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ESTABLISHMENT OF A HAMSTER LYMPHOMA CELL LINE

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Abstract: The establishment of a hamster lymphoma cell line was attempted. Simple mincing and trypsinization of lymphoma tissue resulted in a high degree of cell degeneration. The ascitic tumor cells produced by intraperitoneal transplantation of lymphoma tissue gave a better result. These ascitic cells grew and were cultured successively in medium consisting of RPMI 1640 and 20% fetal calf serum. Cells were round and grew in suspension. Accelerated cell growth was observed one month after starting the culture. In the stained preparations, cells were lymphoblastic. Cells were transplantable into new-born hamsters and produced tumors, but not in young adult hamsters.

In various animals including man, the tissue culture of lymphoma cells and establishment of cell lines from them have been reported. In hamsters, STENBACK *et al.* (1) reported a line of cells from a hamster malignant lymphoma which was established in tissue culture. To compare the surface antigens of adenovirus-induced hamster tumors with those of spontaneous tumors, the author cultured the cells of a hamster lymhoma and established a cell line from them. The results of the comparative study on the surface antigens of these cells have been reported in a previous paper (2). The present report is concerned with tissue culture of cells of a spontaneous hamster lymphoma and the establishment of a cell line from them.

MATERIALS AND METHODS

Tumor: A spontaneous, cutaneous type, malignant lymphoma which developed in one of the hamsters of our colony and maintained by successive transplantations into young adult hamsters (3), was used.

Tissue Culture: The tumorous lymph node from a hamster with transplanted malignant lymphoma were minced and passed through metal meshes in a phosphate buffered saline (PBS). Viable cells were counted by trypan blue dye exclusion test, and 5×10^7 cells were injected into weanling hamsters (3-week-old) intraperitoneally. Within a week, almost all the hamsters injected were found to have massive ascites (over 10 ml). The ascites was collected and resuspended ascitic cells were injected into weanling hamsters. The resulting

ascites was used for tissue culture.

The cells from the ascites were harvested by low speed centrifugation and resuspended. The startig cell concentration was approximately 1×10^7 cells/ml and 4 ml of the cell suspension was seeded into 35 mm glass dishes. The nutrient medium used for suspension cultures consisted of medium RPMI 1640 (4) supplemented with 20% inactivated fetal calf serum (BDH, England), 100U/ml of penicillin, and 100 μg/ml of streptomycin. The culture vessels were placed in a humidified incubator with a constant flow of 5% CO_2 in air. Two days later 2 ml of fresh medium was added and subsequently one-half of the medium was carefuly replaced with fresh medium.

Staining: Cultured cells were smeared on the glass slides. The smear was fixed with methyl alcohol for 5 min, drained and stained with Giemsa stain for 30 min, rinsed with distilled water, air dried, filled with oil and sealed. Some microphotographs of the stained cells were taken.

RESULTS

Tissue Culture: Simple mincing and trypsinization of the tumor tissue resulted in progressive degeneration of the cultured cells in our preliminary study. Hence, the ascitic tumor was produced and used for tissue culture to avoid the mechanical or enzymatic damage of the cells.

Most of the cells explanted appeared round and remained suspended for the first several days. Contaminating erythrocytes degenerated rapidly. Fibroblast-like cells began to emerge several days after culture initiation and formed small patchy sheets on the dish surface. In culture medium consisting of MEM and 20% fetal calf serum, the growth of cells appeared to be poor and serial transfer of suspended round cells was not successful. In culture medium consisting of RPMI 1640 and 20% fetal calf serum, by contrast, cells showed better growth, and after several transfers of the suspended round cells, the fibroblast-like cells were completely eliminated. Accelerated growth of round cells was observed about one month after starting the culture. After this stage, cells grew rapidly in suspension and had a tendency to form cell clumps. The clumps were easily reseparated into single cells by shaking or pipetting. The doubling time of these cells was approximately 48 hr and cellular viability of over 75% was maintained during active growth. The cell line was designated MLP-1.

Cellular Morphology: The stained cells seemed to be lymphoblasts (Fig. 1). The nuclei were round, oval, or indented and nucleoli were prominent. The cytoplasm stained pale to deep blue. Mitotic figures were numerous. All the cells were peroxidase negative.

