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Differential Response of Type C and Intracisternal Type A Particle Markers in Cells Treated with Iododeoxyuridine and Dexamethasone

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Mouse neuroblastoma cells containing intracisternal type A particles were treated with iododeoxyuridine and dexamethasone to induce the release of type C oncornavirus particles. For 5 days after treatment, antigenic markers and DNA polymerase activities specific to particles of each of the two types were assayed in the cells and in pellets obtained by high-speed centrifugation of the culture fluid. There was a marked release of C-particle antigen (p30) and DNA polymerase activity in extracellular particulate form, reaching a maximum on day 3 after treatment and falling thereafter. In contrast, no extracellular A-particle antigen was detected, and A-particle-specific DNA polymerase activity in the medium pellets did not increase from the original very low level. Electron microscopy confirmed the presence of free type C virus particles, but not intracisternal type A particles, in the culture fluid. Although intracellular levels of C-particle antigen rose 20- to 30-fold per milligram of cell protein, intracellular A-particle antigen and DNA polymerase activity did not vary more than two-fold. The relative rate of A-particle synthesis in the treated cells, as judged by incorporation of radioactive amino acids into the major structural protein (P73), was also unchanged over the period of observation. Thus, the induction of type C virus particle formation in cultured neuroblastoma cells had no detectable effect on the quantity, synthesis rate, or location of intracisternal type A particles.

Intracisternal type A particles are found regularly in mouse oocytes and in early embryos produced by normal fertilization or parthenogenetic activation (2, 3, 5, 6). Thus, it appears that the genetic information for production of these particles is vertically transmitted in mice. The particles are seen less frequently in later embryos (5) and rarely in adult tissues (23), but in many mouse tumors A-particle expression is again activated and appears to be a stable property of the transformed cells (9, 13, 20).

A biological function for intracisternal type A particles has not been defined. They exhibit some morphological properties characteristic of known oncogenic RNA viruses (8), and A particles from a variety of tissues contain a common structural protein (14) and characteristic DNA polymerase activity (21, 22). The possibility has been considered that intracisternal type A particles are precursors of known extracellular viruses, such as type C particles, or that they are an alternative form resulting from incomplete or aberrant expression of a viral genome. Expression of type C and A particles is known to vary independently among different cell lines (12) and primary tumors (10, 11). Furthermore, the main A-particle DNA polymerase and structural proteins are biochemically and immunologically distinct from those of type C particles (14, 21, 22). These results suggest that there is no direct precursor-product relationship between intracisternal A particles and extracellular type C particles. Also, a study of interspecies hybrid cells producing both types of particles has shown that the persistence of intracisternal A- and C-particles is dependent on retention of different mouse chromosomes (28).

Preparations of mouse intracisternal A-particles from both myeloma and neuroblastoma cells have been found to contain small amounts of high-molecular-weight RNA (19, 25, 26) and to carry out endogenous RNA-dependent DNA synthesis reactions (19, 25, 27). A possible relationship between type A particles and type C viral particles has recently been suggested by the work of Robertson et al. (19), who found significant homology between the RNA associated with intracisternal A-particles (isolated from mouse myelomas) and the RNAs of several murine leukemia and leukemia-sarcoma viruses. On the other hand, Wong-Staal and coworkers (25) reported that RNAs from both

myeloma- and neuroblastoma-derived A-particles had minimal homology with the RNAs of various mouse type C viruses. The problem of genetic relatedness between the two types of particles thus remains to be resolved.

The present study was undertaken to define more clearly any relationship between C- and A-particle biosynthesis taking place in the same cell. We have exploited the observation of Paran et al. (18) that corticosteroids greatly enhance the production of type C particles by mouse cells treated with iododeoxyuridine (IdU). It has been reported (24) that mouse neuroblastoma cells of the clonal line N4 respond to treatment with IdU plus dexamethasone by releasing C-particles into the culture fluid. Since these cells are also constitutive producers of intracisternal A-particles (17), we asked whether any change in A-particle markers occurs as the cells begin producing extracellular type C virus particles in large numbers. The results indicate that C-particle production is regulated independently of A-particle metabolism in these cells.

MATERIALS AND METHODS

Cells and particle preparation. Neuroblastoma clone N4, a continuous cell line derived from neuroblastoma C1300 (1), was obtained from M. Nirenberg, National Institutes of Health. The cells were grown as monolayers in Falcon T75 flasks in the Dulbecco-Vogt modification of Eagle medium supplemented with 10% fetal calf serum. Purified Rauscher murine leukemia virus (MuLV) was purchased from Electronucleonics, Inc., Bethesda, Md. Type A particles from the neuroblastoma C1300 solid tumor were purified through the isopycnic banding step as described previously (21).

