仔牛型白血病由来Tリンパ系腫瘍細胞の継代培養とその性状

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NOTE

Continuous Cell Culture and Characteristics of T-lymphoid Tumor Cells from Calf Forms of Lymphosarcoma

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Lymphoid tumor cells derived from a calf form case of bovine lymphosarcoma were successfully passaged in nude mice for over 25 passages. The lymphoid tumor cells from this transplantable cell line, BTL-T3, have been continuously cultured *in vitro* for over 6 months, and the descendent cells which had a T-cell marker and was designated as "BTL-C3", expressed a tumor-associated surface antigen (CBTL-ASA), which was not detected on the normal lymphocytes nor the other forms of bovine lymphosarcoma.—*Key words*: Calf T-cells tumor, Tumor-associated antigen.

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Suspension culture cell lines have been established from leukemic tissue of adult bovine [1, 5]. However, T-lymphoid tumor cultured cell line has not yet been established from bovine lymphosarcoma. In the present experiment, T-lymphoid tumor cells derived from calf form of bovine lymphosarcoma (CLS) have been primarily developed as transplantable lymphoid tumor cells using nude mice. Then, the transplantable lymphoid tumor cells were cultured continuously and its characteristics examined. This cell line has been maintained for over 6 months.

Lymphoid tumor cells were originally obtained from a 22 months old Holstein steer, which was negative for bovine leukemia virus antibody and affected with CLS. After bleeding the animal, the lymphoid tumor cells were separated from the tumor nodule of the kidney which measured about 2×2 cm. Tumor proliferations in this calf were observed throughout the lymph nodes, and in viceral organs such as the liver, pancreas, spleen, kidney and lung. However, thymus tissue was not affected with tumor lesions. The tumor tissue was minced in a serum free RPMI 1640 medium with scissors to release the tumor cells and filtered through a 150-mesh stainless steel screen. The tumor cells were separated by Ficoll-Hypaque gradient centrifugation to obtain living cells. The living tumor cells were then centrifuged at 1,000 rpm for 5 minutes and the packed cells were resuspended in the RPMI 1640 medium.

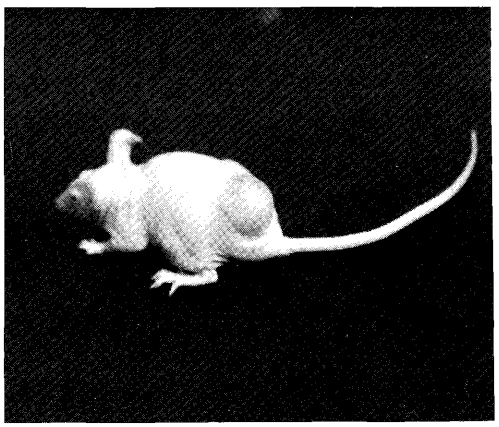

Fig. 1. Transplantable tumor bearing nude mouse. Nude mouse was inoculated with lymphoid tumor cells (BTL-T3) derived from calf form of bovine lymphosarcoma. The tumor developed about 2 weeks after transplantation.

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After counting the cell by trypan blue dye-exclusion method, the living tumor cells (2×10^7) were subcutaneously transplanted into 3 nude mice, respectively, at 3–4 weeks of age which had been given abdominal injection of cyclophosphamide every 2 days before transplantation at a dose of 0.15 mg per lg of body weight. Six weeks after the transplantation, a tumor nodule developed at the site of inoculation in one of the 3 nude mice. Thereafter, this transplantable tumor cells have been passaged every 3 to 4 weeks in nude mice for over 25 passages, and this cell line was designated as transplantable bovine T lymphoma "BTL-T3" (Fig. 1).

BTL-T3 was also cultured in CO_2 incubator to establish an *in vitro* cell line. BTL-T3 cells were placed in 2 different culture media, namely the RPMI 1640 medium and Dulbecco's MEM medium, each containing either 20% horse serum (HS) or 20% fetal calf serum (FCS). Ten different lots of HS and 5 lots of FCS were examined in the present study. After incubation at 37°C in a humidified 5% CO_2 atmosphere, the tumor cells were successfully grown in cultures containing the RPMI 1640 medium supplemented

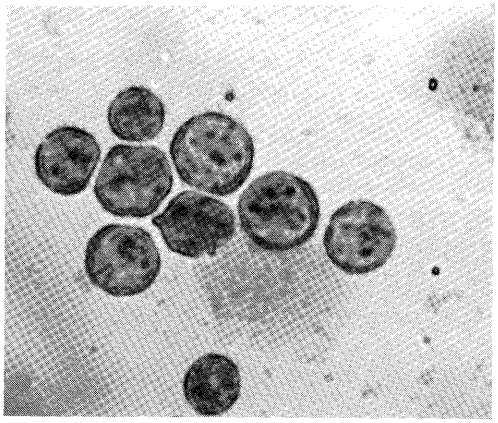

Fig. 2. Smear of continuously cultured tumor cells, BTL-C3, derived from the BTL-T3. Giemsa staining.

