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Characterization of a newly established nonproducer lymphoma cell line for feline leukemia virus

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Abstract

A feline lymphoblastoid cell line (KO-1) was established from a 5-year-old neutered female cat with naturally occurring thymic lymphoma. KO-1 cells had a rearrangement of T-cell receptor β-chain gene and a germ-line configuration of immunoglobulin heavy chain gene, however, they were devoid of T-cell-specific surface phenotype. Cytogenetically, KO-1 cells showed a hyperploidy ($2n = 41$) due to the trisomy of B2, F2 and X chromosomes. Although KO-1 cells were shown to be clonally expanded cells integrated with feline leukemia virus (FeLV) proviruses and expressed its structural proteins in their cytoplasm, they did not produce virus particles as shown by transmission electron microscopy and the absence of the viral protein and reverse transcriptase activity in the culture supernatant. The present study showed that the KO-1 cell line established here was a feline T-cell lymphoma cell line having a unique characteristic as an FeLV nonproducer.

Keywords: Cat; T-cell line; FeLV; Chromosome aberration; Interleukin-2 receptor

1. Introduction

In cats, malignant lymphoma is the most common neoplasm (Dorn et al., 1967; MacVean et al., 1978) and is frequently associated with infection of feline leukemia virus (FeLV) which is a type-C retrovirus horizontally transmitted among domestic cats (Hardy et al., 1973). The majority of FeLV-associated lymphomas show thymic form and consist of clonal population of malignant lymphocytes identified by the retroviral integration, cell surface phenotype or rearrangement of the T-cell receptor (*TCR*) gene

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(Casey et al., 1981; Rojko et al., 1989; Neil et al., 1991; Tsatsanis et al., 1994; Linenberger and Abkowitz, 1995).

Various lymphoma cell lines producing FeLV have been utilized to investigate the tumorigenesis mediated by FeLV (Yamamoto et al., 1986; Miura et al., 1987; Miura et al., 1989; Tsatsanis et al., 1994). In some species, there have been several virus non-producer cell lines derived from tumors induced by oncogenic virus (Zur Hausen and Schulte-Holthausen, 1970; Anand et al., 1981; Itohara et al., 1987). These virus non-producer cell lines can be used as useful materials for studying spontaneous tumorigenesis because of the lack of the effect from superinfection of the virus. There has been no report on the FeLV nonproducer lymphoma cell line integrated with FeLV provirus.

In several human lymphoid tumors, specific chromosomal aberrations have been frequently identified (Showe and Croce, 1987; Korsmeyer, 1992). Translocations between the chromosomes containing T-cell receptor (*TCR*) or immunogloblin (*Ig*) gene locus and those containing loci of genes associated with cellular growth and apoptosis have been observed in such cases. In these human lymphohematopoietic malignancies, chromosomal analysis involving karyotyping, chromosome painting and fluorescence in situ hybridization (FISH) have provided an advantageous tool for studying the molecular mechanism and diagnostic method. In cats, chromosome aberrations have been reported in some malignant neoplastic diseases. Especially, translocation and aneuploidy have been frequently observed in lymphohematopoietic malignancies in cats (Goh et al., 1981; Grindem and Buoen, 1989; Gulino, 1992; Wu et al., 1995).

In the present study, we established an FeLV nonproducer lymphoma cell line, named KO-1, and characterized its virological, immunological and cytogenetic characteristics.

2. Materials and methods

2.1. Case

A 5-year-old spayed female domestic short-hair cat showing anorexia, lethargy, hematuria and dyspnea was referred to Veterinary Hospital, Yamaguchi University. Physical examination revealed dehydration and accumulation of pleural effusion. The cat was serologically positive for FeLV antigen and negative for feline immunodeficiency virus antibody tested with a commercial kit (Petcheck FeLV/FIV) (Idexx Laboratories, Portland, ME). The pleural fluid contained a number of lymphoblastoid cells that were round to oval in shape and were variable in size. They had a basophilic cytoplasm and a polymorphic nucleus with poor chromatin condensation and several large nucleoli. The cat was diagnosed as thymic form lymphoma (non-Hodgkin lymphoma in WHO classification) and died 34 days after diagnosis.

2.2. Cell culture

The lymphoblastoid cells collected from the pleural fluid were cultured in RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 20% fetal bovine serum (FBS) (Cansera, Ontario, Canada), 100 IU of penicillin and 0.1 mg of streptomycin (Sigma) per ml at 37 °C in a humidified atmosphere of 5% CO_2. During the initial cultivation period, addition of fresh medium or medium change was continued every 3–5 days. After the cell growth became apparent, the cells were passed every 3–5 days by adjusting the cell density at 3×10^5 to 1×10^6 cells per ml in the growth medium containing 10% FBS.

2.3. Morphological, cytochemical and ultrastructural analysis

For morphological analysis, KO-1 cells were stained with Wright–Giemsa solution. For cytochemical analysis, cells were tested for myeloperoxidase, α-naphthyl butyrate esterase and naphthol AS-D chloracetate esterase activity according to the methods described previously (Tsujimoto et al., 1983).