Virus induction. Cultures were plated at 10^6 cells per Falcon T75 flask (from cultures in logarithmic growth) and grown in 10 ml of culture fluid for 2 days. The culture fluid was removed, and the cells were treated with 10 ml of culture fluid containing either 40 μg of IdU per ml or 1 μM dexamethasone sulfate, or with a combination of 40 μg of IdU per ml plus 1 μM dexamethasone as described by Paran et al. (18). After 24 h, this medium was removed and fresh culture fluid was added. Cells treated with IdU alone were subsequently grown in the regular culture fluid, whereas those treated with dexamethasone or with IdU plus dexamethasone were grown in culture fluid containing 1 μM dexamethasone. Every 24 h the culture fluid was removed and fresh culture fluid was added.

Preparation of culture fluid pellets. The culture fluid from three to six flasks was pooled and centrifuged at 2,000 $\times$ g for 10 min. The supernatant fraction was removed and from it a particulate fraction was isolated by centrifugation at 78,000 $\times$ g for 30 min. Pellets were resuspended in 200 μl of 50 mM Tris-hydrochloride, pH 7.5, 1 mM EDTA, 5 mM dithiothreitol, and 30% glycerol; this resulted in a 150- to 300-fold concentration of the particulate material in the 2,000 $\times$ g supernatant fraction. Pellets for buoyant density analysis were resuspended in the same solution without glycerol and analyzed by sucrose gradient centrifugation as previously described (16).

Cell extracts. Cell monolayers were washed three times with Hanks saline solution. The cells were then scraped in saline and collected by centrifugation at 200 $\times$ g for 5 min. Cell extracts were prepared by shearing the cells five times through 23-gauge hypodermic needles in 50 mM Tris-hydrochloride, pH 7.5, 1.5 mM $MgCl_2$, 5 mM dithiothreitol. Glycerol was added to a final concentration of 20% after the cells were broken.

Labeling of cells and isolation of A-particle antigen. Cultures were labeled for 4 h with a mixture of four L-^{3}H- or L-^{14}C-amino acids, and Triton X-100: EDTA-resistant pellets were isolated from the cytoplasmic fraction of the labeled cells as previously described (15). The pellets were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (15).

DNA polymerase assays. The assay for C-particle DNA polymerase activity was performed as described previously (16); unless indicated otherwise, each reaction contained: 50 mM Tris-hydrochloride, pH 7.7, at 37°C; 20% glycerol; 400 μg of bovine plasma albumin per ml; 0.05% Nonidet P-40; 2 mM dithiothreitol; 1 mM *p*-hydroxymercuribenzoate; 20 mM glycylglycine; 0.4 mM EDTA; 5 mM magnesium acetate; 40 mM KCl; 40 μg of $(dT)_{12-18}$ per ml; 200 μg of poly(rA) per ml; 0.5 mM [*methyl*-^{3}H]dTTP (300 cpm/pmol); and 5 μl of culture fluid pellet fraction. The assay for A-particle DNA polymerase activity was performed as described previously (21); reactions contained either 3 μl of culture fluid pellet fraction or 1 to 3 μg of cell extract protein. In both DNA polymerase assays, values were obtained in a range of proportionality between the incubation time and amount of enzyme-containing sample in reactions.

Because the catalytic properties of the type A particle and type C particle-associated DNA polymerases are different, reaction conditions may be selected for the specific assay of each enzyme (16, 21, 22). A comparison of the two DNA polymerase assays is shown in Table 1. Activity with the purified neuroblastoma type A particles was about twofold higher under the A-particle assay conditions than under the C-particle assay conditions. The preparation of purified type C particles (MuLV) was not active under the A-particle assay conditions, and under the C-particle assay conditions its activity per microgram of protein was about 500-fold higher than the activity of the purified type A particles. No significant inhibition was observed when the two kinds of particles were mixed.

Immunological assays. Murine type C viral antigen was measured by a microtiter complement fixation assay with an antiserum directed against MuLV p30 that had been purified by isoelectric focusing (16). Intracisternal A-particle antigen was also measured by complement fixation, using antiserum to P73 under conditions that have been described in detail (14).

TABLE 1. *Comparison of DNA polymerase assays and the activities associated with type A and C particles*

Prepn	Rate of DNA polymerase activity[a] (Δ pmol of [^{3}H]dTMP incorporated/min)	
	Type A particle assay conditions	Type C particle assay conditions
6 μg of purified type A particles (from neuroblastoma C 1300)	0.055	0.026
0.7 μg of type C particles (MuLV)	0	1.31
Mixture of 6 μg of type A particles and 0.7 μg of type C particles	0.054	1.15

[a] The values represent the difference between incorporation in reactions incubated with and without enzyme; values in reaction without enzyme were ≤0.007 pmol of [^{3}H]dTMP incorporated/min.

