MJY-alpha cell line, passage 26, day 2, ×233.*

ant density of MMTV virions (1.16 to 1.17 g/ml) was isolated. The relative levels of radioactivity associated with virions isolated from the isopycnic gradients are given in Table 1. The virus preparation from the parent line yielded light-scattering bands of virions and radioactivity at 1.17 g/ml which were strongly positive for MMTV antigens by immunodiffusion. Primary and secondary cultures from MJY-alpha tumors did not yield light-scattering bands, although low peaks of virus-associated radioactivity were present at 1.17 g/ml. Faint precipitin lines were observed when the virions were reacted with rabbit anti-MMTV in immunodiffusion. Supernatant fluids from passages 3 and 4 contained more virions, as indicated by the presence of light-scattering bands of virus in isopycnic gradients, significant increases in the amounts of associated radioactivity, and reactivity with the rabbit antisera in immunodiffusion. Electron microscopic examination of negatively stained samples of the isopycnic material revealed MMTV type B particles; type C retrovirus particles and cellular debris were not observed in the preparations.

Virus preparations were also subjected to SDS-PAGE to characterize the polypeptide profile of the virions. The level of proteins having molecular weights similar to known MMTV polypeptides was negligible in preparations from primary and secondary cultures (Fig. 7). However, SDS-PAGE of virions from the tertiary cultures yielded polypeptide patterns similar to the profiles for MMTV virions. The results of analyses of MMTV production are summarized in Table 2.

Humoral and cellular immune responses of MJY-alpha tumor-bearing hosts. The humoral and cellular immune responses of the MJY-alpha tumor-bearing hosts were examined to determine whether anti-MMTV virion activity was elicited by implantation of the virus-producing cells.

Sera obtained from BALB/c and BALB/cfC3H mice bearing MJY-alpha tumors were analyzed by immunodiffusion assays with MMTV virion antigens purified from MJY-alpha cell cultures or from BALB/cfC3H milk. No reactivity was detected in sera obtained 7 days before implantation or 4 days after implantation. Precipitin lines were formed against MMTV virions with sera obtained 7, 11, 14, 21, and 28 days after implantation of MJY-alpha cells from cell culture. However, reactivity was limited to females receiving cells directly from culture; in subsequent transplantations (tumor generations 2, 3, and 4), sera from animals bearing transplanted MJY-alpha tumors were never positive for antibodies to MMTV.

Spleen cells from both BALB/c and BALB/cfC3H mice bearing primary MJY-alpha cell-induced tumors were able to inhibit MJY-alpha target cell growth in vitro (Table 3). The results show that these animals were reactive to