For ultrastructural analysis, KO-1 cells were fixed with 2.0% glutaraldehyde in 0.1 M phosphate buffer, washed and postfixed with 1.0% osmium tetraoxide in the same buffer. Cells were then dehydrated in an alcohol gradient and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission

electron microscope (JEOL JEM-100S) (JEOL Co. Ltd., Tokyo, Japan).

2.4. Cell surface phenotype analysis

A monoclonal antibody (mAb) against feline IL-2Rα (9F23) (Ohno et al., 1992), and those directed to feline CD4, CD5, CD8α, CD21, CD22 and MHC class II (Rideout et al., 1990; Woo and Moore, 1997) were used as primary antibodies in conjunction with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (H + L) (Jackson ImmunoResearch Laboratories, West Grove, PA). Antibodies against feline IgG-heavy and light chains and IgM-μ chain (BETHYL Laboratories, Montogomery, TX) were also used in conjunction with FITC-conjugated donkey anti-goat IgG (H + L) (Jackson Immuno-Research Laboratories). Samples without primary antibodies were prepared as fluorescence controls. The immunofluorscence of 10,000 cells was analyzed with a flow cytometer (FACScan) (Becton Dickinson, Franklin Lakes, NJ).

2.5. In vitro growth characteristics

To examine the growth characteristics, cells (5×10^5 cells per ml) were cultured in the presence or absence of 100 IU of recombinant human interleukin (IL)-2 (rhIL-2) (Strathmann Biotech, Hannover, Germany) per ml. The number of viable cells was counted by the method of trypan-blue dye exclusion every 24 h.

2.6. Chromosome karyotyping analysis

Cells were cultured in the growth medium with 0.01 mg of colcemid (Demecolcin) (Wako, Osaka, Japan) per ml for 2 h before harvest. Then the cells were treated with 0.075 M potassium chloride at 37 °C for 20 min and fixed with a 3:1 mixture of methanol and acetic acid on ice. After repeating the fixation process more than three times, the fixed cell suspension was dropped onto slides, air-dried and stained with a combined solution of Hoechst 33258 and quinacrine mustard for chromosome HQ-banding. Karyotyping analysis was carried out based on the standard karyotype of domestic cat as previously reported (Cho et al., 1997b).

2.7. Chromosome painting analysis

Painting probes specific for feline A2, B2, B3, C1, D3, D4 and F2 chromosomes that had been previously generated by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) (Wienberg et al., 1997) were kindly provided by Dr. S.J. O'Brien (National Cancer Institute, Frederick, MD). In situ hybridization to the chromosomes was performed as described previously (Fujino et al., 2001a,b). Metaphase cells prepared on the glass slides were denatured in the denaturing solution consisting of 70% formamide and 2× SSC (1× SSC contains 0.15 M sodium chloride and 0.015 M sodium citrate) at 72 °C for 3 min, dipped in 70% ethanol at −20 °C for 3 min, and dehydrated in 100% ethanol at room temperature for 3 min. A 1:14 mixtures of the biotinylated or digoxigenin-labelled PCR products and an hybridization solution consisting of 50% formamide, 1× SSC and 10% dextran sulfate were denatured at 75 °C for 10 min, kept on ice for 5 min, placed onto the denatured metaphase cells on glass slides, and then mounted with parafilms. In situ hybridization was performed at 37 °C for 20 h. After hybridization and washing of the slides, biotinylated chromosome paints were detected with avidin coupled with FITC (Roche Molecular Biochemicals, Mannheim, Germany), and digoxigenin-labelled chromosome paints were detected with anti-digoxigenin rhodamine (Roche Molecular Biochemicals). The identification of chromosomes was facilitated by 4′,6-diamidino-2-phenylindole (DAPI) (Sigma)-Q-banding karyotype concurrently with FISH (Cho et al., 1997b).

2.8. Southern blot hybridization analysis

The exogenous FeLV probe for hybridization prepared from the long terminal repeat (LTR) U3 region of the exogenous FeLV clone, pJ7E2 (Miura et al., 1987), was used. A feline immunoglobulin heavy chain (IgH) constant region clone of 691 bp (IgH Cμ) (FIMC691), a feline T-cell receptor (TCR) β-chain constant region clone of 393 bp (TCR Cβ) (FTBC393) and a feline TCR γ-chain constant region clone of 554 bp (TCR Cγ) (FTGC554) (Cho et al., 1998) were used for analysis of the rearrangement of these genes.

High molecular weight genomic DNA samples were extracted from KO-1 and FT-1 cells derived from

FeLV-positive thymic lymphoma (Miura et al., 1987, 1989) and a feline fetus liver tissue. These specimens were treated with a lysis buffer containing 0.02 mg of proteinase K (Sigma) per ml, 0.01 M Tris–hydrochloride (pH 8.0), 1 mM ehthylenediamine tetraacetic acid (EDTA), 0.5% sodium dodecyl sulfate (SDS) and 0.01 mg of RNase A (Sigma) per ml at 37 °C for 24 h. Then, the DNA samples were extracted with phenol and chloroform, and precipitated with ethanol. The DNA samples (15 μg/lane) were digested with 100 U of restriction enzymes, electrophoresed through 0.8% agarose gels, and transferred onto nylon membranes. The DNA samples on the membranes were hybridized with a ^{32}P-labelled probe in a hybridization solution containing 5× SSC, 1% SDS, 0.05 M Tris–hydrochloride (pH 7.6), 0.1 mg of salmon sperm DNA (Sigma) per ml and 5× Denhardt's solution (1× Denhardt's solution consists of each 0.02% of ficoll type 400, polyvinyl pyrrolidone and bovine serum albumin fraction V) at 62–65 °C for 16 h. After hybridization, the filters were washed three times with a solution containing 1× SSC and 0.1% SDS at 57–60 °C for 30 min and subjected to autoradiogaphy.

