NOTE *Pathology*

Systemic T Cell Large Granular Lymphocyte Lymphoma with Multifocal White Matter Degeneration in the Brain of a Japanese Domestic Cat

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ABSTRACT. A 10-year-old spayed female Japanese domestic cat exhibited clinical symptoms suggesting pancreatitis. One month later the cat exhibited Horner's syndrome and was euthanized. At necropsy, multiple neoplastic masses were found in the intestines, spleen, kidneys, urinary bladder, and lungs. On cytology, many neoplastic lymphocytic cells had fine to large cytoplasmic granules, suggesting large granular lymphocyte (LGL) lymphoma. Histopathological examinations revealed infiltrative proliferation of the neoplastic cells in almost organs. Immunohistochemically, the neoplastic cells were intensely positive for CD3 and granzyme B. In the brain, there were multifocal white matter lesions characterized by diffuse myelin loss with mild infiltration of the neoplastic cells. Based on these findings, the cat was diagnosed as LGL lymphoma presumptively of intestinal origin with systemic involvement.

KEY WORDS: central nervous system, feline, large granular lymphocyte lymphoma, white matter lesion.

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Large granular lymphocyte (LGL) lymphoma is a leukemic disease characterized by the proliferation of large lymphocytic cells with azurophilic granules in their cytoplasm. The neoplastic cells are of either T-cell or natural killer (NK)-cell lineage, which are indistinguishable by cell morphology [4, 9, 11, 19]. Immunohistochemically, however, LGL cells are classified into 2 types; the CD3-positive T-cell type and CD3-negative natural killer-cell type [19]. While LGL lymphomas in dogs and cattle are known as a slowly progressive disease, feline LGL lymphomas are acute and aggressive [11, 19]. Clinical symptoms of feline LGL lymphomas include anorexia, weight loss, vomiting and lethargy [22]. The lymphomas have less favorable prognosis, and are minimally responsive to chemotherapy [15]. They usually emerge in the enteric wall and rapidly metastasize to multiple organs including the regional nodes, liver, spleen, and kidneys with variable levels of leukemia [19]. However, there has been little information concerning the central nervous system (CNS) involvement of the feline LGL lymphoma.

Granzyme is a cysteine protease found in the cytoplasmic lysosome-like granules of the cytotoxic T lymphocyte and natural killer cell [10]. Granzyme A and granzyme B are most abundant within the lytic granules [18]. They are released as a multi-molecular complex and induce apoptosis by caspases-independent or -dependent pathways [12, 21]. Granzyme B is commonly used for a T-cell/NK-cell lymphoma marker in human pathology [6, 16, 24]. However, its utility has not been confirmed in the field of veterinary pathology.

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The present report describes a case of feline systemic T cell/LGL lymphoma with CNS lesions characterized by multifocal white matter degeneration.

A 10-year-old spayed female Japanese domestic cat had been kept as a blood donor at the Veterinary Medical Center of the University of Tokyo. The cat continuously vomited undigested foods. The ultrasonographic examinations revealed corrugated signs in the small intestine. Blood chemistry test revealed elevated value of feline pancreatic lipase (Table 1), suggesting pancreatitis. Neoplastic cells were not observed in the peripheral blood smear. Antibiotics medication and intravenous infusion improved the initial symptoms. Two weeks later anorexia and loose stool appeared, and the antibiotics could not improve such symptoms anymore. Endoscopic examinations revealed multiple

Table 1. The result of complete blood cell count and blood chemistry at the initial symptom

Laboratory parameter		Reference range
RBC ($\times 10^6$ /μl)	11.3	5.0 – 10.0
Hemoglobin (g/d*l*)	13.9	8.0 – 15.0
PCV (%)	44.5	26.0 – 46.0
WBC (/μl)	6,000	5,500 – 19,500
Platelets ($\times 10^3$/μl)	256	300 – 800
TP (g/d*l*)	6.4	5.4 – 7.8
BUN (mg/d*l*)	14.8	17.6 – 32.8
CRE (mg/d*l*)	0.8	0.8 – 1.8
ALP (U/*l*)	79	38 – 165
ALT (U/*l*)	96	22 – 84
Na (mEq/*l*)	145	147 – 156
K (mEq/*l*)	3.7	3.4 – 4.6
Cl (mEq/*l*)	108	107 – 120
Feline pancreatic lipase (μg/*l*)	25.2	4.1 – 12.9

Feline pancreatic lipase showed high value.

