

## Alterations of a Mouse Mammary Tumor Virus Glycoprotein with Interferon Treatment

MARY JANE YAGI,<sup>1\*</sup> NORVAL W. KING, JR.,<sup>2</sup> AND J. GEORGE BEKESI<sup>1</sup>

*Department of Neoplastic Diseases, The Mount Sinai School of Medicine, New York, New York 10029,<sup>1</sup> and New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772<sup>2</sup>*

The effects of exogenous mouse interferon on the MJY-alpha mammary tumor cell line chronically infected with mouse mammary tumor virus (MMTV) were examined. Interferon at concentrations of 25 to 2,000 IU/ml in culture medium did not alter the growth rate or morphology of the cell layers. Electron microscopic examination of interferon-treated cells indicated a decrease in the numbers of A-type and budding B-type particles of MMTV. However, the levels of extracellular MMTV virions in the culture supernatants were not significantly reduced. Profiles of MMTV glycoproteins and nonglycosylated polypeptides obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of virions purified from interferon-treated cultures revealed increases in the relative levels of the 60,000-dalton glycoprotein, gp60.

Interferon is a natural determinant of host resistance to viral infection. There is a strong indication that interferon responses of host cells may complement the host immune system and contribute to host resistance to neoplasia induced by retroviruses (11, 14, 19). *In vivo* studies have suggested that interferon acts by inhibiting development or proliferation of neoplastic cells rather than by decreasing viral infection and replication (8, 11, 26).

Studies examining the effects of interferon on *in vitro* cell cultures have demonstrated that exogenous interferon significantly inhibits production of virus particles in cells infected with murine leukemia virus (MuLV; 1, 25, 35). The mechanism(s) of reduction of viral synthesis is unknown; decreases were observed only when interferon was present in the cultures. Similar reversible depression of murine mammary tumor virus (MMTV) production was reported to occur in the Mm5mt/c<sub>1</sub> C3H mammary tumor cell line when treated with 10 to 100 U of interferon per ml (33). The reduction in MMTV replication was dose dependent and was observed after a 12- to 16-h treatment period. These workers did not observe any changes in expression of MMTV-related antigens in treated cells by immunofluorescence, although cellular MMTV-specific RNA-dependent DNA polymerase levels were significantly reduced. They postulated that interferon inhibition may be due to its action both on MMTV protein synthesis and on viral assembly and maturation.

We have examined the effects of exogenous mouse interferons on the replication of MMTV in the chronically infected mammary tumor cell

line MJY-alpha. The results reported here indicate that interferon does not induce a significant reduction of MMTV virion synthesis but does alter the polypeptide pattern of MMTV proteins.

### MATERIALS AND METHODS

**Cells.** The MMTV-producing MJY-alpha cell line was grown as stationary cultures in T-flasks or petri dishes as previously described (36). Growth medium for all cultures was RPMI 1640 supplemented with 20% fetal calf serum,  $10^{-5}$  M bovine insulin, and antibiotics (36). Cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Cultures were routinely checked for bacterial, fungal, and *Mycoplasma* contamination (36); the results were negative.

**Production and purification of mouse interferon.** Mouse interferon was produced by infection of the murine C-243 cell line with Newcastle disease virus (3). Supernatants from infected cultures were cleared by centrifugation at  $100,000 \times g$  for 2 h, concentrated 2.5-fold by ultrafiltration (Amicon UM-2 membrane with 1,000-molecular-weight exclusion limit), and dialyzed for 20 h against 0.05 M sodium acetate buffer, pH 5.0. Interferon was purified by affinity chromatography using Affi-Gel 202 (Bio-Rad Laboratories) in a column (0.9 by 10 cm) equilibrated with acetate buffer. A flow rate of 17 ml/h was maintained by means of an LKB peristaltic pump. After the breakthrough fraction, the column was equilibrated with 0.02 M sodium phosphate buffer, pH 7.4, followed by elution with 0.2 M sodium phosphate containing 0.5 M sodium chloride, pH 7.4. Portions of the 1.0-ml fractions were assayed for both protein and interferon concentrations. The specific activity of the purified mouse interferon was  $7.0 \times 10^7$  IU per mg of protein.

Mouse interferon samples from two other sources were used for comparative analyses to confirm the results obtained with interferon produced and purified

in our laboratory. Purified interferon, I-F ( $10^5$  IU/ml; specific activity,  $10^7$  IU/mg of protein), was a generous gift from Robert Friedman, Laboratory of Experimental Pathology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Md. (14), and I-LB, concentrated crude interferon ( $10^6$  IU/ml), was purchased from Litton Bionetics, Kensington, Md.

**Titration of mouse interferon.** Purified interferon from C-243 cells was titrated by examining the reduction of cytopathic effects (CPE) induced by vesicular stomatitis virus (VSV) infection of L cells. Interferon samples diluted 1:10 to 1:500 were added to quadruplicate wells of microtest plates containing L cells cultured for 24 h at  $37^\circ\text{C}$ . After 24 h of incubation at  $37^\circ\text{C}$ , culture supernatants were replaced with fresh medium containing  $5 \times 10^4$  PFU of the challenging VSV. Interferon titers are expressed as the reciprocal of the maximum dilution showing a 50% reduction of CPE. The mouse interferon reference standard, indicated to have a titer of 12,000 IU, had a titer of 9,000 U by this method (34). Interferon titers reported in the text have been corrected and are given as international units.

**Titration of mouse interferon in MJY-alpha cells.** The sensitivity of MJY-alpha cells to the antiviral effects of interferon was tested. MJY-alpha cells were cultured in the same manner as L cells (see above), and the reduction of CPE induced by VSV was determined by using various concentrations of interferon. Interferon titers were determined and compared with those obtained from L-cell standards.

