Rescue of a Biologically Active Epstein-Barr Virus from Nonproducer Cells

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ABSTRACT

We recently established an epithelial/hybrid cell line, A2L/AH, by fusion of 8-azaguanine-resistant epithelial cells, Ad-AH, with a nonproducer lymphoblastoid cell line, A2L, using lymphocytes derived from the human nasopharynx transformed with the B95-8 strain of Epstein-Barr virus. The treatment of the parental A2L lymphoid cells with iododeoxyuridine led to the expression of Epstein-Barr virus early antigen, but neither virus capsid antigen or induction of Epstein-Barr virus DNA synthesis was observed. However, the treatment of the A2L/AH hybrid cells with iododeoxyuridine led to early antigen, virus capsid antigen and virus DNA synthesis, and the formation of virus particles. The virus rescued from the A2L/AH hybrid cells transforms human cord blood lymphocytes but is not able to induce early antigen in superinfected Raji cells.

INTRODUCTION

EBV is the etiological agent of infectious mononucleosis (1) and is closely associated with Burkitt's lymphoma (2) and NPC (3, 4). In vitro, EBV can infect B-lymphocytes, but has also been shown to infect normal and transformed epithelial cells (5). The study of the interaction between EBV and NPC has been impeded by the lack of EBV genome-positive epithelial cell lines and the limited susceptibility of epithelial cells to EBV. The tropism of EBV for B-lymphocytes is apparently related, at least in part, to EBV receptors.

Until now, some alternative methods have been used to study the replication of EBV in nonlymphoid cells (6-10). So far, these methods have proved successful in that EBV is able to undergo a lytic replicative cycle in epithelial cells in culture. In some instances, biologically active virus with transforming activity is recovered (11). However, the release of EBV from the epithelial cells is transient, and insufficient virus is recovered for biological and biochemical studies.

The use of EBV genome-positive epithelial/hybrid cells to study the host regulation of EBV expression has proven to be helpful in studying virus cell interactions (12, 13). We have continued studies related to the expression of EBV in epithelial cells by the preparation of new hybrid cells. We recently established an epithelial/hybrid cell line, NPC-KT (14), derived from the fusion of EBV genome-negative human adenoid epithelial cells, Ad-AH (15), and primary EBV genome-positive NPC cells. We succeeded in isolating biologically active virus from the NPC-KT cells (16). We have now established another epithelial/hybrid cell line, A2L/AH, derived from the fusion of human adenoid-derived epithelial cells, Ad-AH, and nonproducing EBV genome-positive lymphoblastoid cells, designated A2L (15). We report here the first successful rescue of biologically active EBV from an epithelial/lymphoblastoid hybrid cell line.

MATERIALS AND METHODS

Cell Lines. The epithelial A2L/AH hybrid cell line was maintained in Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. The A2L/AH cells are epithelial hybrid cells derived from the fusion of the EBV genome-negative epithelial Ad-AH cells derived from a human adenoid, and the nonproducing EBV genome-positive A2L cells. A2L cells are B-lymphocytes obtained from an adenoid removed as part of routine surgery and transformed by B95-8 virus in vitro. Both parental cells, therefore, are derived from human nasopharyngeal tissues (15). A2L/AH cells and the A2L parental cells are positive for EBNA but do not spontaneously express EA or VCA. A2L and B95-8 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics.

Induction of EBV with IdUrd. A2L/AH hybrid cells and A2L parental cells were treated with IdUrd for 3 days and then incubated in growth medium for an additional 7 days at 37°C as previously described (6). The cells were then examined for the expression of EA and VCA using the indirect IF test. The supernatant fluids from IdUrd-treated cells were pooled and concentrated by centrifugation at 28,000 rpm for 90 min in a Beckman Type 30 rotor, and the resulting pellet was resuspended in a small volume of medium in order to achieve a concentration of 100%. Extracellular virus was concentrated using the same procedure from B95-8 cells maintained for 7 days at 37°C without feeding.

Immunofluorescence Test and Electron Microscopy. The indirect IF test was used to detect EA and VCA as previously described, using EA positive/VCA positive and EA negative/VCA positive human sera (16). Detection of virus particles in the cells by electron microscopy were performed by examining thin sections as previously described elsewhere (6, 16).

Analysis of DNA Isolated from A2L/AH Cells. A2L/AH hybrid cells were treated with IdUrd. The medium containing IdUrd was then removed and the cells were incubated in normal medium for an additional 7 days at 37°C. The density of DNA isolated from A2L and A2L/AH cells was analyzed for cellular and virus DNA in CsCl gradients as described elsewhere (17, 18).

Assay for Virus Infectivity. Transforming virus was titered by the method described by Katsuki and Hinuma (19), and EBV-induced EA was assayed using the method described by Sairenji and Hinuma (20).

