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EXPRESSION OF THE EPSTEIN-BARR VIRUS (EBV) RECEPTOR ON THE SURFACE OF CELLS INFECTED WITH EBV DERIVED FROM NASOPHARYNGEAL CARCINOMA

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Summary. — An Epstein-Barr virus (EBV) genome-positive epithelial hybrid cell line, NPC-KT, derived from the fusion of primary nasopharyngeal carcinoma cells with a human epithelial cell line of adenoid origin and a subline of EBV genome-positive Ramos cells, Ramos/NPC, converted after infection with NPC-KT EBV have been previously described (Takimoto *et al.*, 1984; Takimoto *et al.*, 1987). The NPC-KT cells produce virus (NPC virus) with both transforming and lytic properties. In this study, NPC-KT and Ramos/NPC cells were examined for the presence of the EBV receptor as measured by the capacity to absorb radiolabelled P3HR-1 and NPC viruses. It was determined that only P3HR-1 virus can attach to NPC-KT cells. Also, the relative concentration of NPC virus receptors on Ramos/NPC cells was found to be significantly reduced when compared to EBV genome-negative Ramos cells, whereas the relative concentration of receptors for P3HR-1 virus was similar to parental Ramos cells. The results suggest that there are differences at least in part of the receptors for P3HR-1 and NPC viruses.

Key words: *Epstein-Barr virus*; *receptor*; *NPC-KT cells*; *Ramos cells*

Introduction

The Epstein-Barr virus (EBV) is the aetiological agent for infectious mononucleosis (Henle *et al.*, 1968). In addition, it has also been associated with two malignant diseases: Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) (Epstein *et al.*, 1964; Henle *et al.*, 1973).

Previously, we described an EBV genome-positive epithelial/NPC hybrid cell line, designated NPC-KT, that was established by fusion of primary EBV genome-positive NPC epithelial cells with an epithelial cell line derived from human adenoid tissue (Takimoto *et al.*, 1984). The NPC-KT cells produce infectious EBV (designated NPC virus) with transforming and lytic early antigen (EA)-inducing activities (Takimoto *et al.*, 1985). We also described the establishment of an EBV-associated nuclear antigen (EBNA)-positive

subline of Ramos, designated Ramos/NPC, following infection of Ramos cells with NPC virus (Takimoto *et al.*, 1987).

We have recently described the successful superinfection of NPC-KT cells with EBV derived from P3HR-1 cells (Fig. 1) but not of NPC-KT cells with NPC virus (Takimoto *et al.*, 1986). In this study, we have found differences in the relative number of EBV receptors on the surface of NPC-KT and Ramos/NPC cells.

Materials and Methods

Cells. The EBV genome-positive Ramos (Klein *et al.*, 1975), P3HR-1 virus-converted Ramos (AW-Ramos) (Fresen *et al.*, 1976), NPC virus-converted Ramos (Ramos/NPC) (Takimoto *et al.*, 1987), Raji and P3HR-1 cell lines were maintained in RPMI-1640 medium supplemented with 10 % fcetal bovine serum (FBS) at 37 °C. The NPC-KT cells were maintained in Eagle's medium containing 10 % FBS at 37 °C.

Preparations of (^3H)-labelled EBV. P3HR-1 virus was obtained from supernatants of P3HR-1 cell cultures treated with 12-0-tetradecanoylphorbol-13-acetate (TPA) (20 ng/ml) for 7 days at 35 °C as previously described (Takimoto *et al.*, 1986). NPC virus was obtained from supernatants of NPC-KT cell cultures treated with 5-iododeoxyuridine (IUdR) (60 μg/ml) for 3 days, then grown for 7 days in normal medium at 32 °C as previously described (Sato *et al.*, 1986). Each virus was labelled metabolically with (methyl-^3H)-thymidine (specific activity: 60 Ci/mmol) by adding 1 μCi/ml of the isotope at a two-day interval to the virus-producing cultures. Thereafter, each supernatant was centrifuged to pellet the virus as previously described (Sato *et al.*, 1986). Virus pellets were resuspended in RPMI-1640 medium to make approximately 1000 × concentrates, and purification was then achieved by centrifugation in 5 to 30 % Dextran T-10 gradients as previously described (Koide *et al.*, 1980). Biologically active virus was collected from the 17 % dextran fraction.