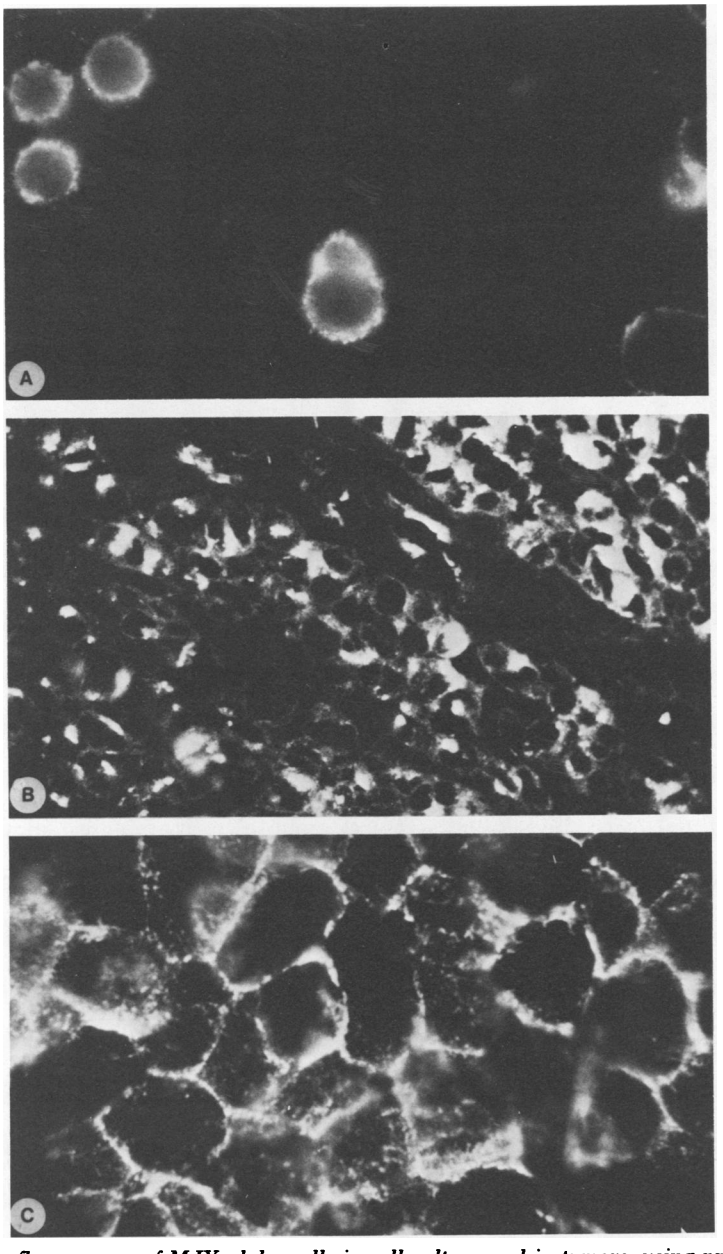

FIG. 3. *Immunofluorescence of MJY-alpha cells in cell culture and in tumors, using rabbit anti-MMTV antiserum. (A) Released cells from MJY-alpha cell line, passage 30, ×997; (B) MJY-alpha tumors, in vivo generation 5 in BALB/c mice, ×455; (C) MJY-alpha cell layer, primary cell culture from in vivo tumors, ×997.*

the MJY-alpha cells by 4 to 7 days after implantation. This reactivity persisted throughout the duration of tumor growth and was still present at 28 days when the mice were sacrificed. The response was directed toward MMTV antigens rather than to antigenicity unique to MJY-alpha cells, since spleen cells from these animals were also reactive to target cells derived from spontaneous, MMTV-induced mammary tumors arising in isogeneic BALB/cfC3H animals. The decrease in the percent survival of these target tumor cells (increase in cytotoxicity) was significant at all time intervals tested, although the level of decrease was similar to that observed with MJY-alpha target cells in hosts bearing MJY-alpha tumors for more than 14 days. In

