Establishment and Characterization of a New Canine B-Cell Leukemia Cell Line

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Abstract. A new cell line derived from a spontaneous canine leukemia was established and designated GL-1. The cells have been cultured in a floating fashion and passaged for over two years. They were round with rich cytoplasm containing many rough endoplasmic reticula and mitochondria. Peroxidase staining was negative. The nuclei of many cells were round, but segmented nuclei were seen frequently. The doubling time of the cells was 27.3 hr and they had 78 chromosomes. Surface marker analysis using monoclonal antibodies (MABs) and flowcytometry revealed that GL-1 possessed CD45 and surface IgG. However, the cells did not react with MABs detecting T-cell markers. These results indicate that GL-1 has a lymphocytic lineage and is derived from a B-cell leukemia. — Key words: canine, cell line, lymphoma/leukemia.


Many established cell lines derived from human and murine leukemic cells have been reported and have played an important role in the biological and therapeutic studies on lymphoproliferative disorders [1, 2, 7]. Some cell lines from canine leukemic cells have been established, but there are few reports on them. We have established a new cell line derived from a spontaneous canine lymphocytic leukemia and investigated some of its characteristics. In this paper, we describe the morphological and cytological features of this cell line.

The neoplastic cells were isolated from a 9-year-old neutered German Shepherd dog with acute leukemia that was referred to the Veterinary Hospital of Yamaguchi University. During hospitalization, the peripheral white blood cell count increased markedly (108,000/µl) and more than 60% of the peripheral leukocytes in blood smear were neoplastic cells. Peripheral mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation from heparinized blood sample. The isolated cells were cultured in a tissue culture flask with RPMI-1640 containing 10% fetal calf serum (FCS) at an initial concentration of 5.0 x 104/ml. The tissue flask was incubated at 37°C in a humidified 95% air-5% CO2 atmosphere. Half of the culture medium was changed once a week. Active proliferation of the neoplastic cells was observed three weeks after culture initiation. These cells were proliferating in a floating fashion and were subcultured every five to seven days. The doubling time of the 33rd passage was 27.3 hr. The cells have been passaged for more than two years and have been proliferating well with a stable doubling time.

This established cell line was designated GL-1 and its morphological and cytological features were investigated. Light microscopy revealed that a large proportion of the cells were round, but some tumor cells showed slightly irregular cell surfaces. The cytoplasm was abundant and slightly basophilic in Giemsa staining (Fig. 1). Some tumor cells contained many vacuoles in their cytoplasm. The nuclei of the cells were mostly ovoid, but sometimes indented or segmented. In cytochemical staining, GL-1 cells were negative for peroxidase, and faintly positive for acid phosphatase and α-naphthyl butyrate esterase (Fig. 2). These cytochemical profiles suggested that GL-1 cells and staining properties of lymphocytic lineage [4, 6]. Electron microscopy revealed abundant rough and smooth endoplasmic reticulum, active Golgi areas, free ribosomes and mitochondria in the cytoplasm, which suggested the cell had a high metabolic rate (Fig. 3). The vacuoles, which were also observed by light microscopy, contained myelin-like structures.

Fig. 1. Light microscopic findings of GL-1 cells (× 400, Giemsa stain). The tumor cells had irregular cell surfaces and abundant cytoplasm, which was slightly basophilic in Giemsa staining (A). Some tumor cells had many cytoplasmic vacuoles (B).
For the chromosomal analysis, cells at the 17th passage were incubated with 0.025 µg/ml colcemide for 3 hr, followed by hypotonic treatment and fixation with 3:1 methanol-acetic acid. Chromosomal preparations were stained with Giemsa and the chromosome number in 200 cells was counted, which yielded a mean of 78 chromosomes per cell (mode: 78, range: 74-80).

Cell surface markers were analysed with anti-dog leukocyte monoclonal antibodies (MABs) and a flowcytometer. The MABs used in this study were anti-dog CD5 (Dog 17-4-8), CD45 (Dog 32-2), Thy-1 (F3-20-7) [9] and a lymphocyte subpopulation (DT-2) [12]. MABs detect CD5 and CD45 were kindly provided by Dr. Christiane Vogl. Anti-dog IgG (heavy and light chain) antibodies (Cappel Research Products, Duham, NC.) were also used to detect surface immunoglobulins. GL-1 cells were incubated with these primary antibodies at 4°C for 30 min, washed three times, incubated with FITC-labeled goat anti-mouse IgG. These cells were analysed with a flowcytometer (Cyto-Ace 150, Japan Spectroscopic Co., Tokyo, Japan).

Figure 4 shows the results of surface marker analysis of GL-1, which showed positive reactions with the MABs against dog CD45 and the lymphocyte subpopulation (DT-2). DT-2 was first reported to recognize a T-lymphocyte...
surface antigen, however, it was recently reported to recognize a B-lymphocyte subpopulation [8]. GL-1 cells also reacted positively with the antibody that detects canine IgG. These surface marker characteristics suggest strongly that GL-1 was derived from B-cell lineage. For more conclusive identification of the lineage of this cell line, rearrangements of immunoglobulin is now under investigation. These chromosomal and surface marker analyses were repeatedly performed with stable results.

Recently, long-time survival has been achieved in human lymphoma/leukemia patients with an intensive chemotherapy. Extensive investigations on tumor biology using established leukemic cell lines may contribute significantly to further improvements [5, 11]. Lymphoma/leukemia is one of the most common tumors in dogs and further work should be carried out using these cell lines to investigate biological features and therapeutic modalities.

Some reports describing the phenotypes of canine lymphoma/leukemia cells have been published. In these papers, many of the lymphoma/leukemia cases had tumor cells with characteristics of B-cell types lineage [3, 8, 10]. Our surface marker analysis results indicated that GL-1 cell line was derived from canine B-cells. Therefore, this cell line may be a useful model for biological and therapeutic investigations on canine lymphoproliferative disorders.

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