EBV binding assay (EBV receptors). We used a direct binding assay to detect the presence and concentrations of EBV receptors by using radio-labelled EBV as previously described (Koide *et al.*, 1980). One thousand cpm of (methyl-^3H)-thymidine-labelled EBV were incubated with 1×10^6 and 10×10^6 cells, respectively, in 1 ml Dulbeccos' balanced salt solution (BSS) containing 1 % bovine serum albumin (BSA). After 30 minutes incubation at 20 °C, the cell suspension was applied to the top of 1 ml 10 % sucrose in BSS. This was spun for one minute at 9,000 g and washed once with 1 % BSA in BSS. The radioactivities of the pellet and supernatants were measured independently. Radiolabelled EBV-binding level (REBL) at a certain cells amount was calculated as follows:

REBL = (Cell-associated counts — cell-free tube-asscciated counts) / (Applied counts — cell-free tube-asscciated counts) × 100.

Electron microscopy. Detection of adsorption of EBV to the cells was performed by examining thin sections as previously described (Takimoto *et al.*, 1985).

Results

Ramos, AW-Ramos, Ramos/NPC, and NPC-KT cells were examined for the capacity to absorb P3HR-1 and NPC viruses using a direct radio-labelled EBV binding assay. As controls, EBV receptor-positive Raji cells and receptor-negative P3HR-1 cells were tested in parallel (Tables 1 and 2).

The binding levels of Ramos, AW-Ramos, and Ramos/NPC cells for radio-labelled P3HR-1 virus varied, with the AW-Ramos cells having the lowest level of the Ramos and two sublines of EBV-converted Ramos cells. The binding level for Raji cells was so far the highest, and P3HR-1 cells the lowest, as expected. The data agreed with the results of Klein *et al.* (1979). The

Table 1. Radio-labelled EBV binding level (REBL) (± S.D.) for P3HR-1 virus

Cell line	REBL*	
	10^6 cells	10×10^6 cells
Ramos	35 (8)	67 (7)
Ramos/B95-8	39 (7)	65 (5)
AW-Ramos	22 (4)**	50 (5)**
Ramos/NPC	31 (5)	62 (4)
Raji	69 (12)	88 (11)
P3HR-1	3 (1)	3 (2)
NPC-KT	12 (3)	25 (4)

* Figures indicate average REBL in five different experiments.
** Significant at $P < 0.05$ when compared with Ramos, Ramos/B95-8, and Ramos/NPC.

binding level of Ramos/NPC cells was similar to that of Ramos cells. On the contrary, the binding level of Ramos/NPC for radio-labelled NPC virus was reduced, compared to Ramos and AW-Ramos. Again, Raji cells showed the highest binding level and P3HR-1 cells the lowest. In addition, the binding level of NPC-KT cells for radio-labelled P3HR-1 virus was low, but higher as compared to P3HR-1 cells. On the contrary, the binding levels of NPC-KT and P3HR-1 cells for radio-labelled NPC virus were similar.

Discussion

We have found in this study that Ramos-NPC cells showed a reduced NPC virus-absorbing capacity, as compared to parental Ramos cells. In addition, we have shown that NPC-KT cells can adsorb P3HR-1 virus (Fig. 1) but cannot adsorb NPC virus.

