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A Comparative Pathological Study on Granulomatous Meningoencephalomyelitis and Central Malignant Histiocytosis in Dogs

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ABSTRACT. Histiocytic proliferative disorders in canine central nervous system (CNS) including granulomatous meningoencephalomyelitis (GME) and malignant histiocytosis were compared pathologically. Lesions of GME mainly existed in the white matter of the cerebrum, brainstem and cerebellum and consisted of characteristic perivascular cuffing, parenchymal granuloma and leptomeningeal infiltrates of mononuclear cells. In malignant histiocytosis, there were two histological patterns, diffuse proliferation of neoplastic histiocytes through the leptomeninges and neoplastic nodule formation in the parenchyma. Neoplastic histiocytes exhibited mild to severe cellular atypia and high ability of invasion into the brain parenchyma. Mitotic and phagocytic figures were also observed. Several histiocytic markers, including lysozyme, α1-antitrypsin and lectin RCA-1, revealed histiocytic origin of both inflammatory and neoplastic cells, however, those were not determinative for the discrimination between GME and malignant histiocytosis. CD3- and PCNA-positive cells existed in the lesions of both diseases. The number of CD3-positive cells in GME tended to be greater than in malignant histiocytosis, while the difference was not statistically significant.

KEY WORDS: canine, CNS primary malignant histiocytosis, granulomatous meningoencephalomyelitis, histiocyte, lymphocyte.

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Histiocytic proliferative disorders in the canine central nervous system (CNS) were previously referred as "reticulosis". In 1972, this syndrome was classified into three categories including (1) inflammatory (granulomatous) reticulosis, (2) neoplastic reticulosis and (3) microgliomatosis [9]. Recently, the term granulomatous meningoencephalomyelitis (GME) tend to be used instead of inflammatory reticulosis [7, 20] and neoplastic reticulosis was re-classified into lymphoma and/or malignant histiocytosis [22]. Although the disseminated form is usually encountered, the focal form, which may result from confluence of the lesions, has been recognized. The focal form of GME may have been previously included in neoplastic reticulosis [2] and veterinary pathologists are in danger of misdiagnosing this form as neoplasm.

Canine malignant histiocytosis is often described as systemic lesions involving the spleen, liver, lymph nodes and bone marrow. Recently, CNS primary malignant histiocytosis has been reported [4]. In CNS primary malignant histiocytosis, the meninges throughout the neuraxis may be diffusely affected, or large tumor nodules are present in the parenchyma [12]. The neoplastic cells have histiocytic features such as phagocytic and multinucleated figures, and are pleomorphic. It is easy to distinguish between inflammatory and neoplastic histiocytic lesions. However, there are actually some difficulty for diagnosing individual cases when we distinguish between the focal form of GME and malignant histiocytosis with low cellular atypia. The purpose of the present study is to clarify pathological differences between the two disorders.

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MATERIALS AND METHODS

Animals and tissue processing: CNS tissues from 9 dogs, including four GME and five malignant histiocytosis cases, were examined. One previously reported case [21] of malignant histiocytosis was included. All the samples were fixed in 10% formalin and paraffin sections of 6 μm-thick were stained with hematoxylin and eosin (HE).

Immunohistochemistry and lectin histochemistry: Immunohistochemistry was performed using Envision polymer reagent (DAKO-Japan, Kyoto, Japan). Lectin histochemistry was performed by the avidin-biotin peroxidase complex method (ABC, Vector Laboratories, Burlingame, CA, U.S.A.). Heating with an autoclave, at 121°C for 5 min, or enzymatic digestion with proteinase K (DAKO-Japan) at room temperature for 10 min were conducted for antigen retrieval procedures. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxidase in methanol at room temperature for 10 min. Sections were then incubated with primary antibodies or lectin RCA-1 (EY Laboratories Inc., San Mateo, CA, U.S.A.) at 37°C for 30 min. The primary antibodies employed were rabbit antibodies against lysozyme (1:300, DAKO-Japan), α1-antitrypsin (1:100, DAKO-Japan), and CD3 (1:20, DAKO-Japan) and mouse monoclonal antibodies against CD68 clone PG-M1 (prediluted, DAKO-Japan), macrophage, myeloid/histiocyte antigen clone MAC387 (prediluted, DAKO-Japan), CD79a clone HM57 (1:20, DAKO-Japan), CD79a clone

JCB117 (prediluted, DAKO-Japan) and proliferating cell nuclear antigen (PCNA, prediluted, Zymed, California, U.S.A.). All sections were incubated with Envision polymer reagent (DAKO-Japan) or ABC reagent (Vector laboratories) at 37°C for 30 min. The reaction products were visualized with 3,3'-diaminobenzidine (Sigma, St Louis, MO, U.S.A.) in Tris-buffered saline. Sections were counterstained with hematoxylin or light green. As positive controls, canine normal spleen and skin with granulomatous dermatitis were prepared.