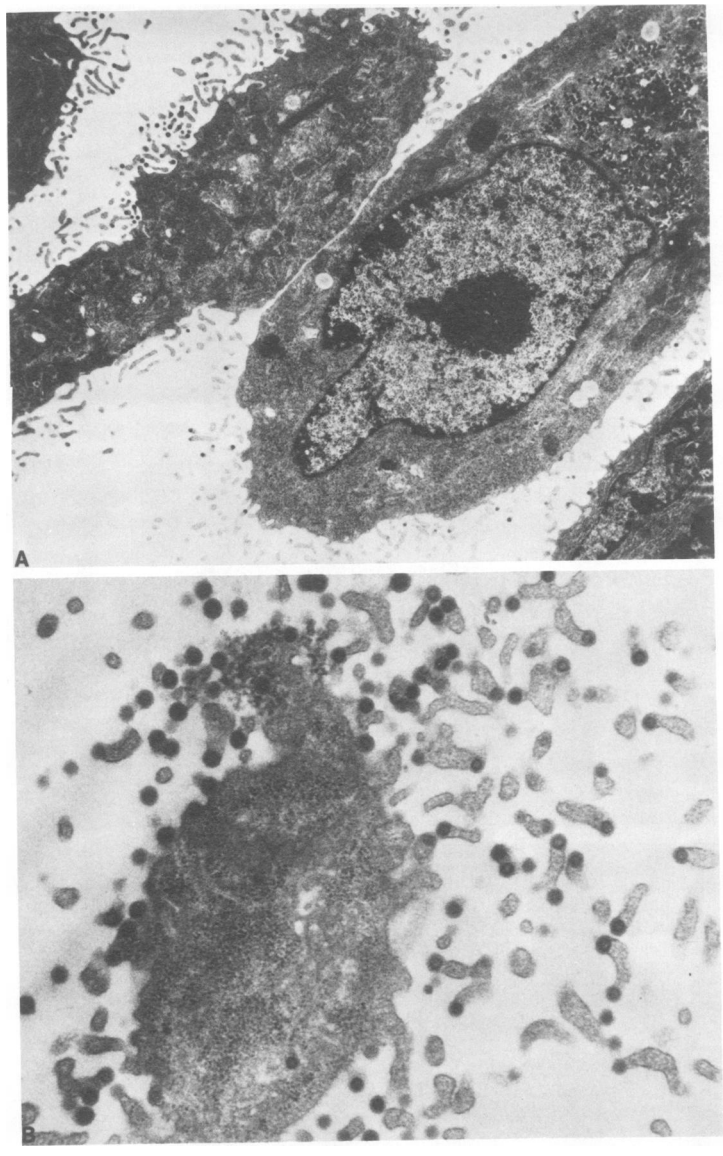

FIG. 4. *Thin sections of MJY-alpha cell line. (A) Passage 16, ×6,492; (B) passage 20, ×15,554.*

addition, spleen cells from other mice (BALB/cfC3H females either without tumors or with spontaneous mammary tumors) which are known to be cytotoxic for MMTV-infected mammary tumor cells also reduced the survival of MJY-alpha target cells. MJY-alpha cells were a sensitive target; in all cases the reduction of target cell survival was greater when MJY-alpha cells rather than cells from primary cultures of spontaneously arising BALB/cfC3H mammary tumors were used as targets. The specificity of these reactivities to MMTV-producing mammary tumors was further demonstrated by the lack of cytotoxicity of the spleen cells for two other isogeneic mammary tumor cell lines which do not express MMTV antigens. The FUKU cell line was initiated from spontaneous mammary tumors arising in BALB/cfC3H females but now produces an endogenous murine leukemia virus; synthesis of MMTV antigens and virions is detectable in only 1 to 5% of the cells (21). The second control target cell was the D1-DMBA3 cell line which originated from a dimethylbenzanthracene-induced BALB/c mammary tumor (10); MMTV and murine leukemia virus have not been detected in this cell line. Positive controls for the sensitivity of these two targets to immunologically specific cytotoxic attack were