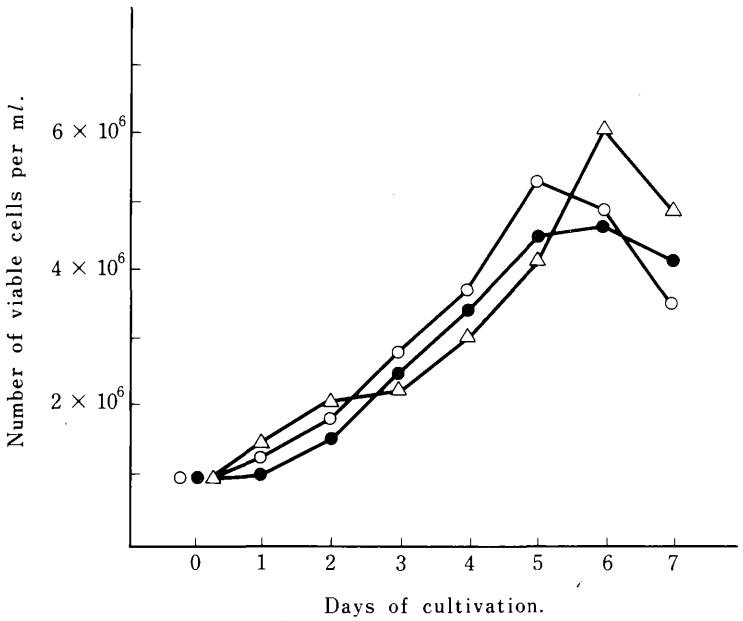

Fig. 3. Growth curves of continuous culture cells, BTL-C3, in suspension culture media. O——O: RPMI 1640 medium containing 20% HS. ●——●: Dulbecco's MEM medium containing 20% HS. △——△: Dulbecco's MEM medium containing 20% FCS.
Each medium was supplemented with 2mM L-glutamine, 1mM sodium pyruvate, 1mM nonessential amino acids, 5×10^{-5}M 2-ME, and antibiotics.

with HS from one of the lots. These cells were cultured further at a density of 1×10^6 cells per m*l* in the RPMI 1640 medium supplemented with the 20% HS, 2mM L-glutamine, 1mM nonessential amino acids, 5×10^{-5}M 2-mercaptoethanol (2-ME), 1mM pyruvate, 50 units/m*l* penicillin, and 50 μg/m*l* streptomycin. From the results, the tumor cells have now grown for over 6 months, and this continuous cell line was designated as cultured bovine T lymphoma "BTL-C3". BTL-C3 cell was round and did not attach to the surface of the culture vessel. After Giemsa staining, the cells were morphologically identified as lymphoblastoid cells of 12–15 μ in diameter (Fig. 2). Growth curves of the BTL-C3 in suspension culture were shown in Fig. 3. The doubling times of the cell cultures were about 45 hr in the Dulbecco's MEM medium contining FCS or HS as well as in the RPMI 1640 medium containing HS.

Living cell surface antigens of the BTL-C3 (CLS) were examined by indirect immunofluorescence techique and compared with those of the normal thymus cells, normal peripheral blood lymphocytes, and lymphoid tumor cells derived from the adult (ALS), thymic (TLS), and skin (SLS) forms of bovine lymphosarcoma using specific rabbit antisera and FITC-conjugated anti-rabbit IgG. The antigens examined were T-cell antigenic marker, cell surface IgM (sIgM) and tumor-associated surface antigen. T-cell marker was detected by a specific antiserum which was prepared by immunizing a rabbit with normal calf thymus cells and absorbing with the same individual bone marrow cells. RBC, liver (acetone powder), and B lymphoid tumor cells obtained from ALS cow. Tumor-associated surface

Table 1. Analysis of cell surface antigens and ANAE staining of the continuous cultured cell line, BTL-C3, and other lymphoid cells

Cells	% of positive cells			
	sIgM[a)]	T-cell marker[b)]	Tumor-associated antigen[c)]	ANAE staining
Normal peripheral blood lymphocytes	25	75	0	75
	27	71	0	70
	21	76	0	73
	24	75	0	77
	28	72	0	76
	23	74	0	72
	29	70	0	76
Normal thymus cell	3	92	0	94
CLS (BTL-C3)	0	100	100	100
ALS	98	0	0	1
	96	3	0	2
	97	2	0	4
SLS	2	70	0	75
TLS	10	86	1	NT[d)]

a) sIgM: Cell surface IgM was detected by indirect immunofluorescence technique using anti-μ chain antibody (rabbit serum) and FITC-conjugated anti-rabbit IgG.

b) T-cell marker: T cell marker was detected by the anti-thymocyte serum.

c) Tumor-associated antigen: Tumor-associated surface antigen was detected by specific antiserum to the BTL-C3.

d) Not tested.

