Large granular lymphoma in a Feline Immunodeficiency Virus-positive and Feline Leukemia Virus-negative cat

CASE REPORT

Large granular lymphoma in an FIV-positive and FeLV-negative cat

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ABSTRACT

The clinical course of a feline leukaemia virus (FeLV)-negative and feline immunodeficiency virus (FIV)-positive cat affected with a large granular lymphocyte lymphoma is presented. Cytological examination showed neoplastic cells in the pleural effusion and in two abdominal masses. Bone marrow and peripheral blood were moderately involved and chemotherapy was used to control the tumour. Cytochemistry, immunohistochemistry and ultrastructural studies were applied to define the cellular lineage; cytochemistry suggested a T-cell lineage.

INTRODUCTION

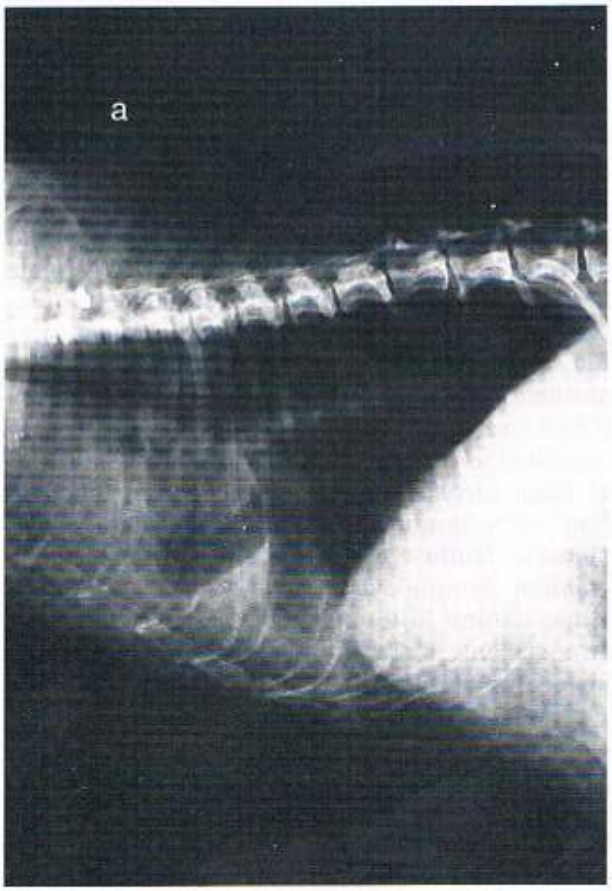
In humans, large granular lymphocytes represent approximately 10 per cent of the peripheral blood lymphocytes (Chan and others 1986a,b, Loughran and others 1988, Duffus 1989). They are characterised by a pale blue cytoplasm and azurophilic cytoplasmic granules of variable number, shape, size and density. Large granular lymphocytes seem to be responsible for most of the natural killer cell activity and play a role in antibody-dependent cell-mediated cytotoxicity (Chan and others 1986a,b, Duffus 1989). They are classified as a subset of T-γ-lymphocytes as most of them carry receptors for the crystalline fraction of γ-immunoglobulins (Chan and others 1986a,b, Duffus 1989). Recently, human large granular lymphocytes have been classified as either natural killer cells (CD3-LGL, with no rearrangement of T-cell receptors) or non major histocomptability complex-restricted cytotoxic T-cells (CSD3-LGL, with rearrangement of T-cell receptors) (Loughran and others 1988). Several large granular lymphocyte disorders have been reported in humans (Chan and others 1986a,b, Loughran and others 1988).

Large granular lymphocytes are also considered in animals as T-γ-lymphocytes with natural killer cell activity (Franks and others 1986, Wellman and others 1989). They are rarely seen in the peripheral blood and bone marrow of normal cats (Jain 1986) and represent 0 to 10 per cent of the canine circulating lymphocytes (Wellman and others 1989). Lymphoproliferative disorders involving large granular lymphocytes have been reported in the rat (Ward and Reynolds 1983), the dog (Wellman and others 1989), the horse (Grindem and others 1989) and the cat (Franks and others 1986, Goitsuka and others 1988, Cheney and others 1990). Three cases of feline globule leucocyte neoplasms are also available (Finn and Schwartz 1972, Honor and others 1986, Kariya and others 1990). Reported cytomorphology of cells of both feline globule leucocyte neoplasms and large granular lymphocyte lymphoma is similar.

