BIKEN JOURNAL Vol. 21, 115-119, 1978

PRELIMINARY REPORT

INFECTION OF AVIAN LYMPHOBLASTOID CELL LINES WITH TYPE 2 HERPES SIMPLEX VIRUS

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(Received February 4, 1978)

Infection of human lymphoblastoid cell lines with herpes simplex virus (HSV) was first carried out by Henle et al. (1969) to see whether the infection influenced the Epstein-Barr virus carrier state. They found that HSV infection did not affect the carrier state in EBV-positive cell lines, or the appearance of EBV antigen-containing cells in EBV-negative lines.

The MSB-1 cell line derived from a lymphoid tumor of Marek's disease is quite analogous to human lymphoblastoid cell lines originating from Burkitt's lymphoma and thus we were interested to do similar experiments to those of Henle et al. using this avian lymphoblastoid cell line. However, before doing these experiments it was necessary to test whether avian lymphoblastoid cell lines are susceptible to human herpesviruses and we examined this problem by electron microscopy and by the fluorescent antibody technique. This preliminary report describes some results of these experiments.

Two avian lymphoblastoid cell lines were used. The one was the 1104 X-5 line, which was established by Hihara et al. (1974) and had the peculiar characteristics of growing both in suspension and attached to the wall of tissue culture bottles. In our laboratory the cells were routinely cultivated in a closed system at 36–37 C. The antigenicity of their surface membranes is thought to be the B cell type (Hihara et al., 1974; Matsuda et al., 1976). The other cells we used were MSB-1 cells, established by Akiyama et al. (1973). The cells of this line grew well only in suspension and had the T cell type character of membrane antigenicity (Nazerian and Sharma, 1975; Matsuda et al., 1976). They were cultivated at 41 C in a humidified atmosphere of about 5% CO_2 in air. Both cell lines were routinely cultivated in RPMI-1640 supplemented with 10% fetal calf serum, while virus infected cells were maintained in RPMI-1640 supplemented with 5% fetal calf serum.

The syn$^-$ variant of the UW 268 strain of type 2 herpes simplex virus (HSV-2) (Went-

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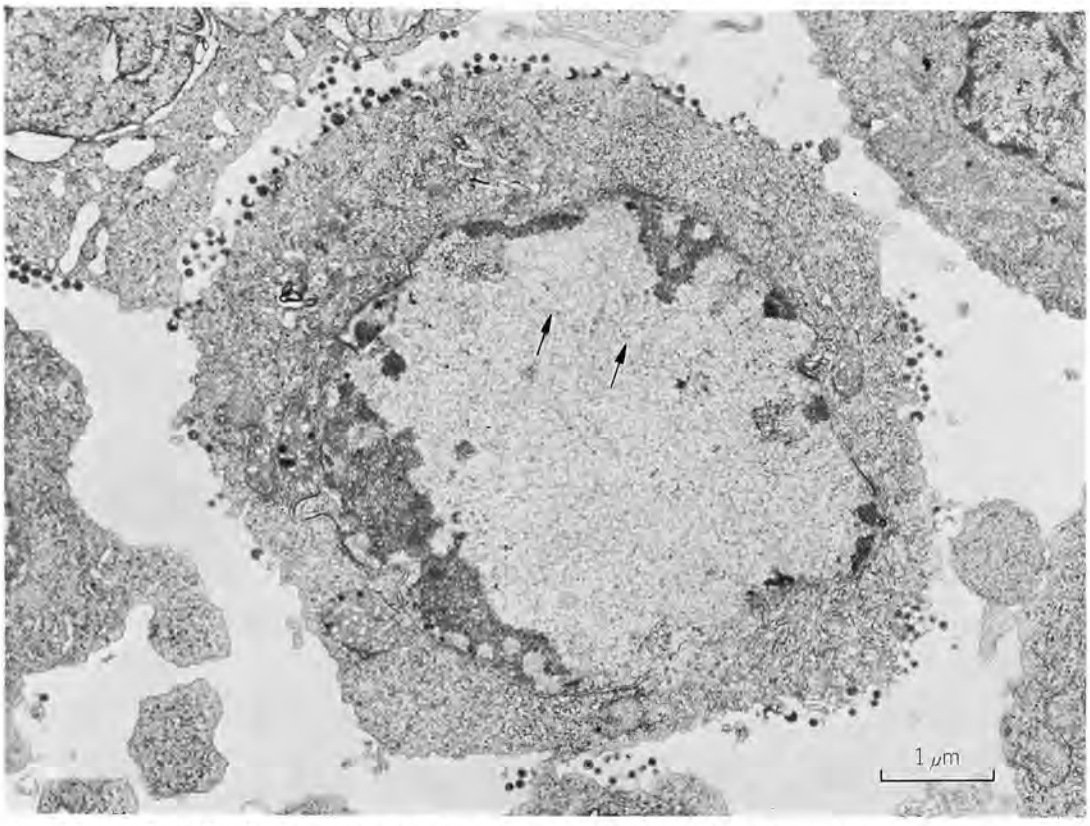