**Interferon treatment of MJY-alpha cell layers.** Confluent, 3- to 5-day-old cell cultures were treated with 25 to 2,000 IU of interferon per ml of culture medium or 7.65 to 300 IU of interferon per  $\text{cm}^2$  of cell layer. Cell layers were treated for 0, 24, or 48 h before viral harvests or labeling with [ $^3\text{H}$ ]glucosamine and [ $^{14}\text{C}$ ]amino acid precursors. Medium and exogenous mouse interferon were replenished daily for periods of 1 to 5 days. Virions were purified from the culture supernatants as described below. Harvested supernatants cleared of cellular debris and virus particles were also assayed for interferon activity in L cells as previously described.

**Virus.** Virions doubly labeled with [ $^3\text{H}$ ]glucosamine and [ $^{14}\text{C}$ ]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 before viral harvests. Cells were labeled with [ $^3\text{H}$ ]glucosamine (10  $\mu\text{Ci/ml}$ ) and [ $^{14}\text{C}$ ]amino acids (12.5  $\mu\text{Ci/ml}$ ) in growth medium containing  $10^{-6}$  M hydrocortisone and 2.5% fetal calf serum. Virions were harvested every 24 h for 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 (36, 38).

**Histology and electron microscopy.** In situ cell cultures were examined daily, and parallel cultures grown on glass cover slips were used for histological examination after staining with May-Grünwald-Giemsa. Petri dishes containing 6 to 10 cover slips each facilitated longitudinal examination of interferon-treated cultures.

MJY-alpha cell layers grown in 25-ml T-flasks or

petri dishes were processed for electron microscopy as previously described (21, 36). Briefly, culture medium was removed from the cell layers and the cells were rinsed with several changes of 0.1 M phosphate buffer, pH 7.4. Layers were then fixed in situ with glutaraldehyde in 0.1 M phosphate buffer for 2 to 3 h followed by further washing with 0.1 M phosphate buffer. Cells were subsequently postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h at  $4^\circ\text{C}$ . After removal of the fixative, the cells were exposed to 1% uranyl acetate before dehydration in a graded series of ethanol solutions. Entire monolayers were embedded in situ by a previously described method (7). En face thin sections were prepared of selected portions of the monolayers, using a diamond knife on a Porter-Blum MT-2 ultramicrotome. Sections were mounted on 200-mesh copper grids, stained with uranyl acetate and Sato lead stain, and examined in a JEOL 100-S electron microscope at 80-kV accelerating voltage (27).

Virions purified from culture supernatants were examined after being applied to carbon-coated Formvar films and stained with 0.1% uranyl acetate or 2% sodium phosphotungstate, pH 6.8, with or without previous fixation in 0.01% glutaraldehyde. Negative-stained preparations were examined in a Philips 301 or JEOL electron microscope.

**SDS-PAGE.** Virus samples were disrupted for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by boiling for 1 min at  $100^\circ\text{C}$  with 1% SDS and 1%  $\beta$ -mercaptoethanol. Electrophoresis was carried out as previously described with 10 to 20% gradient gels and a discontinuous buffer system (40, 41). Gels were processed for determination of radioactivity as described by Compans (12). Levels of radioactivity associated with MMTV polypeptide peaks were determined by using a generalized curve-fit computer program developed by Robbin Bura.

**Chemicals and isotopes.** [ $^3\text{H}$ ]glucosamine (6.3 Ci/mmol) and [ $^{14}\text{C}$ ]amino acid mixture (55 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass. Components for SDS-PAGE and affinity chromatography were obtained from Bio-Rad Laboratories, Rockville Centre, N.Y.

## RESULTS

**Sensitivity of MJY-alpha cells to interferon.** MJY-alpha cells were pretreated with 15 to 600 IU of interferon serially titrated and then challenged with VSV to determine their sensitivity to the protective, antiviral activity of interferon. The results indicate that MJY-alpha cells are 4- to 16-fold less sensitive to interferon than L cells in CPE inhibition assays with VSV.

**Effects of interferon on cell growth and morphology.** Cultures of MJY-alpha cells were continuously treated with 50 to 1,000 IU of interferon per ml from the time of seeding for a period of 10 days to determine whether exogenous interferon exerted any inhibitory effects on cell growth. Spent culture medium was obtained daily and assayed for interferon activity; the results indicated that incubation of interferon

with the cell layers for 24 h at 37°C did not significantly reduce the levels of interferon activity in the medium. The daily addition of interferon at concentrations of 50 to 1,000 IU per ml of medium had no effect on the growth rate or release of viable cells from the monolayers (Fig. 1). The numbers of cells released from interferon-treated and control cultures increased at approximately the same rate until a peak was reached on days 6 to 8. The total numbers of viable cells in interferon-treated cultures were similar to those of the controls during the 10-day period.

Daily histological examination of cell layers from parallel cultures did not reveal any alterations in the cyclic change in cellular shape and the number of mitotic figures observed in the cells treated with 50 to 2,000 IU of interferon per ml. Electron microscopic studies also failed to reveal any differences in the ultrastructure of interferon-treated MJY-alpha cells even at concentrations as high as 2,000 IU/ml. Similar results were obtained with purified and crude

mouse interferon from the other two sources, I-F and I-LB.

