Erythroleukemia in Two Cats Naturally Infected with Feline Leukemia Virus in the Same Household

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ABSTRACT. Erythroleukemia was observed in two unrelated cats infected with feline leukemia virus (FeLV) from the same household. Case 1, a 1-year-old neutered male cat developed erythroleukemia (M6) after a diagnosis of myelodysplastic syndrome (MDS-Er) on the criteria of FAB classification of acute leukemias. Case 2, a 1-year-old neutered female cat, which had close contact with Case 1, also developed erythroleukemia (M6Er). In both cases, marked proliferation of erythroid progenitor cells with disproportionately large numbers of immature forms was observed in the bone marrow. In Case 1, neoplastic proliferation of myeloid cells in the bone marrow was also noted at the terminal stage. Combination chemotherapy with daunomycin was partially effective for treatment of these erythroid neoplasias, but did not induce complete remission. Southern blot analysis using exogenous FeLV-specific probes indicated the clonal origin of these hematopoietic tumor cells. Furthermore, the erythroid and myeloid tumor cells in Case 1 were shown to be derived from independent transformed clones. A variant FeLV was shown to be integrated into the tumor cells in Case 1, while a full-length FeLV was found in both cases. Because these erythroid neoplastic diseases occurred in two unrelated cats kept in the same household and these diseases are rare, they may both have been associated with the same FeLV strain.—**KEY WORDS:** erythroleukemia, feline leukemia virus.

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Erythroleukemia is a type of acute leukemia with aberrant proliferation of cells of erythroid lineage showing defective erythropoiesis leading to severe anemia and marked anisocytosis without accompanying polychromasia [5, 14]. In erythroleukemia, many cases were shown to have neoplastic proliferation of myeloid cell lineage in addition to the erythroid cell lineage. In the French-American-British (FAB) classification of acute myeloid leukemia in dogs and cats [6], erythroleukemia is categorized into M6 whereas erythroleukemia in which erythroid progenitor cells are dominant in the bone marrow is categorized into M6Er. M6 and M6Er of erythroleukemia are rare in cats although several cases have been reported [5, 7, 14]. M6 has been shown to develop after the onset of M6Er in some cats [5, 14]. Many cats with erythroleukemia have been found to be infected with FeLV [5, 7]. Although several FeLV strains have been shown to induce thymic lymphoma [4, 8, 9], no virus strain specifically associated with erythroleukemia has been reported. Therefore, it is not clear whether FeLV is directly involved in the tumorigenesis of cells of erythroid lineage. This paper describes the occurrence of erythroleukemia in two unrelated cats infected with FeLV in the same household. The viral etiology of the disease is discussed.

MATERIALS AND METHODS

Hematological examination: Standard procedures were used to determine the packed cell volume (PCV) value and hemoglobin (Hb) concentration. Nucleated cell and erythrocyte counts were determined with the hemocytometer.

Peripheral blood smears stained with Giemsa were used to determine differential nucleated cell counts and the morphologic features of blood cells. Bone marrow smears stained with Giemsa were also used for morphological evaluation and determination of myelogram including the myeloid cell / erythroid cell ratio (M/E ratio). Preparations stained for peroxidase and alpha-naphthyl acetate esterase were used to identify granulocytic and monocytic lineages, respectively.

Histopathological examination: The liver, spleen, mesenteric lymph nodes and bone marrow were examined. These tissues were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin.

Virological examination: FeLV antigen was detected by screening for FeLV p27 gag protein in sera using Leukassay F kit (Pitman - Moore, Inc., Washington Crossby, NJ). For detection of the FeLV proviral genome, high molecular weight cellular DNAs extracted from the tumor cells were analyzed by Southern blot hybridization. Briefly, DNAs digested with restriction endonucleases were electrophoresed in 0.8% agarose gel and transferred to nylon membrane filters. The filters were hybridized with ³²P-labelled exogenous FeLV LTR U3 probe [8] and then exposed to X-ray films.