2.9. Reverse transcriptase (RT) activity assay

Culture supernatants were obtained 3 days after cultivation of KO-1 cell line, FT-1 cell line (Miura et al., 1987, 1989) and 3201 cell line derived from FeLV-negative lymphoma (Snyder et al., 1978). Each supernatant of KO-1 and FT-1 cell cultures was centrifuged at 750 × *g* for 10 min and filtrated through a 0.45 μm-pore filter (Acrodisc Syringe Filter) (Pall Gelman Laboratory, Ann Arbor, MI) to remove cells, and inoculated onto 3201 cells for 24 h to examine whether cells had produced infectious viruses. Then, culture supernatants of inoculated 3201 cells were obtained 2 and 5 days after inoculation (3 and 6 days after passage). All the supernatants (1 ml per each sample) were first centrifuged at 750 × *g* for 10 min to remove cell debris and then recentrifuged at 20,000 × *g* for 3 h. The resulting pellets were suspended in 10 μl of TNE buffer containing 0.1 M sodium chloride, 0.01 M Tris–hydrochloride (pH 7.6), and 1 mM EDTA, and then mixed with 50 μl of RT buffer containing 5 μg of poly (A) (Sigma) per ml, 0.16 μg of oligo (dT) (Roche Molecular Biochemicals) per ml, 0.1% Nonidet P-40, 0.06 M Tris–hydrochloride (pH 7.8), 0.075 M potassium chloride, 5 mM magnesium chloride, 1 mM EDTA, 1 M dithiothreitol (DTT) and 10 μCi of [^{32}P] dTTP (Amersham Pharmacia Biotech, Buckinghamshire, England) per ml. After incubation at 37 °C for 3 h, 10 μl of the mixtures were spotted onto DEAE filter papers (Wallac Oy, Turku, Finland) and washed four times with 2× SSC and once with 99% ethanol. The filter papers were then dried, and the incorporated radioactivity on the filter papers was measured with a scintillation counter. Each sample was tested in triplicate.

2.10. Detection of FeLV proteins

FeLV proteins were examined by Western blot analysis. KO-1 cells and FT-1 cells (Miura et al., 1987, 1989) were lysed with a lysis buffer containing 0.05 M Tris–hydrochloride (pH 8.0), 0.15 M sodium chloride, 5 mM EDTA, 1% Nonidet P-40, 0.1% SDS, 10% protease inhibitor cocktail (Sigma) and 0.5 mM phenylmethylsulfonyl fluoride at 4 °C for 1 h on a rotary shaker. The protein in the culture supernatant obtained 3 days after cultivation of KO-1 cells was precipitated with 80% aluminum ammonium sulfate and desalted. The protein samples (10 μg/lane) were dissolved in a sample buffer containing 0.06 M Tris–hydrochloride (pH 6.8), 2% SDS, 0.1 M DTT, 2.5% glycerol and 0.01% bromophenol blue, boiled for 5 min, separated by SDS–polyacrylamide gel electrophoresis and then subjected to Western blot analysis using mAbs directed to FeLV p27, gp70 and p15E (Biogenesis, Kingston, NH). The bands were visualized using Lumi-Light^{PLUS} Western Blotting Kit (Roche Molecular Biochemicals).

3. Results

3.1. Morphological, cytochemical and ultrastructural properties

In the Wright–Giemsa-stained preparation, KO-1 cell line was composed of the cells with a large nucleus containing one to several large nucleoli and showing poor condensation of chromatin and markedly basophilic cytoplasm (Fig. 1). KO-1 cells were approximately 15–25 μm in the diameter. KO-1 cells showed weakly positive reactions in the α-naphthyl

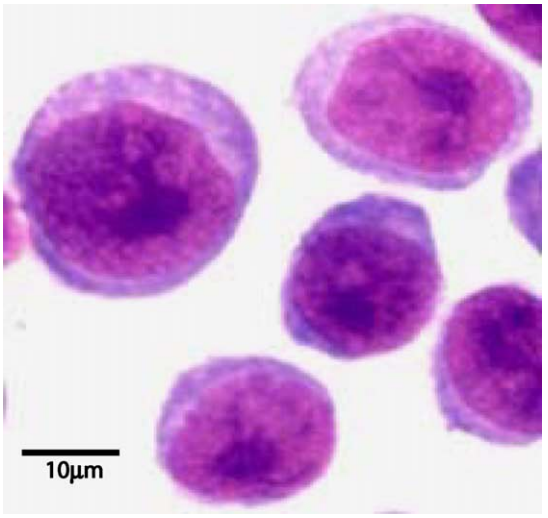

Fig. 1. Morphological appearance of KO-1 cells by Wright–Giemsa stain (×1000). Bar = 10 μm.

butyrate esterase and naphtol AS-D chloroacetate esterase stainings, and were negative for myeloperoxidase.