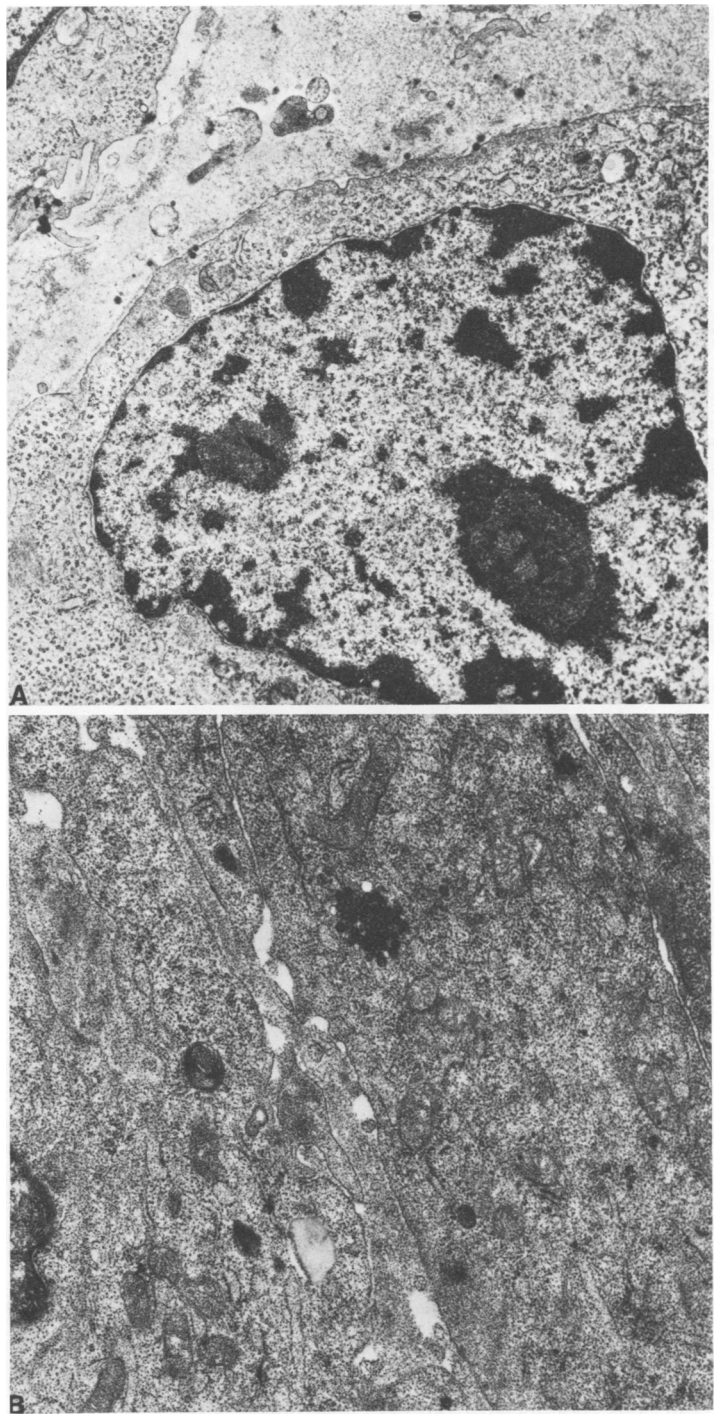

FIG. 5. *Thin sections of MJY-alpha cell-induced tumor in BALB/c host, in vivo generation 1. (A) Budding particles, ×1,855; (B) small nest of A particles, ×3,357.*

provided by tests in which cytotoxicity was demonstrated with spleen cells from BALB/c animals, 8 to 14 weeks old, which carried implants of either FUKU or D1-DMBA3; the spleen cells were reactive only against autologous target cells.

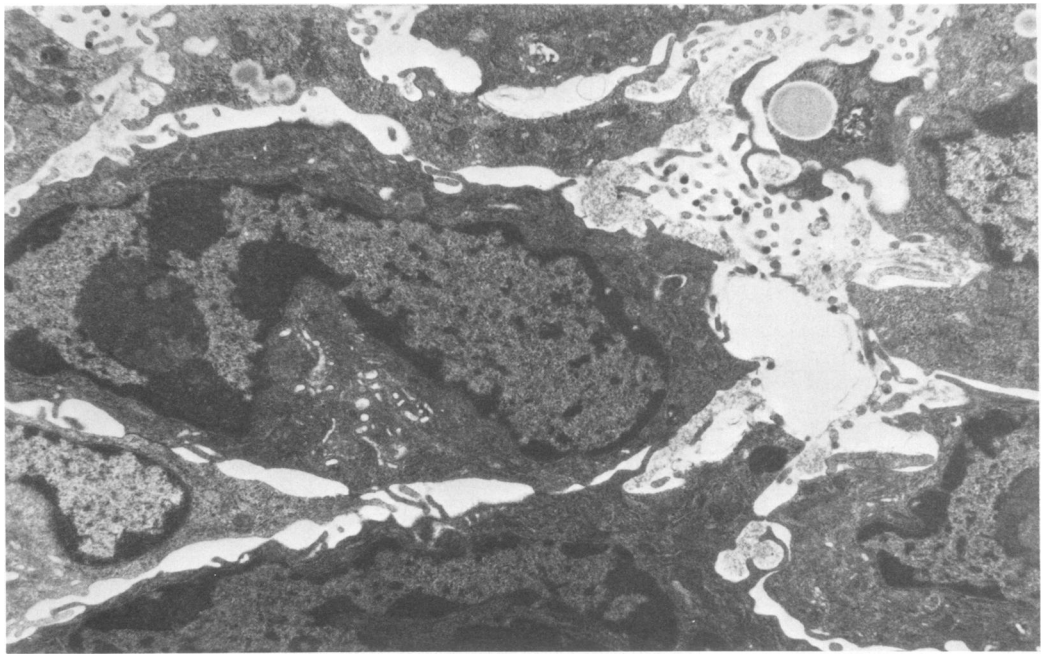

FIG. 6. *Secondary cultures of MJY-alpha initiated from generation 3 of in vivo tumors, ×1,248.*

TABLE 1. *Levels of radioactivity associated with mMTV bands in isopycnic gradients*[a]

Virus prepn	dpm/cm² of cell layer	
	^3H	^{14}C
Alpha cell line	4.4×10^3	9.6×10^2
Cell cultures initiated from MJY-alpha tumors		
Primary	2.0×10^2	4.8×10^1
Secondary	2.7×10^2	6.3×10^1
Tertiary	3.3×10^3	4.4×10^2
Quaternary	4.1×10^3	7.5×10^2

[a] MJY-alpha cell cultures were doubly labeled with [^3H]glucosamine and ^{14}C-amino acids as described in the text. Culture supernatants from the 24-h labeling period and 48-h chase were pooled for purification of MMTV.