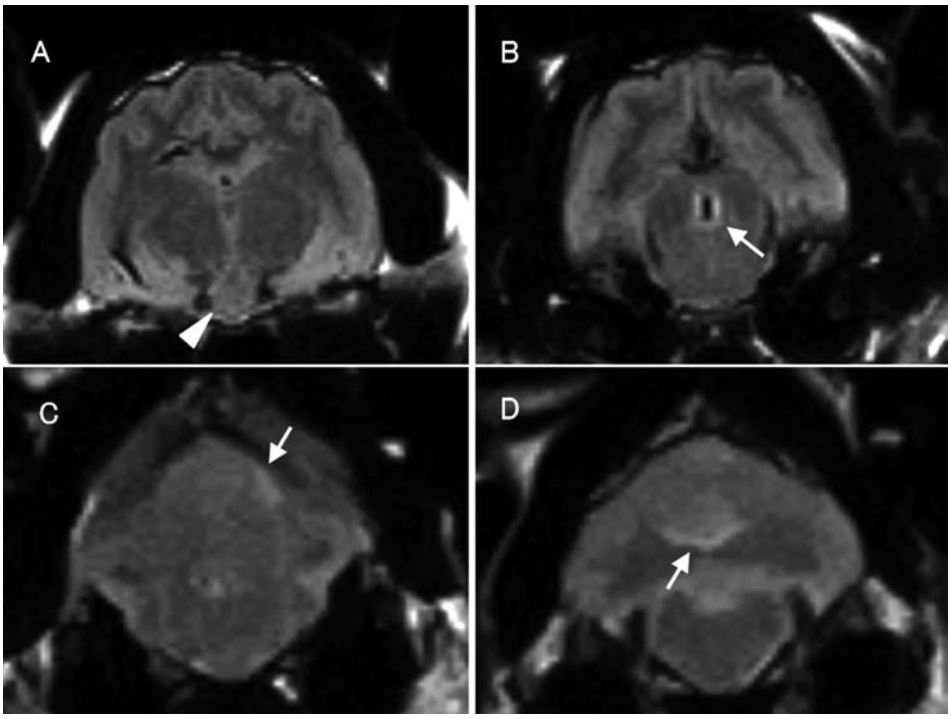

Fig. 1. Transverse fluid-attenuated inversion recovery (FLAIR)-weighted images in magnetic resonance imaging (MRI) at the level of the cerebrum and pituitary gland (A), the mesencephalon (B), and the cerebellum (C and D). Diffuse enhancing lesions are observed in the periventricular regions and leptomeninges (arrows). A mass lesion is also found in the pituitary gland (arrowhead).

ulcerations of the stomach and duodenum. Pathological examination of biopsy specimens revealed severe ulcerative lesions, but typical lesions of lymphoma, such as diffuse infiltrative proliferation of atypical lymphocytes, had not been confirmed. The cat started to exhibit symptoms of Horner's syndrome at the left side, and magnetic resonance imaging (MRI) examinations for the brain revealed diffuse enhancing lesions in the periventricular and leptomeningeal regions, and a mass of the pituitary gland (Fig. 1). One month after the first onset, the cat was euthanized due to aggravated general conditions. Necropsy was performed on the day.

At necropsy, there were severe gastrointestinal multifocal ulcerations and a duodenal perforation. Diffuse thickening of the intestinal wall was also observed. The duodenum, pancreas and right kidney were adhesive each other by fibrinous peritonitis. Multiple neoplastic masses were found in the intestines, kidneys, urinary bladder and lungs (Fig. 2). The masses were white to pink in color, varied in size, and soft.

On the imprint smear specimen from the renal mass stained with May-Grünwald Giemsa, there were many neoplastic lymphocytes characterized by oval or polygonal shaped nuclei, with distinct nucleoli. Such neoplastic lymphocytes had a lot of azurophilic granules in their cytoplasm (Fig. 3). Mitotic figures were frequently observed in the neoplastic cells. Atypical lymphocytes were also observed in the blood smear (18% of the total white blood cells) on the day when the necropsy was performed.