Quantitative analysis: The number of CD3- or PCNA-positive cells was counted in randomly selected 10 fields including lesions under light microscopy, at a magnifications of × 400. The mean values and standard deviations of two disorders were calculated. The mean and standard deviations were calculated and the mean was compared using Student *t*-test.

RESULTS

Case histories and gross findings: The age of the dogs ranged from 2 years and 6 months to 4 years (mean; 3.6 years) in GME and from 6 years to 16 years (mean; 9.4 years) in malignant histiocytosis. Magnetic resonance imaging (MRI) or computed tomography (CT) scanning was performed for 5 cases before necropsy (Cases 1, 3, 4, 8, and 9). These clinical and gross findings of examined cases are summarized in Table 1.

Histopathology: GME; Lesions were distributed widely throughout the CNS, including the brainstem, cerebellum, and cerebrum (Fig. 1). The spinal cord obtained only from Case 1, had similar lesions. The white matter of the cerebrum, brainstem, and cerebellum was affected most severely. However, the cerebral cortex and leptomeninges were affected moderately or mildly. The typical lesions consisted of perivascular cuffing, parenchymal granulomas, hemorrhage and leptomeningeal infiltrates. Histiocytes including epithelioid cells and/or lymphocytes were predominant in the lesions of all lesions (Fig. 2), but the ratio of the cells varied in each lesion. Binucleated or trinucleated cells, plasma cells and neutrophils were occasionally scattered. Mitotic and phagocytic figures were hardly detected. In Case 3, large granuloma was observed at the optic chiasm, and the optic nerves suffered from severe granulomatous neuritis.

Malignant histiocytosis; Two types of proliferation patterns were found by gross observation. In 2 dogs (Cases 5 and 6), the lesions were distributed through the meninges and ventricular wall, sometimes accompanied with parenchymal invasion. In 3 dogs (Cases 7, 8, and 9), neoplastic nodule formation was prominent in the brain parenchyma. In Case 5, the thalamus and hippocampus were most severely affected. In Case 6, the severest lesion was found in the basiarachinoidal area of the spinal cord (Fig. 3). Neoplastic nodule formation was observed in the temporal to parietal lobes of the cerebrum (Case 7), midbrain (Case 8, Fig. 8), thalamus and fornix (Case 9). Neoplastic histiocytes varied in size and had central round or eccentric bizarre

Table 1. Clinical features and gross CNS lesions of 9 dogs

Case No.	Breed	Age	Sex	Clinical course	Gross findings	Pathological diagnosis
1	Golden Retriever	4Y	M	Died after 2 months	Severe disseminated congestion and hemorrhage in the white matter of the cerebellum and brainstem	GME
2	Shih Tzu	4Y	M	Died after 1 day	Mild disseminated congestion and hemorrhage in the white matter of the brainstem	GME
3	Miniature Dachshund	4Y	M	Died after 2 months	Mass at the optic chiasm, swelling of the optic nerves	GME
4	Maltese	2.5Y	F	Euthanized after 2 months	Partial discoloration of cerebral white matter	GME
5*	Tibetern Terrier	8Y	M	Euthanized after 3 months	Severe diffuse discoloration of the spinal white matter	MH
6	Golden Retriever	6Y	M	Euthanized after 4 days	White membranous materials around the cerebellum, brainstem, spinal cord	MH
7	Yorkshire Terrier	16Y	F	Died after 1 month	Mass at the cerebrum	MH
8	Mongrel	10Y	M	Died after 3 weeks	No information	MH
9	Golden Retriever	7Y	F	Died after 1 month	No information	MH

GME: granulomatous meningoencephalomyelitis, MH: malignant histiocytosis,
Y: years, M: male, F: female
* Case No. 5 was previously reported in ref. 29.

