FULL PAPER *Pathology*

Histological and immunohistochemical studies on primary intracranial canine histiocytic sarcomas

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ABSTRACT. Histiocytic sarcoma is a progressive and fatal malignant neoplasm that mainly occurs in middle- to old-aged dogs. This study describes clinicopathological, histological and immunohistochemical characteristics of intracranial histiocytic sarcomas in 23 dogs. Magnetic resonance imaging and/or computed tomography of the brains revealed that the tumors mainly located in the cerebrum, particularly the frontal lobe. Seizure was a predominant clinical sign in most of the cases. Histologically, the tumor cells were morphologically classified into round/polygonal- and spindle-shaped cell types. There was a significant association between tumor cell types and hemophagocytic activity ($P<0.05$). However, there was no significant difference in other clinicopathological parameters and mitotic index between the 2 types. Immunohistochemically, tumor cells were strongly positive for HLA-DR, Iba-1 and CD204 in all the 23 cases, for iNOS in 20, for CD163 in 17, for CD208 (DC-LAMP) in 9, for lysozyme in 8 and for S100 in 5 cases. In addition, the Ki67-proliferative index showed range of 0.50–64.33% (Average 26.60 ± 3.81%). These observations suggest that canine primary intracranial histiocytic sarcomas tend to exhibit both dendritic cell and macrophage phenotypes of histiocytic differentiation.
KEY WORDS: brain, canine, histiocytic sarcoma, immunohistochemistry

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Histiocytic proliferative disorders (HPDs) are currently well documented in human and various animal species, however, the etiology as well as pathogenesis is still unclear [1–3, 13, 15, 28]. In the dog, HPDs were first described in 1970s and recently classified into 3 major types including reactive histiocytosis (cutaneous and systemic forms), cutaneous histiocytoma and histiocytic sarcoma (localized and disseminated forms) depending upon clinical behaviors and pathological features [6, 9, 19].

Canine histiocytic sarcoma (HS) included in HPDs, is a progressive and fatal malignant neoplasm that is mainly documented in middle-age to older purebred dogs, predominantly in the Bernese mountain dog, Retriever and Rottweiler [1, 6, 9, 19, 24]. Moreover, the Pembroke Welsh Corgi, Shetland sheepdog and other purebreds are also described sporadically [2, 11, 29, 30, 33]. In general, the histiocytes are divided into 2 cell types: dendritic cells (DCs) and macrophages. Most of the canine HS cases originate from DCs. Several cases arising from macrophages, namely hemophagocytic HS, are very rare [21, 25]. In accordance with the distribution pattern of tumor, the number of primary organ involved and the evidence of distant metastasis, HSs are classified into localized and disseminated forms. The localized form is recognized as a solitary mass that mainly manifests in the skin and subcutis of the extremities with local invasion to sentinel lymph nodes. In the disseminated form, on the contrary, multiple masses occur preferentially in the spleen, lung and bone marrow with a rapid and widespread metastasis [2].

The incidence of HS with the central nervous system (CNS) involvement is very low in both human and animals. In veterinary literatures, to our knowledge, there have been only 10 publications describing the occurrence of HS with CNS manifestation [5, 11, 12, 16, 26–30, 33]. Like the distribution pattern of HS in the extraneural tissues, both localized and disseminated HSs are being observed in the CNS tissues. Ide *et al.* [11] mentioned that the cellular morphologies of both localized and disseminated HSs in CNS were histologically identical. Moreover, immunohistochemical expression patterns of those were not associated with the tumor cell of origin. HS cases with the CNS involvement exhibited mainly histiocytic markers, such as major histocompatibility complex class II (MHC II), lysozyme and CD18. Currently, most of the histiocytic markers provided to confirm cellular origin of HS are only available for frozen tissue samples. Furthermore, the cellular origin and histogenesis of HS in the CNS are still unclear due to the low incidence. In the present study, therefore, we describe clinicopathological, histological and immunohistochemical (IHC) characteristics of intracranial histiocytic sarcomas in 23 dogs by using conventional diagnostic markers. In addition, inducible nitric oxide synthase (iNOS) and dendritic cell-lysosomal associated membrane protein (DC-LAMP or CD208) were

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employed as macrophage and dendritic cell markers, respectively. The Ki67-proliferative index (PI) was also illustrated in all the samples.