**Effects of interferon on MMTV production.** The effects of exogenous interferon on MMTV replication in MJY-alpha cells were examined in unstimulated cultures and in cultures stimulated with hydrocortisone at the time of interferon addition. Control cells grown without hydrocortisone and interferon contained the lowest numbers of particles, and an increase in virion numbers after the addition of hydrocortisone was observed after 24 h. This increase was progressive, and by day 4 most of the cells had large aggregates of doughnut-shaped intracytoplasmic A-type particles. These cells had numerous microvillous projections of their plasma membrane which contained budding MMTV virions (Fig. 2). Thin sections revealed no detectable differences between these control cultures and cells treated for 1 to 4 days with interferon at concentrations of 20, 50, 100, 200, 500, and 1,000 IU/ml. During the 4-day incubation in the presence of hydrocortisone and interferon, there was a progressive increase in the number of cytoplasmic and budding viral particles in the cells which paralleled that observed in control MJY-alpha cells during comparable time periods. However, in a similar study using 2,000 IU of interferon per ml, a reduction in the numbers of viral particles was observed after 4 days of treatment. This reduction, which was not observed during the first 3 days of incubation with the same concentration of interferon, was most obvious when the numbers of intracytoplasmic A-type particles were compared in the control and interferon-treated cultures at 4 days. There appeared to be fewer and smaller aggregates of A-type particles present in the interferon-treated cells. In addition, a decrease in the number of microvilli and budding particles was also observed (Fig. 3). Interferon treatment did not alter the morphology of the A-type particles and budding particles. Extracellular, B-type MMTV virions purified from culture supernatants and examined after negative staining were identical in interferon-treated and untreated cultures.

Culture supernatants were also examined for MMTV virions. Spent culture fluids were harvested from cells treated with 50, 100, 200, or 1,000 IU of interferon per ml for 24 to 72 h and labeled with [<sup>3</sup>H]glucosamine and <sup>14</sup>C-amino acids. Final sucrose isopycnic gradients were fractionated, and virus-associated radioactivity having the buoyant density of MMTV virions (1.16 to 1.17 g/ml) was isolated (Table 1). The data indicate that interferon treatment caused only small decreases in the levels of MMTV virions.

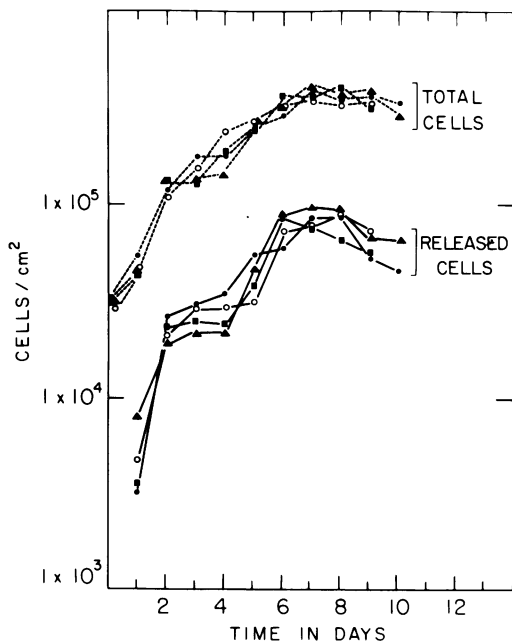


FIG. 1. Growth curves of MJY-alpha spontaneously released cells and total number of cells 1 to 10 days after seeding. Mouse interferon was added daily from the time of seeding at concentrations of 50 (○), 100 (△), and 1,000 (■) IU per ml of medium; (●) untreated cultures. Cells released into the medium were pelleted, resuspended, and counted, using the vital trypan blue stain. Cell layers were removed from the substrate with trypsin-EDTA and processed as above.