Transmission electron microscopy revealed that KO-1 cells were round to elongated with central rounded or oval nuclei (Fig. 2(a)). Their nuclei were moderately large and rarely lobulated in shape and contained one or more nucleoli and peripherally clumped heterochromatin. The cytoplasm contained moderate numbers of organelles, including mitochondria, lysosomes, endoplasmic reticulum, and dilated vacuoles (Fig. 2(b)). Viral particles were not detected.

3.2. Cell surface phenotype

In the flowcytometric analysis for the cell surface phenotype (Fig. 3), KO-1 cells were shown to be composed of a monoclonally expanded cell population positive for MHC class II, IL-2Rα and CD5, and were negative for CD4, CD8α, CD21, CD22, surface IgG and surface IgM.

3.3. Rearrangement of immunogloblin and T-cell receptor genes

To examine the cell lineage of KO-1 cells, rearrangement of *IgH*, *TCRβ* and *TCRγ* genes was analyzed by Southern blot analysis (Fig. 4).

The feline germ-line DNA fragments hybridized with the *IgH Cμ* probe were detected as 2 bands comprising an intense 7.4-kb band and a faint 6.4-kb band in the *Bam*HI digest and as 3 bands comprising an intense 6.5-kb band and 2 other faint bands in the *Eco*RI digest. The DNA sample of KO-1 cells digested with *Bam*HI and *Eco*RI showed the germ-line configuration of *IgH* gene in the Southern blot analysis using the *IgH Cμ* probe (Fig. 4(a)).

Germ-line bands detected by the *TCR Cβ* probe were 4 bands of 6.6, 3.2, 2.2 and 1.8 kb in the *Bam*HI digest and 3 bands of 3.2, 2.2 and 1.8 kb in the *Hin*dIII

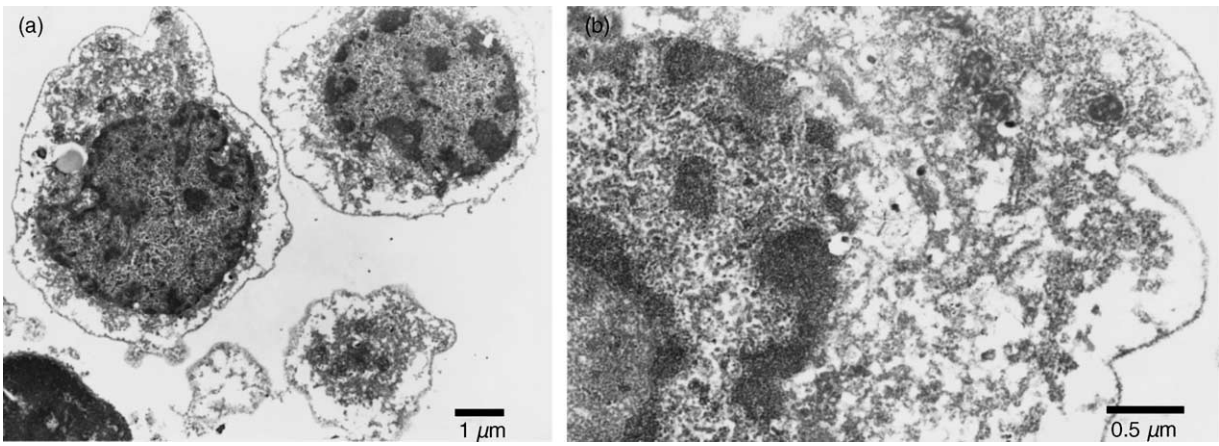

Fig. 2. Ultrastructure of KO-1 cells. The cells were round to elongated with central rounded or oval nuclei (a, ×6000). The cytoplasm contained moderate numbers of organelles (b, ×20,000). Viral particles were not detected. Uranyl acetate and lead citrate. Bar = 1.0 μm (a) and 0.5 μm (b).

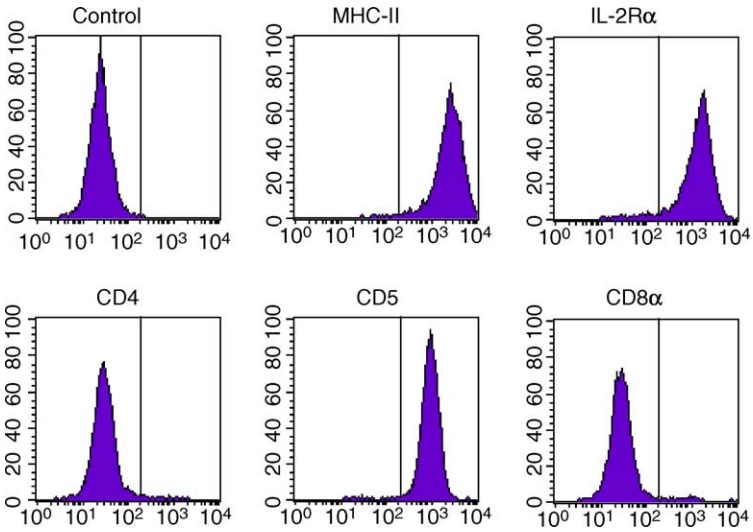

Fig. 3. Flow cytometric analysis of surface markers of KO-1 cells. The single color immunofluorscence staining was performed using MHC class II, IL-2Rα, CD4, CD5 and CD8α antibodies. The sample without primary antibodies was prepared as a fluorescence control. The vertical axis, horizontal axis and vertical bar on each histogram indicated the number of cells, fluorescence intensity and background fluorescence intensity, respectively.

