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Butyrate Increases Type-A and Type-R Virus-Like Particles in BKV-Transformed Hamster Spleen Cells (41701)

T. S. TRALKA, U. FLATOW, S. GOLDSTEIN, AND A. S. RABSON

Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Abstract. A cell line derived from hamster spleen transformed by human papovavirus BK was adapted to grow in 5 m*M* butyrate. Ultrastructurally, the butyrate-adapted cells were found to contain large numbers of type-R and intracytoplasmic type-A virus-like particles (VLP). In contrast, the unadapted cells contained only rare VLP. Treatment of the unadapted cells with nontoxic and toxic concentrations of butyrate for 24 and 48 hr failed to induce VLP, indicating the importance of butyrate adaptation in the induction of VLP. The increase in type-R VLP was stable after removal of the adapted cells from butyrate; however, the increase in type-A VLP was not.

The unique type-R virus-like particle (VLP) has been observed in many types of hamster cells (1–3) since it was first observed by Bernard and Tournier (4) in a BHK-21 clone 13 (C13) line of baby hamster kidney cells. Type-R particles have been classified as endogenous hamster virus particles (5, 6) because of their widespread occurrence in malignant hamster cells and because verticle transmission of type-R VLP in LSH strain of inbred hamsters has been demonstrated (7). The production of type-R particles in hamster tumor cells has been enhanced by dimethyl-sulfoxide (8), 5-bromodeoxyuridine (9), and in some instances by dexamethasone and 5-iododeoxyuridine (10). These compounds are known to be inducers of cell differentiation (11–13) and in some instances also induce murine retrovirus production (14–18). Type-R VLP isolated from culture fluids of BHK-21 clone F (CF) cells (19) and contain a 60–70 S RNA. There are contradictory reports concerning an RNA-directed DNA polymerase associated with these particles (19–21).

The hamster intracytoplasmic type-A particle has been described in Rous sarcoma virus transformed hamster cells (22), in an X-ray-induced hamster lymphoma (23), and in CELO virus-induced hamster tumors (24–26). In the mouse, similar type-A particles are morphological precursors (27, 28) of the murine mammary tumor type B virus. They are also found in chickens and thought to be a precursor of the type-C virion (29). It is unclear what their status is in the hamster.

Sodium butyrate, the salt of a natural 4 carbon fatty acid, is a known "differentiation inducer" (30–34) and can enhance induction of type-C virus by cyclohexamide in a BALB/c cell line (35). It also produces many morphological and biochemical modifications when added to cell cultures in millimolar concentrations (36–58). Many of these effects are reversible (49, 50, 53–55, 58). One of the most carefully studied properties of butyrate is the induction of histone hyperacetylation by inhibition of histone deacetylases (52–57). Butyrate also can inhibit DNA synthesis and cell division (34, 46–48), and cause under-methylation of DNA (59–60).

We have established a butyrate resistant line of a BKV-transformed hamster spleen cell line for the purpose of better understanding the mechanism of the numerous butyrate effects on cells. In examining this cell line by electron microscopy, intracytoplasmic type-A VLP and abundant type-R VLP were observed in almost every butyrate adapted cell in contrast to almost none in the control BKV-transformed cells. By manipulating the growth medium of the adapted cells and comparing them to the control cells, we tried to ascertain whether the enhancement of the type-R VLP was a function of the butyrate adaptation or merely of the presence of the butyrate.

Material and Methods. Hamster spleen cells from newborn Syrian hamsters were transformed *in vitro* by the MM strain of BK virus and maintained in continuous culture for 41 passages. They were grown in RPMI 1640 plus 20% FBS medium with increasing concentration of sodium *n*-butyrate over a period of 6

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months until they could be continually propagated at a final concentration of 5 m*M*. The butyrate-adapted cells (HSpB) differ from the unadapted cells (HSpC) in that they grow more slowly, exhibit contact inhibition, do not grow in soft agar, and morphologically are more flattened and adherent to the plastic surface. The HSpB cells had been passaged 30 times at the time of our electron microscopic studies.