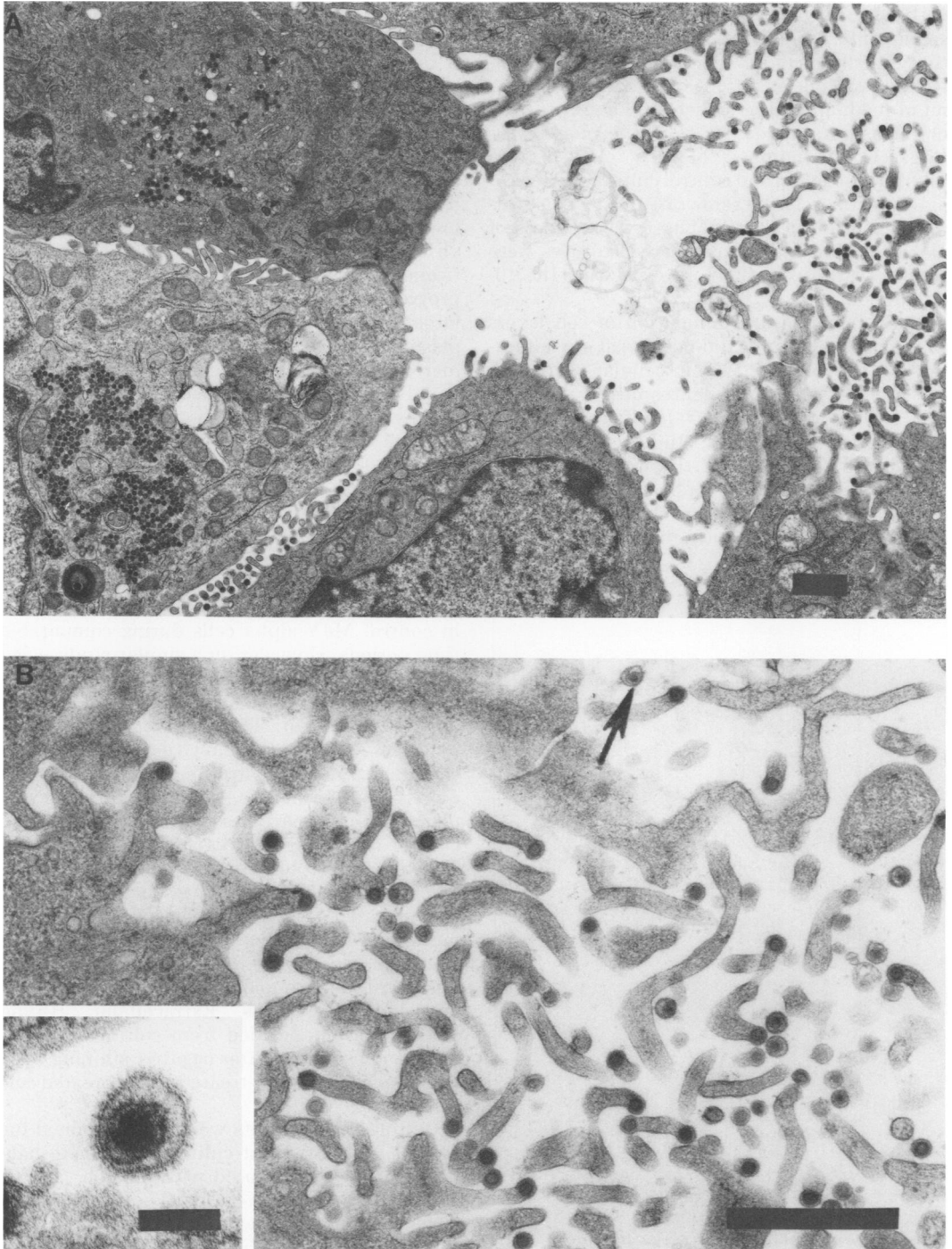
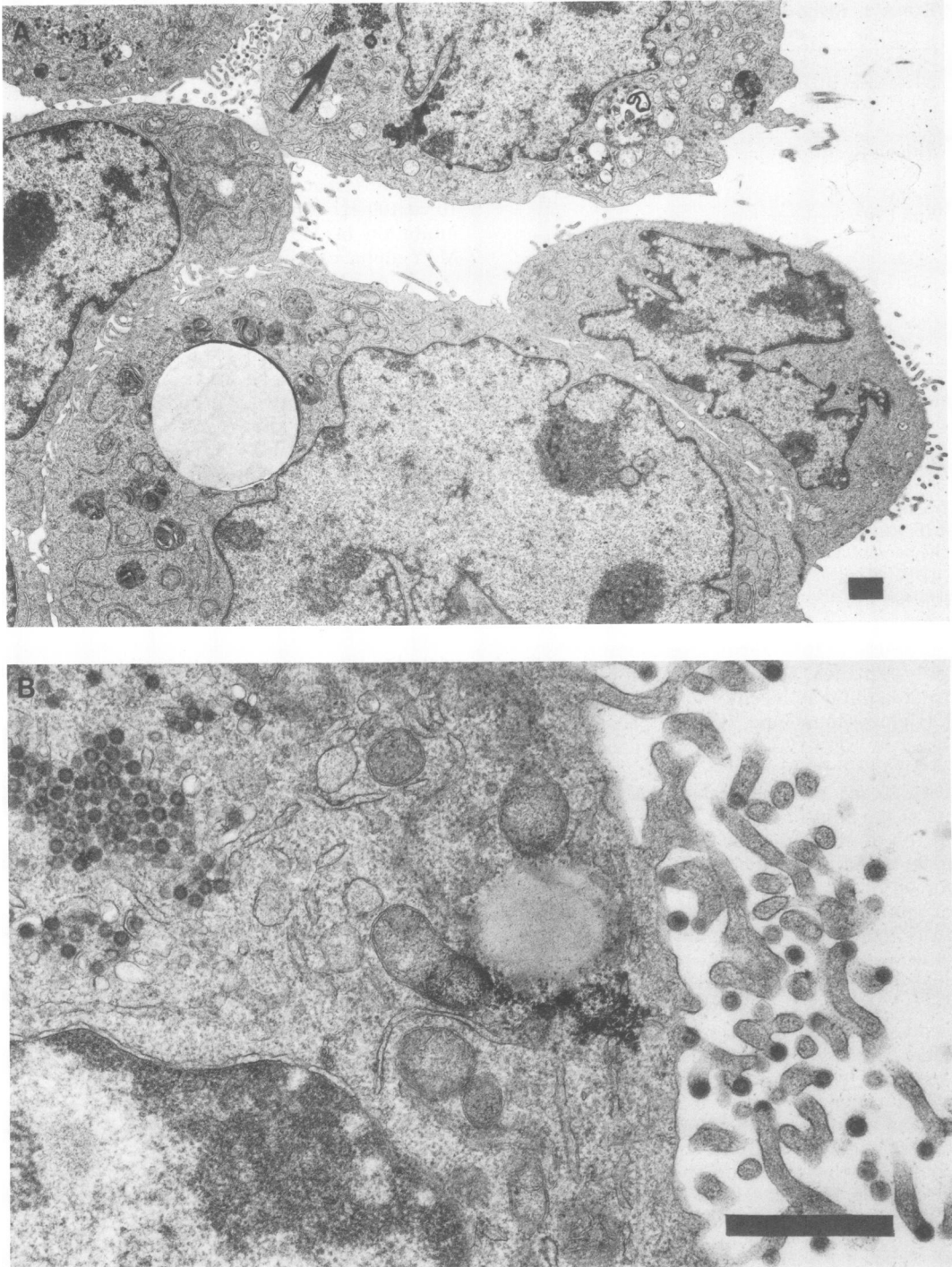


FIG. 2. MJY- $\alpha$  cells stimulated with hydrocortisone for 3 days. (A) Untreated cells depicting aggregates of intracytoplasmic A-type particles and microvilli containing budding B-type particles; (B) untreated cells with budding and mature B-type particles (arrow and inset). Bar = 1  $\mu$ m.