RESULTS

Expression of the EBV Genome in A2L Parental Cells and A2L/AH Hybrid Cells. Early passage (18th) A2L/AH hybrid and parental A2L cells (25th) were found to be positive for EBNA, but did not express EA or VCA spontaneously, as previously described (Table 1) (14). Thereafter, late passage A2L/AH cells (55th) spontaneously expressed EA in <0.1% of cells, whereas A2L cells (at passages 45 and 75) were still EA/VCA negative. The A2L/AH cells and A2L cells were further examined for the induction of EA and VCA with IdUrd using the indirect IF test and the formation of virus particles by electron microscopy. The results are shown in Table 1. A2L cells synthesized EA but not VCA after treatment with IdUrd. In contrast, the A2L/AH cells treated with IdUrd synthesized both EA and VCA after treatment with IdUrd. Moreover, virus particles were detected in the A2L/AH cells when incubated for an additional 7 days after removal of IdUrd (Table 1; Fig. 1).

Titration of Biologically Active EBV from A2L/AH Cells. In
order to determine if the virus rescued from the A2L/AH cells was infectious, we assayed the virus for the ability to transform B-lymphocytes and induce EA in superinfected Raji cells. The results are shown in Table 2. We found that the infection of human cord blood lymphocytes with the virus resulted in the establishment of lymphoblastoid cell lines and that these cell lines were positive for EBNA but negative for EA and VCA. In four transformation experiments with virus from the A2L/AH cells of different passages, the titrations of concentrated supernatant fluids from the mock-treated A2L/AH cells or the IdUrd-treated A2L cells. B95-8 cells grown at 37°C for 7 days without feeding were used as controls. No evidence of spontaneous transformation was observed in uninfected human cord blood lymphocyte cultures. The titers of the supernatant fluid from the IdUrd-treated A2L/AH cells were approximately equal to the titers of EBV from B95-8 cells starved for 7 days at 37°C. The virus preparations from the A2L/AH cells were unable to induce EA in superinfected Raji cells, unlike NPC-KT virus as previously reported (16).

Analysis of DNA Isolated from Either Untreated or IdUrd-treated A2L/AH Cells. Fig. 2 shows EBV DNA replication in IdUrd-treated and control A2L/AH cells. The untreated control A2L/AH cells exhibited only one sharp peak of cellular DNA (density, 1.698 g/cm³). In contrast, the IdUrd-treated A2L/AH cells showed two peaks, one with a density of 1.718 g/cm³ which is the density of EBV DNA, and a second cellular DNA peak.

**DISCUSSION**

In this paper, we demonstrated the rescue of biologically active EBV from an epithelial/lymphoblastoid hybrid cell line derived from the fusion of the nasopharyngeal epithelial cell line (Ad-AH) and nonproducing EBV genome-positive lymphoblastoid cells, A2L.

Human lymphoblastoid cell lines transformed by B95-8 EBV are generally nonproducers. Human nasopharyngeal A2L cells are nonproducer EBNA-positive cells, similar to Raji cells, and like Raji cells, the EBV genome could not be induced to replicate and complete the lytic cycle after treatment with IdUrd (12). The results with the A2L/AH are similar to those previously obtained with D98/Raji hybrid cells with regard to the expression of the EBV genome but in those studies we could not demonstrate that the virus particles rescued from D98/Raji cells were infectious (12).

The virus rescued from the A2L/AH hybrid cells was able to transform human cord blood lymphocytes. To our knowledge, the A2L/AH cells are the first hybrid cell line from which a latent EBV genome (derived from a nonproducer lymphoblastoid cell line) can be induced to synthesize biologically active (transforming) virus. The approach used in this study and a previous study (15) may be useful in obtaining EBV isolates from nonproducer cells such as NPC tumor cells or cell lines which may have unique viral genomes.

The biological activity of the virus released from A2L/AH hybrid cells is the same as that of B95-8 virus which was used to transform the B-lymphocytes to make the A2L cell line. It is possible that different biologically active viruses may be obtained from the other EBV-carrying lymphoid cells using...
EBV replication is at least in part under host cell regulation. Somatic cell hybrids have been used to study the host cell regulation of EBV replication (12, 13, 21, 22). The available data suggest that some factors in nonproducer lymphoblastoid cells depress a complete virus productive cycle and that this block(s) is overcome by the human epithelial cells. Interestingly, in this study we were able to recover transforming EBV after the treatment of A2L/AH cells with IdUrd, which was not the case for IdUrd-treated D98/Raji or D98/HR-1 hybrid cells (6, 12). It may be that some factor(s) necessary for the step(s) to produce biologically active virus in the induced D98/HR-1 or D98/Raji cells is missing, whereas the A2L/AH hybrid cells possess the factor(s) required for the production of biologically active virus. It is possible that Ad-AH cells may be permissive for this function and useful for studying the cell-associated mechanism of the production of biologically active virus.

REFERENCES

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