digest (Fig. 4(b)). The bands detected by the *TCR Cβ* probe in KO-1 cells were 3 bands of 6.6, 3.2 and 2.2 kb in the *Bam*HI digest and 3 bands of 5.0, 3.2 and 2.2 kb in the *Hin*dIII digest (Fig. 4(b)), indicating the apparent rearrangement of *TCRβ* gene.

Germ-line bands detected by *TCR Cγ* probe were 3 bands of 2.1, 3.2 and 7.0 kb in the *Bam*HI digest and 3 bands of 2.4, 3.6 and 6.5 kb in the *Eco*RI digest (Fig. 4(c)). The KO-1 DNA showed the germ-line DNA configuration of *TCRγ* gene in the Southern blot

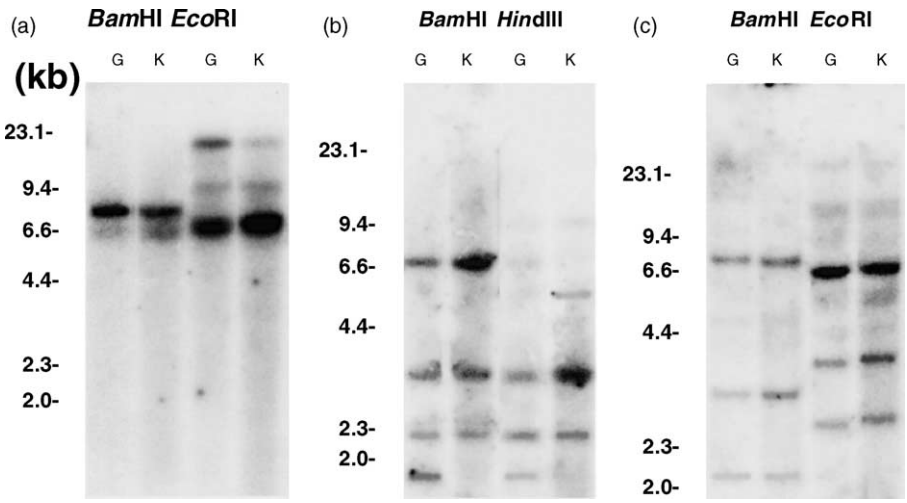

Fig. 4. Genetic status of the *Ig* and *TCR* genes. DNA samples from the liver of a feline fetus as a germ-line (lane G) and KO-1 cells (lane K) digested with *Bam*HI, *Eco*RI or *Hin*dIII were subjected to Southern blot analysis using the probes: (a) *IgH Cμ*; (b) *TCR Cβ* and (c) *TCR Cγ*. Molecular size markers on the left side of each figure were *Hin*dIII fragments of λDNA (kilobase).

analysis using the *TCR Cγ* probe (Fig. 4(c)) in this study.

3.4. In vitro growth characteristics

KO-1 cells had a population doubling time of 50 h and formed floating clumps composed of about 10–50 cells. In the presence of rhIL-2, the KO-1 cells showed a more rapid growth with a population doubling time of 33 h, indicating its responsiveness to IL-2.

3.5. Cytogenetic analysis

A total of 12-well banded metaphases were analyzed by HQ-banded cat karyotyping. Although a normal cat metaphase contain 38 chromosomes (*Felis catus*, $2n = 38$), all of metaphases from KO-1 cells showed hyperdiploid having 41 chromosomes due to the trisomies of B2, F2 and X chromosomes (Fig. 5(a)). By using painting probes specific to feline B2 and F2 chromosomes, the trisomies of these chromosomes were confirmed (Fig. 5(b)–(e)).

3.6. Integration of exogenous FeLV proviruses

Integration of exogenous FeLV proviruses in the KO-1 genome was examined by Southern blot analysis (Fig. 6). In Southern blot analysis using the exogenous FeLV U3 probe, *Kpn*I digest of KO-1 cell DNA gave a dense band of 1.4-kb corresponding to the 3′ FeLV internal fragment and 6 discrete bands of 4.0, 2.5, 2.2, 1.8, 1.5 and 1.2 kb corresponding to the 5′ flanking

