NOTE *Pathology*

Histiocytic Sarcoma in the Brain of a Cat

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ABSTRACT. A mass lesion in the subependymal region of the lateral ventricle in a 13-year-old neutered male mongrel cat with a complaint of somnolence, right circling movement and posture abnormality was examined. The magnetic resonance image examination revealed a relatively large T1-hypointense and T2-hyperintense mass lesion in the left interventricular foramen region, and there were no abnormalities in the chest and abdominal x-ray radiographic, funduscopic, and electric retinogram findings. The cat was died 43 days after the initial referral, and the post-mortem examinations revealed a poorly demarcated subependymal mass. Histologically, the brain lesion consisted of complex proliferation of highly pleomorphic cells resembling histiocytes with atypia and abundant mitotic figures. Moderate infiltrates of small reactive lymphocytes were admixed with the pleomorphic cell population. Gemistcytic astrocytes were also intermingled with the periphery of neoplastic foci. Immunohistochemically, most of the pleomorphic cells were positive for HLA-DR alpha-chain and ionized calcium binding adaptor molecule 1, and few were positive for lysozyme and alpha-1 antichymotrypsin. The atypical pleomorphic cells were negative for CD3, IgG (H and L), glial fibrillary acidic protein and neurofilament, suggesting monocytic/histiocytic-origin of the cells. The number of Ki-67-positive cell nuclei was extremely large, reflecting the high growth activity of these cells. Based on the findings, the lesion was considered as histiocytic sarcoma.

KEY WORDS: brain, feline, histiocytic sarcoma, immunohistochemistry.

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In dogs, histiocytic disorders have been classified into 3 major categories: canine cutaneous histiocytoma; canine reactive histiocytoses (both cutaneous and systemic); and histiocytic sarcoma complex (both localized and disseminated histiocytic sarcoma) [10]. Cats, in contrast, are rarely affected by these diseases [6]. Histiocytic sarcoma (HS) in cats has been reported to date in the intestinal wall, femur, tarsus, spleen and liver, and mediastinal and vertebral canals [1, 5, 11, 13, 16]. A brief description of an HS in the central nervous system (CNS) of a cat was reported only in a review article [19], while malignant histiocytic disorders have been well demonstrated in the canine CNS [2, 4, 14, 18, 20]. This report documents the features of a brain tumor in a cat. The histological and immunohistochemical natures characterized by the proliferation of highly pleomorphic cells resembling histiocytes and immunoreactivity for HLA-DR and ionized calcium binding adaptor molecule 1 (Iba1) are consistent with HS [7, 8, 15].

A 13-year-old neutered male mongrel cat was presented to a private animal hospital with a complaint of somnolence, blindness, right circling movement and posture abnormality. The neurological examinations revealed a postural reaction decrease, a left mydriasis and disappearance of a physiologic nystagmus. Complete blood cell count and serum biochemical analyses were within normal ranges, but an enzyme-linked immunsorbent assay (ELISA) test detected feline leukemia virus (FeLV) specific antigen and feline immunodeficiency virus (FIV) specific antibodies. There were no abnormalities in the chest and abdominal x-ray radiographic, funduscopic, and electric retinogram findings. In order to confirm the intracranial lesions, the magnetic resonance image (MRI) examination was carried out. There was a relatively large T1-hypointense and T2-hyperintense mass lesion in the left interventricular foramen region with a strong and heterogenous post contrast enhancement, and post contrast T1-weighted images revealed a poorly defined margin of the lesion (Fig. 1 a, b and c). And there was edema in the left thalamus and cerebral white matter adjacent to the mass lesion. Cytology of the cerebrospinal fluid and the surgical extirpation were unavailable because of the risk of foraminal herniation in this case. Then, the chemotherapy with prednisolone and cytosine arabinoside was chosen considering the possibility of lymphoma. The level of consciousness was recovered after two days. Following the repetitive administration of the prednisolone, clinical symptoms had been improved for a month, but had recurred and progressed afterward. Then, the cat had been insensitive to cytosine arabinoside and doxorubicin, and died 43 days after the initial referral, and the post-mortem examinations revealed foraminal herniation by edema around the mass and obstructive hydrocephalus. Whole brain fixed in 10% formalin solution, was then submitted to the Department of Veterinary Pathology, the University of Tokyo for pathological examinations. Grossly, a focal mass lesion, approximately 1 cm in diameter, was found in the subependymal region of the lateral ventricle. The transverse cut surface of the mass in the body of fornix was round-shaped, poorly demarcated and discolored (Fig. 2).

