Establishment of a Lymphoid Cell Line from Tumor of Bovine Skin Leukosis
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Bovine leukemia is classified into enzootic bovine leukemia (EBL) caused by bovine leukemia virus (BLV) and sporadic bovine leukemia (SBL) [2]. SBL is subdivided into calf, thymic and skin forms of bovine lymphosarcoma. SBL is rare and its etiological agent has not been identified. Tumor from EBL is of B cell origin [11, 17], while tumor cells from SBL appear to be of non-B-cell lineage. The establishment of continuous cell suspension cultures has been reported in EBL cases [3, 5, 12] and SBL cases [4, 7]. Recently we established a suspension culture cell line, SBL-1, derived from bovine skin tumor. The cells appear to have T-cell character and no integration of bovine leukemia provirus. In this communication we describe the characteristics of the lymphoid cell line.

The tumor specimen used for the suspension culture was obtained from a 24 month-old Holstein cow with skin leukemia. The cow was negative for BLV isolation in peripheral blood lymphocytes by synctium assay and negative of BLV antibodies. Diagnosis of lymphosarcoma was confirmed by clinical and histological examinations. Fresh tumor materials were collected in Dulbecco’s minimal essential medium (DMEM) containing 10% calf serum. Tumor specimens were teased with scissors and forceps to make a single suspension of cells.

Surface immunoglobulin (sIg) immunofluorescent test for detection of B cells [16] and E rosettes test for detection of T cells [8] were performed by the methods described previously. For α-Naphthyl acetate esterase (ANAE) staining, cells were fixed for 10 min with 2.5% glutaraldehyde (pH 7.2) and stained in an incubation mixture at 37°C for 3 hrs as described previously [6].

Hybridization probe was obtained from molecularly cloned λ BLV-1 [14] using Bgl II digestion, labeled with 32P by nick translation and used for hybridization probe as described previously [9]. High molecular weight DNA was extracted from the cell line [13]. The extracted DNA was digested with Sac I, electrophoresed on 0.8% agarose gels and transferred to nitrocellulose filters. The filters were hybridized with the probe by the method of Southern [15].

Single viable cells (5×10³/ml) from tumor specimens were suspended with DMEM supplemented with 10% fetal calf serum and seeded in glass plate. The cells began to proliferate as a single cell suspension and the first transfer was carried out 7 weeks later. The suspension culture line designated SBL-1, has been maintained for over 1.5 years. The culture grew as single-floating cells that did not attach to the glass surface. The cells were 15 to 22 μm in diameter and showed large atypical lymphocytes (Fig. 1 and 2). Electron microscopically, they had a relatively broad rim of cytoplasm containing a clustered dense body of lysosomes. The cytoplasmic margin was irregular with short microvilli. They had no virus-like particles (Fig. 2). By ANAE staining, all cells showed granular (nodular) positive staining as shown in Fig. 3. No sIg positive cells could be demonstrated on the surface of SBL-1 cells. Only 2.5% of the cells was found to be E-rosettes positive. Although our results showed the lack of cell surface marker on SBL-1 cells, the results of ANAE staining suggested a T-cell origin of the cell line. This cell line might have lost its normal T cell surface marker during neoplastic transformation. In previous papers, established cell lines from calf and skin leukemia were thought to be of T cell origin [4, 7].

Table 1 summarizes the properties of the cell line. Chromosome analysis showed that the cell line was derived from a female bovine. Cells contained an average of 59 mostly acrocentric chromosomes. The cell line showed linear growth during the initial 48 hrs after seeding the cells at a density of 1×10⁵/ml and the population doubling time was about 26 hrs. SBL-1 cells could be
grown in semi-solid agar medium and colony-forming efficiency was 18.5%. The cells formed solid tumors when $5 \times 10^6$ of the cells were inoculated into athymic nude mice. This established the malignancy of the cell line. BLV and BLV antigens in acetone-fixed cells could not be
LYMPHOID CELL LINE OF BOVINE SKIN LEUKOSIS

Table 1. Properties of SBLC-1 cell line

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SBLC-1 Cell Line Properties</th>
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<tbody>
<tr>
<td>Morphology</td>
<td>Large atypical lymphocytes</td>
</tr>
<tr>
<td>ANAE staining</td>
<td>Positive</td>
</tr>
<tr>
<td>sg positive cells</td>
<td>0</td>
</tr>
<tr>
<td>E-rosettes positive cells</td>
<td>2.5%</td>
</tr>
<tr>
<td>Chromosome and number</td>
<td>Bovine female, 59</td>
</tr>
<tr>
<td>Doubling time</td>
<td>26 hrs</td>
</tr>
<tr>
<td>Colony formation in semi-solid agar medium (colony efficiency)</td>
<td>Yes (18.5%)</td>
</tr>
<tr>
<td>Tumorigenicity in nude mice</td>
<td>Yes</td>
</tr>
<tr>
<td>Detection of BLV and BLV antigens</td>
<td>Negative</td>
</tr>
<tr>
<td>Bovine leukemia provirus</td>
<td>Negative</td>
</tr>
<tr>
<td>Reactivity with c143 antibody</td>
<td>Yes (cytotoxic index 88.5)</td>
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detected. Furthermore, no integration of bovine leukemia provirus was detected by hybridization to the probe. These results indicated that BLV was not involved in skin leukosis. These results support the evidence for lack of relatedness between BLV and SBL, reevaluating the previous Southern blotting analysis using molecularly cloned BLV DNA [10].

Expression of tumor-associated antigen was determined by using c143 monoclonal antibody against EBL tumor cells by complement-dependent antibody cell cytotoxicity test. Monoclonal antibody c143 has been previously characterized and it reacted with all EBL tumor cells but not with normal bovine lymphocytes nor BLV antigens [1]. Most of SBLC-1 cells were killed in the presence of c143 and rabbit complement, and the cytotoxic index of the cell line was 88.5. In the preliminary experiments, we obtained monoclonal antibodies against SBLC-1 cells and all of these antibodies reacted with the SBLC-1 cells but not with normal bovine lymphocytes nor EBL tumor cells. Furthermore, some antibodies to SBLC-1 cells and c143 showed cytotoxic activity to bovine fetal thymus cells (Our unpublished data). These results suggest that the SBLC-1 cells expressed a specific antigen which differed from that of EBL tumor cells and common antigen to bovine fetal thymus cells. Characterization of tumor-associated antigens of the SBLC-1 cells defined by monoclonal antibodies and the relationship between the antigens expressed on EBL and SBLC-1 cells are now under investigation.

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REFERENCES

要約

ヒフ型ウシ白血病腫瘍由来のリンパ系細胞株の樹立（短報）：小沼邦，西英穂，岡田洋之1，桐沢力雄・千早豊1，川上善三（酪農学園大学家畜微生物学教室，1家畜病理学教室）——ヒフ型ウシ白血病の腫瘍から、T細胞様性状を示し浮遊した状態で増殖するリンパ系細胞株を樹立した。この細胞は1.5年以上継代され，ヌードマウスに移植腫瘍を示したが，そのDNAにはウシ白血病ブロウイルスは含まれていなかった。