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SHORT COMMUNICATION

EFFECT OF CULTURE TEMPERATURE ON THE PRODUCTION OF MAREK'S DISEASE VIRUS ANTIGENS IN A CHICKEN LYMPHOBLASTOID CELL LINE

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Lymphoblastoid cell lines from Marek's disease lymphomas are quite analogous to those established from Burkitt's lymphoma or normal human lymphoid cells tranformed by EB virus and 1–2% of the cell population produce viral antigens and particles (Akiyama et al., 1973; Akiyama and Kato, 1974).

Dunn and Nazerian (1977) studied the spontaneous production of MDV-specific antigens in one of the cell lines, MSB-1, and also the induction of antigens by IdUrd in these cells. They found that in some cultures less than 1% of the cells showed spontaneous antigen production, while in other cultures 2–5% of the cells were positive for MDV antigens; the former cultures were called low producers, and the latter high producers. In their laboratory cultures passaged more than 80 times were characteristically low producers. They also found that the number of antigen-positive cells was increased by low concentrations of IdUrd.

During continuous cultivation of MSB-1 cells we also observed that on serial culture of high producers many times at 41 C, the cells gradually became low producers. Moreover, we found that when low producers at 41 C were passaged at the lower temperature of 33 C they became high producers for several months at least. This paper briefly describes these findings.

MSB-1 cells in the early stage of in vitro cultivation were used; that is, cells were used after they had been cultivated for 56 days, stored frozen in liquid nitrogen for more than three years and recultivated again just before experiments. They were grown in RPMI-1640 medium supplemented with 10% fetal calf serum and kept routinely at 41 C in a humidified atmosphere of 5% CO_2 in air. For subculture, cells were seeded at a concentration of 5×10^5—1×10^6/ml. Usually this was done by diluting well grown cultures 1: 5—1: 10 by volume with fresh medium. Cells were subcultured once a week.

For immunofluorescence staining, cell smears were prepared as follows. About 6 ml of MSB-1 cell suspension was centrifuged and resuspended in 0.2 ml of PBS. Small drops of the resulting suspension were smeared on

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coverslips and air dried at room temperature. The coverslips were fixed in acetone at about −20 C for 15 min, air dried and then stained with anti-MDV fluorescent antibody. Direct staining was performed by the method of Naito et al. (1969). On examinations, the total numbers of cells and the number of viable cells, determined by trypan blue exclusion, were counted in the same materials as cells staining with fluorescent antibody.

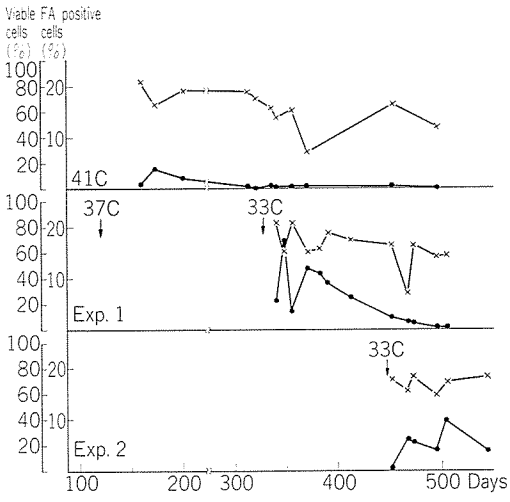


FIGURE 1. Percentages of viable cells (×——×) and MDV antigen positive cells (●——●) in MSB-1 cells cultivated at 41 C and 33 C (Exp. 1 and 2). Experimental procedures for Exp. 1 and 2 are described in detail in the text and are also shown in Fig. 2.

The results of occasional fluorescent antibody staining of MSB-1 cells routinely cultivated at 41 C in a CO_2 incubator are shown in Fig. 1 with the percentage of viable cells and the total number of days of in vitro cultivation. After about 200 days of cultivation, about 2% of the cells were still positive for MDV antigens, after 300 days less than 1% were antigen-positive, and after 450 days very few fluorescent cells were detectable. Thus the MSB-1 cultures seemed to be high producers for 200 days, but to become low producers after 300 days.

Two experiments on cultivation of MSB-1 cells at lower temperatures were successful and the results of both are shown schematically in Fig. 2.