Table 2. Radio-labelled EBV binding level (REBL) (± S.D.) for NPC virus

Cell line	REBL*	
	10^6 cells	10×10^6 cells
Ramos	48 (7)	68 (6)
Ramos/B95-8	44 (5)	69 (8)
AW-Ramos	39 (7)	62 (7)
Ramos/NPC	23 (5)**	49 (6)**
Raji	77 (9)	88 (8)
P3HR-1	5 (3)	4 (3)
NPC-KT	6 (4)	4 (2)

* Figures indicate average REBL on five different experiments.
** Significant at $P < 0.05$ as compared with Ramos, Ramos/B95-8, and AW-Ramos.

Previously, Klein *et al.* (1978) suggested that P3HR-1 EBV receptor-positive cells were selected against by the cytopathic effect of P3HR-1 virus and that receptor-negative cells were protected from lysis by the reduction or loss of their EBV receptors. The P3HR-1 virus-receptor concentrations on the surface of P3HR-1 virus converted Ramos cell lines were also significantly reduced, in comparison with the parental Ramos cell line.

Recently, we reported that using a high multiplicity of infection (m.o.i.) NPC virus inhibited the transformation of human cord blood lymphocytes (Sato *et al.*, 1986). It is likely that higher m.o.i. with NPC virus, like P3HR-1 virus, exert a cytopathic effect on NPC virus receptor-positive cells. In addition, we found that NPC-KT cells could be superinfected with P3HR-1 virus, but not with NPC virus (Takimoto *et al.*, 1986).

In this study, we have found that NPC-KT cells possess receptors for P3HR-1 virus, but not for NPC virus and also that the relative numbers of NPC virus-receptors on Ramos/NPC cells were reduced when compared to the parental Ramos cells, but the P3HR-1 virus-receptor concentration was similar to that of Ramos cells. Our results suggest that the reduction in the relative numbers of NPC virus-receptors on Ramos/NPC or the loss of NPC virus-receptors on NPC-KT cells may be the result of the cytopathic effect of the NPC virus and also that there might be, at least in a part, different P3HR-1 virus- and NPC virus receptors.

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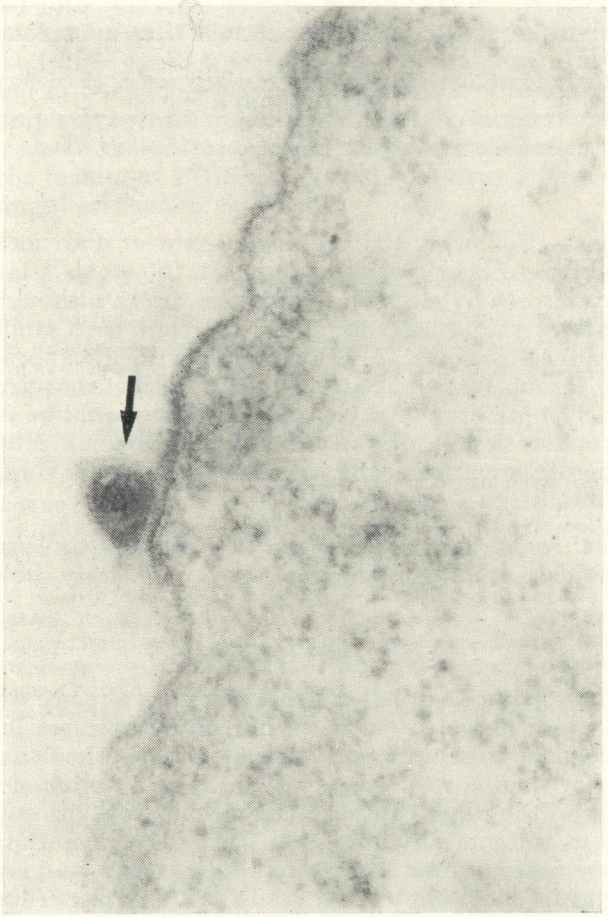

Fig. 1.
Electron micrograph showing adsorption of P3HR-1 EBV (arrow) to a NPC-KT cell] (×45,000).