FIGURE 1. 1104 X-5 cells 32 hr after infection with type 2 herpes simplex virus. Arrows in one nucleus indicate intranuclear particles with capsids and cores of low density. Marginal condensation of chromatin is clearly seen in the same nucleus.

worth and French, 1969) was used. Samples of infected cells were prepared as follows: 1104 X-5 cells attached to the wall of the bottles were covered with 5 ml of a viral sample at an input multiplicity of about 5. After adsorption for 1 hr at 37 C, the inoculum was replaced by maintenance medium and the culture was incubated at the same temperature. Thirty-two hours after virus inoculation the infected cells were scraped off the bottle and collected by low speed centrifugation. For infection of MSB-1 cells, the cells growing in suspension were collected by low speed centrifugation, resuspended in a virus solution and incubated at 37 C. The input multiplicity of infection was about 4. After adsorption for 1 hr the cells were collected by centrifugation, resuspended in the maintenance medium and incubated at 37 C for 15 hr. Then the cells were collected by centrifugation.

Samples for electron microscopy were prepared essentially in the same way as described in a previous report (Nii and Yasuda, 1976).

The following observations were made. In sections of control 1104 X-5 cells numerous C type particles were observed extracellularly and a few similar particles were also found budding at the cell surface. These findings suggest that these cells formed C type particles endogenously. No other kind of virus particles was observed.

On the other hand, in sections of 1104 X-5

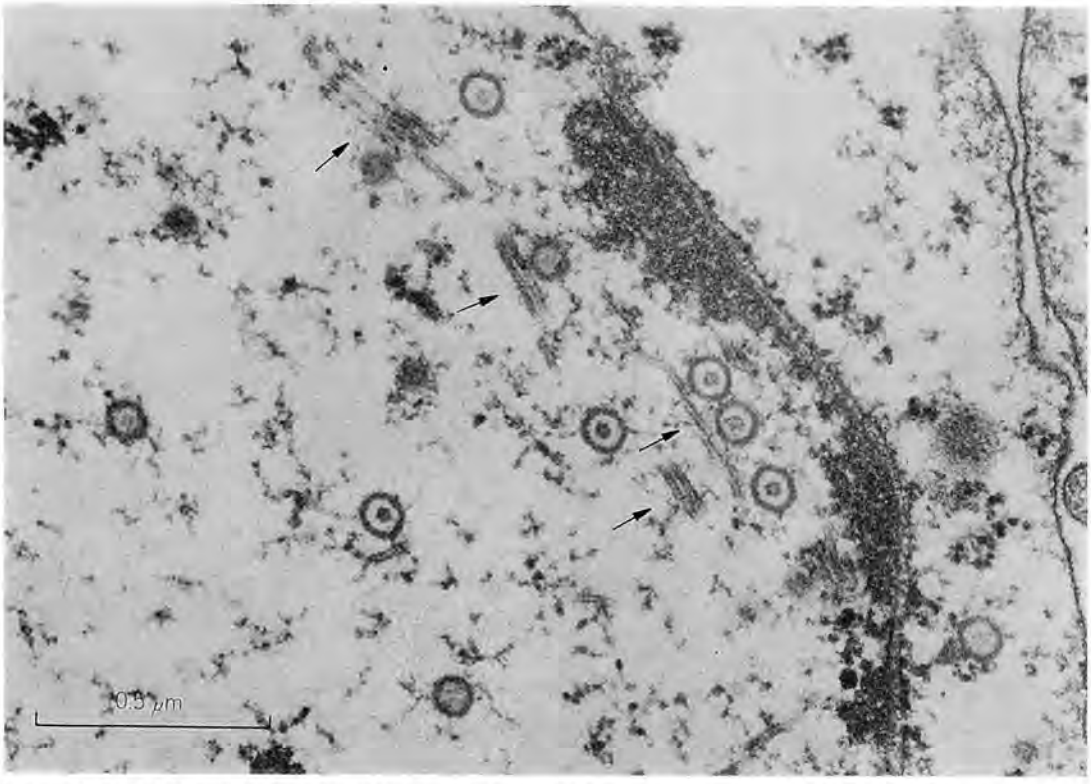

FIGURE 2. MSB-1 cells 15 hr after infection with type 2 herpes simplex virus. Most of the photo is occupied with parts of the nucleus and cytoplasm of one cell.