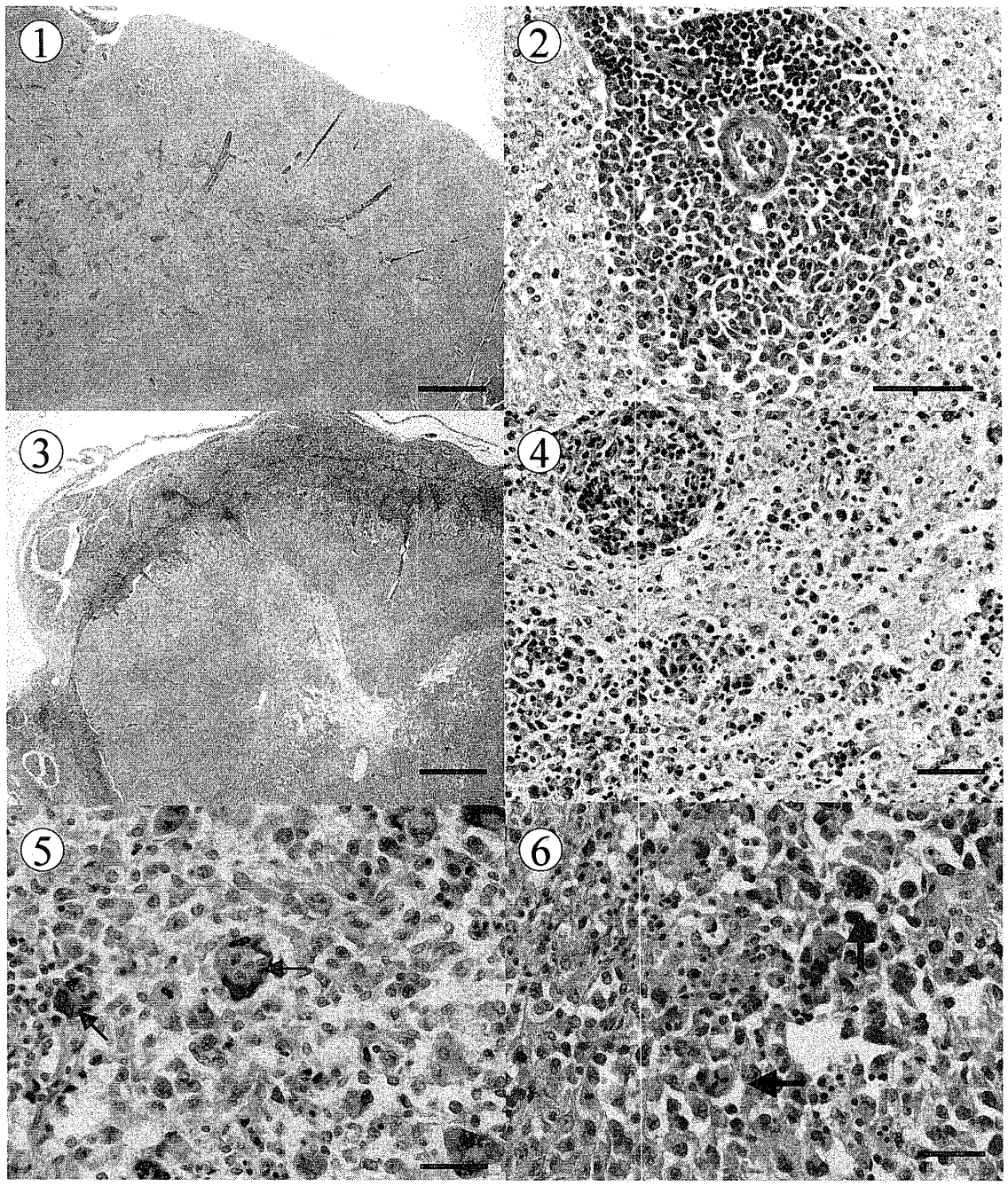

Fig. 1. Cerebrum; Case 2. Typical low power magnification view of GME. The white matter is dominantly involved in severe perivascular cuffing. HE Bar=1,000 μm.

Fig. 2. Cerebrum; Case 2. High power magnification view of GME. Perivascular infiltration consisting of histiocytes and lymphocytes. HE. Bar=50 μm.

Fig. 3. Spinal cord; Case 7. Leptomeningeal malignant histiocytosis. Diffuse lesions throughout the meninges with parenchymal invasion. HE. Bar=1,000 μm.