MATERIALS AND METHODS

Samples: Formalin-fixed canine brain tumor samples including 20 tumor biopsies and 3 necropsies between 2009 and 2014 were pathologically examined at the Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, the University of Tokyo. All the cases were histologically diagnosed as HS. The signalment, neurological signs and tumor location of the 23 dogs are summarized in Table 1.

Histology: Two to four-μm thick paraffin tissue sections were stained with hematoxylin and eosin (HE). The tumors were morphologically divided into 2 categories (round/polygonal and spindle cell types) as described previously [6]. In the present study, conversely, multinucleated giant cells were included in round/polygonal cell type. In order to determine the mitotic index (MI), 10 highest densities of mitotic figure areas were randomly selected, and then, the total number of mitoses was counted per 10 high power fields (hpf; 400X).

Immunohistochemistry: Primary antibodies used for immunohistochemistry (IHC) and antigen retrieval methods are detailed in Table 2. In order to block non-specific reactions, all tissue sections were immersed in 10% hydrogen peroxide (H_2O_2) in methanol at room temperature for 5 min and then incubated in 8% skim milk at 37°C for 30 min. All tissue sections were applied with each primary antibody at 4°C overnight. The Envision$^+$ system-HRP labeled polymer reagent (DAKO, Tokyo, Japan) was then applied at 37°C for 40 min. For the detection of CD208, tissue sections were applied with a biotinylated secondary antibody (1:400, anti-rat IgG (H+L) antibody, KPL, Gaithersburg, MD, U.S.A.) at 37°C for 1 hr and then incubated with streptavidin/HRP reagent (1:300, DAKO) at room temperature for 40 min. All sections were rinsed with Tris-buffered saline (TBS) prior to treat with 3–3′-diaminobenzidine solution containing 0.03% H_2O_2 and the counterstained with Mayer's hematoxylin (Muto Pure Chemicals, Tokyo, Japan). Normal canine tissues were used as positive controls, whereas negative controls were performed through applying with TBS instead of the primary antibodies. Positive tumor cells were counted in randomly selected areas (hpf; 400X). Semiquantitative scores included 4 categories as follows: − (Negative)= no positive tumor cells; + (Weakly positive)= 1–25% positive tumor cells; ++ (Moderately positive)= 26–50% positive tumor cells; +++ (Strongly positive)= >50% positive tumor cells. In addition, the Ki67 expression was also determined by counting the number of nuclear positive in the HS cells among total numbers of HS cells in 10 random hpf fields (400X). The average percentage of those was defined as Ki67-PI.

Statistical analyses: Chi-square or Fisher's exact test was used to assess the association between clinicopathological features together with hemophagocytic activity and necrosis, and morphological difference of tumor cells, as appropriate. The percentage of Ki67-positive tumor cells was demonstrated as range and mean ± standard error of the mean (SEM). In addition, Mann-Whitney *U* test was performed to determine the significance of difference of mean MI and Ki67-PI between two cell types. Two-sided significant level was used that *P*-value <0.05 was considered statistically significant.

RESULTS

Tumor occurrence: Twenty three dogs examined were 14 males and 9 females with the median age of 9 years (4 years to 14 years). Breeds were comprised of Pembroke Welsh Corgi (n=11), Shetland sheepdog (n=3), Labrador retriever (n=2), Beagle (n=2), mixed breed (n=2), Flat coated retriever (n=1), Miniature schnauzer (n=1) and Siberian husky (n=1). Various neurological signs were recorded in 19 dogs, which included seizure (n=12), altered level of consciousness (n=5), circling (n=5), abnormal basic vision test (n=4), gait abnormalities (n=4), proprioceptive deficits (n=4), hemiplegia and paralysis (n=3), disorientation (n=2), head pressing (n=2), head tilt (n=2), tremor (n=2), behavioral change (n=1) and somnolence (n=1). Brain magnetic resonance imaging (MRI) and/or computed tomography (CT) were also performed in all the cases to detect tumor distribution. Most of the tumors (n=21) were observed in the cerebrum, whereas two cases (Case Nos. 4 and 15) were in the cerebellum. Complete postmortem examination was performed only in Case Nos. 4, 5 and 15, and as far as examined in the 3 cases, tumor invasions to distant organs were not detected.