Paraffin sections of 3–4 μm thick were stained with

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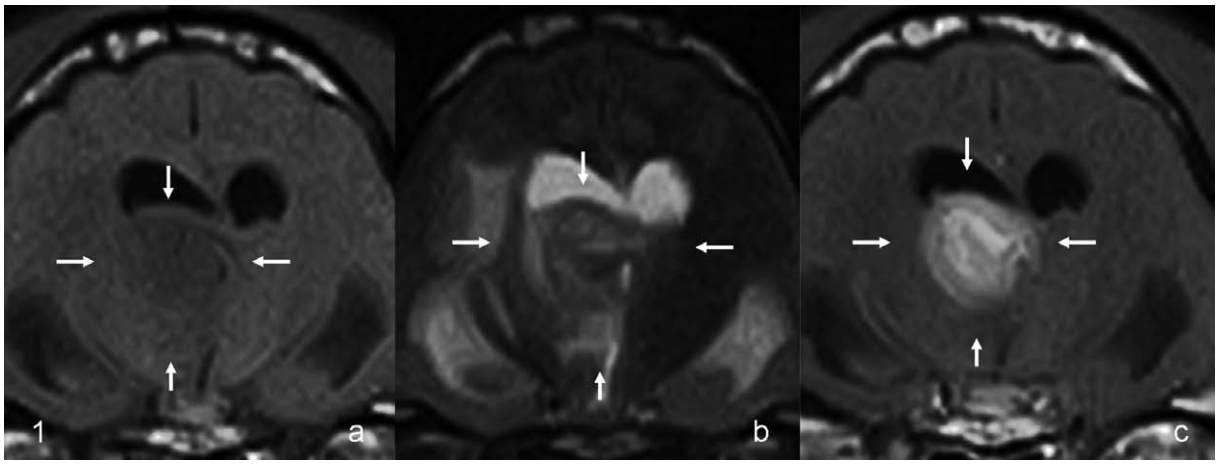

Fig. 1. Transeverse views of a fast spin-echo T1-weighted MRI (a), a fast spin-echo T2-weighted MRI (b), and a postcontrast spin-echo T1-weighted MRI (c), showing a mass lesion at the left interventricular foramen region (arrows).

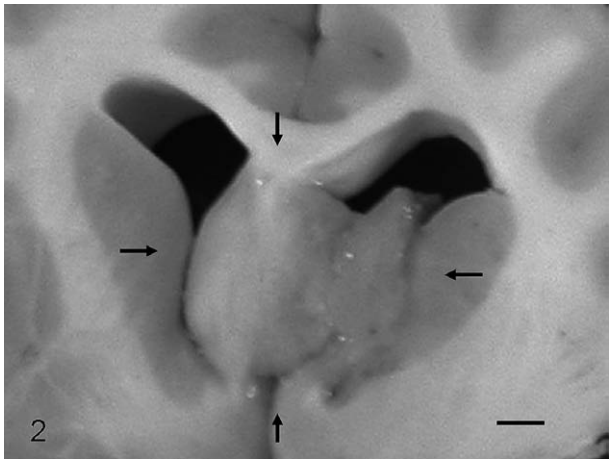

Fig. 2. A mass lesion on the transverse cut surface (arrows). The mass in the body of fornix is round-shaped, poorly demarcated and discolored. Bar=0.2 cm.

hematoxylin and eosin (HE), Periodic acid-Schiff (PAS) and Ziehl Neelsen stain. Immunohistochemistry was performed by the Envision polymer method (Dako Japan, Kyoto, Japan). The panel of antibodies used, dilution rate, and antigen retrieval methods are listed in Table 1. After antigen retrieval, tissue sections were treated with 1% hydrogen peroxide/methanol for 30 min, and then incubated in 8% skim milk/Tris buffered saline (TBS, pH7.4) at 37°C for 40 min. The tissue sections were incubated at 4°C overnight with the primary antibodies diluted in 8% skim milk/TBS. Following 3 washing in TBS, the sections were then incubated with Envision polymer reagent (Dako-Japan) at 37°C for 40 min. Finally, the reaction products were visualized with 0.05% 3–3'-diaminobenzidine (DAB) and 0.03% hydrogen peroxide in Tris/HCl buffer, followed by a counterstain with Mayer's hematoxylin.