This report is the first documented case of large granular lymphocyte lymphoma in a cat that was feline leukaemia virus (FeLV)-negative and feline immunodeficiency virus (FIV)-positive.

CASE HISTORY

An 11-year-old male mixed-breed cat was referred to the University Veterinary Clinic of Turin on July 3, 1990. The cat was thin with a rectal temperature of 39·3°C. Increased and



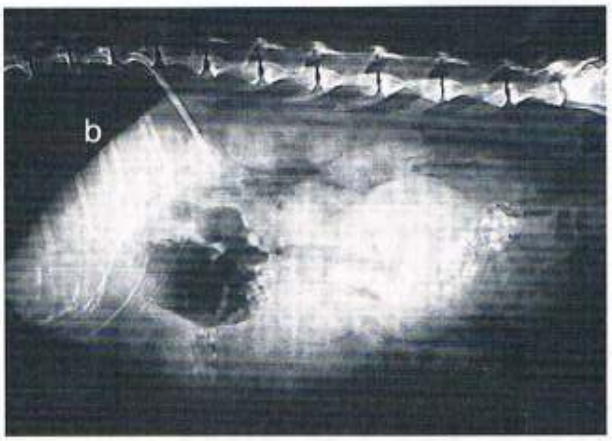


FIG 1. (left) Lateral view of the thorax: presence of a pleural effusion. (above) Lateral view of the abdomen: evidence of two abdominal masses

decreased sounds in the upper and lower lung fields, respectively, were heard bilaterally. Gingivitis and ulcers of the nose were noted. Palpation revealed two 8 cm × 6 cm × 4 cm masses in the mid-abdomen which were rounded with irregular surfaces, hard, freely movable and not painful. These were interpreted as mesenteric lymph nodes.

Radiographs showed some pleural effusion (Fig 1 left) and the presence of two abdominal masses (Fig 1 right). Blood was submitted for a complete blood count and chemistry profile. FeLV, FIV, feline infectious peritonitis virus (FIPV) and toxoplasma infections were also tested. Air-dried smears from both fine needle aspirates of the two abdominal masses and pleural fluid were stained with May-Grünwald Giemsa and cytologically evaluated. The former were highly cellular with a monotonous population of round discrete mononuclear cells ranging from 10 to 20 μm in diameter. Anisocytosis was moderate and few cells larger than 20 μm were present. The cytoplasm was scant to moderate and deeply basophilic. Variation in nuclear:cytoplasmic ratios was mild to moderate. The majority of these cells had numerous and often polar azurophilic cytoplasmic granules which varied greatly in number, shape and size and were generally less than 1 μm in diameter (Fig 2). At the time of presentation, 16 per cent of the lymphocytes in the peripheral blood showed the same granules seen in the cells from the mass aspirates. Pleural effusion cytology revealed the same cell type seen in both mass aspirates and blood. A diagnosis of large granular lymphocyte lymphoma was made.

On July 10, the cat was re-examined. Smears from a bone marrow biopsy stained with May-Grünwald Giemsa showed only a few neoplastic large granular lymphocytes.

Therapy began on July 10 and consisted of a combination of intravenous vincristine, intraperitoneal L-asparaginase, prednisone by mouth, intravenous cyclophosphamide and intravenous methotrexate (Hardy and MacEwen 1989). The cat was treated for two months and the follow-up was provided by periodic physical examination and laboratory monitoring. On September 12, the cat was found again in a poor condition, with larger abdominal masses. *Microsporum canis* was cultured from some cutaneous lesions. Radiographs showed some fluid in the pleural and peritoneal cavities. On September 15, the owner