Histological examination: Microscopically, brain masses of all cases were poorly demarcated and invading to the brain parenchyma. The tumor cells were classified into 2 types in accordance with cellular morphology. The first type was defined by round- to pleomorphic-shaped cells with eosinophilic cytoplasm and distinct border. Cytoplasmic vacuolation was occasionally found. These cells had eccentric, round to ovoid nuclei with prominent nucleoli (1–2 nucleoli/nucleus). Marked anisocytosis and anisokaryosis were noted with various numbers of atypical mitoses. Multinucleated giant tumor cells were frequently found. Hemophagocytic activity was commonly observed in almost all the cases of this type (Fig. 1 and Table 3). The second type was defined by spindle- and fusiform-shaped cells with indistinct border. These cells arranged in irregular pattern. Their nuclei were ovoid to spindle shaped and concentrically located. The nucleoli were obscure. Mild anisocytosis and anisokaryosis, and atypical mitoses were noted (Fig. 2). Hemophagocytosis was seen in only one dog (Case No. 23). In both tumor types, moderated to marked infiltration of small lymphocytes was notably observed surrounding small-sized blood vessels and scattering throughout the neoplastic lesions. Moderate necrosis was occasionally found. Moreover, we found statistically significant associations between tumor cell types and hemophagocytic activity (*P<0.05*). However, there were no significant differences in other clinicopathological parameters (age, sex and necrosis) and MI between the two cell

Table 1. Information of 23 primary intracranial canine histiocytic sarcomas

Tumor cell morphology	No.	Breed	Age [a]	Sex [b]	Neurological sign [c]	Tumor localization [d]	Sample collection
Round/polygonal cell type	1	Pembroke Welsh Corgi	11Y	FX	Gait abnormalities, depression, worsening respiratory status	Cerebrum (temporal lobe)	Biopsy
	2	Pembroke Welsh Corgi	11Y5M	F	Right hemiplegia, seizure	Cerebrum (left frontal to parietal lobe)	Biopsy
	3	Pembroke Welsh Corgi	7Y2M	M	Seizure, subconscious, circling, aimless pacing, somnolence, torticollis, left eye vision loss	Cerebrum (right temporal to occipital lobe)	Biopsy
	4	Pembroke Welsh Corgi	5Y2M	FX	Gait abnormality, lateral recumbence	Cerebrum (right temporal lobe) and cerebellum	Necropsy
	5	Pembroke Welsh Corgi	10Y	FX	n/d	n/d	Necropsy
	6	Pembroke Welsh Corgi	12Y	F	n/d	Cerebrum (right temporal lobe)	Biopsy
	7	Labrador retriever	8Y	M	Seizure, progressive depress	Cerebrum (left frontal lobe)	Biopsy
	8	Labrador retriever	8Y6M	M	Seizure, circling, head pressing, proprioceptive deficit, head tilt, slow blink reflex	Cerebrum (right occipital lobe)	Biopsy
	9	Mixed breed	9Y	F	Circling, proprioceptive deficit, head pressing, right eye vision loss	Cerebrum (left parietal lobe)	Biopsy
	10	Mixed breed	4Y	MX	Anorexia, negative blink reflex, mydriasis	Cerebrum (frontal lobe)	Biopsy
	11	Shetland sheepdog	9Y	M	Behavioral change (aggressive), Gait abnormality (wobble)	Cerebrum (frontal lobe)	Biopsy
	12	Shetland sheepdog	11Y11M	FX	Circling, walking difficulty	Cerebrum (occipital lobe)	Biopsy
	13	Beagle	14Y	MX	Seizure	Cerebrum (base of brain to olfactory bulb)	Biopsy
	14	Flat coated retriever	12Y	M	Seizure, confusion, subconscious, lateral recumbence	Cerebrum (right temporal and occipital lobe)	Biopsy
	15	Siberian husky	11Y	FX	n/d	Cerebellum	Necropsy
Spindle cell type	16	Pembroke Welsh Corgi	11Y5M	MX	Seizure, stupor	Cerebrum (fornix)	Biopsy
	17	Pembroke Welsh Corgi	9Y	MX	Seizure, paralysis, tremor, proprioceptive deficit, inactivity	Cerebrum (right frontal lobe)	Biopsy
	18	Pembroke Welsh Corgi	8Y	FX	Proprioceptive deficit	Cerebrum (right frontal and temporal lobe)	Biopsy
	19	Pembroke Welsh Corgi	8Y	M	n/d	Cerebrum (right frontal lobe)	Biopsy
	20	Pembroke Welsh Corgi	9Y9M	M	Seizure, drooling, tremor	Cerebrum (left temporal lobe)	Biopsy
	21	Beagle	10Y11M	M	Seizure, paralysis, circling	Cerebrum (left frontal lobe)	Biopsy
	22	Miniature schnauzer	4Y	M	Seizure, progressive depress	Cerebrum (left temporal lobe)	Biopsy
	23	Shetland sheepdog	11Y	M	Seizure	Cerebrum	Biopsy