For histological examinations, tissue samples were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin wax, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Some selected sections were also stained with luxol fast blue (LFB), toluidine blue and phosphotungstic acid hematoxylin (PTAH). Immunohistochemical stains were also performed using the paraffin sections by the method of labeled streptavidin biotinylated antibody (LSAB) or envision polymer. Primary antibodies used were anti-CD3 (1:50; Dako, Glostrup, Denmark), granzyme B (1:100; Spring Bioscience, Fremont, CA, U.S.A.), BLA-36 (1:50; Dako), HLA-DR (1:100; Dako), neurofilament (NF, pre-diluted; Dako), and glial fibrally acidic protein (GFAP, 1:50; Dako). Sections were then counterstained with Mayer's hematoxylin, LFB, or nuclear fast red. Since the immunoreactivity of anti-granzyme B antibodies for feline cytotoxic/NK lymphocytes was unclear, the immunochemistry was performed without setting positive control. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method for detecting DNA fragmentation and apoptotic bodies was also performed using the Apoptag kit (Oncor, Gaithersburg, MD, U.S.A.) according to the manufacturer's protocol.

Histopathologically, diffuse proliferation of round to oval neoplastic lymphocytes was observed throughout the intes-

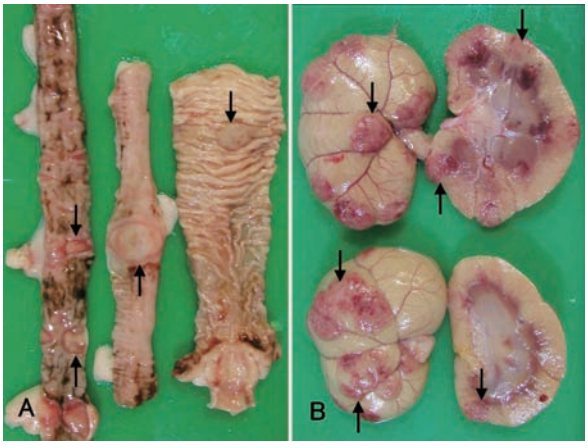

Fig. 2. Multiple white- to pink-colored nodules are observed in the intestines (A) and kidneys (B) (arrows).

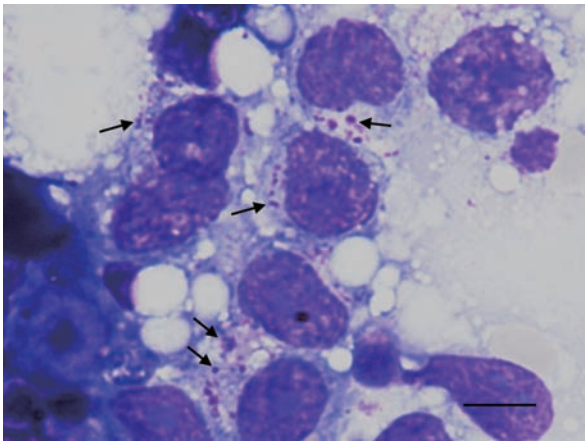

Fig. 3. Imprint smear from the renal mass. Large round-shaped neoplastic cells contain azurophilic granules in their cytoplasm (arrows). Bar =10 μm. May-Grünwald Giemsa stain.

tinal masses (Fig. 4A). The neoplastic cells showed a distinct epitheliotropism. The nuclei of the neoplastic cells were pale and had several prominent nucleoli. Mitotic figures were prominent (4 to 5 per high power field). Multifocal or diffuse proliferation of the neoplastic lymphocytes was also found in the heart, lungs, pancreas, renal cortex, urinary bladder, stomach, adrenal glands, thyroid glands, pituitary gland, brain and iris of the left eyeball. Neoplastic lesions in the spleen and bone marrow were not evident. Most neoplastic lymphocytes were strongly positive for CD3 (Fig. 4B) and granzyme B (Fig. 4C), and negative for BLA-36 and HLA-DR. The cytoplasmic granules detected on the smear specimen were not distinct on the HE sections, and negative for toluidine blue and PTAH. Besides neoplastic lesions, severe fat necrosis in the adipose tissue around the pancreas, interstitial pneumonia, and proliferation of glandular epithelium with squamous metaplasia in the pituitary gland were also observed, respectively.

