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Short communication

Establishment of a novel feline leukemia virus (FeLV)-negative B-cell cell line from a cat with B-cell lymphoma

Hiroyuki Mochizuki[a], Masashi Takahashi[a], Kazuo Nishigaki[b], Tetsuya Ide[c], Yuko Goto-Koshino[a], Shinya Watanabe[b], Hirofumi Sato[a], Masahiko Sato[a], Yukiko Kotera[a], Yasuhito Fujino[a], Koichi Ohno[a], Kazuyuki Uchida[c], Hajime Tsujimoto[a,*]

[a] Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan
[b] Laboratory of Molecular Immunology and Infectious Disease, Faculty of Agriculture, Yamaguchi University, Japan
[c] Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan

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ABSTRACT

We established a novel feline B-cell line, MS4, from the neoplastic pleural effusion of a cat with cutaneous B-cell lymphoma. Immunophenotype staining of the MS4 cells was positive for CD20, CD79α, and IgA and negative for CD3, CD4, CD5, CD8α, CD18, CD21, CD22, IgM, IgG, Ig light chain, and MHC class II. PCR analysis for immunoglobulin heavy chain gene rearrangements revealed a monoclonal rearrangement, whereas no clonal rearrangement of the T-cell receptor γ gene was detected. Southern blotting with an exogenous feline leukemia virus (FeLV) U3 probe revealed no integration of exogenous FeLV provirus. The MS4 cell line is the first FeLV-negative feline B-cell lymphoma cell line, and may be used to investigate the pathogenesis of spontaneously occurring feline lymphoma and the development of new therapies.

1. Introduction

Lymphoma is the most common hematopoietic malignancy in domestic cats, accounting for 90% of hematopoietic tumors in this species (Dorn et al., 1967). The occurrence of lymphoma in cats has been strongly associated with feline leukemia virus (FeLV) infection (Cotter et al., 1975). In recent decades the widespread use of the FeLV testing and vaccine has reduced the frequency of FeLV infection. With the decline in FeLV infection rate, the prevalence of FeLV-negative lymphoma has increased significantly, presenting with changes in tumor cell immunophenotypes, anatomical sites and prognoses (Teske et al., 2002; Louwerens et al., 2005). Therefore, investigation of FeLV-negative lymphoma in cats is required for understanding cancer cell biology and the development of new therapies in the post-FeLV era.

Pathological studies and development of new therapies in various malignancies would benefit greatly from cell lines derived from naturally occurring neoplasms. *In vitro* research on feline lymphoma is severely limited despite the high prevalence of this disease because of the lack of well-characterized feline lymphoma cell lines. Most currently available feline lymphoma cell lines are FeLV-positive T-cell lymphoma cell lines, which were derived from the most common type of feline lymphoma in the FeLV era (Rickard et al., 1969; Theilen et al., 1969; Miura et al., 1987). There is no published evidence of a B-cell lymphoma cell line that could account for 50–70% of all feline lymphoma (Gabor et al., 1999; Waly et al., 2005).

To accelerate research on spontaneously occurring feline lymphoma, we established a FeLV-negative B-cell

* Corresponding author at: Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.
Tel.: +81 3 5841 5403; fax: +81 3 5841 5640.
E-mail address: atsuji@mail.ecc.u-tokyo.ac.jp (H. Tsujimoto).

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lymphoma cell line, designated as MS4. This cell line will contribute to further research on feline lymphoma.

2. Materials and methods

2.1. Patient history

A 9-year-old castrated male Abyssinian cat was presented to the referring veterinarian with subcutaneous mass in June 2009. Two discrete masses (6.0 cm × 4.5 cm × 4.0 cm and 4.0 cm × 2.5 cm × 1.5 cm) were noted during physical examination. Both masses were removed surgically and evaluated for histopathology and immunohistochemistry. Staining was performed as described below. Cutaneous large B-cell lymphoma was diagnosed based on World Health Organization classification criteria (Valli et al., 2002). The cat was referred to the Veterinary Medical Center of the University of Tokyo in July 2009 for consultation and additional treatment. The cat was negative for FeLV antigen and feline immunodeficiency virus (FIV) antibody (FIV-FeLV Snap Combo Test for FeLV p27 antigen and specific antibodies to FIV; IDEXX, Westbrook, ME). The cat was treated with the multi-agent therapy, including lomustine, L-asparaginase, cytosine arabinoside, and prednisolone. At an admission 91 days after initiation of chemotherapy, pleural effusion was observed and collected by thoracocentesis to establish the MS4 cell line. The cat died due to disease progression 2 weeks after pleural effusion was observed.