a) Y=Year(s); M=Month(s), b) M=Male; F=Female; X=Sterilization, c) n/d=No data, d) Tumor locations were confirmed by magnetic resonance imaging (MRI) and/or computed tomography (CT); n/d=No data.

Table 2. Primary antibodies used in immunohistochemical examination

Antibody	Type [a]	Dilution [b]	Antigen retrieval for IHC [c]	Expression	Source
HLA-DR	mAb, (TAL.1B5)	1:50	HIER (Citrate buffer, pH 6.0), 121°C, 10 min	Antigen presenting cells	Santa Cruz, CA, U.S.A.
Iba-1	pAb	1:250	HIER (Citrate buffer, pH 6.0), 121°C, 10 min	Microglia, macrophage	Wako, Osaka, Japan
CD204	mAb, (SRA-E5)	1:100	HIER (Tris/EDTA buffer, pH 9.0), 121°C, 10 min	Monocyte, macrophage	TransGenic, Kobe, Japan
CD163	mAb, (AM-3K)	1:100	HIER (Citrate buffer, pH 2.0), 121°C, 10 min	Histiocyte	TransGenic, Kobe, Japan
iNOS	pAb	1:200	HIER (Citrate buffer, pH 6.0), 121°C, 10 min	Macrophage	Abcam, Tokyo, Japan
Lysozyme	pAb	1:1,000	PIER (Proteinase K), room temperature, 30 min	Monocyte, macrophage	Dako, Tokyo, Japan
S100	pAb	1:1,000	HIER (Citrate buffer, pH 6.0), 121°C, 10 min	Dendritic cell	Dako, Tokyo, Japan
CD208 (DC-LAMP)	mAb, (1010E1.01)	1:100	HIER (Citrate buffer, pH 6.0), 121°C, 10 min	Dendritic cell	Dendritics, Lyon, France
Ki67	mAb, (MIB-1)	RTU	HIER (Citrate buffer, pH 6.0), 121°C, 10 min	–	Dako, Tokyo, Japan

a) pAb=Polyclonal antibody; mAb=Monoclonal antibody, b) RTU=Ready-to-use, c) IHC=Immunohistochemistry, HIER=Heat-induced epitope retrieval; PIER=Proteolytic-induced epitope retrieval.