Electron microscopy. All fixation procedures were performed at 4°C. Cells were washed with Hanks saline solution and collected by scraping and centrifuging at 800 × *g* for 10 min. A solution containing 2% glutaraldehyde, 0.067 M sodium cacodylate buffer, pH 7.4, and 1% sucrose was added to the cell pellets and to culture fluid pellets. After 60 min, the pellets were cut into smaller pieces and fixed overnight. The fragments were washed thoroughly with 0.2 M sodium cacodylate buffer, pH 7.4, and then postfixed in Dalton's chrome-osmium for 50 min (7). After being washed with the 0.2 M sodium cacodylate buffer, the tissue was dehydrated by successive exposure for 10 min to 30, 50, 70, and 95% acetone and for 30 min to 100% acetone before being embedded in Epon-Araldite. Sections were placed on Formvar- and carbon-coated 200-mesh copper grids, stained with uranyl acetate and lead citrate, and photographed in a Siemens Elmiskop 1A at 80 kV.

RESULTS

Type C particle induction in neuroblastoma N4 cells. From electron microscopy, A-particle antigen assays, and DNA polymerase assays, it was known that the neuroblastoma N4 cells used in the current study contained abundant intracisternal type A particles. To establish that release of type C particles was induced by IdU and dexamethasone in this cell line, the experiment shown in Fig. 1 was performed. Measurement of the amount of type C particle DNA polymerase activity in the culture fluid pellets after treatment of neuroblastoma N4 cells with 40 μg of IdU per ml plus 1 μM dexamethasone revealed that the enzyme activity increased 29-fold during the period of 1 to 3 days of culture and then by day 5 had fallen to a level similar to that on day 1 after treatment (Fig. 1). This induction of C-particle DNA polymerase activity and its dependence on both IdU and dexamethasone were essentially identical to the results of Paran et al. (18) obtained with mouse fibroblast cells.

Some properties of the DNA polymerase activity in the culture fluid pellet fraction isolated after day 3 of treatment were examined in the experiment shown in Table 2. The properties of the DNA polymerase activity were as expected for C-particle DNA polymerase and were similar to those of the sample of MuLV that served as a reference enzyme; these reaction properties were different from those of the A-particle DNA polymerase, because the A-particle enzyme was relatively inactive with Mn^{2+} and was not inhibited by either 250 mM KCl or MuLV DNA polymerase antiserum (22).

Attempts to determine the buoyant density of the structure containing the type C particle DNA polymerase activity were unsuccessful due to extensive loss of enzyme activity during centrifugation. Such extensive loss of activity is unusual, since it had not been observed with the DNA polymerase associated with A-particles or with the various other C-particles tested.

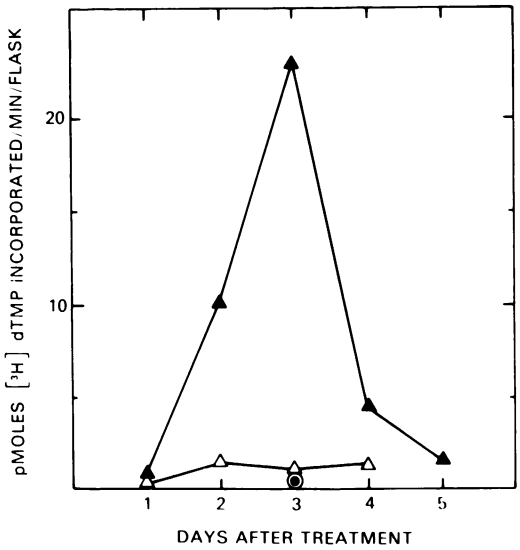

FIG. 1. *Effect of time in culture on the amounts of type C particle DNA polymerase activity in the culture fluid pellets from neuroblastoma N4 cells. Treatments of cells, preparation of pellets, and enzyme assays were performed as described in Materials and Methods. On each day, pellets were prepared from the culture fluids of cells that had been treated with IdU dexamethasone (▲) or IdU alone (△). Also shown are values for pellets prepared on day 3 from culture fluids of control cells (○) and cells treated with dexamethasone alone (●).*

Comparison of A- and C-particle markers during induction. As noted above, the reaction properties of the DNA polymerase associated with A- and C-particles are different, and conditions have been selected for the specific assay of each enzyme in samples containing a mixture of the two particles. Intracisternal A- and C-particles also have distinct antigenic markers; intracisternal A-particles contain a major structural protein of 73,000 daltons (P73), which is localized in the detergent-resistant inner particle shell (14) and carries a specific antigenic determinant not detected in type C particles (14). Conversely, intracisternal A-particles do not react with antisera against C-particle p30 antigen. This combination of markers was used to evaluate production of both kinds of particles after treatment of cells with IdU plus dexamethasone (Table 3).