In the brain, CD3- and granzyme B-positive neoplastic lymphocytes were found in the choroid plexus, leptomeninges and brain parenchyma. Severe myelin loss and macrophage infiltration were multifocally observed in the cerebral and cerebellar white matter, and in the medulla oblongata. In the cerebellum, infiltration of both CD3- and granzyme B-positive neoplastic lymphocytes was found in the Purkinje and granular layers adjacent to white matter lesions (Fig. 5). Some neoplastic lymphocytes were closely contact with Purkinje cells (Fig. 5, inset). Purkinje and granular cells in the lesions were TUNEL-negative, while some of glial cells in the white matter lesions were positive (Fig. 6). Proliferation of GFAP-positive astrocytes was minimal in the white matter lesions. NF-positive spheroids representing axonal degeneration were scattered in the white matter.

Clinical and pathological features of the present case are almost identical to those in the previous reports of feline LGL lymphomas [4, 9, 10, 13, 15, 17]. The diagnosis of LGL lymphoma was confirmed by the results of cytology and immunohistochemistry. Although the cytoplasmic

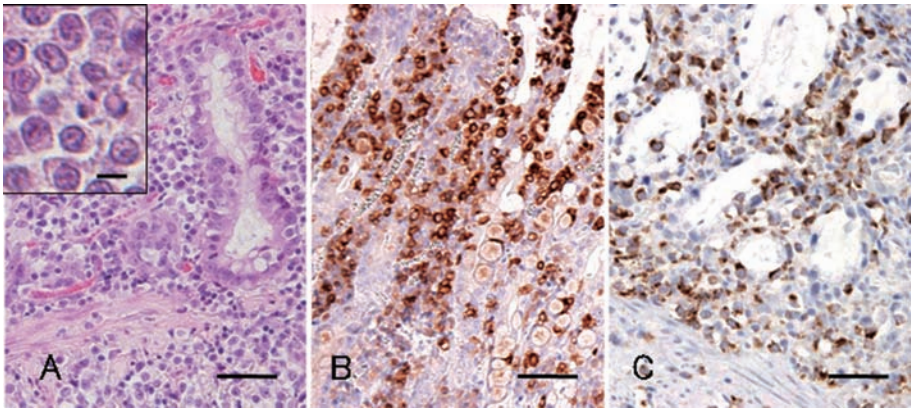

Fig. 4. (A); Diffuse proliferation of neoplastic lymphocytes throughout the lamina propria and serosa of the ileum. Bar =50 μm. Inset: Higher magnification of neoplastic cells. Bar =10 μm. HE stain. (B), (C); Neoplastic cells are positive for CD3 (B) and granzyme B (C). Bar =50 μm. Immunostain.

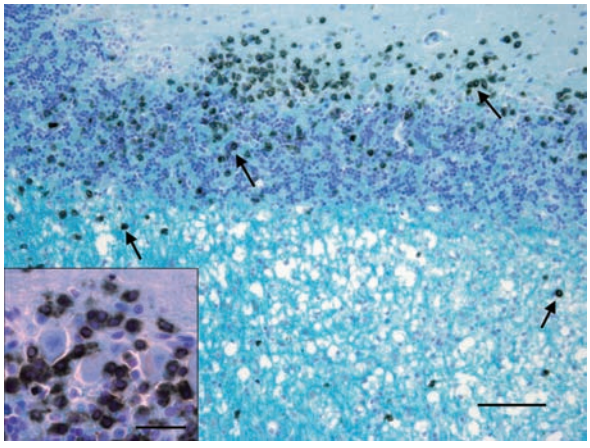

Fig. 5. Severe myelin loss in the white matter of the cerebellum. Neoplastic cells (arrows) are observed in the Purkinje and granular layers, and also in the white matter. Bar =200 μm. Inset: Higher magnification of the Purkinje layer. A lot of neoplastic lymphocytes are present around Purkinje cells. Bar =10 μm. LFB and CD3 immnostain.