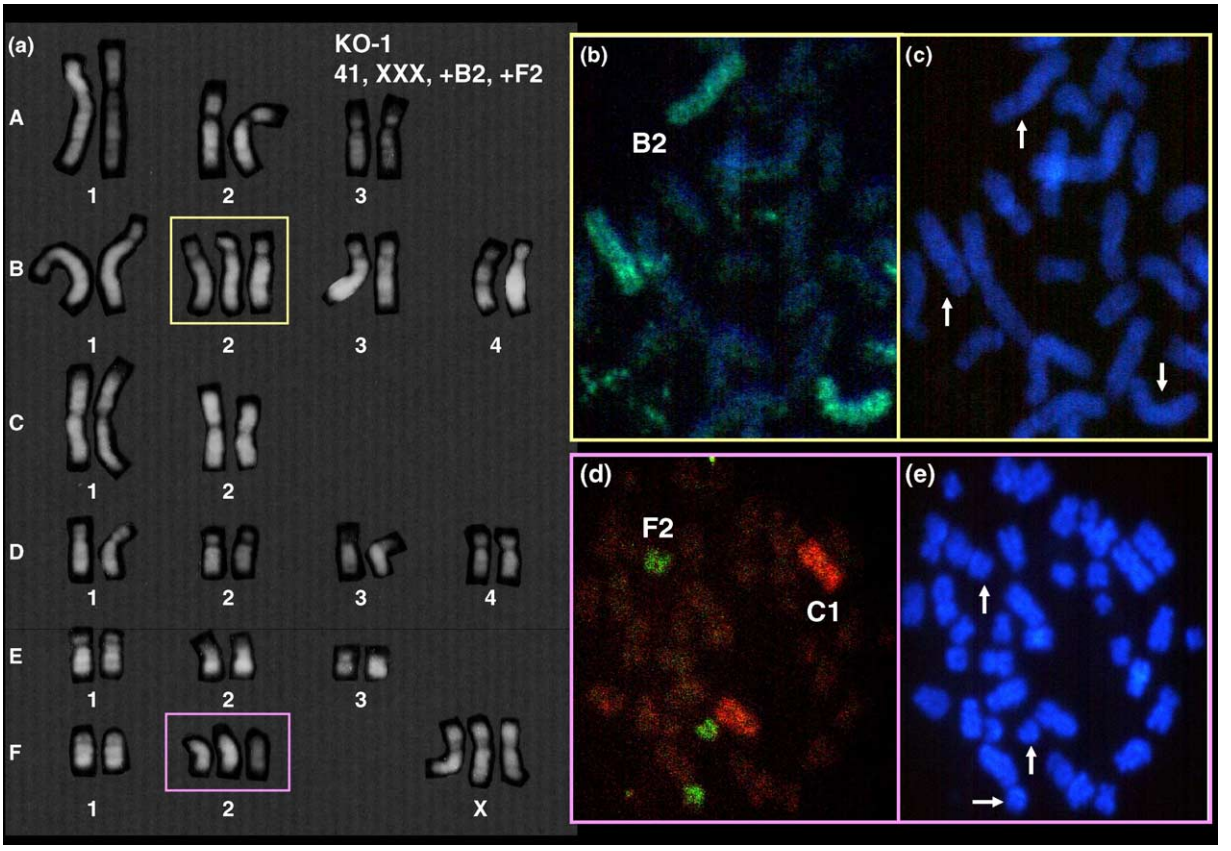

Fig. 5. Cytogenetic analysis of the KO-1 cell line. The karyotype was interpreted as [41, XXX, +B2, +F2] by HQ-banding (a). Each extra copy of chromosomes B2 and F2 was confirmed by feline chromosome painting. Three copies of chromosomes B2 (green (b)) and F2 (green (d)) were visualized with FITC in contrast to normal two copies of chromosome C1 painted with rhodamime (red (d)). Each chromosome preparation was counterstained with DAPI ((c) and (e) arrows indicated trisomies).

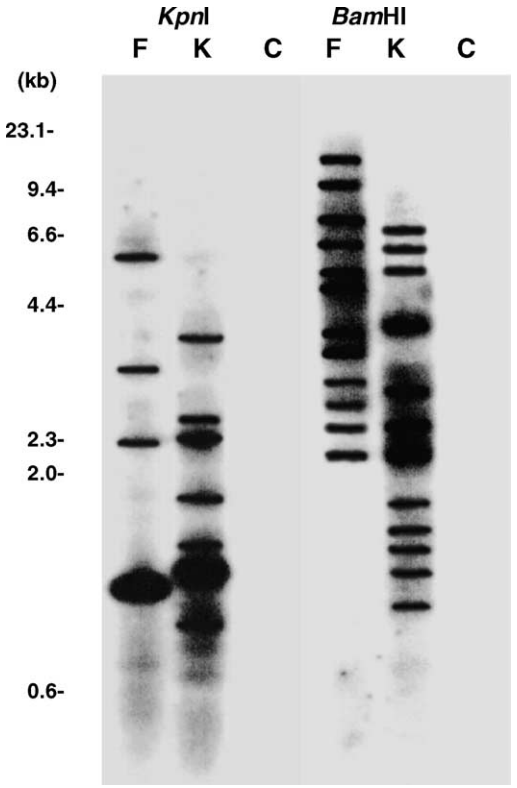

Fig. 6. Detection of exogenous FeLV proviral genome by Southern blot analysis. DNA samples from FT-1 cells as a positive control (lane F), KO-1 cells (lane K) and the liver of a feline fetus as a negative control (lane C) digested with *Kpn*I and *Bam*HI are subjected to Southern blot analysis using the exogenous FeLV U3 probe. Molecular size markers on the left side of each figure were *Hin*dIII fragments of λDNA (kilobase).