Fig. 4. Midbrain; Case 8. Focal mass-forming malignant histiocytosis. Invasion of tumor cells into the brain parenchyma. HE. Bar=70 μm.

Fig. 5. Spinal cord; Case 7. Malignant histiocytosis. Multinucleated tumor cells and phagocytic figures (arrows). HE. Bar=30 μm.

Fig. 6. Spinal cord; Case 8. Malignant histiocytosis. Atypical mitotic figures (arrows) are scattered. HE. Bar=30 μm.

nuclei of variable sizes. Multinucleated giant cells were scattered (Fig. 4). Only in Case 5, cellular atypia was mild to moderate. Mitotic figures were frequent. Atypical mitotic figures were also occasionally observed (Fig. 5). Phagocytic cells were often found and phagocytic vacuoles contained cellular fragments (Fig. 6). Neoplastic histiocytes invaded into the parenchyma in all cases. Reactive leukocytic infiltration varied among cases or lesions. Leukocytic infiltrates were composed of lymphocytes, plasma cells and neutrophils, and the infiltration pattern was disseminated or perivascular.

Immunohistochemistry and lectin histochemistry: Both infiltrative histiocytes in GME and neoplastic cells in malignant histiocytosis were positive for lectin RCA-1. Histiocytes in 4 GME cases consistently exhibited intense reactivity (Fig. 7). However, the intensity varied according to cases of malignant histiocytosis. For example, in Cases 5, 7 and 8, almost neoplastic cells showed intense reactivity (Fig. 8). Besides, half population of neoplastic cells including multinucleated giant cells was negative in Case 6. Mild to intense reactivity was observed in Case 9. Microglia and vascular endothelial cells were positive for RCA-1 in all cases. Lysozyme expression was detected in infiltrative histiocytes in all cases of GME. In Cases 1 and 2, the lysozyme immunoreactivity was moderate and diffuse, and limited number of positive histiocytes infiltrated in Cases 3 and 4. On the contrary, a part of neoplastic cells exhibited and mild to moderate immunoreactivity to lysozyme in malignant histiocytosis. Infiltrative histiocytes of all cases except for Case 5 showed mild to intense immunoreactivity to α1-antitrypsin. Actually, the neurons, astrocytes and spheroids as well were sometimes positive for lysozyme and α1-antitrypsin. Monocytes in the blood vessels and granurocytes showed intense immunoreactivity to MAC387, but most histiocytes including control slides, exhibited no reaction. Antibody to CD68-antigen could not labeled canine histiocytes.

In GME, CD3-positive lymphocytes scattered in the perivascular cuffs and in the parenchymal lesions (Fig. 9). In malignant histiocytosis, CD3-positive lymphocytes were