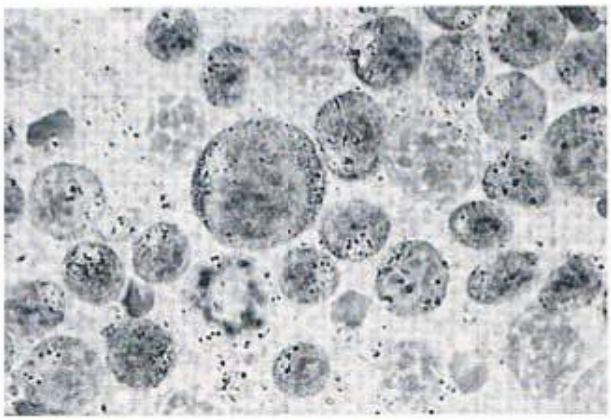


FIG 2. Fine needle aspirate smear from the abdominal mass: many free granules from disrupted cells are also present. May-Grünwald Giemsa. ×1000

refused any further treatment. On October 6, the cat died and no necropsy was allowed.

Methods

ELISAs for FeLV, FIV and FIPV infections were performed on sera from the cat with the large granular lymphocyte lymphoma and four cats from the same household (Pedersen and others 1987, Ishida and others 1988). Sera both from the lymphoma cat and a control were also tested for FIV infection via Western blotting assay. Crandell feline kidney (ATCC CCL 94) cells infected with the FIV-Petaluma strain were used as antigen (Pedersen and others 1987). The total protein extract was separated in SDS-PAGE and then transferred on to nitrocellulose paper. Filters were incubated and ^{125}I protein A was used to detect the antigen-antibody complex (Towbin and others 1979).

A direct haemagglutination test was applied to detect anti-toxoplasma antibodies.

Mass fine needle aspirates and peripheral blood buffy coats were stained for acid phosphatase, acid phosphatase with tartrate inhibition, α-naphthyl acetate esterase, acid α-naphthyl acetate esterase, N-acetyl-β-glucosaminidase, alkaline phosphatase, β-glucuronidase, peroxidase and chloroacetate esterase activities. Toluidine blue, Sudan black B, periodic acid-Schiff, and phosphotungstic acid haematoxylin stains were also utilised. As a control, buffy coats from four healthy cats were used to compare cytochemical data.

Immunohistochemistry was performed on air-dried abdominal mass aspirates fixed in acetone analytical reagent for 10 minutes and chloroform for 10 minutes. The primary antibodies applied were rabbit anti-human CD3 polyclonal (dako) (that recognise human T lymphocytes and seem to cross-react with similar feline antigens, unpublished data), monoclonal anti-UCHL-1 (dako), polyclonal anti-kappa and lambda light chains (ortho), and polyclonal anti-lysozyme and anti-S 100. The secondary antibody used was anti-mouse and anti-rabbit lgG biotinylated (dako). The antigen-antibody reaction was amplified by AB complex (vector) and stained with 3-amino-9-ethyl-carbazole. Finally, the section was counterstained with Harris' haematoxylin (Hsu and Raine 1981).

Lymphocytes obtained from several abdominal mass fine needle aspirates were suspended in 9 per cent sodium chloride fixed in 3 per cent glutaraldehyde and centrifugated. Then, cells were dehydrated in ethanol and embedded in Epon-araldite resin. Thin sections, obtained with a Reichert Jung ultracut E, were stained with uranyl acetate and lead citrate and examined with a Zeiss 109 electron microscope.

RESULTS AND DISCUSSION

L-asparaginase administration resulted in a 50 per cent size reduction of the two abdominal masses and in a decrease of the pleural effusion. After two months, they returned to their original size indicating treatment failure and progressive disease. As necropsy was not allowed, the authors were unable to document any visceral involvement other than the presence of large granular lymphocytes in the pleural fluid. All large granular lymphocyte neoplasms reported in animals and humans manifested as a systemic disease. In this cat, total proteins were always high (8 to 9·3 g/dl) and albumin decreased dramatically at the time of the third blood withdrawal (3·5 to 2·35 g/dl): this represented a very poor prognostic sign, together with an increase in alanine aminotransferase (ALT) activity (102 to 156 U/litre), probably compatible with liver involvement and 'progressive disease'. The increase in lactate dehydrogenase (LDH) activity (up to 401 U/litre) is analogous to that reported by Goitsuka and others (1988). In particular, the increase of the LDH-3 isoenzyme (13·19 to 25·13 per cent) was considered significant. This 'non-specific' behaviour was first reported in human oncological patients (D'Arrigo and others 1980).