Transplantation of Cultured Cells into Hamsters: To test the tumorigenicity of the cultured cells, 5×10^7 cells from the 31st transfer generation were

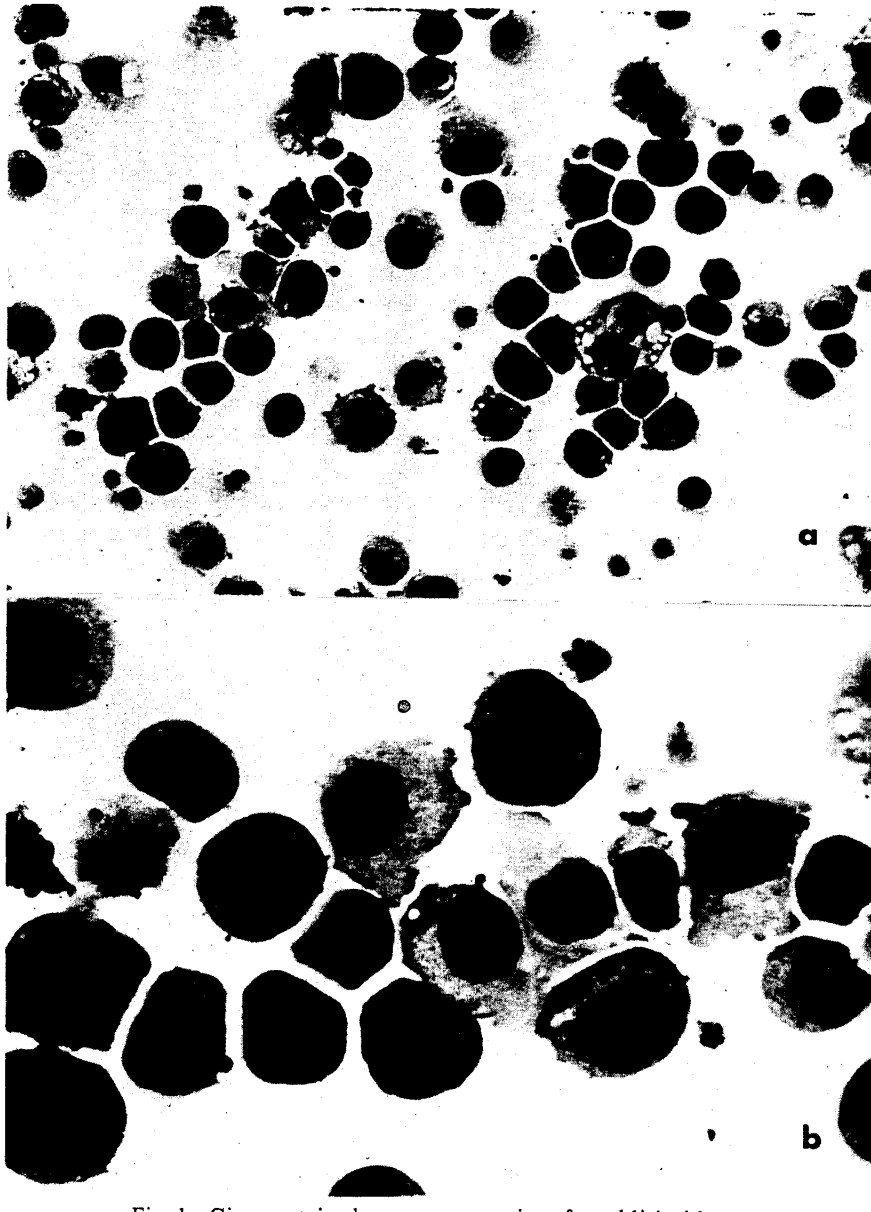

Fig. 1. Giemsa-stained smear preparation of established hamster lymphoma cells (MLP-1)
a: Low magnification (×320)
b: High magnification (×970)

inoculated intraperitoneally into hamsters. When inoculated into weanling hamsters, as shown in Table 1, no tumor was detected at autopsy 45 days

TABLE 1 TRANSPLANTATION OF MLP-1 IN HAMSTERS

Host	Cell Dose[1]	Route	Period of Observation	Tumor/Hamsters
Weanling hamster (3-week-old)	5×10^7	i.p.[2]	45 days	0/5
New-born hamster	5×10^7	i.p.	7 days	9/9

1) Cells from the 31rt culture passage
2) Inoculated intraperitoneally

after inoculation. When newborn hamsters were inoculated with the same dose of cells, however, all hamsters developed tumors within a week.

DISCUSSION

STENBACK *et al.* (1) reported a successful establishment of a tissue culture cell line from a hamster malignant lymphoma by trypsinization of a tumor and by culturing cells in MEM supplemented with 10% fetal calf serum. In the present study, our effort by a similar method resulted in the degeneration of cells. An attempt to maintain the continued growth of cells using MEM containing 20% fetal calf serum also gave no successful result. A successful result was obtained only when the ascitic cells produced by intraperitoneal tumor transplantation was used as the cell source and RPMI 1640 as the tissue culture medium. The lymphoblastic nature of the cell line established in the present study is evident from its growth pattern in the tissue culture and the cellular morphology of the stained preparations. Hence, the difference of results in these attempts to establish tissue culture cell lines might be due to the difference of the lymphomas used. In any event, the method employed in the present study would be another method suitable for the establishment of a tissue culture cell line of lymphoma.

The original tumor was highly transplatable into young adult hamsters. However, after long-term *in vitro* cultivation, the tumor cells were transplantable only into neonatal hamsters, suggesting their reduced transplantability. The mechanism for this reduction of transplantability is obscure at present, and needs further studies.

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