Table 3. Histological and immunohistochemical features of primary intracranial canine histiocytic sarcomas

No.	Breed	Tumor cell morphology[a)]	MI[b)]	Hemophagocytosis[c)]	Necrosis[d)]	IHC results[e)]								
						HLA-DR	Iba-1	CD204	CD163	iNOS	Lysozyme	S100	CD208	Ki67 (%)
1	Pembroke Welsh Corgi	Round/polygonal cell type	4	+	–	+++	+++	+++	–	+++	–	–	+++	30.08
2	Pembroke Welsh Corgi	Round/polygonal cell type	33	–	+	+++	+++	+++	+++	+++	+++	+	+++	53.29
3	Pembroke Welsh Corgi	Round/polygonal cell type	56	+	+	+++	+++	+++	++	+++	+++	+	–	26.00
4	Pembroke Welsh Corgi	Round/polygonal cell type	22	+	+	+++	+++	+++	+++	+++	+++	–	–	29.52
5	Pembroke Welsh Corgi	Round/polygonal cell type	8	+	–	+++	+++	+++	++	+	+++	–	–	3.00
6	Pembroke Welsh Corgi	Round/polygonal cell type	32	–	+	+++	+++	++	+++	+++	+++	–	+	64.33
7	Labrador retriever	Round/polygonal cell type	39	+	–	+++	+++	+++	+++	+++	+++	–	++	32.86
8	Labrador retriever	Round/polygonal cell type	37	–	+	+++	+++	+++	++	+++	–	+	–	15.68
9	Mixed breed	Round/polygonal cell type	70	+	+	+++	+++	+++	+++	+++	–	–	–	49.05
10	Mixed breed	Round/polygonal cell type	44	–	+	+++	+++	+++	–	+	–	–	–	5.67
11	Shetland sheepdog	Round/polygonal cell type	24	+	–	+++	+++	+++	–	+	–	–	+	38.41
12	Shetland sheepdog	Round/polygonal cell type	62	+	+	+++	+++	++	++	–	–	–	–	6.50
13	Beagle	Round/polygonal cell type	4	+	+	+++	+++	+++	+++	–	–	+	++	0.50
14	Flat coated retriever	Round/polygonal cell type	39	+	–	+++	+++	+++	++	+	–	–	–	40.75
15	Siberian Husky	Round/polygonal cell type	0	+	+	+++	+++	+++	–	+++	+++	–	++	2.00
16	Pembroke Welsh Corgi	Spindle cell type	58	–	+	+++	+++	+++	+++	+++	+++	–	–	23.37
17	Pembroke Welsh Corgi	Spindle cell type	26	–	–	+++	+++	+++	+++	++	–	–	–	32.99
18	Pembroke Welsh Corgi	Spindle cell type	11	–	+	+++	+++	+++	–	–	–	–	–	42.38
19	Pembroke Welsh Corgi	Spindle cell type	10	–	+	+++	+++	+++	+++	+	–	–	–	18.88
20	Pembroke Welsh Corgi	Spindle cell type	44	–	+	+++	+++	+++	+++	++	–	–	–	48.82
21	Beagle	Spindle cell type	7	–	–	+++	+++	+++	++	+	–	–	++	26.68
22	Miniature schnauzer	Spindle cell type	6	–	+	+++	+++	+++	+++	+	–	–	++	18.35
23	Shetland sheepdog	Spindle cell type	12	+	+	+++	+++	+++	–	++	–	+	–	2.75
Total						23	23	23	17	20	8	5	9	

a) Round/polygonal cell type=>50% of tumor cell population are neoplastic histiocytes and multinucleated giant cells; Spindle cell type =>50% of tumor cell population are spindle-shaped cells, b) Mitotic index=Number of mitotic figures per 10 high power fields, c) Hemophagocytosis score: +=Hemophagocytosis is present; −=Hemophagocytosis is absent, d) Necrosis score: +=Necrotic area is observed; −=No necrotic area is observed, e) Immunohistochemical scoring: − (Negative)=Negative tumor cells; + (Weakly positive)=1–25% positive tumor cells; ++ (Moderately positive)=26–50% positive tumor cells; +++ (Strongly positive) =>50% positive tumor cells.

types (Table 4). Based on histological results, the diagnoses of HS were made in all the cases.