Effects of antibody treatment on MJY-alpha cell line. MJY-alpha cell cultures were treated with antiserum against MMTV virion antigens to determine whether the decrease of viral synthesis and production observed in vivo could be induced in vitro under more defined conditions. Electron microscopic examination of serial thin sections of cells treated with rabbit anti-MMTV antiserum for 24, 48, and 72 h revealed a decrease in the numbers of all MMTV-associated particles over that observed with either untreated controls or cell cultures treated with normal rabbit serum. Clusters of aggregated MMTV particles were not observed in the extracellular spaces in any of the cultures examined. The ultrastructure of the serum-treated cells was similar to that of untreated controls; no cytotoxic effects were observed.

Cell-free virions were purified from supernatants of cultures doubly labeled with radioactive precursors. There was a significant decrease in the levels of virions recovered from cultures treated with anti-MMTV antiserum. A three- to fivefold decrease in both ^3H and ^{14}C radioactivity was observed in the single peak of activity detected at the 1.16- to 1.18-g/ml buoyant density region of the gradients (Table 4). SDS-PAGE analyses of these preparations also revealed alterations in the virion polypeptide patterns of MMTV from antiserum-treated cultures. MMTV virions from cultures treated with rabbit anti-MMTV antiserum contained significantly higher levels of the 60,000-dalton glycoprotein, gp60, as shown in Fig. 8 and Table 5. This glycoprotein is very sensitive to proteolytic cleavage and was present in extremely low levels in the normal rabbit serum controls. SDS-PAGE also revealed a change in the electrophoretic mobilities of the 37,700- to 33,000-dalton polypeptides in virions from cultures treated with anti-MMTV antiserum. The major peak of ^{14}C-amino acid radioactivity is present at 33,000 daltons, whereas the peak of [^3H]glucosamine radioactivity is at 37,700 daltons. This is in contrast to the usual profile observed with MMTV virions, in which the major peak of both labels

is coincident and present at 37,700 daltons; gp33 is detected as a doubly labeled small peak or shoulder. The profiles of virions harvested from cultures treated with normal rabbit serum are similar to those of untreated MMTV particles, although virion glycoproteins gp37.7 and gp33 were poorly separated on the gel. These results show that a change is induced in the glycoproteins of MMTV virions and that this change is specific for the anti-MMTV antibody.