TABLE 2. *Comparison of properties of the DNA polymerase activities associated with MuLV and with the culture fluid pellet from N4 cells grown for 3 days after treatment with IdU plus dexamethasone*

Modification of reaction	Amt of DNA polymerase activity (%)[a]	
	Culture fluid pellet from day 3, IdU plus dexamethasone-treated cultures	MuLV
None	100 (131)[b]	100 (136)
(+) 1 μl of antiserum to MuLV DNA polymerase	20	22
(+) 1 μl of control serum	70	82
(+) 210 mM KCl	0.2	0.5
(−) Mg^{2+}, (+) 1.5 mM Mn^{2+}	136	
(−) poly(rA)·$(dT)_{12-18}$, (+) poly(dA)·$(dT)_{12-18}$	0.5	0.5

[a] Unless otherwise indicated, reactions were performed as described in Materials and Methods, using 1 μg of MuLV or 3 μl of the culture fluid pellet fraction.

[b] Values in parentheses indicate the Δ pmol of [^{3}H]dTMP incorporated per reaction.

The increase in C-particle DNA polymerase activity in culture fluid pellets after treatment with IdU plus dexamethasone was accompanied by an increase in the amount of p30 antigen in the culture fluid pellets as well as in the cells. The peak in release of C-particles from the cells, as judged by the increase in both markers, occurred on day 3 after treatment. The amount of enzyme activity per unit of antigen was not constant during the induction period, however, showing that enzyme and p30 were not coordinately expressed in the extracellular particles.

The low levels of A-particle DNA polymerase activity found in culture fluid pellets did not increase after treatment, and there was little change in the activity in the cells. No A-particle P73 antigen was detected in culture fluid pellets, and the P73 antigen levels in the cells were unchanged. Thus, there was a dramatic increase in the C-particle markers both in the cell and in the culture fluid, whereas the A-particle markers remained relatively constant.

Treatment of cultures with IdU alone re-

TABLE 3. *Comparison of intracisternal A- and C-particle markers in the cells and in the culture fluid pellets after treatment with IdU plus dexamethasone*[a]

Days[b]	Cell protein (mg/flask)	C particle			A particle			
		DNA polymerase activity (medium pellet)	p30 antigen		DNA polymerase activity		p73 antigen	
			Medium pellet	Cells	Medium pellet	Cells	Medium[c] pellet	Cells
Treated cells								
1	1.22	0.7	79	49	0.10	1.87	<13	785
2	1.82	5.6	106	264	0.08	1.00	<9	528
3	1.42	16.2	723	519	0.11	0.75	<23	1,040
4	1.00	4.6	410	519	0.11	0.76	<26	1,038
5	1.26	1.4	NT	1,015	0.16	0.70	<80	1,020
Untreated cells								
3	4.10	0.07	62	15	0.09	0.69	<31	480

[a] Values in table are expressed as picomoles of [^{3}H]dTMP incorporated per minute per milligram of cell protein for DNA polymerase and as complement-fixing units per milligram of cell protein for antigen measurements. One unit of complement fixation is equivalent to the reciprocal of the end point dilution (14). NT, Not tested.

[b] Cells were grown as described in Materials and Methods, and on day 0 IdU and dexamethasone were added to the treated cells.

[c] Complement fixation was equal to or less than the values shown, which represent the level of anticomplementary activity of the samples.

sulted in a much smaller increase in C-particle DNA polymerase activity in culture fluid pellets than did treatment with IdU plus dexamethasone (Fig. 1). The levels of p30 in these culture fluid pellets were also only slightly increased, but total levels of p30 in cultures (cells and culture fluid pellets) appeared to be increased to almost the same extent as in IdU plus dexamethasone-treated cultures (870 and 1,240 complement-fixing units/mg of cell protein, respectively, compared to 77 units/mg of cell protein in untreated cultures). IdU treatment alone caused a slight increase in A-particle antigen (threefold) and A-particle DNA polymerase activity in the cells, but this increase did not appear to be the result of increased synthesis of A-particles (see below). Treatment of cultures with dexamethasone alone had no effect on C- or A-particle markers in the culture fluid pellets or in the cells.