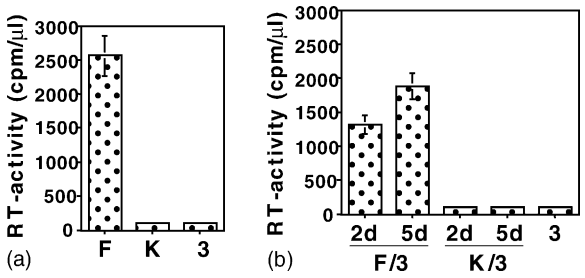

Fig. 7. RT activities in FT-1 (F), KO-1 (K) and 3201 (3) cells. Each supernatant of FT-1 and KO-1 cell cultures was inoculated onto 3201 cells (F/3 and K/3, respectively). RT activities in the culture supernatants were measured after (a) 3 days or (b) 2 and 5 days cultivation. The columns and bars showed mean and standard deviations, respectively, obtained from the data in triplicate samples.

DNA fragments containing FeLV U3 region. In the Southern blot analysis, *Bam*HI digest of KO-1 cell DNA gave 12 discrete bands of 6.6, 6.0, 5.0, 4.1, 2.7, 2.3, 2.1, 1.7, 1.6, 1.5, 1.4 and 1.3 kb corresponding to 5′ and 3′ flanking DNA fragments containing FeLV U3 region. In the FT-1 cell DNA, similar band patterns were observed but the number and the length of the bands were different from those detected in KO-1 cell DNA. No hybridization was shown in the normal cat liver DNA. These results indicated that the KO-1 cell line was composed of a clonal cell population with integration of exogenous FeLV proviruses of six or more copies.

3.7. RT activity

Although RT activity was obviously detected in the culture supernatant of FeLV-producing FT-1 cells, not in that of KO-1 cells (Fig. 7(a)). The radioactivity incorporated into DNA by the KO-1 culture supernatant sample was as low as that by FeLV-negative 3201 culture supernatant sample. To examine whether KO-1 cells produced infectious viruses or not, each supernatant of KO-1 and FT-1 cell cultures was inoculated onto 3201 cells (abbreviated as K/3 and F/3, respectively). Both the radioactivities incorporated into DNA by the supernatants obtained 2 and 5 days after inoculation (3 and 6 days after passage) of K/3 cells were as low as that by 3201 cultures supernatant whereas those of F/3 cells were apparently detected (Fig. 7(b)). These data indicated that KO-1 cells did not produce retrovirus particles having RT activity into the culture supernatant.

3.8. Expression of FeLV proteins

The production of FeLV structural proteins in KO-1 cells was examined by Western blot analysis (Fig. 8). The lysate of FT-1 cells gave 2 bands corresponding to $Pr65^{gag}$ and p27 detected by anti-p27 antibody (Fig. 8(a)), 2 bands corresponding to $gPr85^{env}$ and gp70 detected by anti-gp70 antibody (Fig. 8(b)), and 2 bands corresponding to $gPr85^{env}$ and p15E detected by anti-p15E antibody (Fig. 8(c)), showing the presence of authentic FeLV proteins and their precursors. However, the lysate of KO-1 cells gave 2 bands of 33 and 30 kDa detected by anti-p27 antibody (Fig. 8(a)), 2 bands of 75 and 36 kDa detected by

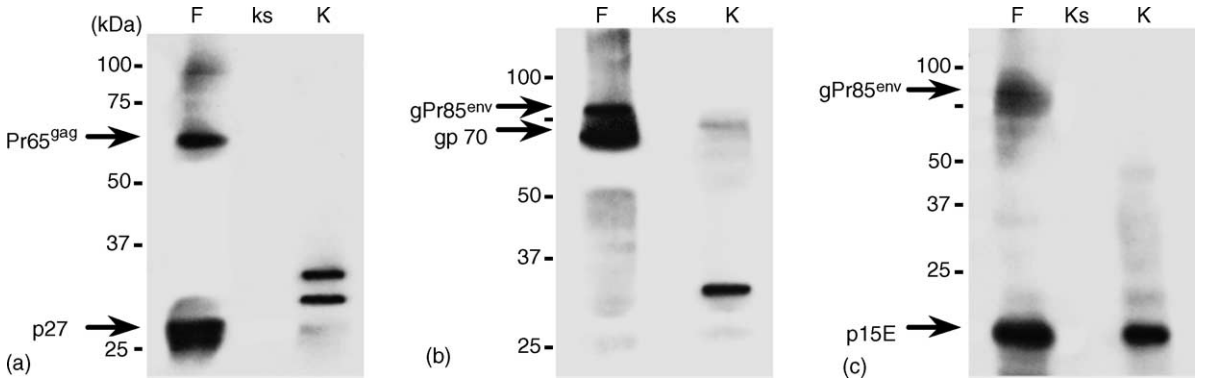

Fig. 8. Detection of FeLV structural proteins by Western blot analysis. The lysates of FT-1 (lane F) and KO-1 (lane K) cells cultured for 3 days, and the protein from the culture supernatant of KO-1 cells (lane Ks) were subjected to Western blot analysis using mAbs of (a) anti-FeLV p27 (b) gp70 and (c) p15E. Protein size markers were shown on the left of each figure (kilodalton).

anti-gp70 antibody (Fig. 8(b)), and a band of 15 kDa detected by anti-p15E antibody (Fig. 8(c)). The concentrated protein sample of the culture supernatant of KO-1 cells gave no detectable band by using any of these antibodies (Fig. 8(a)–(c)) and was also negative for FeLV p27 using a commercial kit (Petcheck FeLV/FIV) (Idexx Laboratories). These results indicated that KO-1 cells produced variant or incomplete FeLV proteins in the cytoplasm and did not release detectable FeLV particles into the culture supernatant.