**FIG. 3.** *MJY-alpha* cells stimulated with hydrocortisone for 3 days and treated with 2,000 IU of interferon per ml for 4 days. (A) General reduction of intracytoplasmic A-type (arrow) and budding particles; (B) presence of aggregates of A-type particles and budding B-type virions. Bar = 1  $\mu$ m.

TABLE 1. *Relative levels of radioactivity associated with MMTV virions<sup>a</sup>*

Treatment (IU of interferon/ml)	% of untreated control virions	
	<sup>3</sup> H	<sup>14</sup> C
Without hydrocortisone		
100	105	110
1,000	99	100
With hydrocortisone		
100	97	87
1,000	96	89
50 (I-F)	81	82
100 (I-F)	87	80
50 (I-LB)	88	83
100 (I-LB)	89	94

<sup>a</sup> MJY-alpha cultures were treated with interferon for 48 h before and during labeling. Cultures were doubly labeled with [<sup>3</sup>H]glucosamine and <sup>14</sup>C-amino acids as described in the text. Culture supernatants after a 24-h labeling period were quantitatively harvested and purified; the final isopycnic gradients were fractionated, and total radioactivity at a density of 1.16 to 1.17 g/ml was determined. Values represent the percentage of radioactivity associated with virions in treated cultures as compared with parallel untreated controls.

**Effects of interferon treatment on MMTV polypeptides.** MMTV virions from interferon-treated MJY-alpha cultures doubly labeled with [<sup>3</sup>H]glucosamine and <sup>14</sup>C-amino acids were analyzed by SDS-PAGE. The polypeptide profiles of MMTV obtained after treatment of cultures with 25, 50, 100, and 1,000 IU of interferon per ml of medium for 24 to 72 h revealed increases in the levels of the 60,000-dalton glycoprotein, gp60 (Fig. 4). The increase of gp60 was greater for virions from cultures treated with 1,000 IU of interferon per ml than for virions from cultures treated with the lower concentrations. However, the differences were not proportional, suggesting that the effect exerted by interferon on the glycoprotein of MMTV is not dose dependent. The increase of gp60 relative to the other MMTV polypeptides and glycoproteins was observed in virions from cultures pretreated with interferon for 24 to 48 h before labeling as well as in virions from cultures which also contained hydrocortisone in the medium. Similar changes in the level of gp60 were observed when interferons I-F and I-LB were used (Fig. 4 and Table 2).

## DISCUSSION

The results presented here demonstrate that treatment of MJY-alpha cells with 25 to 2,000 IU of interferon per ml did not significantly reduce extracellular production of MMTV viri-

ons. In contrast, a previous *in vitro* study using the mammary tumor cell line Mm5mt/c<sub>1</sub> chronically infected with MMTV detected a 5- to 10-fold reduction in MMTV replication by quantitation of extracellular RNA-dependent DNA polymerase activity (33). This inhibition was observed when the glucocorticoid-stimulated cultures were treated for periods of 12 to 16 h with 10 to 100 IU of interferon per ml. The lack of inhibition of MMTV virions observed in the MJY-alpha cell line is not attributable to the source or purity of interferon, since similar results were obtained with interferon purified in our laboratory as well as interferon obtained from two other sources. The differences in inhibition of MMTV replication may be due to the sensitivity of the two cell lines to the antiviral action of mouse interferons. CPE induced by encephalomyocarditis virus was reduced in Mm5mt/c<sub>1</sub> cells at a 3- to 10-fold-lower level by interferon than in Ly mouse cells, whereas CPE induced by VSV in MJY-alpha cells was reduced 4- to 16-fold when compared with L cells examined in parallel. Similar phenomena have been reported for mouse cell lines chronically infected with MuLV. In these studies, the degree of reduction of retrovirus replication appears to be related to the sensitivity of the cell line to interferon-induced protection against cytopathic viral infection (4, 6, 10, 15, 16, 25). Our results also demonstrate the variability in the degree of interferon sensitivity of mouse mammary tumor cells chronically producing MMTV. Further examination of primary or short-term mammary tumor cell cultures is necessary to determine whether newly arising mammary tumors also exhibit a range of sensitivity to interferon.

Electron microscopic examination of interferon-treated MJY-alpha cells did not reveal detectable ultrastructural changes in cellular organelles or in the appearance of MMTV A-type or of budding or mature B-type particles that would account for the elevated levels of the specific virion-associated glycoprotein, gp60. The numbers of virions detected in control and parallel cultures treated with 100 to 1,000 IU of interferon per ml were also similar. Cultures treated with interferons I-F and I-LB, in which 6 to 20% reductions in the levels of virion-associated radioactivity were observed in preparations of extracellular virions, were also morphologically identical to controls. Examination of thin sections of cells from these cultures suggested that the reduction was not due to a blockage of a late step in MMTV maturation. There was no evidence of accumulations of budding particles or aggregations of mature virions at cellular surfaces as previously described in stud-