2.2. Establishment of the feline B-cell lymphoma cell line MS4

Cells (5×10^6) obtained from the plural effusion on day 91 were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 20% fetal bovine serum (Biowest, Nuaille, France), 100 IU/ml of penicillin and 0.1 mg/ml of streptomycin (Sigma–Aldrich, St. Louis, MO) at 37 °C in a humidified atmosphere of 5% CO_2. For the first 4 weeks, half of the medium was changed once a week. When cell growth became apparent after 4 weeks, the cells were passaged at 3- or 4-day intervals by cell density to 2×10^5–2×10^6 cells/mL. After 20 passages, MS4 cells were cultured in RPMI 1640 with 15% FBS. The MS4 cell line has been maintained in continuous culture for more than 12 months with over 90 passages.

2.3. Cytology and cytochemistry

MS4 cells were stained with Wright-Giemsa solution for morphological analysis. For cytochemical analysis, cells were tested for myeloperoxidase, alpha-naphthol AS-D chloroacetate esterase activity according to previously described methods (Tsujimoto et al., 1983).

2.4. Generation of growth curve

MS4 cells were seeded at a density of 4×10^5 cells in 2 mL culture medium per well of a 24-well plate (Corning, Lowell, MA). Cells in triplicate wells were counted by trypan-blue dye exclusion every 24 h. Cell counts were plotted on a log-linear scale. The population-doubling time was determined from the exponential phase of the growth curve.

2.5. Flow cytometry and immunocytochemical staining

For each flow cytometric analysis, 1×10^6 cells were incubated with the 7 mouse monoclonal antibodies against feline CD4 (Clone: FE1.7B12), feline CD5 (Clone: FE1.1B11), feline CD8α (Clone: FE1.10E9), canine CD18 (Clone: CA1.4E9), canine CD21 (Clone: CA2.1D6), feline CD22 (Clone: FE2.9F2), and feline MHC class II (Clone: 42.3) for 30 min on ice. Antibodies were purchased from the Leukocyte Antigen Biology Laboratory (Davis, CA). After a washing step, the samples were labeled with FITC-conjugated anti-mouse IgG_1 antibody (BD Biosciences, San Jose, CA) and incubated for an additional 30 min on ice. Finally, labeled cells were washed twice and analyzed on a flow cytometer (FACSCalibur; BD Biosciences). The expression of seven intracytoplasmic antigens was assessed on cytospin smears of MS4 cells. MS4 cells were labeled with 6 polyclonal antibodies against human CD3 (DAKO, Glostrup, Denmark), human CD20 (Neomarkers, Fremont, CA), feline IgA (Bethyl, Warren, NJ), feline IgG (Bethyl), feline IgM (Bethyl) and feline Ig light chain (Bethyl) and 1 mouse monoclonal antibody against human CD79α (Clone: HM57; Dako). After fixation with acetone (for CD3, CD20, and CD79α) or methanol (for IgM, IgG, IgA and Ig light chain), slides were washed 3 times in Tris-buffered saline (TBS, pH 7.4), and treated with 1% hydrogen peroxide/methanol for 30 min. Following three washes in TBS, the slides were incubated in 8% skim milk/TBS at 37 °C for 40 min. The slides were incubated with primary antibodies diluted in 8% skim milk/TBS at 37 °C for 1 h. Following three washes in TBS, the slides were then incubated with Envision polymer reagent (Dako) at 37 °C for 40 min. Finally, the reaction products were visualized with 0.05% 3-3′-diaminobenzidine tetrahydrochloride (DAB) and 0.03% hydrogen peroxide in 0.05 M Tris/HCl buffer (pH 7.5), followed by a counterstain with Mayer's hematoxylin. Formalin-fixed paraffin-embedded lymph node and spleen from a cat, which died of intestinal obstruction served as a positive control for these antibodies.

2.6. PCR for antigen receptor gene rearrangements

PCR for antigen receptor gene rearrangements (PARR) analysis was performed on extracts of total genomic DNA from the primary neoplastic pleural effusion and 1×10^7 MS4 cells using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany).

The DNA samples were assayed by amplifying immunoglobulin heavy chain (IgH) gene rearrangements and T-cell receptor gamma (TCRγ) gene rearrangements with previously described primer sets (Henrich et al., 2009; Moore et al., 2005). The PCR products were analyzed by 10% polyacrylamide Tris–borate EDTA gel electrophoresis and stained with ethidium bromide. A reaction was considered monoclonal if a discrete PCR product was present on the gel after electrophoresis. To compare the IgH or TCRγ gene rearrangements between cells in the