JOURNAL OF VIROLOGY, July 1974, p. 174-176
Vol. 14, No. 1
Printed in U.S.A.
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Host Cell Regulation of Induction of Epstein-Barr Virus

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Received for publication 14 February 1974

When Epstein-Barr virus (EBV) negative cells (Raji) were treated with iododeoxyuridine, only the early antigen (EA) component was induced. There was no significant increase in EBV DNA and no virus particles were observed. Somatic-cell hybrids were prepared from the fusion of Raji and D98 cells (D98/Raji). When these cells were treated with iododeoxyuridine, early antigen EBV DNA, and virus particles were synthesized. These data suggest cellular control over the expression of the EBV genome.

In some human lymphoblastoid cell lines derived from patients with Burkitt's lymphoma only a few cells (2 to 10%) spontaneously synthesize Epstein-Barr virus (EBV) even though all the cells in the population in these producer cultures (e.g., HR-1) are capable of allowing expression of the EBV genome spontaneously, as determined by cloning experiments (5). However, other lymphoblastoid cell lines (i.e., Raji) do not spontaneously synthesize EBV antigens, though they also contain EBV DNA, as determined by nucleic acid hybridization techniques (6, 7).

The somatic-cell-hybrid, D98/HR-1 (2) produced by the fusion of HR-1 cells (producer) to a human HeLa cell variant (D98) contains EBV DNA, but the EBV genome is repressed (i.e., no detectable EBV specific-antigens are synthesized). When the D98/HR-1 cells were treated with bromodeoxyuridine (BUdR) or iododeoxyuridine (IUdR), the cells were induced to synthesize EBV DNA, early antigen (EA), virus capsid antigen (VCA), and virus particles (3, 4). When Raji cells were treated with BUdR or IUdR, only the EA component was induced (1; B. Hampar, W. Henle, and K. Traul, personal communication). No other virus marker was detected by the immunofluorescence (IF) test. In this report we present data that show that the EBV genome in Raji cells, which codes only for EBV EA after treatment of Raji cells with IUdR, can code for other virus-specific markers when the virus genome is transferred to another cell.

Somatic-cell-hybrids were prepared by fusing Raji cells with D98 cells by the procedure already published (2). Confirmation of cell hybridization was determined by chromosome analysis. Raji cells were maintained in RPMI 1640 medium and the hybrid cells were maintained in HAT selective medium (2, 3). The modal number of chromosomes for D98 cells was 61–62 (2) and the modal number for Raji cells was 46. As can be seen in Table 1, the resulting three clones of hybrid cells, D98/Raji, had modal numbers from 83 to 88 when examined at two different passage levels.

Induction studies were performed by growing Raji cells in RPMI 1640 medium containing 60 μg of IUdR per ml for 3 days at 37 C. The cells were centrifuged and resuspended in fresh RPMI 1640 medium containing no drug for an additional 3 days. Cells were examined for EBV EA and VCA by IF by using acetone-fixed smears, and EA- or VCA-specific-antisera (obtained from Werner Henle) (3, 4). In addition, IUdR-treated Raji cells were harvested on the same day and examined for EBV genomes by nucleic acid hybridization tests. The procedure for the DNA-cRNA assay has been previously published (4, 6). D98/Raji hybrid cells were treated with IUdR in a similar way, and identical IF and nucleic acid hybridization assays were performed. IUdR-treated Raji and D98/Raji cells were also fixed for electron microscopy 7 days after removal of drug, to determine if virus particles had been synthesized (3).

The results of the IF and electron microscopic assays are shown in Table 2. Raji cells synthesized only EA after treatment with IUdR as previously shown by others. No virus particles were detected by electron microscopy. D98/Raji cells were negative for EBV specific-antigens by IF. When the D98/Raji cells were treated with

IUdR, EA, VCA, and virus particles were induced within 7 days after removal of IUdR (Table 2 and Fig. 1). In addition, as shown in Table 3, there was no significant increase in EBV DNA in Raji cells after treatment with IUdR whereas a large increase in hybridizable EBV DNA was detected in the D98/Raji hybrids.

The induction of EBV-specific markers in the D98/Raji cells is similar to the data obtained by using D98/HR-1 hybrid cells (3, 4). However, the D98/HR-1 hybrids contain EBV genomes derived from an EBV-producer cell line, HR-1, whereas the D98/Raji cells contained EBV genomes derived from the EBV-negative Raji cell line.