Comparison of particle markers in optimally induced and control cultures. Examination of A-particle markers after induction of cultures with IdU plus dexamethasone revealed that no major changes had occurred on any of the days after treatment. Also shown in Table 3 is a comparison of the levels of A- and C-particle markers in control cultures and in cultures on day 3 after IdU plus dexamethasone treatment, when C-particle markers had reached their peak. C-particle DNA polymerase activity in the culture fluid pellets was markedly increased, whereas the A-particle DNA polymerase activity was unchanged. The C-particle p30 antigen was increased 34-fold in the cells and 12-fold in the culture fluid pellet after IdU plus dexamethasone treatment of the cultures, whereas the A-particle P73 antigen in the cells had increased only slightly and was not detected in culture fluid pellets before or after treatment. Cell division was inhibited in the treated cultures, accounting for the lower amounts of cell protein compared to the untreated cells.

Synthesis of A-particle structural protein. To further investigate the effect of the drug treatment on A-particle metabolism, the relative rate of synthesis of P73 was compared in treated and control cells. Cultures were labeled for 4 h with ^{3}H- or ^{14}C-amino acids, and the cells were then fractionated to yield pellets of Triton X-100:EDTA-resistant cytoplasmic material. We have previously shown that this fraction contains 95% of the total cellular A-particle antigen and displays P73 as a prominent component when the proteins are subjected to electrophoresis in sodium dodecyl sulfate-containing polyacrylamide gels (15). In the present experiments, Triton X-100:EDTA-resistant pellets from control cells and from cells cultured for 3 days after IdU plus dexamethasone treatment contained similar proportions (8.1 and 8.3%, respectively) of the total cellular incorporated radioactivity. The electrophoretic patterns of the pellet proteins are shown in Fig. 2. The radioactivity profiles were essentially identical, and P73 represented about 19% of the protein label in each case. Thus, in both control and treated cells, synthesis of the major A-particle structural protein accounted for approximately 1.5% (8% × 19%) of the total protein synthesis. Cells labeled on the other days after treatment with IdU plus dexamethasone also were analyzed this way, and no differences in the proportion of P73 synthesized were found. Treatment of cells with IdU alone, or dexamethasone alone, also had no effect on the relative rate of P73 synthesis 3 days after treatment.

Electron microscopic observations. Three days after treatment with IdU plus dexamethasone, neuroblastoma cells still contained large numbers of intracisternal A-particles (Fig. 3a). In addition, large numbers of type C particles were observed between the cells, both in singular form and in clusters (Fig. 3b). Immature type C particles (also referred to as enveloped A particles but distinct from intracisternal A-particles) were observed between cells, but budding particles were seen only occasionally (Fig. 3c). Examination of culture fluid pellets from these cells revealed clusters of C-particles and scattered immature C-particles (Fig. 3d). Fragments of rough endoplasmic reticulum containing intracisternal A-particles (Fig. 3e) were occasionally observed, and this may have accounted for the low levels of intracisternal A-particle DNA polymerase activity found in the culture fluid pellets.

DISCUSSION

The kinetics of induction of type C particle release from neuroblastoma cells after treatment with IdU plus dexamethasone were identical with those found by Paran et al. (18) for induction of C-particles from mouse fibroblasts. The peak of particle release occurred the same number of days after treatment whether assessed by antigen levels or by DNA polymerase activity. Budding and release of type C particles from the cells were confirmed by electron microscopy. In addition, the reaction properties of the DNA polymerase activity associated with the culture fluid pellets in these experiments were those expected for murine C-particle DNA polymerase. Our inability to band the enzymatically active material suggests, however, that the extracellular particles induced from neuro-

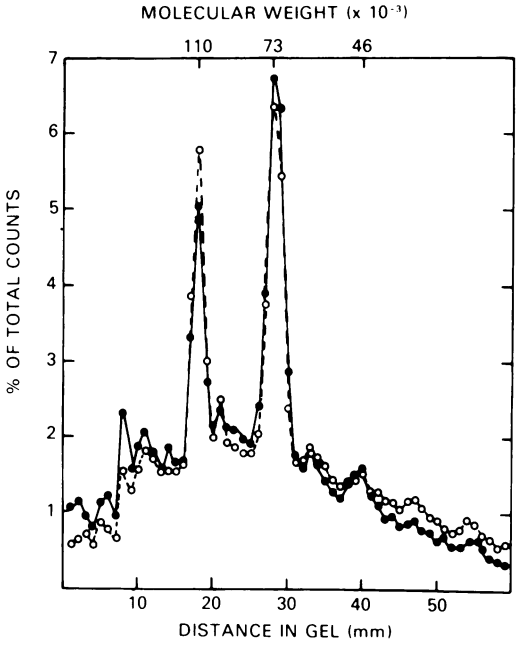

FIG. 2. *Labeling of A-particle structural protein (P73) in treated and control cells, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Triton X-100:EDTA-resistant pellets were isolated from neuroblastoma cells grown for 3 days after treatment with IdU plus dexamethasone and then labeled with ^{3}H-amino acids for 4 h (●), or from untreated cells similarly labeled with ^{14}C-amino acids (○). The pellets were dissolved in sodium dodecyl sulfate and mercaptoethanol, and samples containing 19,000 cpm of ^{3}H and 15,800 cpm of ^{14}C were subjected to co-electrophoresis on the same gel. The label in each gel fraction is expressed as percentage of total ^{3}H or ^{14}C label in the gel. Recovery of radioactivity from the gels was 110% for ^{3}H and 120% for ^{14}C. Molecular weights were obtained by reference to known standard proteins analyzed simultaneously on separate gels.*