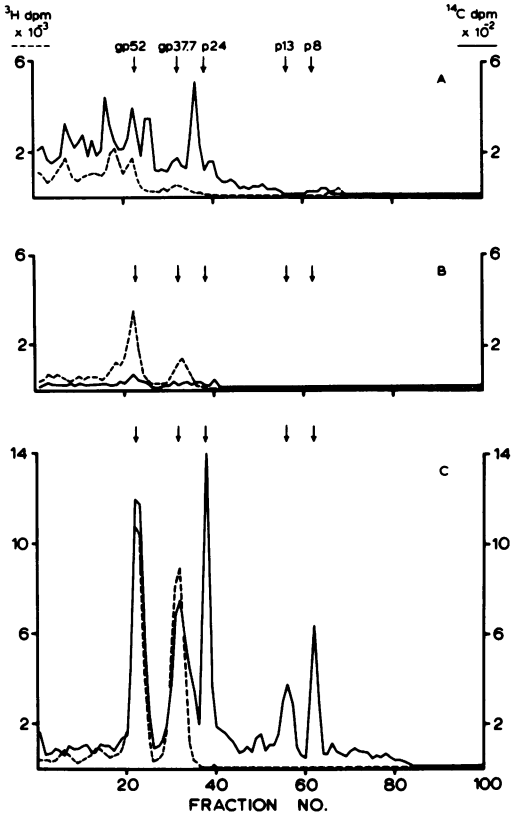

FIG. 7. *SDS-PAGE of virions from MJY-alpha cell cultures initiated from in vivo transplantation generation 3 of the cells in BALB/c mice. Cultures were labeled when confluent with [^{3}H]glucosamine (----) and ^{14}C-amino acids (——) for 24 h. Material having a buoyant density of 1.16 to 1.17 g/ml was analyzed. (A) Primary cultures; (B) secondary cultures; (C) tertiary cultures. Each sample analyzed contained 3.0 × 10^4 to 3.8 × 10^4 cpm of ^{3}H radioactivity and 1.0 × 10^4 to 1.8 × 10^4 cpm of ^{14}C radioactivity.*

DISCUSSION

The study presented here demonstrates that the production of MMTV virions by the MJY-alpha cell line was reversibly suppressed by in vivo implantation of the cells into isogeneic MMTV-free BALB/c or MMTV-infected BALB/cfC3H mice. This suppression was rapid; the decrease in MMTV particles was observed as early as 7 days after implantation and remained constant with further in vivo passage of the MJY-alpha tumors. Cell cultures initiated from these tumors also produced extremely low numbers of virions during the first two passages; after this, the level of MMTV virion synthesis increased and in later subcultures was similar to the parent cell line. The cause of virion particle suppression in vivo is unknown. It may have occurred as a consequence of changes in the levels or types of nutrients and hormones accessible to the tumor cells. However, it is also possible that virion synthesis was altered as a result of humoral and cellular immune reactivities of the tumor-bearing hosts. Both BALB/c and BALB/cfC3H hosts possess antibodies against MMTV virion antigens and spleen cells cytotoxic for MMTV-infected mammary tumor cells. These immunological reactivities, which were detected as early as 4 to 7 days after implantation, could have caused a selective pressure, giving survival advantage to those cells in the MJY-alpha cell population which were producing low levels of MMTV virions; because the MJY-alpha cell line has not been cloned, cells expressing a range of MMTV viral production could have been present in the inoculum. Return of MMTV virion synthesis in vitro would then be explained by an increase in the cell population of virus-producing cells once the selective pres-

TABLE 2. *Characteristics of MJY-alpha cell line in vivo and in vitro*

Virus prepn	Immunofluorescence assay for MMTV antigens (%)	Immunodiffusion for cell-free MMTV antigens	Electron microscopy			
			A	Budding	Cell-free B	Cell-free C
MJY-alpha cell line	+(95–99)	+++	+++	+++	+++	–
MJY-alpha tumors (generations 1–5)	+(90–95)	Not done	Rare	Rare	Rare	–
Cell cultures from MJY-alpha tumors						
Primary and secondary	+(90–95)	+/–	+	+	Rare	–
Tertiary and quaternary	+(90–95)	++	++	++	++	–

TABLE 3. *Activity of spleen cells from BALB/c and BALB/cfC3H females against mammary tumor cells*

Spleen donors	Target cell survival (%)			
	MJY-alpha cell line	BALB/cfC3H spontaneous mammary tumors	FUKU cell line	D1-DMBA3 cell line
BALB/c hosts bearing MJY-alpha tumors				
4 days[a]	38–43	88–94	100[b]	
7 days	36–51	67–91	100[b]	
11 days	41–62	79–85		
14 days	40–56	56		
21 days	52–60	58		
28 days	41–55		99[b]	100[b]
BALB/cfC3H hosts bearing MJY-alpha tumors				
7 days[a]	40–66	84–88		
14 days	49–59	50		
21 days	46–50	50		
28 days	29–46		100[b]	100[b]
Controls				
BALB/c hosts with FUKU tumors	100[b]		62	100[b]
BALB/c hosts with D1-DMBA3 tumors	98–100[b]	96[b]	99[b]	38
BALB/c (8–14 weeks of age)	94–96[b]	100–102[b]		
BALB/cfC3H (8–14 weeks of age)	84–88	94[b]		
BALB/cfC3H with spontaneous mammary tumor	24–41	58		

[a] Number of days after implanation of MJY-alpha cells, primary in vivo generation.
[b] The decrease in target cell survival was not statistically significant.