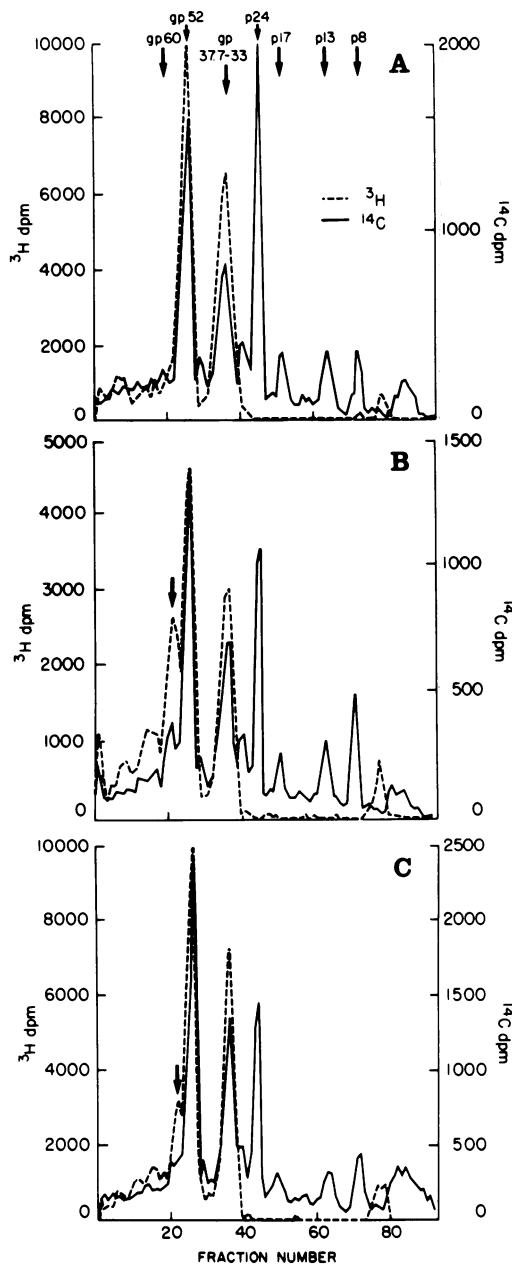


FIG. 4. SDS-PAGE of MMTV virions from MJY-alpha cultures stimulated with  $10^{-6}$  M hydrocortisone for 24 h before labeling with [ $^3$ H]glucosamine (----) and  $^{14}$ C-amino acids (—) for 24 h. (A) Untreated control cultures; (B) cultures pretreated with 1,000 IU of interferon per ml for 24 h before addition of isotopic labels; (C) cultures pretreated with 1,000 IU of interferon (I-F) per ml for 24 h before labeling. Each sample examined contained  $4.6 \times 10^4$  to  $5.1 \times 10^4$  dpm of  $^3$ H and  $2.2 \times 10^4$  to  $2.9 \times 10^4$  dpm of  $^{14}$ C radioactivity.

ies of interferon-treated murine leukemia cells chronically infected with MuLV (5, 10). The morphological evidence of a reduction in the number of intracytoplasmic A-type particles and budding virions observed in MJY-alpha cells treated with high levels of interferon (2,000 IU/ml) for 4 days deserves further investigation by more precise quantitative methods. It is possible that prolonged treatment of MJY-alpha with high levels of interferon would exert an inhibitory effect on MMTV replication.

The effects of interferon on the synthesis of viral polypeptides were examined by comparing polypeptide profiles obtained by SDS-PAGE. Analyses of virions doubly labeled with carbohydrate and amino acid precursors revealed that MMTV virions from interferon-treated cultures contained elevated levels of gp60. The increases were detected over the entire range of interferon concentrations studied, 25 to 2,000 IU/ml. The results suggest that the level of gp60 increases with increasing doses of interferon, although there was no direct correlation between dose and the amount of gp60 observed. We have previously shown that this glycoprotein is very susceptible to proteolytic cleavage (40) and that its level in extracellular virions can be altered by culture conditions (40) as well as by treatment

TABLE 2. Percentage of radioactivity associated with MMTV virion polypeptides<sup>a</sup>

MMTV polypeptides	Radioactivity (%)				
	Untreated	Interferon treated			
		100 IU/ml	100 IU of I-F/ml	100 IU of I-LB/ml	1,000 IU/ml
gp60					
$^3$ H	3.8	17.7	14.7	11.2	26.1
$^{14}$ C	2.7	7.1	7.4	6.8	10.6
gp52					
$^3$ H	47.0	39.4	45.0	44.5	40.1
$^{14}$ C	27.5	28.8	32.7	25.6	31.1
gp37.7-33					
$^3$ H	49.2	42.9	40.3	44.3	33.8
$^{14}$ C	23.8	27.1	23.9	21.9	22.4
p24					
$^{14}$ C	21.8	22.8	18.7	20.4	18.7
p17					
$^{14}$ C	5.1	4.3	4.2	5.9	2.9
p13					
$^{14}$ C	8.1	6.8	6.3	8.1	6.7
p8					
$^{14}$ C	11.0	3.1	6.8	11.3	7.6

<sup>a</sup> Values for the polypeptides are percentages of total virion-associated radioactivity as determined by quantitation of the polypeptides separated 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  $4.6 \times 10^4$  to  $5.1 \times 10^4$  dpm of  $^3$ H and  $2.2 \times 10^4$  to  $2.9 \times 10^4$  dpm of  $^{14}$ C radioactivity.

of the cells with specific antiserum against MMTV B-type particles (39). Antibody treatment of the cells (2%, vol/vol) induced a similar increase in gp60 relative to control virions as observed with interferon treatment (39).