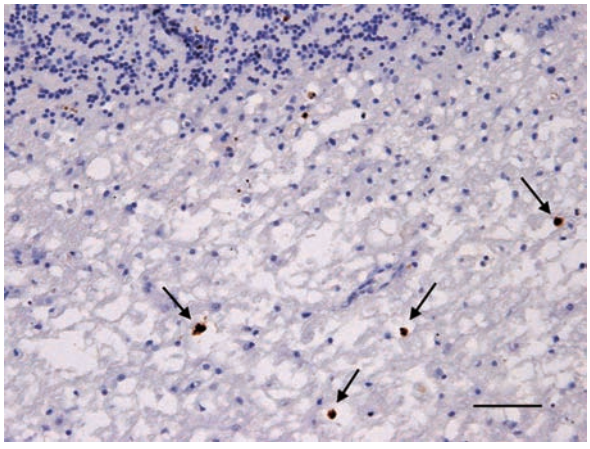

Fig. 6. TUNEL stain of the cerebellar white matter. TUNEL-positive cells (arrows) are observed in the white matter. Bar =100 μm.

granules in the neoplastic cells were not identified in the HE specimens, those were intensely positive for granzyme B. This fact indicates that the neoplastic cells originated from the T cell/NK cell lineage, supporting the diagnosis of LGL lymphoma. The utilization of granzyme B-immunohistochemistry has been confirmed in human medicine for the identification of cytotoxic T-cells or NK-cells [10], and has been used for refined classification of lymphomas [6, 16, 24]. However, little information about granzyme B involvement in lymphocytes and lymphomas is available in the veterinary field. The present case indicates that the combination of CD3- and granzyme B-immunostains is useful for the pathological diagnosis of feline LGL lymphoma.

Previous studies [4, 9, 10, 13, 15, 17] dealing with feline systemic T cell/LGL type lymphomas mentioned that the alimentary tract might be the primary region, although the exact origin of the tumor is not confirmed. In the present case, neoplastic cells were observed in almost all organs, including the CNS. In general, the metastatic CNS lesions of a systemic lymphoma are characterized by the perivascular accumulation of neoplastic cells [14]. The neoplastic lymphocytes of the present case diffusely infiltrated also in the brain parenchyma. Although the pathological changes in the gray matter associated with neoplastic cell invasion were mild, lesions in the white matter such as severe myelin loss and mild axonal degeneration were observed in the case. Horner's syndrome can be explained by loss of myelin and secondary axonal damage in the brain stem.

For the interpretation of this unique phenomenon, generation of autoantibodies is suspected as a cause of paraneoplastic symptoms [8] associated with lymphoma. The association of focal demyelinating lesions along the neuroaxis has been noted in cases of lymphoma, both in medical [20, 23] and veterinary [3] field, although the lesions were only observed in the peripheral nerves in most cases. One report concerning a paraneoplastic demyelination in the CNS accompanied by cytotoxic T cell lymphoma in the heart was found in medical field. The demyelinating lesions in the cerebellum are quite similar to that of the present case [7]. Paraneoplastic demyelination is supposed to be mediated through antigen-specific T cells that have received the antigen from apoptotic neoplastic cells [5]. Two pathogenic mechanisms were suggested in such phenomenon; (a) demyelination as a result of paraneoplastic oligodendroglial cytotoxic phenomenon [1], or (b) immune-mediated lymphocytic response to demyelination, which later transforms into a neoplastic process [2]. However, in the present case, the morphology and immunoreactivity of the lymphocytes infiltrating into the brain parenchyma were quite similar to the neoplastic lymphocytes proliferating in the intestine. Furthermore, no autoantibody against feline CNS tissues was detected by indirect fluorescent antibody (IFA) and Western blot methods (Data not shown). Thus, the paraneoplastic syndrome-like pathogenesis is unlikely to be a cause of the white matter degeneration in the present case.

We, therefore, suspected that the white matter lesions are induced by the direct tissue injury by infiltrated LGL lymphoma cells. The neoplastic LGL lymphoma cells in the present case contained cytoplasmic granzyme B. And in fact, some glial cells in the white matter lesions were TUNEL-positive, indicating apoptotic activity of the neoplastic lymphocytes. Although the pathogenesis remains unclear, these findings suggest that the direct cell injury to oligodendroglial cells by infiltrated LGL lymphoma cells might cause the severe myelin loss in the present case.

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