BIKEN JOURNAL Vol. 16, 177–179, 1973

PRELIMINARY REPORT

CONTINUOUS CELL CULTURE FROM LYMPHOMA OF MAREK'S DISEASE

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(Received July 7, 1973)

Many human cell lines associated with Epstein-Barr virus (EBV), such as the EB-1 cell line (Epstein and Barr, 1965) and P3HR-1 cell line (Hinuma et al., 1967) have been reported. Unlike in studies on EBV, a big difficulty in research on Marek's disease virus (MDV), has been the lack of tumor-derived cell lines, as pointed out by Klein (1972). This difficulty made it impossible to compare the virus-cell interactions of MDV and EBV.

In this work, a cell line derived from an ovarian lymphoma of a 8-week-old SPF chick which had been inoculated with a virulent strain of MDV, BC-1 at one day of age, has been obtained. The virulent strain was kindly supplied by Dr. A. Okaniwa, Tanabe Pharmaceutical Co. The ovarian lymphoma was minced with surgical knives, passed through 6 layers of gauze and centrifuged at 1,000 rpm for 5 min. The packed cells were resuspended in the culture medium described below at a final count of 5×10^6 cells/ml. Fifteen ml of the cell suspension were seeded into a petri dish (90×20 mm).

Cells were cultured at 41 C in a humidified atmosphere of 5% CO_2-in-air. The culture medium was nutrient mixture PRMI 1640 supplemented with 10 to 20% fetal calf serum. The cell line, designated as the MOB line, consists of lymphoblastoid cells (Fig. 1). These cells grew singly and did not become attached to the surface of the culture vessel. They grew better at 41 C than 37 C. These cells have now grown well for over 23 weeks.

Direct immunofluorescence tests were performed on MOB to detect viral (VA) and cell surface antigen (CSA) of MDV by the method of Naito et al. (1969) and of Ishikawa et al. (1972), respectively. There were always a few percent of VA positive cells but only very few CSA positive cells. The percentages of viable cells and cells with VA are plotted in Fig. 2. Electronmicroscopic examination showed that the nuclei of a few percent of the cells contained several particles which seemed to be nucleocapsids and empty capsids of herpes type virus while no enveloped particles have yet been observed in any cells (Fig. 3). No C type particles were observed in any cells. The line gave a negative COFAL test and RIF tests for subgroup A, B, C and D of avian leucosis virus.

The cells (1×10^5 to 1×10^8) were inoculated either into the yolk sac of 7 day-old SPF 20

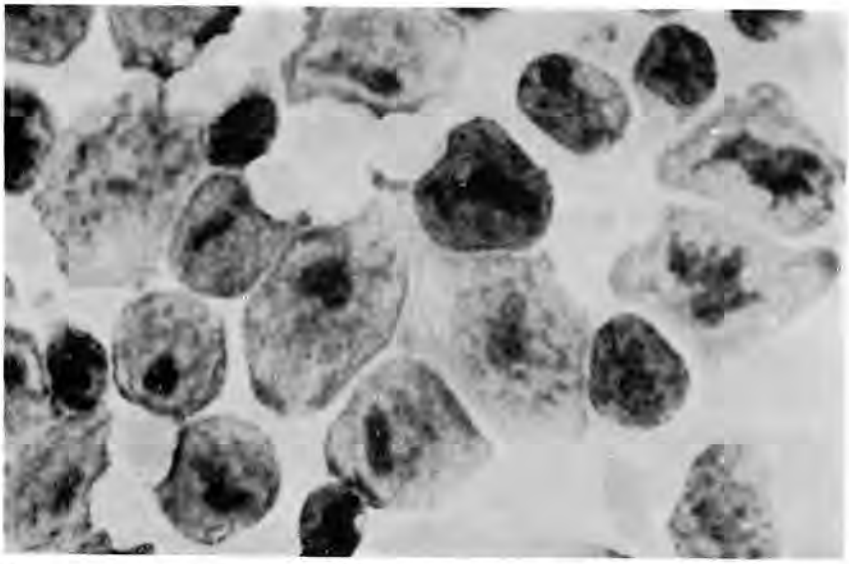

FIGURE 1. *Smear preparation of cells of the MOB line after 16 weeks cultivation. Fixed with methanol, stained with Giemsa solution. The line consists of lymphoblastoid cells. Many mitotic cells are observed.*