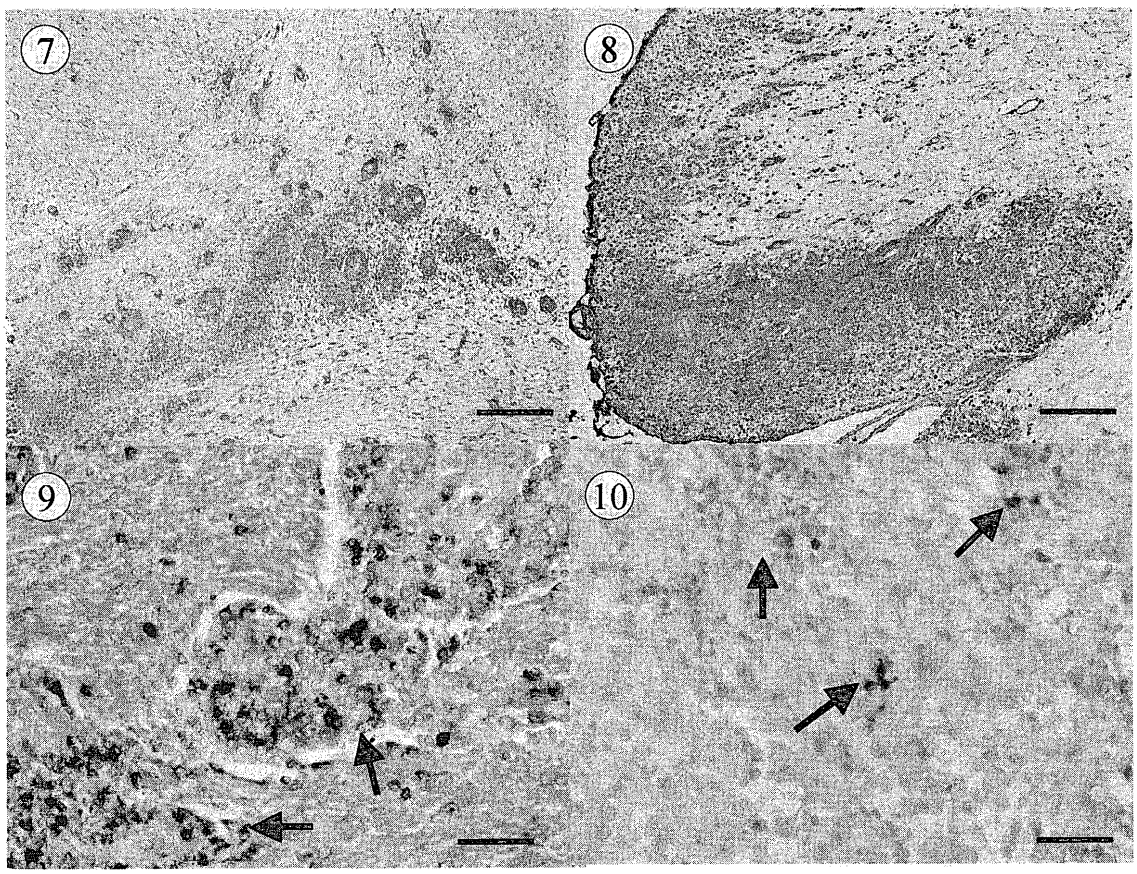

Fig. 7. Cerebrum; Case 1. GME. Lectin RCA-1-positive cells in perivascular cuffings and granulomas. ABC method. Bar=125 mm.
Fig. 8. Cerebrum; Case 5. Malignant histiocytosis. Lectin RCA-1-positive neoplastic cells. ABC method. Bar=125 μm.
Fig. 9. Cerebrum; Case 2. GME. CD3-positive lymphocytes within perivascular cuffing. Envision Polymer method. Bar=20 μm.
Fig. 10. Spinal cord; Case 7. Malignant histiocytosis. CD3-positive reactive lymphocytes in the neoplastic foci. Bar=20 μm. Envision Polymer method.

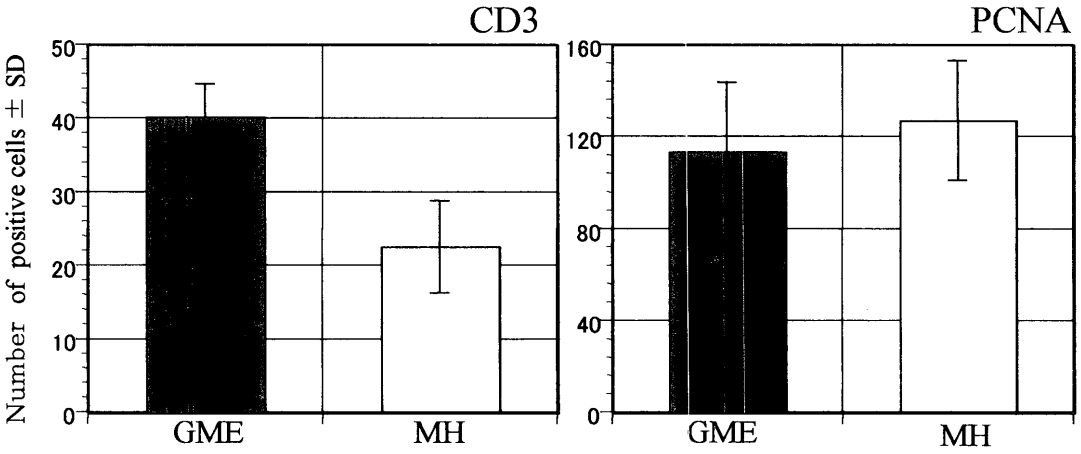

Fig. 11. The mean values and standard deviations of the number of CD3- (left) and PCNA- (right) positive cells. MH; malignant histiocytosis.

present in all cases (Fig. 10), but the number of reactive lymphocytes varied among cases. Neoplastic cells were negative for CD3. Antibodies to CD79a, both clone HM57 and JCB 117, could not label any lymphocytes in the brain. Only in positive control section from canine spleen, we could find CD79a (clone HM57)-positive lymphocytes. Nuclear immunoreactivity to PCNA was observed in the majority of inflammatory and neoplastic histiocytes. PCNA-positive glial cells also scattered in the brain parenchyma except for Case 7. Figure 11 shows the number of CD3- or PCNA-positive. The number of CD3-positive cells in GME tended to be greater than that in malignant histiocytosis, although the difference was not significant.