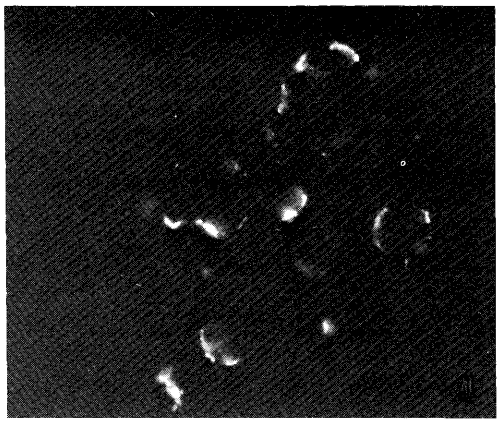

Fig. 4. T-cell antigenic marker on the culture cells, BTL-C3. Stained by indirect immunofluorescence technique.

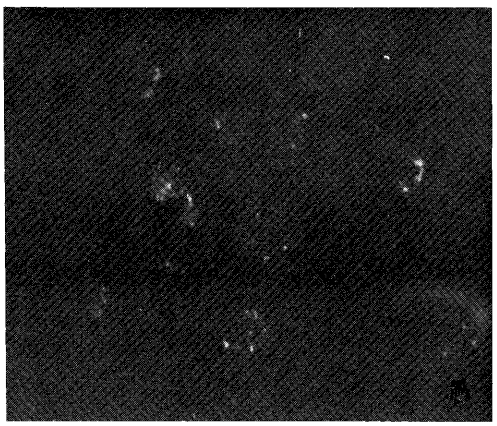

Fig. 5. Tumor-associated surface antigen (CBTL-ASA) of the BTL-C3 detected by indirect immunofluorescence technique using the specific antiserum.

antigen was detected by a specific antiserum which was made by immunizing a rabbit with the transplantable tumor cells, BTL-T3, and absorbing with the autologous thymus cells (normal cells), liver (acetone powder), and lymphoid tumor cells from ALS cows. The lymphoid tumor cells examined were separated from 3 ALS cows, 1 SLS cow, and 1 TLS calf. As shown in Table 1, the BTL-C3 expressed T-cell marker on their membranes (Fig. 4), but did not possess sIgM, and thus differed from the tumor cells derived from the ALS. The similar result was obtained with normal thymus cells. Moreover, acid α-naphthyl acetate esterase (ANAE) staining, done as a T-cell marker by the method of Knowles *et al.* [2], showed that 100 percent of the BTL-C3 cells, and less than 4 percent of the tumor cells from 3 ALS cows were positively stained respectively (Table 1). Tumor-associated surface antigen (unrelated to the histocompatibility antigen) was detected on the BTL-C3 cells using the specific antiserum to the tumor cells (Fig. 5), but not on cells such as the normal thymus cells, peripheral blood lymphocytes, and bovine lymphosarcoma cells except CLS (Table 1). It is suggested that this antigen may be different from that of those expressed on lymphoid tumor cells of ALS as reported by some investigators [3, 4]. Thus, this antigen is provisionally designated as calf form of bovine T lymphoma-associated surface antigen (CBTL-ASA).

In conclusion, it is indicated that; 1) the transplantable tumor cell line, BTL-T3, derived from the CLS cow can be successfully passaged in the nude mice for over 25 passages, 2) the continuous cell line, BTL-C3, cultured from the BTL-T3 is actually a bovine T lymphoid tumor cell, and 3) the T lymphoblastoid cells, BTL-C3, expressed tumor-associated surface antigen (CBTL-ASA), which is not detected on lymphoid tumor cells derived from the ALS, SLS or TLS.

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要　約

仔牛型白血病由来 T リンパ系腫瘍細胞の継代培養とその性状（短報）：石井　博・大木与志雄（日本獣医畜産大学生理化学教室）——BLV 抗体陰性の仔牛型白血病例由来リンパ系腫瘍細胞をヌードマウスに25代にわたって継代移植し，さらにこの継代株（BTL-T3）を６ケ月以上にわたって in vitro で継代培養した細胞株（BTL-C3）の性状について検討した。すべての培養腫瘍細胞に，間接蛍光抗体法により T-cell marker が証明された。また培養腫瘍細胞に対する抗血清を用いて検討したところ，組織適合抗原とは関係なく，正常リンパ球および仔牛型白血病以外の牛白血病例の腫瘍細胞には存在しない腫瘍関連表面抗原（CBTL-ASA）が検出された。