4. Discussion

Though KO-1 cell line was derived from a cat lymphoma case infected with FeLV, Western blot analysis showed the production of abnormal FeLV structural proteins in the cytoplasm and no detectable FeLV protein in the culture supernatant. Moreover, no detectable viral particle was observed by transmission electron microscopy and also RT activity was not detected in the culture supernatant of KO-1 cells. However, clonal integration of exogenous FeLV was detected by Southern blot analysis. These results indicated that the KO-1 cell line had unique characteristics as an FeLV nonproducer lymphoma cell line and the FeLV proteins in KO-1 cells might be unable to assemble at the cell surface to be released from the cells because of the defectiveness of the *gag* and *env* genes.

Southern blot analysis using exogenous FeLV U3 probe indicated some of the structural abnormality of the FeLV proviruses integrated in KO-1 cells. As shown in the restriction endonuclease map of standard FeLV proviruses, approximately 3.4-kb *Kpn*I band corresponding to the env and 3′-LTR-U3 can be detected in most of FeLV-infected cell lines (Mullins et al., 1980; Casey et al., 1981; Stewart et al., 1986; Overbaugh et al., 1992; Tsujimoto et al., 1993). However, the band corresponding to the 3′ part of FeLV in KO-1 cells was 1.4 kb long, suggesting the variation of the *Kpn*I site or deletion in the proviral genome. Moreover, more than half of the *Bam*HI fragments hybridized with FeLV U3 probe were shorter than 3.1 kb in KO-1 cell DNA although they are usually detected as bands longer than 3.1 kb in tumor cells integrated with standard FeLV proviruses because of the length of internal proviral fragment, suggesting the presence of proviral genomes with some nucleotide change or deletion in KO-1 cells. Variant FeLV proviruses with mutations, deletions, and recombinations with various cellular genes have been demonstrated in feline lymphoma cells (Onions et al., 1987; Terry et al., 1992; Rohn et al., 1994, 1996). These data in Southern blot analysis suggested the presence of various FeLV proviral genomes in KO-1 cells that might be responsible for the inability to produce FeLV particles although molecular cloning of the proviral genome in KO-1 cells is needed to characterize them.

Chromosomal aberrations, such as anomalous chromosome numbers or translocations, have been reported in feline lymphoma and leukemia cells (Goh et al., 1981; Grindem and Buoen, 1989; Gulino, 1992;

Wu et al., 1995). Hyperdipoidy due to extra copies of B3, B4, C2, D1, D3, E1, E3 or sex chromosome have been observed previously (Goh et al., 1981; Grindem and Buoen, 1989; Gulino, 1992). In the present study, hyperdiploidy due to trisomies of B2, F2 and X chromosomes was observed in KO-1 cells using karyotyping and chromosome painting analyses. Feline chromosomes B2 and F2 contain some tumor-associated genes in feline lymphoma. For instance, *fit-1* and *pim-1* known as loci of FeLV common insertion sites in lymphoid tumors are mapped on chromosome B2 (Tsujimoto et al., 1993; Tsatsanis et al., 1994), and c-*myc*, which has been reported to have a close association with FeLV-induced tumorigenesis, is mapped on chromosome F2 (Cho et al., 1997a). The trisomies of B2 and F2 chromosomes in KO-1 cells might have an association with the tumorigenesis of KO-1 cells via enhanced expression of these genes or some other genes mapped on B2 and F2 chromosomes.

In previous studies on immunophenotyping of feline lymphomas (Rojko et al., 1989; Day, 1995; Jackson et al., 1996; Gabor et al., 1999; Wang et al., 2001), many of thymic lymphomas were shown to be T-cell origin. Naturally occurring thymic lymphomas associated with FeLV infection frequently showed rearrangement of *TCRβ* gene (Tsatsanis et al., 1994). In this study, the flowcytometry analysis showed that the KO-1 cells were composed of clonally expanded cells. Though the lineage of KO-1 cells could not be identified by the phenotypic analysis, rearrangement of TCR β-chain gene detected by Southern blot analysis strongly suggested that the KO-1 cell line was originated from αβ T-cell lineage (non-Hodgkin T-cell neoplasm in WHO classification). The expression of CD5, IL-2Rα and MHC class II on the cell surface indicated that the KO-1 cells might be in the activated phase. The presence of rhIL-2 in the growth medium accelerated the proliferation of KO-1 cells, indicating that the KO-1 cells retained an ability to respond to IL-2, and the IL-2Rα on the cell surface was functional to transduce IL-2 signal.

The present study reported an establishment of a novel feline lymphoma cell line, KO-1. The KO-1 cell line established here was shown to have several unique characteristics, and it will be useful for understanding the tumorigenesis associated with FeLV and developing novel diagnostic and therapeutic procedures in lymphoid tumors.

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