Immunohistochemistry: Intense cell membrane and/or cytoplasmic immunoreactivities to HLA-DR, Iba-1 and CD204 were observed in all 23 tumors (100.00%). Diffuse cytoplasmic staining for iNOS was detected in 20 cases (86.96%). Tumor cells in 17 cases (73.91%) were positive for CD163 with strong membrane staining. Nine tumors (39.13%) exhibited focal to diffuse cytoplasmic staining for CD208. Eight tumors (34.78%) exhibited cytoplasmic staining for lysozyme. Variable or weak cytoplasmic S100 immunoreaction was noted in 5 cases (21.74%) (Fig. 3 and Table 3). Ki67-PI of HS with CNS involvement ranged 0.50−64.33% (average 26.60 ± 3.81%). However, there was no significant difference in Ki67-PI between round/polygonal and spindle cell types.

DISCUSSION

Despite canine histiocytic sarcoma has been well documented over the past several years, there have been only 10 publications demonstrating the occurrence of HS in the CNS. In the present study, HS in the brain was frequently found in Pembroke Welsh Corgis, which is consistent with the results of previous studies [11, 16, 29]. Seizure is the major neurological sign of the cases of HS in the brain, while other clinical signs were found sporadically. A variety of the clinical signs might be associated with the affected areas of the brain. Based on clinical histories and diagnostic imaging results, the tumor invasion and metastasis to other distant organs were not detected, supporting that the brains are the primary site of HS in all the present 23 cases. Furthermore, in accordance with tumor distribution, only the localized pattern was observed in all the present cases, supporting that localized HS might be main form of intracranial HS in dog as described previously [5, 11, 29, 33].

Tumor cells were infiltrated to brain parenchyma in all cases. The term of primary intracranial canine HS, therefore, applies to the present study. McMenamin *et al.* [18] demonstrated that antigen presenting cells were commonly found in the meninges and choroid plexus of normal rat brains and that the cells have similar immunophenotype and ultrastructural characteristics to DC. In accordance with the results of the present study, we postulate that the cellular origin of canine HS in the brain is possibly resident DC in either the meninges or choroid plexus.

The lesions of canine HS in the brain can be histologically classified into 2 types (round/polygonal and spindle cell types) like those in the spleen and extremities described previously [6]. Interestingly, the present results showed hemophagocytic activity of round/polygonal cell type was significantly higher than that of spindle cell type, suggesting

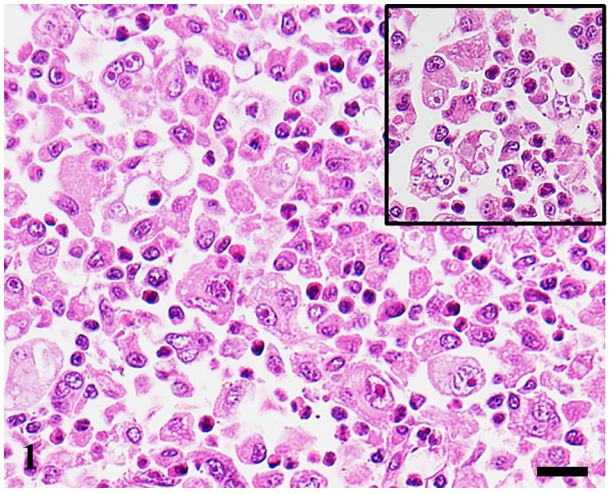

Fig. 1. Cerebellum. Dog. Case No. 4. Histiocytic sarcoma. Numerous polygonal to pleomorphic shaped neoplastic histiocytes proliferate in the brain parenchyma. Hemophagocytosis is commonly seen (inset). HE. Scale bar=20 μm.

