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NOTE

Pathology

Disseminated histiocytic sarcoma with hemophagocytosis in a rabbit

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ABSTRACT. A 7-year-old female domestic rabbit suffered from labored respiration, poor appetite, mild anemia and thrombocytopenia. Radioscopic examination revealed masses in multiple locations including the intrapleural cavity and spleen. Forty-three days after the first visit to a private veterinary clinic, the rabbit died of severe respiratory distress. Microscopically, all of the masses were composed of round to polygonal neoplastic cells with distinct cell borders that were arranged in a sheet pattern. Multinucleated giant neoplastic cells were often observed. Some neoplastic cells had phagocytozed one or more erythrocytes. Immunohistochemical staining revealed that the neoplastic cells expressed vimentin, CD204, Iba-1 and lysozyme, but not CD163. Based on the morphological and immunohistochemical findings, this case was diagnosed as disseminated histiocytic sarcoma with hemophagocytosis.

KEY WORDS: CD204, hemophagocytosis, histiocytic sarcoma, rabbit

Histiocytic proliferative disorders in dogs have been well characterized. These disorders are classified as reactive lesions (i.e., cutaneous reactive histiocytosis and systemic reactive histiocytosis) or neoplasms (i.e., cutaneous histiocytosis and histiocytic sarcoma [HS]) [10, 15]. Canine HS is a rare malignant hematopoietic neoplasm characterized by morphological and immunohistochemical features similar to those of human HS [2, 15]. Canine HS occurs in various tissues, including the spleen, lung, lymph nodes, bone marrow, skin, brain and appendicular joints, and it originates from histiocytes, which are further divided into interstitial dendritic cells and macrophages [10]. Hemophagocytosis is often observed in HS of a macrophage origin. Moreover, two cases of HS in the lung and skin arising from dendritic cells have been reported in rabbits [6, 8]. To our knowledge, there have been no reports of HS with hemophagocytosis in rabbits. Herein, we describe the histological and immunohistological features of disseminated HS with hemophagocytosis in a domestic rabbit.

A 7-year-old female domestic rabbit suffering from labored respiration and poor appetite for 5 days was presented to a private veterinary clinic for evaluation. A physical examination, complete blood count analysis and routine serum biochemical profiling revealed the presence of mild anemia, thrombocytopenia and elevated alkaline phosphatase levels. Radioscopic examination revealed masses in multiple locations, including the intrapleural cavity, spleen, liver and kidney, and increased opacity throughout the lung. The rabbit was treated with a diuretic and tracheal relaxant, but the symptoms did not recover. Forty-three days after the first visit, the rabbit died due to severe respiratory distress and was subsequently subjected to necropsy by clinical veterinarians. Tissue samples were submitted to the Department of Veterinary Pathology, Nippon Veterinary and Life Science University for diagnostic examination.

Several tissue samples from the intrapleural mass, lung, liver, kidney, spleen, heart, trachea and esophagus were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin and eosin (HE) and Prussian blue. Immunohistochemistry was performed on serial sections using the labeled streptavidin-biotin method with

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Table 1. Primary antibodies used in the current case

Antibody	Clone	Dilution	Source	Antigen retrieval	Positive control rabbit tissue
Cytokeratin	AE1/AE3	1:200	Dako, Glostrup, Denmark	121°C for 20 min in citrate buffer, pH 6.0	Skin
Vimentin	V9	1:100	Dako, Glostrup, Denmark	121°C for 20 min in citrate buffer, pH 6.0	Spleen
CD163	AM-3K	1:100	TransGenic, Kumamoto, Japan	Microwave for 10 min in citrate buffer, pH 2.0	Spleen
CD204	SRA-E5	1:100	TransGenic, Kumamoto, Japan	Microwave for 10 min in citrate buffer, pH 2.0	Spleen
E-cadherin	36/E-cadherin	1:150	BD Biosciences, Franklin Lakes, NJ, U.S.A.	121°C for 20 min in citrate buffer, pH 6.0	Skin
Ki-67	MIB-1	1:100	Dako, Glostrup, Denmark	121°C for 20 min in citrate buffer, pH 6.0	Trichoblastoma
CD3	F7.2.38	1:300	Dako, Glostrup, Denmark	121°C for 20 min in citrate buffer, pH 6.0	Lymph node
BLA36	A27-42	1:300	BioGenex, San Ramon, CA, U.S.A.	121°C for 20 min in citrate buffer, pH 6.0	Lymph node
Iba-1	polyclonal	1:1,000	Wako, Osaka, Japan	Microwave for 20 min in citrate buffer, pH 6.0	Spleen
Lysozyme	polyclonal	1:300	Dako, Glostrup, Denmark	Microwave for 20 min in citrate buffer, pH 6.0	Spleen