In humans, large granular lymphocyte proliferation has been associated with lymphocytosis and neutropenia of unknown origin (Chan and others 1986b). In this case there was a slight neutrophilia (up to 14,270/mm^3 over 20,100 white blood cells), also shown by Goitsuka and others (1988) who correlated it with a presumptive stimulation of the bone marrow by interleukin-1 and interleukin-3.

Ultrastructurally, large granular lymphocytes from this cat contained moderate amounts of cytoplasm, with frequent cytoplasmic blebs. Nuclei were large and often indented and mitochondria were few, with a tendency to cluster; the endoplasmic reticulum was moderate. Many round-shaped cytoplasmic granules surrounded by a simple membrane, sometimes double in appearance, were seen. Granules often had an electron-dense core, surrounded by a light band variably extended or absent.

Some linear electron-dense crystalline-like structures in a variably dense granular matrix were enclosed into vacuoles (Fig 3). These cells were morphologically similar to the large granular lymphocytes described in earlier reports (Ward and Reynolds 1983, Chan and others 1986a,b, Franks and others 1986, Grindem and others 1989, Wellman and others 1989).

The positivity for the acid phosphatase, α-naphthyl acetate esterase, acid α-naphthyl

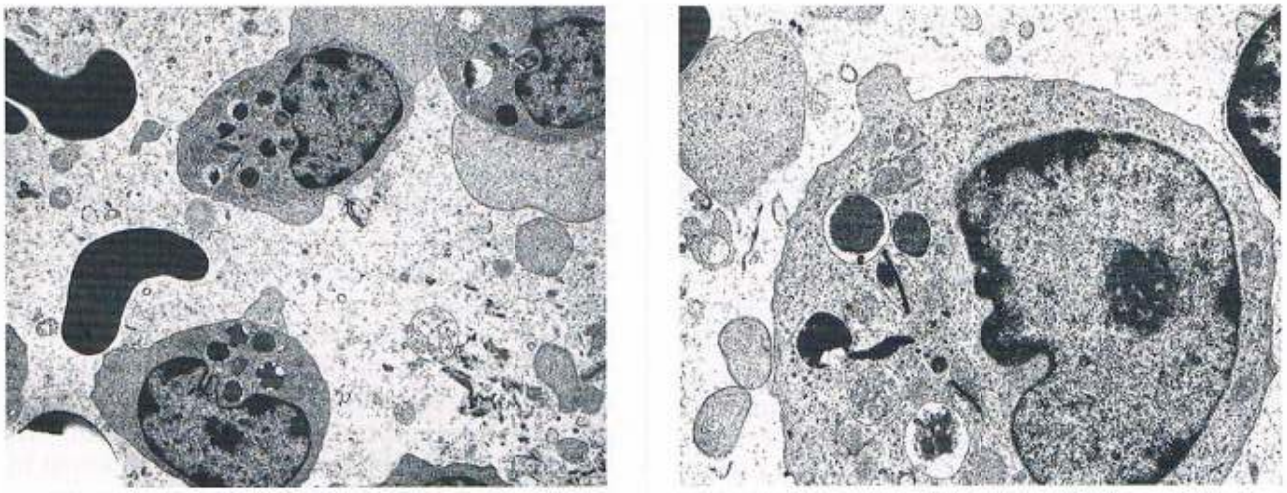


FIG 3. Ultrastructural morphology of large granular lymphocytes from fine needle aspirates from the two masses. (left) ×7500 and (right) ×14,000

acetate esterase and N-acetyl-β-glucosaminidase activities observed in this cat is suggestive of a T-cell lineage, as also shown in man (Invernizzi and Perugini 1987, Hayhoe and Quaglino 1988), the cat (Dockrell and others 1978) and the dog (Wulff and others 1981). All mass and blood large granular lymphocytes were positive for acid phosphatase activity, with a localised coarse granular pattern (Fig 4). Positivity has also been documented both in human and feline large granular lymphocyte lymphoma (Reynolds and Foon 1984, Chan and others 1986a, Franks and others 1986, Grindem and others 1989) and in peripheral blood large granular lymphocytes (Grogan and others 1981, Grossi and others 1982). After tartrate inhibition, 20 per cent of the neoplastic cells from the mass were faintly positive. This is not easy to explain as in man tartrate resistant acid phosphatase is characteristic of 'hairy cells' leukaemia and, at present, there are not enough data on cats. In this case, a focal coarse granular positivity for the α-naphthyl acetate esterase activity was observed both in the mass and in the blood large granular lymphocytes, and a strong dot-like pattern of acid α-naphthyl acetate esterase positivity in the mass large granular