blastoma cells were less stable than those induced from fibroblasts (18).

Induction of C-particles from neuroblastoma cells had no detectable effect on extracellular A-particle levels. The low levels of A-particle DNA polymerase activity found in culture fluid pellets probably resulted from A-particle-containing fragments of endoplasmic reticulum derived from damaged cells. Such fragments were observed in medium pellets from both treated and control cultures, whereas free A-particles of the intracisternal type were not seen. There was no evidence from antigen assay, enzyme measurements, or electron microscopy that intracisternal type A particles were actively released from cells either before or after treatment. If the failure of these particles to become extracellular under usual growth conditions results from lack of some essential cell or viral component(s), this component was not provided by activation of C-particle production in the cells.

Intracellular levels of A-particle antigen and DNA polymerase activity also showed little or no change during C-particle induction, and the relative rate of synthesis of the main A-particle structural protein was not altered in the treated cells. The twofold increase in A-particle antigen per milligram of cell protein seen in Table 3 was of questionable significance, since variations of this magnitude are within the limit of precision of the complement fixation assay. If real, this increase could well have resulted from preferential loss of other cell components in the treated cultures, since N4 cells exposed to IdU (with or without dexamethasone) ceased dividing and showed ultrastructural changes indicative of cell damage.

The lack of significant increase in A-particle markers after drug treatment might be attributed to the fact that the genetic information for these particles is already fully expressed in the untreated neuroblastoma cells. Billiau et al. (4) have described a fourfold increase in the concentration of A-particles visualized by electron microscopy in transformed mouse fibroblasts exposed to bromodeoxyuridine and dimethyl sulfoxide. However, more marked changes in intracisternal A-particle levels or induction of A-particle expression in previously negative mouse cells have not been demonstrated after treatment with halogenated pyrimidines. Intracisternal A-particles may differ characteristically from type C viruses in the extent to which their expression is augmented or activated by this class of compounds.

In conclusion, the synthesis rate, quantity, and localization of intracisternal A-particle markers remained relatively constant during a time when extracellular C-particles increased markedly and when there were high levels of C-particle-specific protein in the cells. The possibility remains that intracisternal A-particles are in some way related to an endogenous type C virus that did not respond to IdU-dexamethasone induction in the neuroblastoma cells. Nevertheless, the present results do not support the concept that murine intracisternal type A particles are precursors of extracellular type C virus and are consistent with earlier studies showing independent regulation of type C- and intracisternal type A-particle production in mouse cells (10–12, 28).