Several capsids with cores of low density are seen in the nucleus. These cores are about 40 nm in diameter. Arrows indicate microtubules located nearby the virus particles.

cells exposed to HSV-2, herpes type viral particles could be seen in a few cells (Fig. 1). The nuclei containing virus were usually mainly of low electron density with a marginal area of condensed chromatin. Intranuclear particles with capsids and cores of low density (arrows) were seen in regions of low density. Capsids with a core of low or high density were occasionally seen in the cytoplasm.

Many enveloped particles were seen at or very close to the surface of single infected cells, and most of these particles contained electron dense material but were devoid of a nucleocapsid. In other words enveloped particles containing nucleocapsids as well as electron dense material were rarely seen. Extracellular particles of herpes simplex virus occasionally coexisted with C type particles, but the latter were usually seen singly or in groups without accompanying extracellular herpes virus particles.

In sections of control MSB-1 cells, herpes virus of Marek's disease could be observed in less than 1% of the cells. The most common particles in nuclei harboring virus were capsids with a ring shaped core of low density (or double shell particles). This core (or inner shell) was about 60 nm in diameter.

Cells containing herpes type virus were slightly more frequent, i.e. 2–3%, in MSB-1 cells exposed to HSV-2 than in control cells. Most virus-infected nuclei contained capsids

with a core of low density of about 40 nm diameter.

Capsids with a core of about 60 nm diameter were less frequent. The capsids with a 40 nm core have so far been observed most frequently and rather specifically in cells productively infected with HSV, but they are extremely rare in cells infected with MDV. Therefore, it is very likely that the capsids with 40 nm cores observed in HSV-2 infected MSB-1 cells were particles of HSV-2, not of MDV.

Additional evidence for this conclusion is the appearance of microtubules in the nuclei of these cells, as shown by arrows in Fig. 2, because these structures are characteristic of HSV-2 infection (Couch and Nahmias, 1969; Nahmias et al., 1971). In fact, 18 strains of HSV-2 so far examined in our laboratory produced microtubules in the nuclei of FL cells, indicating a close correlation between the appearance of microtubules and HSV-2 infection (unpublished data). Moreover, these structures have never been observed on infection with other herpesviruses, including HSV-1.

We also studied the appearance of viral antigens in infected 1104 X-5 and MSB-1 cells. The 1104 X-5 cells were infected on a coverslip in replicate Leighton tubes with an input multiplicity of about 8, while the MSB-1 cells were infected in suspension with an input multiplicity of about 4, as described for making samples of infected cells for electron microscopy. After adsorption for 1 hr, the virus inocula were replaced by maintenance medium. With MSB-1 cells this was done by resuspending the infected cells in maintenance medium and transferring the resulting cell suspension to replicate Falcon flasks (each with a flat area of 25 cm^2). The replicate cultures of the two kinds of cells were used at hourly intervals to test for the presence of fluorescent antigens in infected cells.

In both cells the antigen began to appear 4 hr after infection and the percentage of antigen-positive cells increased as infection proceeded. However, at most the percentage reached about 20% in 1104 X-5 cells by 9 hr and about 9% in infected MSB-1 cells by 10 hr. Further cultivation of parallel cultures for more than 1 week with occasional change of the medium never resulted in total destruction of the cells, and instead the infection gradually seemed to decrease in these cultures.

FL cells, which are routinely used for preparation of viral samples of HSV-2, were also examined for comparison with these two lymphoblastoid cell lines. When the FL cells in Leighton tubes were inoculated with an input multiplicity of about 8, the percentage of fluorescent cells reached about 100% as early as 6 hr after infection.

These results, obtained by electron microscopy and fluorescent microscopy, show that the two avian lymphoblastoid cell lines are fairly susceptible to a human herpesvirus.

It is unknown why most cells of both avian lymphoblastoid cells were so resistant to infection with HSV-2, even when exposed to a high dose of the virus.

Experiments to see the effects of superinfection with herpes simplex virus on expression of viral genomes of MDV are in progress, and these experiments together with further details of the present studies will be reported elsewhere.

ACKNOWLEDGMENTS

We wish to thank Prof. S. Kato for his advice and encouragement throughout this work and also Dr. K. Akatsuka for his help in preparation of this manuscript.

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