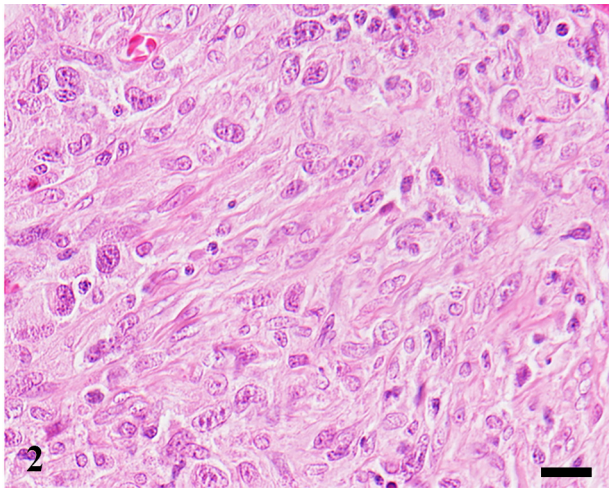

Fig. 2. Cerebrum. Dog. Case No. 20. Histiocytic sarcoma. Most of the tumor cells are spindle-shaped. HE. Scale bar=20 μm.

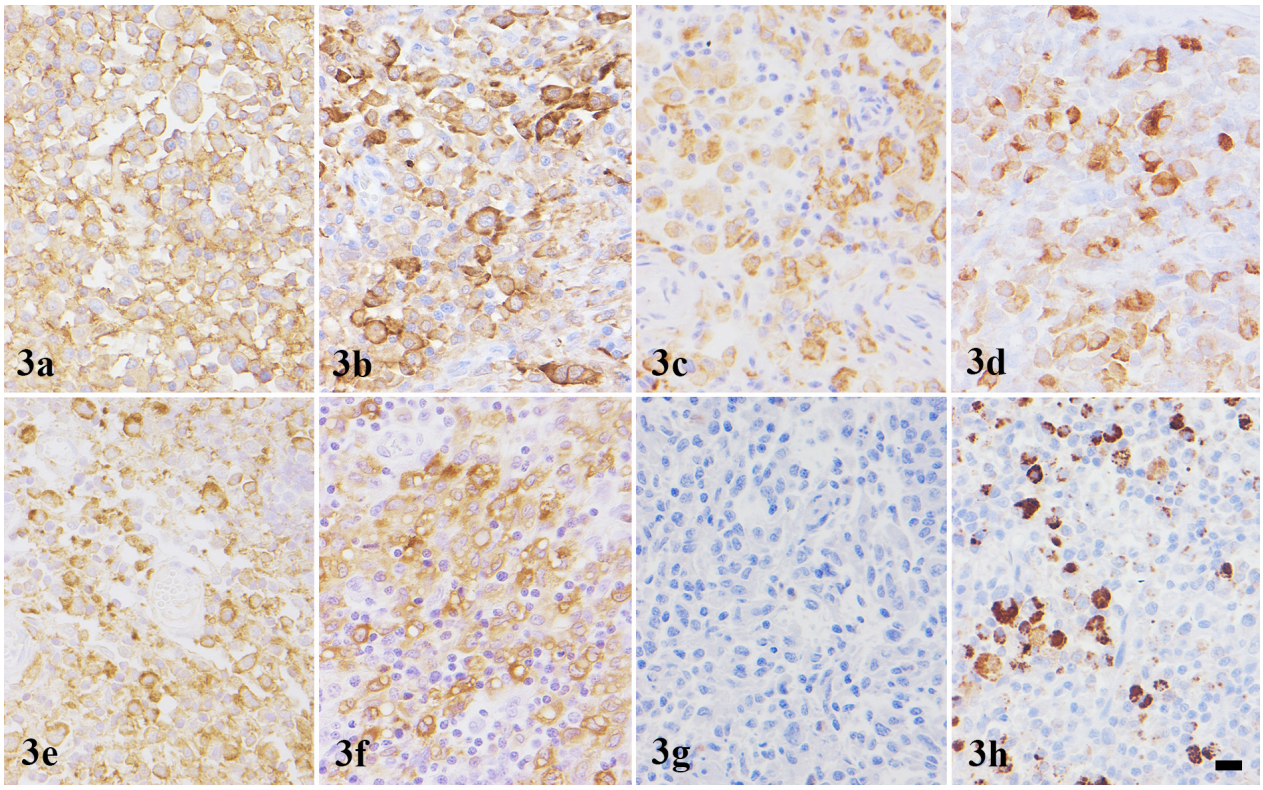

Fig. 3. Histiocytic sarcoma. Cerebrum; Dog. Case No. 6. Neoplastic histiocytes are positive for (a) HLA-DR, (b) Iba-1, (c) CD204, (d) CD163, (e) iNOS, (f) lysozyme and (h) CD208, but negative for (g) S100. IHC. Hematoxylin counterstain. Scale bar=10 μm.

that the biological behaviors of round/polygonal-shaped cell type are probably more aggressive than another. However, there were no significant differences in sex, age, the presence of necrosis, Ki67-PI and the expression of immunohistochemical markers of tumor cells (data not shown) between the 2 types. These results support that the difference of tumor cell morphology cannot be used as histological predictive parameter for primary intracranial HS in dog, unlike HS

Table 4. The association between clinicopathological characteristics and cellular morphology of primary intracranial canine histiocytic sarcoma

Variable [a]	Round/polygonal cell type (n=15)	Spindle cell type (n=8)	*P*-value [b]
Sex			
Male	7	7	0.086
Female	8	1	
Age range			
<3 years	0	0	1.000
≥3 years, <6 years	2	1	
≥6 years	13	7	
Tumor location			
Cerebrum	13	8	0.558
Cerebellum	1	0	
n/d	1	0	
Hemophagocytosis (Presence)	11	1	0.009 [b]
Necrosis (Presence)	10	6	1.000

a) n/d=No data, b) *P<0.05.*

cases of extraneural tissues [6].

Lysozyme is widely used as a histiocytic marker in both human and animal to substantiate a diagnosis of histiocytic disorders. In human histiocytic disorders, the tumors that originated from macrophage lineage exhibited high expression of lysozyme, whereas those arose from DC had low expression or devoid of this molecule [4, 10, 17, 20, 31]. In the present study, intense lysozyme-immunoreactivity was observed in 8 dogs, supporting that these tumors had macrophage phenotype. On the other