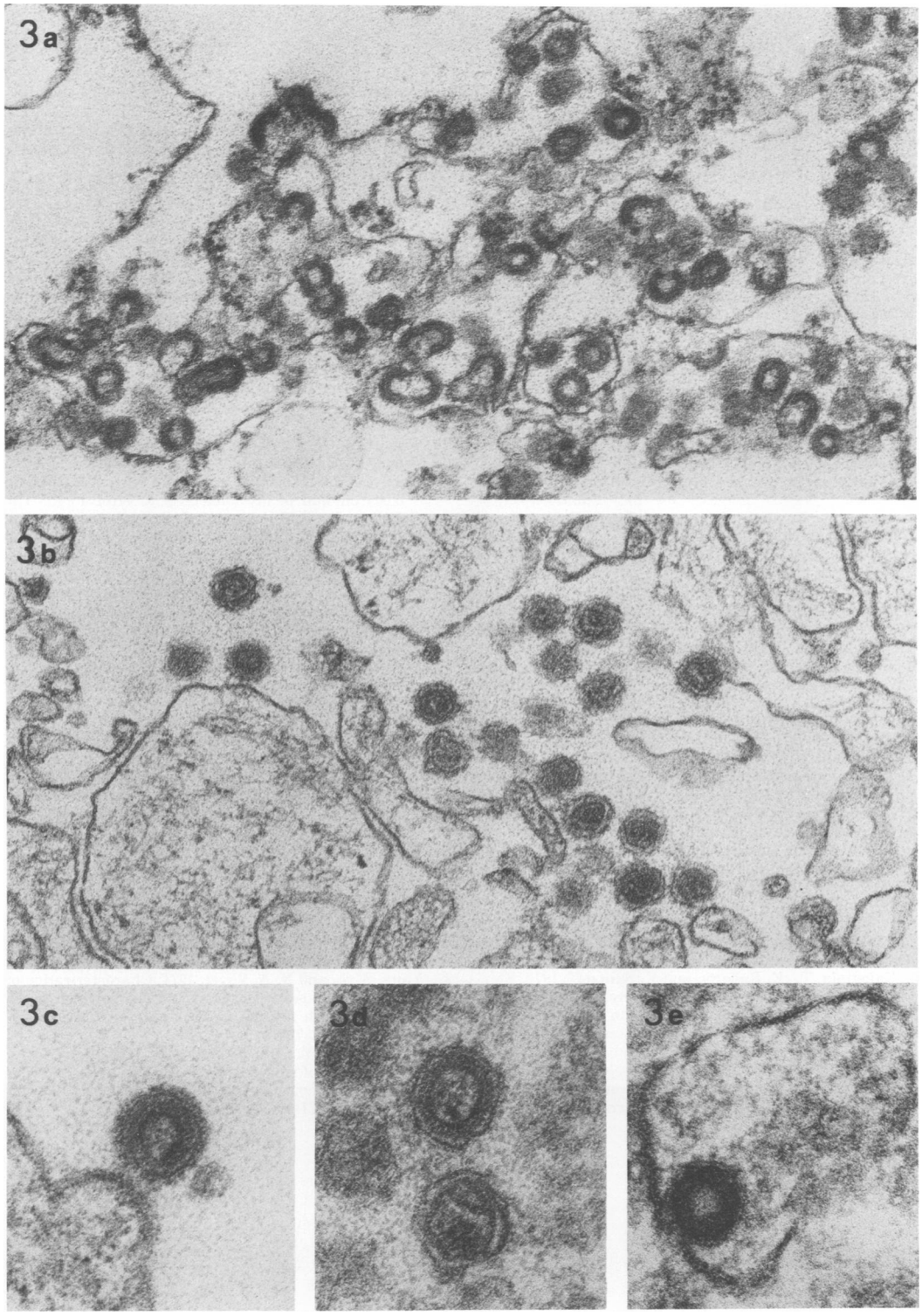

FIG. 3. *Electron micrographs of thin-sectioned material from neuroblastoma N4 cultures treated with IdU plus dexamethasone. (a) Rough endoplasmic reticulum containing intracisternal A particles in a cell 3 days after treatment. Magnification, ×70,000. (b) Cluster of C particles between two cells 3 days after treatment. Magnification, ×70,000. (c) Typical particle budding from the plasma membrane of a treated cell. Magnification ×140,000. (d) Immature C particle and a C particle in the culture fluid pellet from cells 4 days after treatment. Magnification, ×140,000. (e) Fragment of rough endoplasmic reticulum containing an intracisternal A particle in a culture fluid pellet from cells 3 days after treatment. Magnification, ×140,000.*