DISCUSSION

Lesions of GME are usually distributed widely throughout the CNS, especially in the white matter of the cerebrum, brainstem, cerebellum and spinal cord and are composed of granulomatous inflammatory cell accumulation and/or proliferation around vessels [2, 3, 7, 21]. The lesions of GME in this study had the same features. Kipar *et al.* [11] discussed that GME might be T cell-mediated delayed-type hypersensitivity of an organ-specific autoimmune disease from the immunomorphorogic features of this disease. The distribution pattern suggests that the antigen would associate with the central white matters and the lesion of optic nerve would also support this hypothesis. The distribution of malignant histiocytosis was pretty different from that of GME. Diffuse proliferation of neoplastic cells through the meninges and neoplastic nodule formation in the parenchyma are the characteristic patterns in malignant histiocytosis. Although some pathologists say that malignant histiocytosis is a primary multicentric malignancy [1], we adopted the histopathological classification of WHO, in which the diffuse and nodular distribution patterns are described [12]. Infiltrative histiocytes observed in both disorder possessed morphologic features of histiocyte but most neoplastic cells in malignant histiocytosis exhibited cellular atypia and/or highly infiltrative nature compared with inflammatory histiocytes in GME.

Although neoplastic reticulosis in canine CNS was previously diagnosed according to morphologic features of histiocytic tumor cells, the criterion is applicable also to lymphoma and the focal form of GME at present [2, 22]. Moreover, the former classification of malignant histiocytosis in human had also been reclassified into "true malignant histiocytosis", lymphoma and carcinoma [16]. This suggests that specific cell markers for histiocyte are indispensable for employ for the diagnosis of histiocytic tumors. In the present study, several commercially available histiocytic markers were applied to all cases. Lysozyme and α1-antitrypsin are classical histiocytic markers [17, 19]. Lysozyme is reported as a useful marker to identify macrophages, however, while the absence of lysozyme antigen does not necessarily preclude a histiocytic origin [17]. Moreover, the neoplastic cells of primary malignant histiocytosis of the canine brain reported by Chandra *et al.* [4] did not react for α1-antitrypsin. On the contrary, lectin RCA-1 was the most sensitive to inflammatory and neoplastic histiocytes of all histiocytic markers in the present study. Although there have been few reports to employ for canine tissue, it is well known that RCA-1 is a good marker for detecting microglia and macrophage in human brain [8, 14]. Antibodies to MAC387 and CD68, human histiocytic markers [5, 6, 15, 18, 23], labeled very limited number of canine histiocytes. All these histiocytic markers used in this study could not clarify any differences between the two disorders. If leukocyte-specific antibodies including CD1, CD11, MHC class II and ICAM-1 could be applied, immunoreactive profiles of histiocytes between GME and malignant histiocytosis would be confirmed [1].

The specificity of CD3 antigen for the T cell lineage is established in canine tissues [10]. In the lesions of GME,

lymphocytes are one of the dominant cell population and the majority of lymphocytes expressed CD3 antigen [11]. Most lymphocytes in present GME cases were also positive for CD3. The number of CD3-positive lymphocytes in GME was greater than that in malignant histiocytosis, while the difference was not significant. This tendency will not be definitive for diagnosing the actual cases because many inflammatory cells were observed in some malignant histiocytosis. On the other hand, antibody to CD79a (clone HM57 and JBC 117) is a specific B-lymphocyte marker, which can be applied to formalin-fixed and paraffin embedded canine tissues [15]. However, B-lymphocytes did not react to these markers in almost canine sections in this study.

PCNA is synthesized in early G1 and S phase of the cell cycle and serve as a good marker for proliferating cells. It has been well known that various types of neoplastic cells exhibit positive reactivity to PCNA [13]. As well as neoplastic cells, a number of inflammatory histiocytes and lymphocytes in GME were positive for PCNA in the present study, while there was no significant differences of PCNA-positive cell number between GME and malignant histiocytosis. It could be also concluded that PCNA is not a useful marker to distinguish between the two disorders.

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