The interrelationship of gp60 and the other polypeptides of MMTV has not yet been established. There is evidence from our previous study (40) and that of Sheffield et al. (31) that the cleavage products, gp22 and gp36, are derived from gp60 or gp52, and we have suggested that gp60 may be an uncleaved form of the major virion glycoproteins. Our cumulative findings indicate that the presence of gp60 in mature B-type virions is related to the environment of the MJY-alpha cells, although the mechanism by which exogenous antibody and interferon increase gp60 is unknown. Investigators studying the effects of interferon on Moloney murine leukemia virus replication in a fibroblastic cell line (TB) have also reported elevated levels of a large glycoprotein, gp85, associated with mature virions (9). These workers suggested that gp85 was related to a glycoprotein precursor of the major viral glycoprotein gp69/71, and that interferon acted by inhibiting a cleavage step in the maturation of C-type particles.

It is probable that such inhibition of proteolytic cleavage is related to or is the consequence of the action of interferon on the plasma membranes of the infected cells (2, 9). It is not known whether exogenous antibody against the virion polypeptides exerts its action in a similar manner. Unlike interferon treatment, addition of antiviral antibody to MJY-alpha cell cultures induces a significant and rapid decrease in MMTV production (39). Studies examining the effects of antibody against measles virus have shown that exogenous immunoglobulins do induce cytoplasmic changes in the synthesis and processing of viral polypeptides as well as removal of virions from the cell surfaces (17). In light of these results, further studies are needed to determine whether interferon and antibody treatments also induce detectable changes in the composition of the plasma membranes of MJY-alpha cells as well as in the viral components in the cytosol.

Our *in vitro* studies indicate that interferon does alter MMTV replication in mammary tumor cells. The biological consequences of increased levels of gp60 in MMTV B-type particles are unknown, although it was reported that MuLV virions containing interferon-induced increases of gp85 had reduced levels of infectivity (9). It is possible that interferon treatment of MMTV-infected cells could effectively reduce viremia and subsequent secondary infection and

neoplastic transformation of mammary parenchyma by decreasing the levels of MMTV replication and by altering the infectivity of the virions (13, 24, 30). Previous studies by Came and Moore (8) and Bekesi et al. (3) indicate that exogenous interferon is effective in delaying the development and growth of mammary tumors as well as in restoring the hosts' cell-mediated immunity and abrogating the suppressor cell population. It is probable that the protection afforded the host by interferon treatment is mediated by the dual action of interferon on viral replication and the immune system (18, 20, 23, 29, 32). Studies are in progress to determine whether *in vitro* treatment of MJY-alpha cells with interferon alters the tumorigenicity of the cells in syngeneic hosts and to analyze the viral and cellular compositions of MJY-alpha-induced tumors in hosts treated with interferon.

#### ACKNOWLEDGMENTS

We acknowledge Roland E. Stutzman, Stephen B. Lambert, and Svetlana Salova-Perova for their technical assistance and John J. MacKey for his technical assistance in preparing the ultrathin sections.

This work was supported by contract NO1-CP4-3225 from the National Cancer Institute Biological Carcinogenesis Branch, by Public Health Service grant RR-00168-17 from the National Institutes of Health, and by the T. J. Martell Memorial Foundation for Leukemia Research.

#### LITERATURE CITED

1. Aboud, M., R. Shoor, and S. Salzberg. 1978. Effect of interferon on exogenous murine leukemia virus infection. *Virology* **84**:134-141.
2. Ankel, H., C. Chany, B. Galliot, M. J. Chevalier, and M. Robert. 1973. Antiviral effect of interferon covalently bound to sepharose. *Proc. Natl. Acad. Sci. U.S.A.* **70**:2360-2363.
3. Bekesi, J. G., J. P. Roboz, E. Zimmerman, and J. F. Holland. 1976. Treatment of spontaneous leukemia in AKR mice with chemotherapy, immunotherapy, or interferon. *Cancer Res.* **36**:631-639.
4. Billiau, A., V. G. Edy, H. Sobis, and P. De Somer. 1974. Influence of interferon on virus-particle synthesis in oncornavirus-carrier lines. II. Evidence for a direct effect on particle release. *Int. J. Cancer* **14**:335-340.
5. Billiau, A., H. Heremans, P. T. Allen, J. De Maeyer-Guignard, and P. De Somer. 1976. Trapping of oncornavirus particles at the surface of interferon-treated cells. *Virology* **73**:537-542.
6. Billiau, A., H. Sobis, and P. De Somer. 1973. Influence of interferon on virus particle formation in different oncornavirus carrier cell lines. *Int. J. Cancer* **12**:636-653.
7. Brinkley, B. R., P. Murphy, and L. C. Richardson. 1967. Procedure for embedding *in situ* selected cells cultured *in vitro*. *J. Cell Biol.* **35**:279-283.
8. Came, P. E., and D. H. Moore. 1971. Inhibition of spontaneous mammary carcinomas of mice by treatment with interferon and poly I:C. *Proc. Soc. Exp. Biol. Med.* **137**:304-305.
9. Chang, E. H., and R. M. Friedman. 1977. A large glycoprotein of Moloney leukemia virus derived from interferon-treated cells. *Biochem. Biophys. Res. Commun.* **77**:392-398.