Three replicates each of the following cell types were mechanically harvested and processed for electron microscopy: (i) The control hamster spleen (BKV-transformed) cells (HSpC); (ii) HSpC cells grown for 24 and 48 hr in 0.25 m*M*, 0.5 m*M*, 0.75 m*M*, 1 m*M*, 2 m*M*, and 3 m*M* butyrate; (iii) the 5 m*M* butyrate-adapted cells (HSpB); and (iv) HSpB-washed, trypsinized, and passed four times in control period of 2 weeks. All samples were thin sectioned, double stained with uranyl acetate and lead citrate, and examined in a Philips 400 electron microscope. In addition, 10^7, 10^6, and 10^5 HSpC and HSpB cells were injected subcutaneously on the dorsal surface of 25 Nu/Nu mice. The HSpB cells were much less tumorigenic than the HSpC cells. At Day 55 postinjection of 10^7 HSpB cells, 50% of the mice had tumors in contrast to 100% of animals injected with 10^7 HSpC cells. Samples of tumors arising after 40, 70, and 90 days were also looked at by electron microscopy. Approximately 300 cells were examined in all replicates from each type of cell treatment and from tumors derived from HSpB and HSpC.

Type-R VLP were enumerated per complete cell profile (118 HSpC and 120 HSpB profiles) using a Hewlett–Packard digitizer and computer.

Results. No type-R VLP were observed in 88% of HSpC cells and 12% contained one to five VLP (Table I). No intracytoplasmic type-A VLP were seen. Abundant type-R VLP measuring approximately 100 nm were seen in cisternae of the rough endoplasmic reticulum in most of the butyrate resistant cells (Fig. 1). As many as 39% of the HSpB adapted cells contained 11–45 type-R VLP (Table I). Many cells also contained intracytoplasmic type-A VLP which measured 80–90 nm (Fig. 2). Often the type-A particles were in close juxtaposition to the mitochondrial membrane.

TABLE I. TYPE-R VIRUS-LIKE PARTICLES (VLP) IN HAMSTER SPLEEN CONTROL (HSpC) AND HAMSTER SPLEEN BUTYRATE-ADAPTED (HSpB) CELL PROFILES

No. of VLP	Percentage HSpC (118 cells)	Percentage HSpB (120 cells)
0	88	0
1–5	12	25
6–10	0	33
11–45	0	39
46–65	0	3

When the butyrate-adapted cells were grown in control media for a period of 15 days, the type-R VLP were still abundant but only a few type-A particles were evident. Also type-R particles were still seen in abundance in the tumors produced by injecting butyrate resistant hamster spleen cells into nude mice; however, no type-A particles were visible. Only a few type-R particles and no type-A particles were observed in the tumors produced from the control hamster spleen cells.

No VLP were visible in the short-term treatment of HSpC cells with nontoxic concentrations (0.25 m*M*, 0.5 m*M*, and 0 .75 m*M*) of butyrate for 24 or 48 hr. In 1 m*M* butyrate, more than one-half of the cells were nonviable, but no VLP were seen. Severe cytotoxicity was evident with the short exposure to 2 and 3 m*M* butyrate and again no VLP were found.

Discussion. Effects of butyrate on several virus systems have been studied. In spite of its inhibition of cellular DNA replication it can permit viral DNA replication in SV40 (61), and adeno-5 infected cells (62). Butyrate can also enhance activation of integrated viral DNA as in type C virus in Balb/C cell line (35) and EBV, latent in P_3HR-1, Burkitt lymphoma cell lines (63). The mechanism of induction is unclear.