In the first experiment MSB-1 cells were cultivated at 41 C for a total of 121 days with

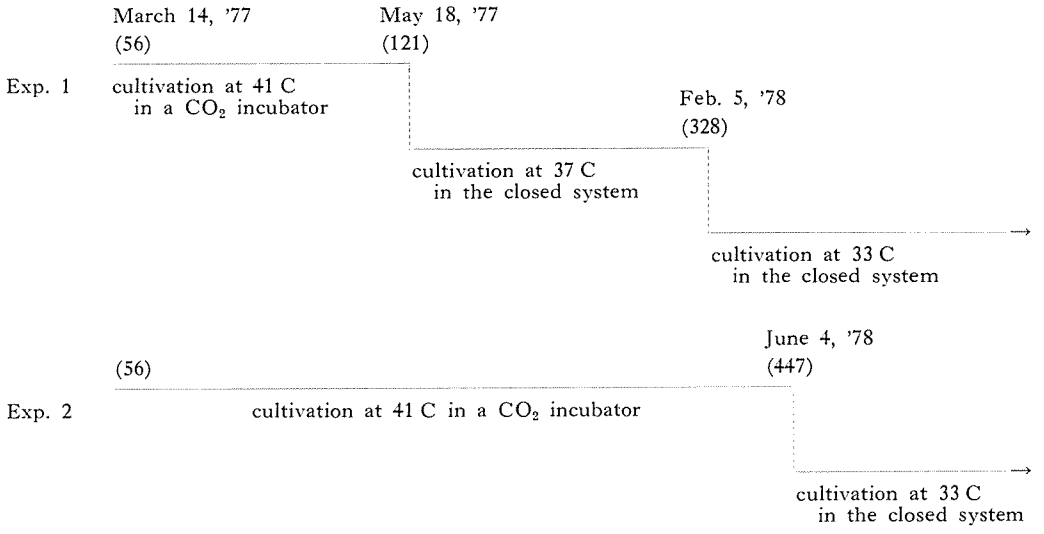


FIGURE 2. Process of lowering the culture temperature at 33 C. Numbers in parenthesis are days of in vitro cultivation of MSB-1 cells.

one interval of freezing. Then they were cultured at 37 C for about 200 days, from day 121 to 328 of in vitro cultivation, in the closed system in 30-ml Falcon flasks. For the first 3 months of culture under the latter conditions, subcultures were performed every 3 or 4 days as follows: suspensions of cells in the used medium were diluted two-fold or three-fold with fresh medium and the resulting cell suspensions were introduced into new flasks. From 3 months after the beginning of culture the cells were subcultured once a week by 4- or 5-fold dilution. Examination of the extent of spontaneous antigen production at intervals during this cultivation period at 37 C showed that 2–5% of the cells were always antigen-positive (data not shown). After about 200 days cultivation at 37 C, the temperature was lowered further to 33 C. For about 3 months after the start of cultivation at 33 C, subculture was performed once a week by 2- to 3-fold dilution of samples of the cultures with fresh medium. After 3-months cultivation at this temperature the suspensions were diluted 4- or 5-fold. Results on fluorescent antibody staining of the cells are shown in Fig. 1. For about the first 80 days of cultivation at 33 C, more than 5% antigen-positive cells were consistently detected and it is especially noteworthy that 17.3% of the cells were antigen-positive after 20-days cultivation at 33 C.

A second experiment was carried out to determine whether the above phenomenon was reproducible. As shown in Fig. 2, MSB-1 cells were cultivated for about 450 days at 41 C. At the end of this long cultivation period scarcely any antigen-positive cells were detectable. These very low producers were then cultured at 33 C in 30-ml Falcon flasks. After about 450 days, the cells were cultured at this temperature in a closed system. Subcultures were performed in the same way as described in the former experiment and numbers of total cells and viable cells and the percentage of fluorescent cells were determined at intervals. As shown in Fig. 1, the percentage of antigen-positive cells gradually increased, reaching about 10% after 2 months.

The present findings indicate that MSB-1 cells can be grown at 37 C, or even at 33 C, and that the frequency of spontaneous production of antigen-positive cells increases on cultivation of cells at this temperature.

Analogous phenomena have been reported for EBV-associated cell lines; namely, when P_3HR-1 cells were cultivated at temperatures of 29, 32, 35 and 37 C, the percentage of immunofluorescence-positive cells was highest at 32 C (Hinuma et al., 1967) and the amount of infective virus produced was higher in cells at 33 C than in those at 37 C (Nagoya and Hinuma, 1972). Extracellular particles were rarely found in MSB-1 cell cultures, whereas they were produced in large amounts by P_3HR-1 cells, but apart from this, the mechanisms operating in spontaneous production of viral antigens in MDV- and EBV-associated lymphoblastoid cell lines are essentially the same.

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