hands, S100 and CD208 are used as a marker for human DCs. The S100 molecule is specifically expressed by DC lineage except for follicular DCs, whereas the latter is exclusively expressed by human mature DCs and closely associated with DC differentiation and maturation [7, 23]. In the present study, S100 and CD208 immunoreactivities were observed in 5 and 9 tumors, respectively, supporting that these tumors had DC phenotype. HLA-DR, Iba-1 and CD204 immunoreactivity was detected in all 23 cases, confirming that the tumors originated from histiocytes [9, 14, 22]. In 20 cases, iNOS was detected, and CD163 in 17 cases; as the two molecules are widely used as M1 and M2 macrophage markers, respectively [8, 32]. The results showed that 15 cases of primary intracranial HS showed both $iNOS^+$ and $CD163^+$ (15/23), suggesting that the HS cells of the brain belong to the M1 and M2 macrophage phenotypes. However, some of intracranial HS cases (7/23) exhibited either M1 or M2 macrophage phenotype, and one case was negative for both macrophage and DC markers. These observations suggest that variable immunophenotypic features might be associated with the differentiation stage of the tumor cells.

Primary HS of the CNS is an aggressive malignant neoplasm, which has a worse prognosis. This tumor is the leading cause of cancer-related death in both human and animal. In accordance with all the present results, we can conclude that canine HS in the brain may in part possess the features of both macrophage and DC. However, M1 and M2 types are relatively predominant compared to the DC phenotype. This phenomenon was also found in HS cases of extraneural tissues, but was an uncommon event [19]. Therefore, a number of samples including fresh/frozen primary brain tumor tissues and further *in vitro* studies are required in order to further verify cellular origins of canine HS arising in the CNS.

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