Butyrate produces such a multitude of effects on different cells, it is likely to have more than one mechanism of action. It causes hyperacetylation of histones by inhibition of histone deacetylases (54, 56, 57). Gene activation and transcription was thought to be induced via histone acetylation (64) because of the preferential solubilization of hyperacetylated chromatin following nuclease treatment of nuclei from butyrate-treated cells (65, 66). However, recently Perry and Chalkley (67) have shown that histone acetylation increases solubility of nucleosome oligomers and it is

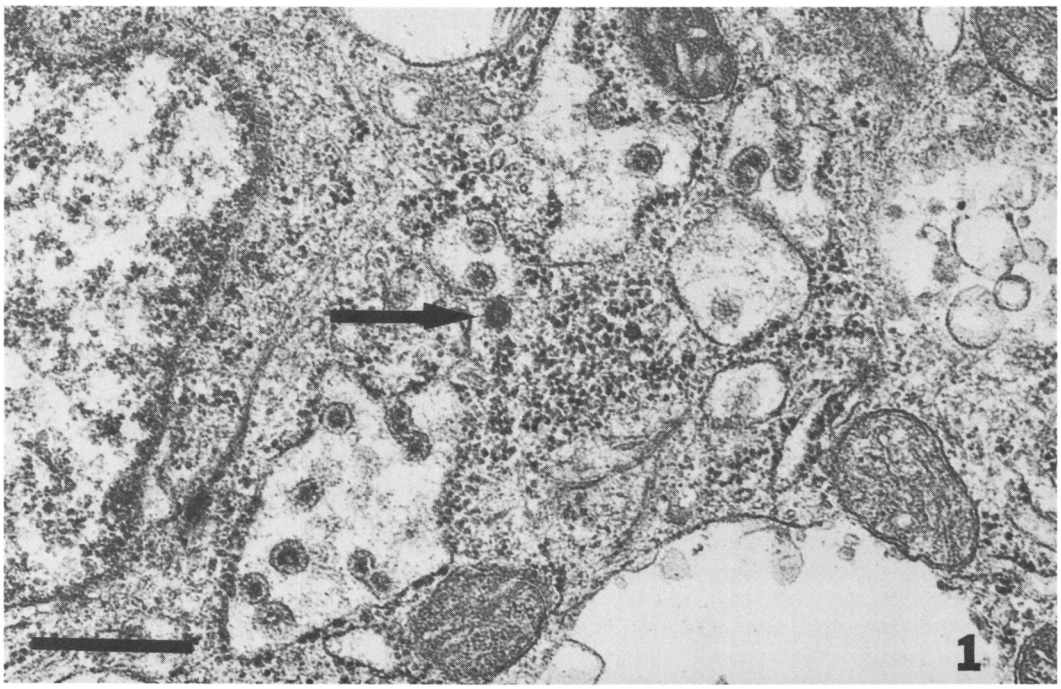

FIG. 1. Type-R VLP in RER of butyrate-adapted BKV-transformed hamster spleen cells. Type-A VLP in cytoplasm (arrow). ×42,000 Bar = 5 μm.