TABLE 4. *MMTV-associated radioactivity from MJY-alpha cell cultures treated with rabbit antisera*[a]

Treatment	dpm/cm² of cell layer	
	^{3}H	^{14}C
Control		
Day 1	500	280
Day 2	1,800	530
Normal rabbit serum		
Day 1	430	270
Day 2	1,400	540
Rabbit anti-MMTV antiserum		
Day 1	110	50
Day 2	410	130

[a] Rabbit antiserum was added 24 h before and throughout the labeling procedure. Cell cultures were doubly labeled on day 0 with [^{3}H]glucosamine and ^{14}C-amino acids as described in the text. Culture supernatants were collected 24 h after labeling (day 1) and after a 24-h chase period (day 2).

sure was removed. An alternative hypothesis to cellular selection is that viral expression was temporarily repressed or was modulated in the cells. This is supported by several lines of evidence. First, both MJY-alpha tumor and cultured cells contained MMTV antigens; the only change detected was a reduction in the numbers of viral particles. Decreases in MMTV virion production are usually accompanied by concomitant decreases both in the number of MMTV-positive cells and in the level of MMTV antigens detected in the infected cells (11, 21). Second, in vitro treatment of MMTV-producing cells with anti-MMTV antiserum for 48 h resulted in a three- to fivefold decrease in the amount of extracellular MMTV virions present in culture supernatants. Reduction in the numbers of A and budding particles was also observed in these cells by electron microscopy. These decreases were not due to the use of heterologous antiserum (as indicated by the normal rabbit serum controls), to a reduction in cell numbers in the treated cultures, or to the aggregation of virion particles at the surfaces of the cells. Modulation in the expression of cell surface antigens as a result of specific antibodies has been well documented with thymus-leukemia (TL) antigens (4–6, 12, 14). TL antigen disappears from the cell surface in the presence of anti-TL antibody and reappears in its absence. Modulation of a similar nature would explain the scarcity of MMTV particles in MJY-alpha tumors and in cultures treated with antiserum to MMTV. Further investigations will be made to determine