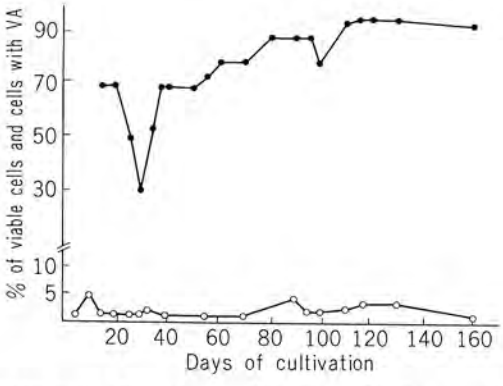

FIGURE 2. *Percentages of viable cells (●——●) and cells with VA (○——○) in cultures of the MOB line during cultivation for 23 weeks.*

chick embryos or into the abdominal cavity of one-day-old 20 SPF chicks. Lymphomas, histologically similar to MD lesions, appeared in many organs, such as the liver, spleen, lungs, kidneys and peripheral nerves, 10 days after inoculation of chick embryos and 4 to 8 weeks after inoculation of one-day-old chicks. Lymphoid cells could easily be cultured again from these lymphomas using the same procedures as before. The recultured cell line again contained a few VA-positive cells.

This first MD-lymphoma cell line should be useful in comparative research on herpes related lymphoma and human diseases possibly related to herpes, such as Burkitt lymphoma.

ACKNOWLEDGEMENTS

We wish to thank Prof. K. Toyoshima of this Institute for advice on the RIF test. Electronmicrography was kindly carried out by Mr. T. Naito, Osaka Medical College and the COFAL test by Mr. O. Tanishita, Kanonji Institute, The Research Foundation for Microbial Diseases of Osaka University, Kanonji. This work was supported in part by a Scientific Research Grant from the Ministry of Education.

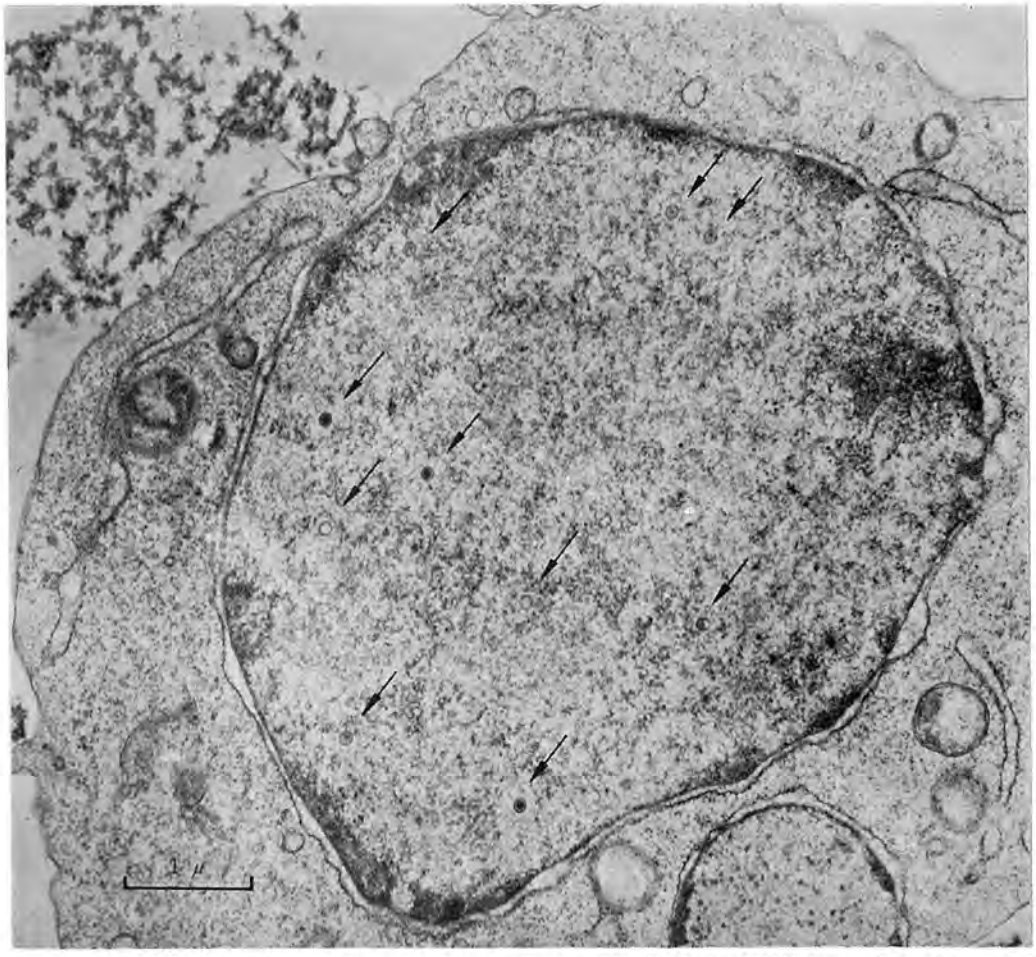

FIGURE 3. *Thin section electron micrograph of a lymphoblastoid cell of the MOB line. Several intranuclear herpes type capsid structures (arrows) are observed.*

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