RESULTS

Clinical and hematological findings: Case 1- A 1-year-old, neutered male, Japanese domestic cat (Case 1) was brought to the Veterinary Medical Center (VMC), Facul-

Table 1. Hematological findings in Case 1

Parameter	First admission	After 1 month	After 5 months
Peripheral blood			
RBC ($\times 10^6$ cells/ μ l)	3.18	2.19	4.32
Packed cell volume (%)	19.0	14.0	20.0
Hemoglobin (g/dl)	6.2	4.8	7.6
Reticulocytes (%)	1.3	1.0	0.2
WBC ($\times 10^3$ cells/ μ l)	29.7	25.0	69.0
Band neutrophils	0	1.7	15.9
Segmented neutrophils	21.1	20.0	50.3
Lymphocytes	7.4	1.7	0.7
Monocytes	0.6	0.8	0.7
Eosinophils	0.6	0.8	1.4
Nucleated erythroid cells ($\times 10^3$ cells/ μ l)	0	81.4(11.0 ^a)	17.0(17.6 ^a)
Immature myeloid cells ($\times 10^3$ cells/ μ l)	0	0	1.91(10.0 ^b)
Platelets ($\times 10^3$ cells/ μ l)	128	57.0	32.0
Bone marrow			
M/E ratio	NT ^c	0.61	1.01
Erythroid cells (%)			
Rubriblasts	NT	11	41
Prorubricytes	NT	11	5
Rubricytes	NT	23	3
Metarubricytes	NT	18	2
Myeloid cells (%)			
Myeloblasts	NT	1	31
Promyelocytes	NT	2	9
Myelocytes	NT	4	3
Metamyelocytes	NT	7	2
Band neutrophils	NT	10	2
Segmented neutrophils	NT	13	2

a) Percentage of rubriblast.

b) Percentage of myeloblast.

c) Not tested.

ty of Agriculture, the University of Tokyo, with complaints of salivation and anorexia for 1 week. At the time of first admission, clinical findings included pale mucous membranes, enlargement of the submandibular lymph nodes and gingivitis. Hematological examination revealed marked anemia and leukocytosis (Table 1). Nucleated erythroid cells were not found in the peripheral blood. The cat was treated with prednisolone, antibiotics and vitamin B complex for 1 month, but did not respond to these treatments.

About 1 month later, the second hematological examination indicated that the cat had more profound anemia and many nucleated erythroid cells in the peripheral blood. These cells included rubriblasts, prorubricytes, rubricytes and metarubricytes. The rubriblasts and prorubricytes had an immature chromatin arrangement with nucleoli and basophilic cytoplasm and showed negative stainings for peroxidase and alpha-naphthyl acetate esterase. In the bone marrow, marked proliferation of erythroid progenitor cells (M/E ratio, 0.61) were observed with disproportionally large numbers of immature form (Table 1, Fig. 1A). The morphological and cytochemical features of these immature erythroid cells were similar to those of the cells found in the peripheral blood. Some binuclear rubricytes and metarubricytes were

observed. Furthermore the rubricytes and metarubricytes showed discrepancy in their degrees of maturation of the nucleus and cytoplasm. Because the proportion of the blasts to all nucleated cells was less than 30% at this stage, the cat was diagnosed as having myelodysplastic syndrome with hyperplasia of erythroid cells (MDS-Er) on the criteria of the French-American-British (FAB) classification of acute leukemias in dogs and cats [6].

The cat responded well to low-dose cytosine arabinoside chemotherapy (20 mg/m²) [10] for 1 week, but relapse of the disease became evident 2 months after the first admission. The cat was then given BHAC-DMP (BHAC, 100 mg/m²; daunomycin, 20 mg/m²; 6-mercaptopurine, 70 mg/m²; prednisolone, 40 mg/m²) combination chemotherapy, which is widely used for the treatment of human acute myelogenous leukemia [11, 13]. However, complete remission could not be achieved. Five months after the first admission, in parallel with increase of erythroid progenitor cells, some immature myeloid cells appeared in the peripheral blood. These cells stained positively for peroxidase, but negatively for alpha-naphthyl acetate esterase. In the bone marrow, disproportionately large numbers of rubriblasts (41% in all nucleated cells) and myeloblasts (31% in all nucleated cells) were detected (Table 1). The morphological features of the rubriblasts

Fig. 1. Morphology of bone marrow cells in Case 1 and Case 2. (A) Disproportionally large numbers of erythroid progenitor cells were found in Case 1 (at the stage of MDS-Er) 1 month after the first admission. (B) Proliferation of myeloid and erythroid blasts in Case 1 (at the stage of erythroleukemia, M6) 5 months after the first admission. (C) Marked proliferation of erythroid progenitor cells in Case 2 with erythroleukemia (M6Er) 6 months after the first admission. ($\times 1,500$, Giemsa stain)

were similar to those of the cells observed in the bone marrow 4 months before. The myeloblasts had a large nucleus with finely stippled chromatin and some prominent nucleoli (Fig. 1B), and stained positively for peroxidase, but negatively for alpha-naphthyl acetate esterase. Because of the involvement of both erythroid and myeloid cell lineages, the cat was diagnosed as having erythroleukemia, that would be classified as M6 by FAB system, at this stage. Chemotherapy was not effective at this stage and the cat died 6 months after its first admission.