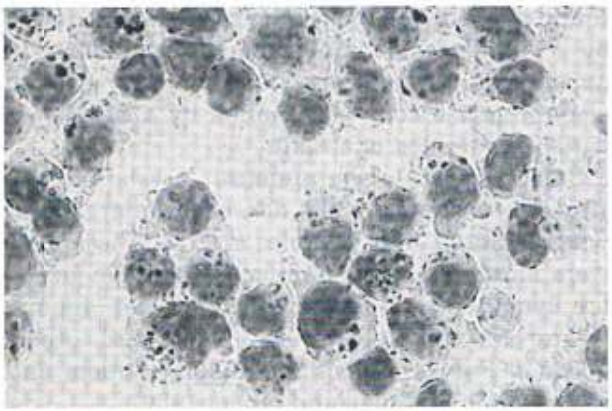


FIG 4. Acid phosphatase. Abdominal mases: strongly positive large granular lymphocytes. ×1000

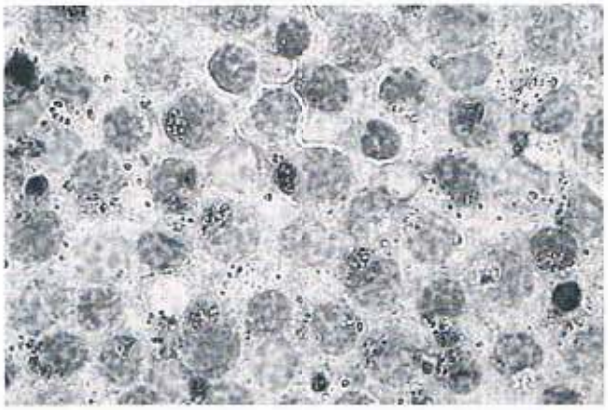


FIG 5. Acid α-naphthyl-acetate esterase: focal positivity in large granular lymphocytes from the masses. × 1000

lymphocytes (Fig 5) and in the majority of the blood lymphocytes (80 per cent). N-acetyl-β-glucosaminidase positivity was shown by several small granules at a pole of large granular lymphocytes both from the masses and peripheral blood.

Scattered positivity with a granular pattern was detected in the mass large granular lymphocytes for the β-glucuronidase activity; in the blood, 76 per cent of cells were both focal (46 per cent) and granular (30 per cent) positive, whereas in the healthy cats a very low number of positive lymphocytes was seen; this was also shown by Tsujimoto and others (1983). A common finding in large granular lymphocyte lymphoma of man (Reynolds and Foon 1984, Chan and others 1986b) and rat (Ward and Reynolds 1983) is the positivity for both acid phosphatase and β-glucuronidase activities.

Large granular lymphocytes from this cat were positive for toluidine blue (Fig 6), which stains mast cell and basophil leucocyte granules metachromatically. This metachromasia may be a common feature of large granular lymphocytes and mast cells due to similar granular components.

Negativity of both peroxidase, chloroacetate

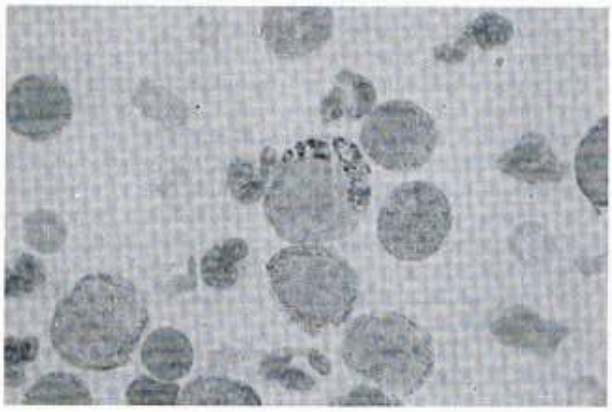


FIG 6. Large granular lymphocyte-lymphoma buffy coat stained with toluidine blue: the only positive lymphoid cell is a large granular lymphocyte. × 1000

esterase and alkaline phosphatase activities and Sudan black B and periodic acid-Schiff stains indicated absence of myelocytic features (Jain 1986). Besides, phosphotungstic acid haematoxylin negativity excluded the possibility of a globule leucocyte neoplasm.