Histologically, the mass lesion was loosely demarcated from the surrounding tissue, and was composed of mixed proliferation of pleomorphic histiocytic cells and lymphocytes, and astrocytes at the periphery. Most histiocytic cells had abundant eosinophilic cytoplasm varied in size with fairy well delineated cell margins. Multinucleated giant cells were scattered (Fig. 3), and some had epithelioid cell-morphology. These histiocytic cells had centrally located round or eccentric bizarre nuclei of various sizes and frequent mitotic figures (1–2 mitoses/high-power field), indicating high pleomorphism and atypia. Phagocytic figures were occasionally observed and phagocytic vacuoles con-

Table 1. Primary antibodies used for immunohistochemical evaluation of feline histiocytic sarcoma

Antibody*		Dilution	Source**	Antigen retrieval	Results
HLA-DR Alpha-Chain	Mouse anti-human Mab, TAL.1B5	1:50	Dako	Autoclave	+
Iba1	Rabbit anti-human Pab	1:250	Wako	Autoclave	+
Lysozyme	Rabbit anti-human Pab	1:1000	Dako	Proteinase K	+
Alpha-1 Antichymotrypsin	Rabbit anti-human Pab	1:1000	Dako	Autoclave	+
CD3	Rabbit anti-human Pab	1:50	Dako	Autoclave	–
IgG (H and L)	Rabbit anti-cat Pab	1:200	Bethyl	Autoclave	–
GFAP	Rabbit anti-cow Pab	1:400	Dako	None	–
Neurofilament	Mouse anti-human Mab, 2F11	Ready-to-use	Dako	None	–
Ki-67	Mouse anti-human Mab, MIB-1	Ready-to-use	Dako	Autoclave	41%

* Mab=monoclonal antibody, Pab=polyclonal antibody.
** Dako=Dako Japan Ltd., Japan. Wako=Wako Pure Chemical Industries Ltd., Japan. Bethyl=Bethyl Laboratories Inc.

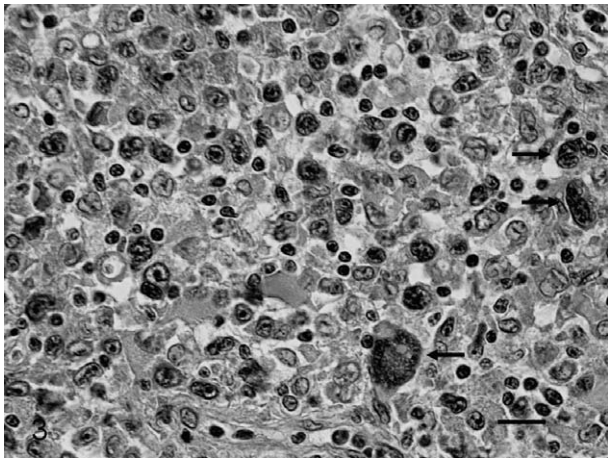

Fig. 3. The mass is composed of proliferation of highly pleomorphic cells resembling histiocytes with occasional multinucleated giant cells (arrows). Moderate infiltrates of small lymphocytes are admixed. HE stain. Bar=10 μm.

tained cell debris. The pleomorphic histiocytic cells occasionally accumulated around the small arterioles within and around the mass lesion. Moderate numbers of small reactive lymphocytes were admixed with the cell population. Gemistcytic astrocytes were also intermingled at the periphery of the mass lesion. PAS and Ziehl Neelsen stains could not reveal any bacterial or fungal organisms in the lesion.

Immunohistochemically, most pleomorphic histiocytic cells were immunopositive for HLA-DR and Iba1 (Fig. 4 a and b), and a few of such cells morphologically resembling macrophages but epithelioid cells were positive for lysozyme and alpha-1 antichymotrypsin. Many CD3-positive T lymphocytes and few IgG-positive lymphocytes and plasma cells were infiltrated in the lesion. GFAP-positive reactive astrocytes and neurofilament-positive neurites were also found in the lesion, while the anaplastic pleomorphic histiocytic cells were completely negative for these antibodies. As described previously [12], Ki-67 labeling index was investigated, and the number of Ki-67-positive neoplastic cells including multinucleated giant cells was approximately 41% of the nuclei, indicating high proliferating activity. The immunohistochemical and morphological natures of the neoplastic cells indicated that the cells were monocytic/histiocytic origin.