Case 2- A 1-year-old neutered female cat (Case 2) was brought to the VMC with complaints of dyspnea, cough, and anorexia. This case lived in the same household as Case 1 for 1 year, but had no familial relationship with Case 1. On the first admission, Case 2 showed enlarge-

ment of the submandibular and popliteal lymph nodes. No abnormality was detected on hematological examination of the peripheral blood (Table 2). Thoracic radiography indicated the presence of an anterior mediastinal mass. Pleural fluid aspirated from the thorax contained a large number of abnormal lymphocytes with prominent nucleoli. These findings led to a diagnosis of thymic-form malignant lymphoma. Combination chemotherapy with cyclophosphamide (50 mg/m^2), vincristine (0.5 mg/m^2), cytosine arabinoside (100 mg/m^2) and prednisolone (40 mg/m^2) resulted in remission after 2 weeks. Six months after its first admission, however, the cat was readmitted because of non-regenerative anemia (Table 2). No atypical cells were found in the peripheral blood, but disproportionately large numbers of erythroid progenitor cells were observed in the bone marrow (Fig. 1C). These cells

Table 2. Hematological findings in Case 2

Parameter	First admission	After 6 months	After 9 months
Peripheral blood			
RBC ($\times 10^6$ cells/ μ l)	8.09	3.36	4.32
Packed cell volume (%)	32.0	17.0	25.0
Hemoglobin (g/dl)	14.3	6.0	8.7
Reticulocytes (%)	NT ^{a)}	1.0	0.2
WBC ($\times 10^3$ cells/ μ l)	11.1	8.7	0.8
Band neutrophils	0	0	0
Segmented neutrophils	8.7	6.5	0.8
Lymphocytes	1.4	1.9	0
Monocytes	0.1	0.1	0
Eosinophils	0.9	0.2	0
Nucleated erythroid cells ($\times 10^3$ cells/ μ l)	0	0	1.8(5.0 ^{b)})
Platelets ($\times 10^3$ cells/ μ l)	198	121	51
Bone marrow			
M/E ratio	NT	1.01	0.05
Erythroid cells (%)			
Rubriblasts	NT	24	98
Prorubricytes	NT	17	2
Rubricytes	NT	9	0
Metarubricytes	NT	1	0
Myeloid cells (%)			
Myeloblasts	NT	1	0
Promyelocytes	NT	3	0
Myelocytes	NT	5	0
Metamyelocytes	NT	8	0
Band neutrophils	NT	12	0
Segmented neutrophils	NT	20	0

a) Not tested.

b) Percentage of rubriblasts.

showed marked discrepancy in the degrees of maturation of the nucleus and cytoplasm. Their morphological and cytochemical features were similar to those of the erythroid progenitor cells observed in Case 1. Case 2 was diagnosed as M6Er at this stage. Although BHAC-DMP and DOAP (daunomycin, vincristine, cytosine arabinoside, and prednisolone) combination chemotherapies were used, complete remission could not be achieved. After 3 months, erythroid precursors became apparent in the peripheral blood. At that time, the bone marrow was almost completely replaced by immature erythroid cells showing a typical myelogram of erythroleukemia (M6Er). Despite intensive chemotherapy, the cat died 9 months after its first admission.

Pathological findings: On autopsy of Case 1, enlargements of the liver, spleen, and mesenteric lymph nodes were apparent. Histopathological examination revealed proliferation and infiltration of a large number of tumor cells (erythroid cells and myeloid cells) in these organs. The bone marrow was almost completely replaced by a large number of tumor cells and contained some wide areas of hemorrhagic necrosis. At the time of necropsy, the tumor cells were almost all immature myeloid cells rather than erythroid cells.

The pathological findings in the liver, spleen, mesenteric lymph nodes and bone marrow in Case 2 were similar to those in Case 1. In the bone marrow, diffuse proliferation

of a large number of erythroid progenitor cells was observed. The alimentary tract showed hemorrhage of the mucous membranes from the jejunum to the ileum.

Virological examination: Case 1 was positive for both FeLV antigen and feline immunodeficiency virus (FIV) antibody, whereas Case 2 was positive for FeLV antigen, but not for FIV antibody.