TABLE 1. *Number of chromosomes in D98/Raji hybrid cells*

Clone	Subculture level[a]	No. of chromosomes[b]	
		Range	Modal no.
3	8	86-89	87-88
	34	82-88	86
4	8	82-89	85-86
	34	80-86	83
16	8	80-119	85-87
	34	81-89	88

[a] All clones were grown in HAT medium.
[b] Metaphase chromosomes were prepared by the procedure already described (2).

Spontaneous expression of antigen production is repressed in D98/Burkitt hybrid cells derived from both producer and nonproducer Burkitt cell lines. Whether or not this phenomenon is due to a specific repressor or inhibitor of the EBV genome is presently being explored. Though spontaneous EBV antigen production is repressed in D98/Raji hybrid cells the regulatory mechanism controlling virus induction appears to be different from the regulatory mechanism in Raji cells.

TABLE 2. *Induction of EBV antigens and virus particles in Raji and D98/Raji cells as measured by the indirect IF test[a] and electron microscopy[b]*

Cell line	EBV antigens[c]		Virus particles[d]
	EA	VCA	
Raji	+	−	−
D98/Raji[e]	−	−	−
D98/Raji	+	+	+

[a] As previously described (3, 4).
[b] As previously described (3).
[c] Treated with 60 μg of IUdR per ml for 3 days at 37 C. Cells were maintained in medium for an additional 3 days (D98/Raji cells in Eagle medium and Raji cells in RPMI 1640 medium).
[d] Treated with 60 μg of IUdR per ml for 3 days at 37 C. Cells were maintained in normal medium for an additional 7 days.
[e] Control, no IUdR.

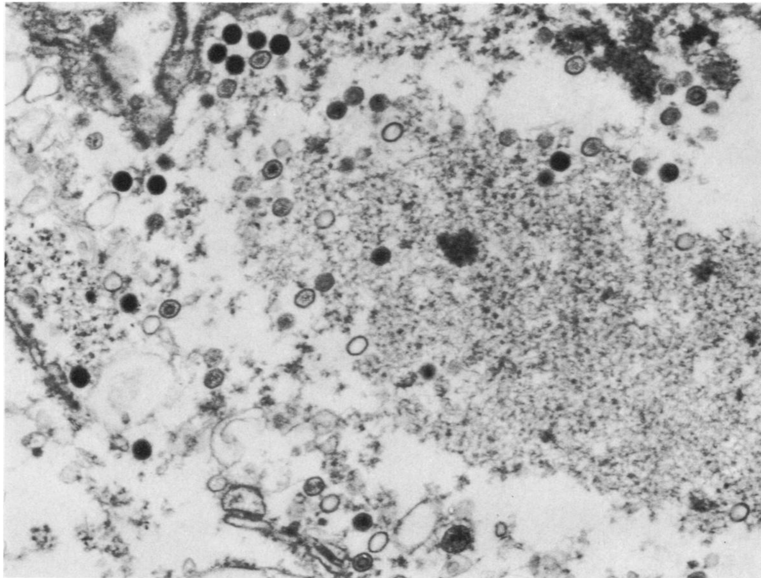

FIG. 1. *Electron micrograph of D98/Raji cells treated with 60 μg of IUdR per ml, 7 days after replacement of medium containing IUdR with normal medium. Note particles with both empty and dense cores. Magnification approximately ×30,000.*

TABLE 3. *DNA-RNA hybridization tests*

DNA on filter	cRNA hybridized (counts per min per 50 μg of DNA)	Estimated no. of genome equivalents per cell
Raji	5,000	50
Raji[a]	5,206	54
D98/Raji clone 4	143	3
D98/Raji[a] clone 4	27,672	346

[a] Treated with 60 μg of IUdR per ml for 3 days, followed by growing the cells in normal medium for additional 3 days.

We had previously suggested that either qualitative or quantitative differences in EBV genomes in EBV-positive and EBV-negative lymphoblastoid cell lines could account for differences in expression of the EBV genome (4). Whether this is true remains to be clarified. However, we have now shown that the EBV genome associated with a negative lymphoblastoid cell and which can only be induced to synthesize EBV EA after induction with IUdR, can be induced to specify more virus components when the genome is associated with another cell. These data suggest cellular control over the degree of induction of the latent virus.

We thank Ross Farrugia and Martin Nau for excellent technical assistance. The interest, support, and constructive criticism of the manuscript by Fred Rapp is gratefully acknowledged.

This study was conducted under Contract no. 70-2024 and Contract no. NOI-CP-33219 within the Virus Cancer Program of the National Cancer Institute and a grant from the Illinois division of the American Cancer Society no. 73-23 and a Public Health Service grant from the National Cancer Institute (no. CA15281-01).

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