Based on these findings, the diagnosis of histiocytic sarcoma in the brain was made, though the differential diagnoses by imaging analysis were choroid plexus tumor, ependymoma, glioma and meningioma from the localization [3], and considering the positivity of FeLV specific antigen, central lymphoma. Proliferating histiocytes were considered as neoplastic rather than reactive because of the remarkable mitotic activity, high Ki-67 nuclear staining, and high cellular atypia and pleomorphism. In dogs, CD1, CD11b, CD11c, CD14, CD68, and canine MHC class II are known as the reliable markers for monocytic/histiocytic lineage [2]. However, most of these antibodies are not available in cat tissues and formalin-fixed materials [11]. As formalin-fixed tissues were available in the present case, CD3, IgG, HLA-DR and Iba1 were used to identify nonlymphoid, monocytic/histiocytic lineage [7, 17]. Differing from neoplastic lymphocytes, neoplastic histiocytes in the present case may remain phagocytic activity and occasionally epithelioid cell-like morphology as features observed in the previous cases [4, 18]. In addition, the neoplastic histiocytes were intensely positive for Iba1 and HLA-DR, partly positive for lysozyme and alpha-1 antichymotrypsin, and completely negative for CD3, IgG, and GFAP. Among them, Iba1, which is known to mediate calcium signals in the monocytic/histiocytic lineage, is predominantly expressed on the cells, but not on astrocytes and lymphocytes in humans [7, 8]. Although a use of Iba-1 antibody to identify feline and canine monocytic/histiocytic lineage has not yet been elucidated, it is indicated in the present study that the molecule might be a possible marker for feline histiocytes. In a previous report [14], many inflammatory

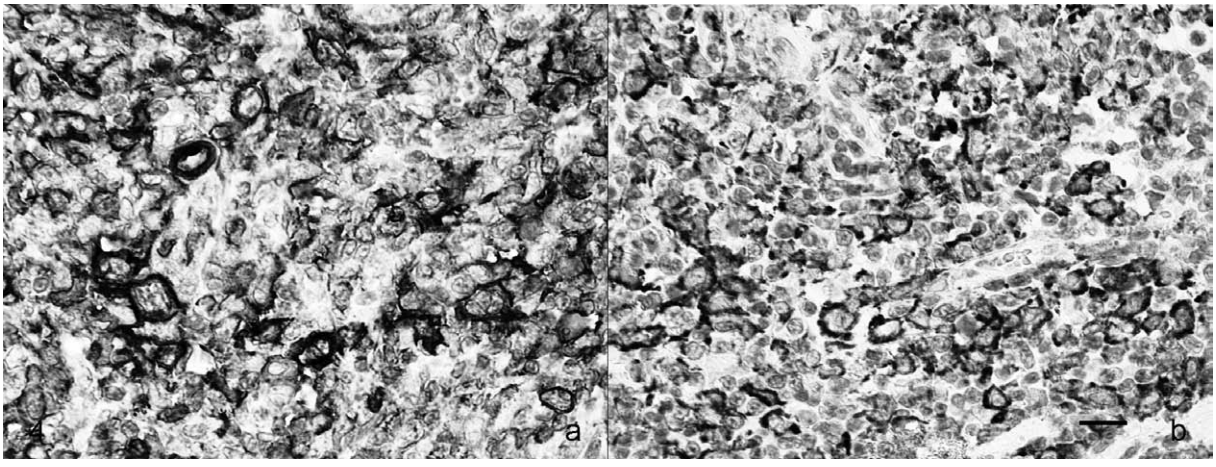

Fig. 4. Most pleomorphic histiocytic cells are immunopositive for HLA-DR (a), and Iba1 (b). Mayer's hematoxylin counterstain. Bar=10 μm.

lymphocytes were frequently observed in some canine malignant histiocytosis as in the present case.

Since apparent breed predilection (Bernese mountain dogs) has been confirmed in canine HS, some hereditary factor may exist. FeLV infection are considered to be a crucial factor for the malignancy in young age [9, 10]. Since the present cat was also FeLV and FIV positive, FeLV and FIV infection may play certain roles on tumorigenesis of feline histiocytic sarcoma. To establish adequate classification of these types of feline histiocytic tumors in the CNS, a number of further case studies and more clinical and histological information will be needed.

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