For detection of integration of exogenous FeLV into the tumor cells, the chromosomal DNAs were digested with *Bam*HI, which has one cutting site in the *pol* region of the standard FeLV genome [3, 12], and were subjected to Southern blot analysis with FeLV U3 probe [8]. In both Case 1 and Case 2, the *Bam*HI digests gave several discrete bands, indicating clonal expansion of the tumor cells with respect to the integration site of FeLV (Fig. 2). In Case 1, the integration sites of FeLV proviruses in the tumor cells at the stage of MDS-Er (at the time of bone marrow biopsy 60 days after the first admission) (Fig. 2, lane 1) were clearly different from those in tumor cells when myeloid cells were predominant at the time of necropsy (Fig. 2, lane 2). This result indicated that the erythroid and myeloid tumor cell clones observed in Case 1 were independent cell clones. On Southern blot analysis using DNAs digested with *Eco*RV, which has cutting sites in the LTR U3 region and several *pol* regions of the FeLV genome [3, 12], a discrete 3.7-kb fragment containing the 5'-portion of the FeLV genome should be found in

Fig. 2. Detection of exogenous FeLV genome in tumor cells of Case 1 and 2 by Southern blot hybridization. High molecular weight tumor cell DNAs from Case 1, 2 months after the first admission (lane 1) and at necropsy (lane 2), and from Case 2 at necropsy (lane 3) were digested with *Bam*HI and subjected to Southern hybridization using an exogenous FeLV U3 probe. The tumor cell DNAs from Case 1 (lane 4) and Case 2 (lane 5) were digested with *Eco*RV and probed with the exogenous FeLV U3 probe. The positions of size markers in a *Hind*III-digest of lambda DNA are shown on the left.

prototype FeLVs. In Case 1, beside the 3.7-kb band, a 5.6-kb *Eco*RV fragment was also found, indicating infection of a variant FeLV. On the other hand, only the prototype 3.7-kb *Rco*RV fragment was detected in tumor DNA of Case 2.

DISCUSSION

In this paper, two feline cases of erythroid neoplasia are described. There are several reports of transition from erythremic myelosis to erythroleukemia or reticuloendotheliosis (undifferentiated myeloproliferative disease) [5, 7, 14]. Transition of the disease was also observed in the present two cases. In Case 1, pre-leukemic state was observed at the first admission, and subsequently MDS-Er developed 1 month later and erythroleukemia (M6) 5 months later. Previous reports describing transition from erythremic myelosis to erythroleukemia did not show

whether the erythroid and myeloid tumor cells originated from a single tumor cell clone or from different cell clones. In this study, however, Southern blot analysis of tumor cells in Case 1 clearly indicated that the erythroid tumor cells and myeloid tumor cells were derived from independent transformed clones, and thus that two independent transformation events occurred in this case. On the other hand, Case 2 was first diagnosed as a case of thymic-form malignant lymphoma and remission was achieved by chemotherapy. However, erythroleukemia (M6Er) developed 6 months after the first admission. Conceivably, in Case 2, the tumor cell clones were generated in different hematopoietic organs such as the thymus and bone marrow.

It should be noted that these erythroid neoplastic diseases developed in two pet cats kept in the same household. Southern blotting analysis indicated that in both cases the tumor cells were clonally expanded cells after infection with FeLV. A variant FeLV was detected in Case 1, and the full-length FeLV was detected in both Case 1 and 2. In preliminary experiments on molecular cloning of the full-length FeLV, we found a characteristic nucleotide mutation in the LTR in both Case 1 and 2, indicating transmission of specific FeLV strain from one cat to the other. Because these erythroid neoplastic diseases occurred in two unrelated pet cats kept in the same household and erythroid neoplasia is a rare disease, the diseases may have been associated with the FeLV strain commonly infecting both cats. For this possibility, further analysis of pathogenicities of the FeLV isolates from Case 1 and Case 2 is needed.

The prognosis of erythroleukemia is in general poor, so it seems necessary to develop some suitable chemotherapeutic protocols for treatment of these diseases. Low-dose cytosine arabinoside chemotherapy was effective without serious side effects and induced remission in Case 1 at an early stage of MDS-Er. This therapy is known to induce cell differentiation in humans [1, 2]. Recently, more effective combination chemotherapies such as BHAC-DMP has been recommended for treatment of the MDS in humans [10]. Although BHAC-DMP and DOAP combination chemotherapies decreased the numbers of tumor cells in the peripheral blood and the bone marrow of the present two cats, they did not achieve complete remission and had marked side effects. Therefore, it seems necessary to apply an effective combination chemotherapy from an early stage of these erythroid neoplastic disease.

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