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LITERATURE CITED

1. **Amano, T., E. Richelson, and M. Nirenberg.** 1972. Neurotransmitter synthesis by neuroblastoma clones. Proc. Natl. Acad. Sci. U.S.A. 69:258–263.
2. **Biczysko, W., M. Pienkowski, D. Solter, and H. Koprowski.** 1973. Virus particles in early mouse embryos. J. Natl. Cancer Inst. 51:1041–1050.
3. **Biczysko, W., D. Solter, C. Graham, and H. Koprowski.** 1974. Synthesis of endogenous type-A virus particles in parthenogenetically stimulated mouse eggs. J. Natl. Cancer Inst. 52:483–489.
4. **Billiau, A., H. Sobis, and P. DeSomer.** 1973. Influence of interferon on virus particle formation in different oncornavirus carrier cell lines. Int. J. Cancer 12:646–653.
5. **Calarco, P. G., and D. Szollosi.** 1973. Intracisternal A-particles in ova and preimplantation stages of the mouse. Nature (London) New Biol. 243:91–93.
6. **Chase, D. G., and L. Piko.** 1973. Expression of A- and C-type particles in early mouse embryos. J. Natl. Cancer Inst. 51:1971–1973.
7. **Dalton, A. J.** 1955. A chrome-osmium fixative for electron microscopy. Anat. Rec. 121:281.
8. **Dalton, A. J.** 1972. RNA tumor viruses—terminology and ultrastructural aspects of virion morphology and replication. J. Natl. Cancer Inst. 49:323–325.
9. **Dalton, A. J., M. Potter, and R. M. Merwin.** 1961. Some ultrastructural characteristics of a series of primary and transplanted plasma-cell tumors of the mouse. J. Natl. Cancer Inst. 26:1221–1267.
10. **de Harven, E.** 1966. Primary methylcholanthrene-induced sarcomas of mice: an integrated ultrastructural and immunological study, p. 221–222. *In* R. Uyeda (ed.), Sixth International Congress on Electron Microscopy, Kyoto. Maruzen Co. Ltd., Tokyo.
11. **Gardner, M. B., J. E. Officer, R. W. Rongery, J. D. Estes, H. C. Turner, and R. J. Huebner.** 1971. C-type RNA tumor virus genome expression in wild house mice. Nature (London) 232:617–620.
12. **Hall, W. T., J. W. Hartley, and K. K. Sanford.** 1968. Characteristics and relationship between C particles and intracisternal A particles in clonal cell strains. J. Virol. 2:238–247.
13. **Kakefuda, T., E. Roberts, and V. Suntzeff.** 1970. Electron microscopic study of methylcholanthrene-induced epidermal carcinogenesis in mice: mitochondrial dense bodies and intracisternal A-particles. Cancer Res. 30:1011–1019.
14. **Kuff, E. L., K. K. Lueders, H. L. Ozer, and N. A. Wivel.** 1972. Some structural and antigenic properties of intracisternal A-particles occurring in mouse tumors. Proc. Natl. Acad. Sci. U.S.A. 69:218–222.
15. **Lueders, K. K., and E. L. Kuff.** 1975. Synthesis and turnover of intracisternal A-particle structural protein in cultured neuroblastoma cells. J. Biol. Chem. 250:5192–5199.
16. **Minna, J. D., A. F. Gazdar, G. M. Iverson, T. H. Marshall, K. Stromberg, and S. H. Wilson.** 1974. Oncornavirus expression in human × mouse hybrid cells segregating mouse chromosomes. Proc. Natl. Acad. Sci. U.S.A. 71:1695–1700.
17. **Minna, J. D., K. K. Lueders, and E. L. Kuff.** 1974. Expression of genes for intracisternal A-particle antigen in somatic cell hybrids. J. Natl. Cancer Inst. 52:1211–1217.
18. **Paran, M., R. C. Gallo, L. S. Richardson, and A. M. Wu.** 1973. Adrenal corticosteroids enhance production of type-C virus induced by 5-iodo-2′-deoxyuridine from cultured mouse fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 70:2391–2395.
19. **Robertson, D. L., N. L. Baenziger, D. C. Dobbertin, and R. E. Thach.** 1975. Characterization of DNA polymerase and RNA associated with A-type particles from murine myeloma cells. J. Virol. 15:407–415.
20. **Schubert, D., S. Humphreys, C. Baroni, and M. Cohn.** 1969. *In vitro* differentiation of a mouse neuroblastoma. Proc. Natl. Acad. Sci. U.S.A. 64:316–323.
21. **Wilson, S. H., E. W. Bohn, A. Matsukage, K. K. Lueders, and E. L. Kuff.** 1974. Studies on the relationship between deoxyribonucleic acid polymerase activity and intracisternal A-type particles in mouse myeloma. Biochemistry 13:1087–1093.
22. **Wilson, S. H., and E. L. Kuff.** 1972. A novel DNA-polymerase activity found in association with intracisternal A-particles. Proc. Natl. Acad. Sci. U.S.A. 69:1513–1536.
23. **Wivel, N. A., and G. H. Smith.** 1971. Distribution of intracisternal A-particles in a variety of normal and neoplastic tissues. Int. J. Cancer 7:167–175.
24. **Wivel, N. A., and S. S. Yang.** 1974. Induction of infectious virus in different clones of mouse neuroblastoma cells. J. Cell Biol. 63:751.
25. **Wong-Staal, F., M. S. Reitz, Jr., C. D. Trainor, and R. C. Gallo.** 1975. Murine intracisternal type A particles: a biochemical characterization. J. Virol. 16:887–896.
26. **Yang, S. S., and N. A. Wivel.** 1973. Analysis of high-molecular-weight ribonucleic acid associated with intracisternal A particles. J. Virol. 11:287–298.
27. **Yang, S. S., and N. A. Wivel.** 1974. Characterization of an endogenous RNA-dependent DNA polymerase associated with murine intracisternal A particles. J. Virol. 13:712–720.
28. **Yotsuyanagi, Y., and B. Ephrussi.** 1974. Behavior of three types of ribovirus-like particles in segregating hamster × mouse somatic hybrids. Proc. Natl. Acad. Sci. U.S.A. 71:4575–4578.