10. **Chang, E. H., S. J. Mims, T. J. Triche, and R. M. Friedman.** 1977. Interferon inhibits mouse leukemia virus release: an electron microscopic study. *J. Gen. Virol.* **34**:363-367.
11. **Chirigos, M. A., and J. W. Pearson.** 1973. Cure of murine leukemia with drug and interferon treatment. *J. Natl. Cancer Inst.* **51**:1367-1368.
12. **Compans, R. W.** 1973. Influenza virus proteins. II. Association with components of the cytoplasm. *Virology* **51**:56-70.
13. **Drohan, W., R. Kettmann, D. Colcher, and J. Schlom.** 1977. Isolation of the mouse mammary tumor virus sequences not transmitted as germinal provirus in the C3H and RIII mouse strains. *J. Virol.* **21**:986-995.
14. **Friedman, R. M.** 1978. Interferons and cancer. *J. Natl. Cancer Inst.* **60**:1191-1194.
15. **Friedman, R. M., E. H. Chang, J. M. Ramseur, and M. W. Myers.** 1975. Interferon-directed inhibition of chronic murine leukemia virus production in cell cultures: lack of effect on intracellular viral markers. *J. Virol.* **16**:569-574.
16. **Friedman, R. M., and J. M. Ramseur.** 1974. Inhibition of murine leukemia virus production in chronically infected AKR cells: a novel effect of interferon. *Proc. Natl. Acad. Sci. U.S.A.* **71**:3542-3544.
17. **Fujinami, R. S., and M. B. A. Oldstone.** 1979. Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell. *Nature (London)* **279**:529-530.
18. **Gidlund, M., A. Orn, H. Wigzell, A. Senik, and I. Gresser.** 1978. Enhanced NK cell activity in mice injected with interferon and interferon inducers. *Nature (London)* **273**:759-761.
19. **Gresser, I.** 1972. Antitumor effects of interferon. *Adv. Cancer Res.* **16**:97-140.
20. **Gresser, I., C. Maury, and D. Brouty-Boye.** 1972. Mechanism of the antitumor effect of interferon in mice. *Nature (London)* **239**:167-168.
21. **King, N. W., Jr., H. Barahona, M. D. Daniel, J. G. Bekesi, and T. C. Jones.** 1978. The effect of phosphonoacetic acid on the ultrastructure of cell cultures infected with simian oncogenic herpesviruses. *Lab. Invest.* **38**:181-187.
22. **Klenk, H. D., R. Rott, M. Orlich, and J. Blodorn.** 1975. Activation of influenza viruses by trypsin treatment. *Virology* **68**:426-439.
23. **Lindahl, P., P. Leary, and I. Gresser.** 1972. Enhancement by interferon of the specific cytotoxicity of sensitized lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **69**:721-726.
24. **Michalides, R., G. Vlahakis, and J. Schlom.** 1976. A biochemical approach to the study of the transmission of mouse mammary tumor viruses in mouse strains RIII and C3H. *Int. J. Cancer.* **18**:105-115.
25. **Pitha, P. M., W. P. Rowe, and M. N. Oxman.** 1976. Effect of interferon on exogenous, endogenous, and chronic murine leukemia virus infection. *Virology* **70**:324-338.
26. **Rossi, G. B., G. P. Matarese, C. Grappelli, F. Belardelli, and A. Benedetto.** 1977. Interferon inhibits dimethyl sulphoxide-induced erythroid differentiation of Friend leukemia cells. *Nature (London)* **267**:50-52.
27. **Sato, T.** 1968. A modified method for staining of thin sections. *J. Electron Microsc.* **17**:158.
28. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of sendai virus. *Virology* **57**:475-490.
29. **Schellekens, H., W. Weimar, K. Cantell, and L. Stitz.** 1979. Antiviral effect of interferon *in vivo* may be mediated by the host. *Nature (London)* **278**:742.
30. **Schlom, J., D. Colcher, W. Drohan, R. Kettmann, R. Michalides, G. Vlahakis, and J. Young.** 1977. Differences in mouse mammary tumor viruses: relationships to early and late occurring mammary tumors. *Cancer* **39**:2727-2733.
31. **Sheffield, J. B., C. M. Zacharchuk, N. Taraschi, and T. M. Daly.** 1976. Effect of trypsin on mouse mammary tumor virus. *J. Virol.* **19**:255-266.
32. **Strannegard, O., I. Larsson, E. Lundgren, H. Miorner, and H. Persson.** 1978. Modulation of immune responses in newborn and adult mice by interferon. *Infect. Immun.* **20**:334-339.
33. **Strauchen, J. A., N. A. Young, and R. M. Friedman.** 1974. Interferon-mediated inhibition of mouse mammary tumor virus expression in cultured cells. *Virology* **82**:232-236.
34. **Tilles, J. G., and M. Finland.** 1968. Microassay for human and chick cell interferons. *Appl. Microbiol.* **16**:1706-1707.
35. **Wong, P. K. Y., P. H. Yuen, R. MacLeod, E. H. Chang, M. W. Myers, and R. M. Friedman.** 1977. The effect of interferon on *de novo* infection of Moloney murine leukemia virus. *Cell* **10**:245-252.
36. **Yagi, M. J.** 1973. Cultivation and characterization of BALB/cfC3H mammary tumor cell line. *J. Natl. Cancer Inst.* **51**:1849-1860.
37. **Yagi, M. J.** 1974. Further observations on the production of oncornaviruses by MJY-alpha cell line. *J. Natl. Cancer Inst.* **53**:1383-1385.
38. **Yagi, M. J.** 1975. Characteristics of mammary tumor cultures from four mouse strains infected with mammary tumor virus. *Cancer Res.* **35**:370-373.
39. **Yagi, M. J., P. B. Blair, and M. A. Lane.** 1978a. Modulation of mouse mammary tumor virus production in the MJY-alpha cell line. *J. Virol.* **28**:611-623.
40. **Yagi, M. J., and R. W. Compans.** 1977. Structural components of mouse mammary tumor virus. *Virology* **76**:751-766.
41. **Yagi, M. J., R. E. Stutzman, B. H. Robertson, and R. W. Compans.** 1978b. Structural components of mouse mammary tumor virus. II. Isolation and purification of virion polypeptides. *J. Virol.* **26**:448-456.