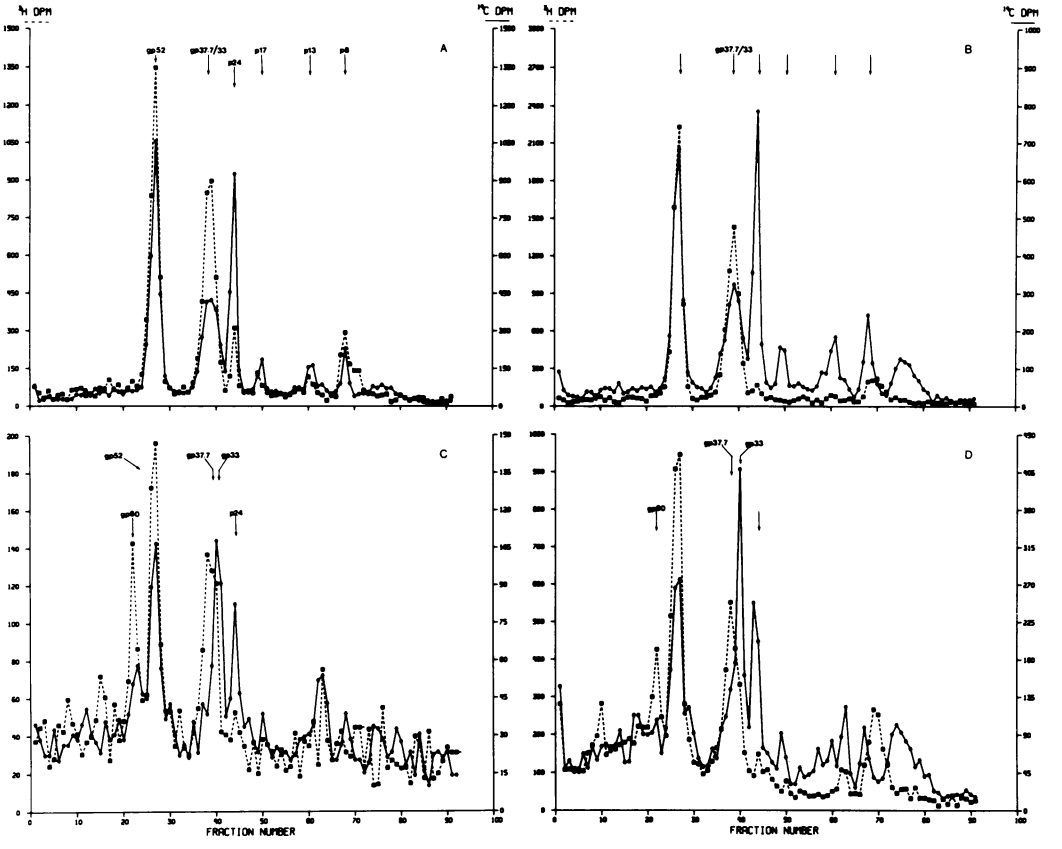

FIG. 8. *SDS-PAGE of virions from MJY-alpha cell cultures treated with normal rabbit serum (A and B) and rabbit anti-MMTV antiserum (C and D). (A) and (C) represent treatment with sera for 24 h before labeling with [^{3}H]glucosamine (----) and ^{14}C-amino acids (——) for 24 h; (B) and (D) represent treatment for 48 h with sera before addition of cold chase after labeling.*

whether MMTV production increases to control levels after removal of the rabbit antiserum and whether similar changes in MMTV production are observed when isogeneic anti-MMTV antiserum against MMTV virions or MMTV polypeptides or sera from tumor-bearing hosts are utilized.

If modulation of MMTV production did occur, the mechanism of this change or suppression has yet to be determined. Immunofluorescent studies demonstrated that MMTV viral antigens continued to be synthesized by the cells. However, MMTV polypeptides may be differentially represented in the cellular cytoplasm. Further examination of these tumor cells must be carried out using monospecific antisera rather than antiserum against the entire complement of MMTV antigens. It is possible that the synthesis of one or more virion proteins or cellular precursors was altered (9). Such an alteration either qualitative or quantitative could have resulted in the blockage of virion maturation. MMTV polypeptide profiles of extracellular MMTV particles from cultures treated with anti-MMTV antiserum indicated that the level of the glycoprotein gp60 is increased. It is uncertain whether this relative increase of gp60 is related to a reduction of virion synthesis since the level of this labile glycoprotein increases under other defined culture conditions (24). The shift in the migration rate of the ^{14}C-amino acid peak observed in the 37,700- to 33,000-dalton region is similar to that observed when MMTV virions are treated with proteolytic enzymes such as trypsin or chymotrypsin. However, under these conditions there is a concomitant decrease in the levels of gp60 and gp52 and an appearance of a 22,000-dalton component (20, 24). Because antibody treatment did not result in these other changes, the shift in gp37.7 to gp33 could be due to a mechanism other than proteolytic cleavage of gp60 and gp52. Further analyses must be carried out to characterize the changes in mature virion polypeptides and to determine whether

TABLE 5. *Percentage of radioactivity associated with MMTV virion polypeptides*[a]

MMTV polypeptides	Radioactivity (%)			
	Normal rabbit sera		Rabbit anti-MMTV antiserum	
	Day 1	Day 2	Day 1	Day 2
gp60				
^{3}H	1.5	<0.5	19.4	13.5
^{14}C	1.0	<0.5	11.3	8.9
gp52				
^{3}H	42.8	48.0	38.8	42.4
^{14}C	33.6	27.7	29.9	30.6
gp37.7-33				
^{3}H	41.5	42.5	37.4	28.3
^{14}C	27.5	22.9	29.2	29.6
p24				
^{14}C	21.2	20.6	15.1	19.9
p17				
^{14}C	3.7	4.9	<0.5	<0.5
p13				
^{14}C	5.7	8.2	13.8	6.4
p8				
^{14}C	4.6	8.7	0.5	4.6

[a] The values reported for the polypeptides are percentages of total virion-associated radioactivity as determined by quantitation of the polypeptides isolated by SDS-PAGE. Cultures were doubly labeled with [^{3}H]glucosamine and ^{14}C-amino acids as described in the text. Each sample examined by SDS-PAGE contained 3.0×10^{4} to 3.8×10^{4} dpm of ^{3}H and 1.0×10^{4} to 1.8×10^{4} dpm of ^{14}C radioactivity.

they are related to immunological suppression of MMTV synthesis.

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