Epstein-Barr Virus: Stimulation By 5′-Iododeoxyuridine or 5′-Bromodeoxyuridine in Human Lymphoblastoid Cells From a Rhabdomyosarcoma*

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ABSTRACT

Epstein-Barr virus was observed by electron microscopy in human lymphoblastoid cells derived from a rhabdomyosarcoma. Replication of virus particles was stimulated approximately 10-fold after treatment of the cells with either 5′-iododeoxyuridine or 5′-bromodeoxyuridine. The evidence supports the concept that the entire Epstein-Barr virus genome persists in some cells of this type. The data also are consistent with the notion that this type cell cannot grow in continuous culture unless they carry at least part of the Epstein-Barr virus genome.

Introduction

Herpesviruses have been implicated as etiologic agents in certain chicken, primate and frog neoplasms. The Epstein-Barr virus (EBV), a member of the herpes group, has been discovered in cell cultures derived from Burkitt’s lymphoma (BL), identified as a causative agent of infectious mononucleosis and has a suspected role in the etiology of certain human lymphomas and nasopharyngeal carcinoma. Nucleic acid hybridization studies with BL and nasopharyngeal carcinoma biopsies have shown that EBV-DNA is consistently associated with these two tumors in vivo, whereas certain other tumors derived from EBV-seropositive patients with different types of malignancies gave no evidence of detectable EBV-DNA associated with tumor. Furthermore, EBV particles, early antigen and viral capsid antigen generally are not found in tumor cells of BL; however, EBV-associated membrane antigen regularly is detected in the tumor biopsies.

In the course of electron microscope screening of human sarcoma biopsies and cell cultures derived from these specimens, virus particles have been observed in certain cell cultures. This study presents some observations of virus particles, identified as EBV, in normal and chemically stimulated lymphoblastoid cells derived from a human alveolar cell rhabdomyosarcoma.

Materials and Methods

CELL CULTURE

Lymphoblastoid cells derived from a human rhabdomyosarcoma were propagated as static suspension cultures in McCoy’s 5A medium supplemented with 20 percent

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fetal bovine serum (FBS), penicillin (100 units per ml), streptomycin (100 µg per ml), and kanamycin (50 µg per ml).

**Chemical Stimulation**

The technique of Lowy et al. was used with slight modification. Cells were treated with medium containing 20 µg per ml of either 5′-iododeoxyuridine (IdU) or 5′-bromodeoxyuridine (BrdU) for three days. The cells then were resuspended in fresh, drug-free medium for three days, harvested, and processed for electron microscopy.

**Electron Microscopy**

Cell pellets were fixed in glutaraldehyde, osmium tetroxide, and embedded in epon 812. Thin sections were prepared with a Porter-Blum MT2B ultramicrotome. Sections stained with uranyl acetate and lead citrate were examined with a Siemens Elmiskop I electron microscope.

**Results**

Virus particles observed in untreated cells are presented in figure 1. The chromatin is diffuse and the nuclear inclusion is well defined. The virions are predominantly of double-shell morphology. Isolated, immature particles were occasionally observed in the cytoplasm, and one mature particle was seen in untreated cells (inset, figure 1). Moreover, the particles were observed in association with an otherwise healthy appearing cell. The virions in untreated cells have been identified as EBV.1

The morphology of cells containing virus particles and treated with either IdU or BrdU is in sharp contrast to the untreated cells. Virus particles were usually found in degenerating or completely disrupted cells. Note the extreme clumping and electron density of the chromatin in figure 2. The nuclear membrane, where visible, is thickened and very electron dense (figure 3).

Several morphological types characteristic of naked herpesviruses were observed in chemically stimulated cells. Single and double-shell particles with electron lucent centers and single shell virions with large, electron dense centers are illustrated in figures 2 and 4. The diameter of the naked particles averaged 100 to 110 nm and occasional mature particles were seen extracellularly (inset figure 4). Virus-containing, cytoplasmic inclusions were observed but these appeared to be contained in a phagocytic vacuole. An early stage of phagocytosis of virus-containing nuclear debris is illustrated in figure 3.

More than 1,000 untreated cells were screened; only two examples of virus particles were observed. Approximately the same number of chemically treated cells were screened and 21 of these elicited virus. Furthermore, electron microscopic analysis of the biopsy specimen failed to detect virus particles.

**Discussion**

The results reported here demonstrate a 10 fold increase in number of cells containing EBV after treatment with IdU or BrdU. Furthermore, the degenerative appearance of chemically treated cells containing EBV suggests a more progressive, lytic infection than in untreated cells. From these data, it is concluded that latent EBV genomes contained in these cells were chemically stimulated. The significance of these observations with respect to the etiology of this type tumor is unknown.

Gerber and Hampar et al. demonstrated the activation of infectious virus by BrdU in Raji cells. Their results suggested that the entire viral genome may persist in at least some cells. Hybridization studies of EBV RNA and DNA with Raji cell DNA have given impetus to the notion that human lymphoblastoid cells cannot grow in continuous culture unless they carry at least part of the EBV genome.
Figure 1. Epstein-Barr virus in the nucleus of an untreated lymphoblastoid cell derived from a human rhabdomyosarcoma. The chromatin is diffuse and the nuclear inclusion well defined. (×16,800) Inset: Mature virion located outside untreated cell. (×62,400) Figure 2. IdU treated cell showing the degenerated nucleus containing immature EBV. Chromatin is clumped and the nuclear membrane, where visible, is thickened. (×40,000)
Figure 3. IdU treated cells showing virus-containing nuclear debris of a disrupted cell being engulfed by a neighboring cell. (×13,000) Figure 4. Naked EBV particles associated with a degenerating cell. The particles are primarily single shell with a dense center. (×46,000) Inset: Mature virion located outside IdU treated cells. (×85,000)
Although the studies presented here deal with a lymphoblastoid cell line from a different type tumor, the data support this latter hypothesis and further indicate that the entire EBV genome persists in at least some cells.

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References

1. DALTON, A. J.: Personal communication.