Contrasted with the cytochemical results are those obtained by immunohistochemistry that showed no positivity to the antibodies applied. Because of the negativity of the reaction with both the rabbit anti-human CD3 polyclonal and the UCHL-1 monoclonal antibodies, a B- or a non-B/non-T lymphoma (with no expression of any specific membrane antigen) may be suggested (Bocchini and others 1989). The negativity of a reaction for B cell origin may be due to an early stage of growth of these cells.

Data from ELISAs for FeLV, FIV and FIPV are presented in Table 1. Western blotting, revealing bands of both p24-28 and gp40 viral proteins, confirmed FIV infection (Ishida and others 1988, Pedersen and others 1989). Feline lymphoma is frequently associated with a FeLV infection; this was not detected in any of the five cats tested. Because of the localisation of lesions, this lymphoma can be classified as an alimentary lymphoma that, in 78 per cent of cases, occurs in FeLV-negative cats (Reinacher 1989). Only some previously reported feline large granular lymphocyte lymphomas and globule leucocyte neoplasms were FeLV-negative (Franks and others 1986, Honor and others 1986), one was not tested (Finn and Schwartz 1972) and two were positive (Cheney and others 1990, Kariya and others 1990). FeLV-negative but FIV-positive lymphomas have been documented (Ishida and others 1989, Yamamoto and others 1989, Rosenberg and others 1991). As suggested for human AIDS, sometimes associated with tumours such as Kaposi's sarcoma and non-Hodgkin's lymphoma (Kaplan 1988), FIV may allow a malignant monoclonal expansion of a cell type because of a dysfunction of the T-cells (Rosenberg and others 1991). The cell lineage often involved in AIDS is the B-cell, with an initial generalised persistent lymphoadenopathy (Abrams 1988, Kaplan 1988). This mechanism could also be evoked for other cell types (Ishida and others 1988, 1989, Pedersen and others 1989). However, tumour development may also have no association with these infections. At present, no particular relationship between FIV and FIPV can be suggested (Ishida and others 1989). Finally, the serum positivity for *Toxoplasma gondii* (up to dilution 1:1024) is not surprising in relation to the FIV infection (Ishida and others 1989, Pedersen and others 1989) and the oncological condition.

Table 1. Results of ELISAs for FeLV, FIV and FIPV infections from the sera of five cats from the same household

Cats	Sex	Clinical state	FeLV	FIV	FIPV
1	M	LGLymphoma	–	+*	–
2	M	Healthy	–	–	–
3	M	Healthy	–	+†	+(1:29)
4	M	Healthy	–	+†	+(1:29)
5	F	Healthy	–	–	–

*Strongly positive, † Weakly positive

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ABSTRACT

Thoracolumbar vertebral osteochondroma in a young dog

A SEVEN-month-old German shepherd bitch had a progressive hindlimb weakness. When examined she was found to be ataxic and had slightly atrophied high limb muscles. There were proprioceptive deficits in both hindlimbs, slight increase in patella reflexes and an adherent mass on palpation of the spine at the thoracolumbar junction. Radiographs demonstrated a circumscribed, calcified mass at T13/L1, involving the articular processes. Myelography indicated extradural compression associated with this lesion. Surgical removal of the mass en bloc, together with a margin of normal tissue, was undertaken via a laminectomy. Histology of the mass confirmed it to be an osteochondroma. Immediately postoperatively the bitch could walk, however next day she was unable to support weight. Over the next week the neurological status returned to that of before surgery and steady improvement occurred over the next month. After four weeks the animal rolled on her back, cried out in pain and became severely ataxic. Instability, due to a fracture at T13, was confirmed radiographically. Euthanasia was performed. Post mortem examination confirmed a recent stress fracture at T13. Intraoperative stabilisation of the laminectomy might have prevented this.

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