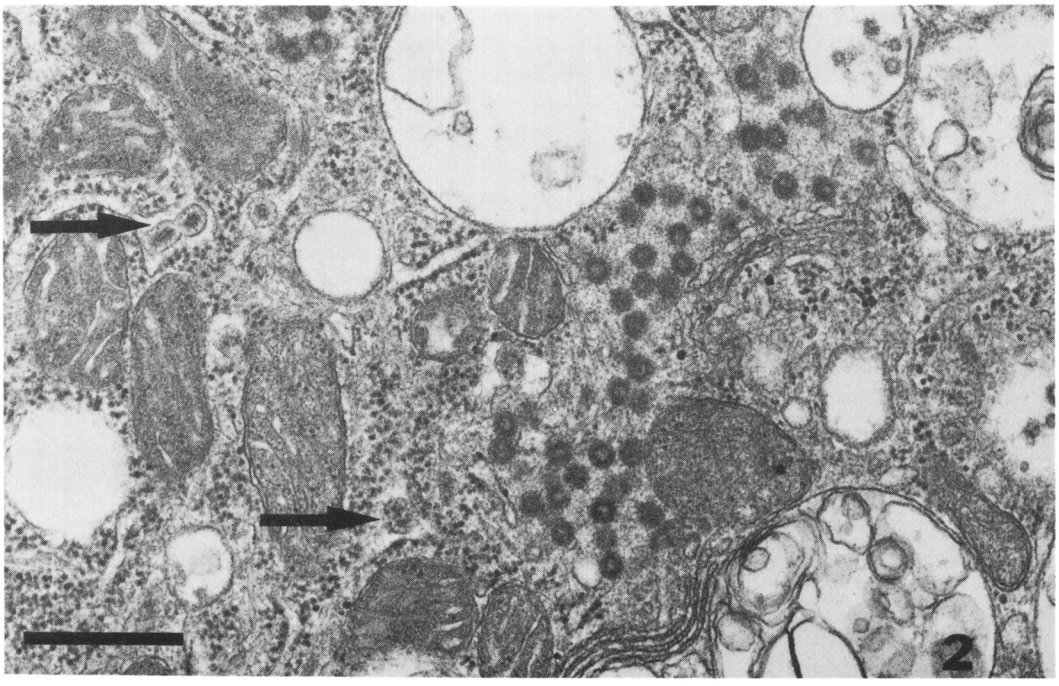

FIG. 2. Intracytoplasmic type-A VLP in butyrate-adapted BKV-transformed hamster spleen cells. Type-R VLP also present in RER (arrows). ×42,000. Bar = 5 μm.

comparable in both transcribed and nontranscribed chromatin. Butyrate also has the capacity to cause undermethylation of DNA (59, 60) which has been shown to be related to gene expression (68, 69).

Hyperacetylation occurred in the HSpC cells treated with butyrate for 48 hr and also in the HSpB cells growing in butyrate medium (manuscript in preparation); however, type-R VLP were not present in the butyrate short-term treated HSpC cells. It would seem then that the hyperacetylation did not directly cause type-R VLP induction.

It has been shown that infection of BHK cells by rubella virus (70) and also, transformation of BHK cells by polyoma virus either increases the number of cells producing the type-R VLP or increases the number of VLP per cell. In our hamster spleen cells the transformation with BKV apparently was not sufficient to induce type-R VLP.

Butyrate by itself did not enhance the type-R VLP even in the HSpC cells containing the BKV genome. It appears that the adaptation to butyrate was necessary to induce type-R VLP in the hamster cells.

In order to determine whether the induction of the type-R and type-A VLP by butyrate adaptation was reproducible, a BKV-transformed hamster ependymoma cell line was also examined after adaptation to growth in butyrate. There was a similar induction of both type-R and type-A VLP in the butyrate-adapted cells.

The intracytoplasmic type-A VLP was only observed in abundance in the butyrate-resistant mutant hamster spleen cells, seen less in those cells removed from the butyrate media, and not seen in HSpC cells or nude mouse tumors produced by injection of either HSpC or HSpB. The combination of the butyrate in the media, the presence of the integrated BK virus transforming gene and the butyrate-adapted phenotype all may be necessary to induce type-A VLP in the HSpB cells. This is unlike the type-R VLP which are still abundant in the absence of butyrate whether in tissue culture cells or in the tumors produced in nude mice.

The mechanism of the induction of the type-R VLP in the butyrate-adapted hamster spleen cells is still unclear. It is possible that only the cells with productive replication of type-R VLP at levels so low they were undetectable by electron microscopy were able to survive the butyrate adaptation; therefore, an enriched producer population remained. It is possible that during the time (6 months) in which the HSpC cells were being adapted to butyrate, the hamster spleen cells became irreversibly committed to type-R particle production due to a direct effect of butyrate on the integrated viral genome. The possibility also exists that the butyrate adaptation may induce production of a substance such as a butyrate resistant histone deacetylase which can directly stimulate the virus replication.

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