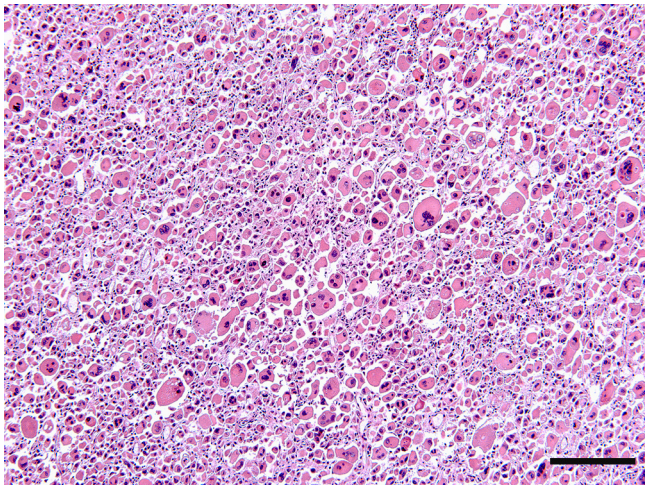

Fig. 1. Histiocytic sarcoma in a rabbit. The masses composed of round to polygonal neoplastic cells that arranged in a sheet pattern. HE. Bar=200 μm.

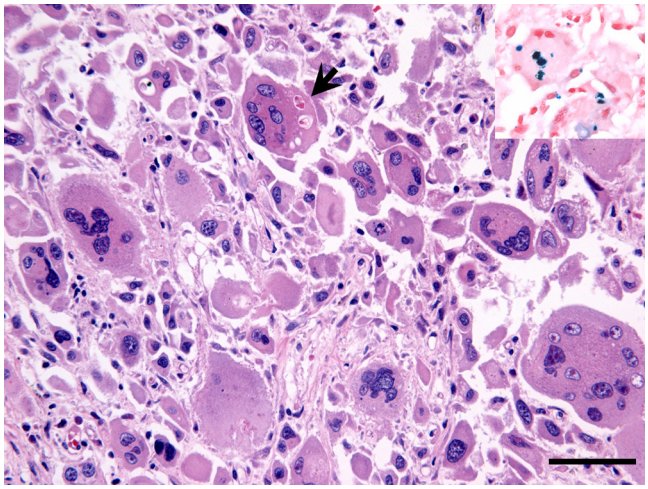

Fig. 2. Histiocytic sarcoma in a rabbit. The neoplastic cells had large, round or bizarre nuclei and abundant cytoplasm, showing hemophagocytosis (arrow). Multinucleated giant cells were also observed. HE. Inset: Prussian blue stain. Bar=50 μm.

the primary antibodies listed in Table 1. The sections were treated with 0.03% H_2O_2 in 33% methanol at room temperature for 30 min to block endogenous peroxidase, and then underwent antigen retrieval treatment (Table 1), followed by an incubation in a 4% milk solution at room temperature for 30 min. Finally, the reaction to each antigen was visualized by the addition of 3,3′-diaminobenzidine tetrahydrochloride chromogen and counterstaining with hematoxylin. The antibodies used were validated by the positive normal or neoplastic tissues listed in Table 1, and normal mouse or rabbit immunoglobulins (Dako) were used as a negative control.

Grossly, a large mass measuring 7.5 × 5.5 × 4.0 cm was located in the intrapleural cavity stuck to the lung. Four masses measuring 0.5–3.5 cm in diameter were randomly distributed throughout the liver. A mass with the size of a rice grain was observed in both the kidney and spleen. The cut surfaces of the masses were homogeneously gray-white in color.

Microscopically, all of the masses were composed of round to polygonal neoplastic cells with distinct cell borders arranged in a sheet pattern (Fig. 1). The neoplastic cells had large, round or bizarre hyperchromatic nuclei containing large prominent nucleoli and an abundant eosinophilic cytoplasm (Fig. 2). Multinucleated giant neoplastic cells were frequently observed. Approximately 0–3 mitotic figures were observed in each high power magnification (400×) field. Some neoplastic cells had phagocytized one or more erythrocytes, cellular debris or hemosiderin showed blue by Prussian blue stain (Fig. 2). Diffuse infiltration of lymphocytes and neutrophils was observed in the masses. Neoplastic cells were observed in the intrapleural mass, liver, spleen, kidney and lung.

Immunohistochemically, the neoplastic cells were positive for vimentin (Fig. 3), CD204 (Fig. 4), ionized calcium binding adaptor molecule-1 (Iba-1) (Fig. 5) and lysozyme, but not for cytokeratin, CD163, E-cadherin, CD3 and BLA36. The Ki-67 index of the neoplastic cells was 11.7%. The immunohistochemical features of all of the neoplastic masses were similar. On the basis of the morphological and immunohistochemical findings, this case was diagnosed as disseminated HS with hemophagocytosis. In addition to the neoplasms, the rabbit presented with chronic pulmonary congestion, splenic extramedullary hematopoiesis, hemosiderosis in the lung, kidney and spleen, and calcification in the lung, liver and kidney.

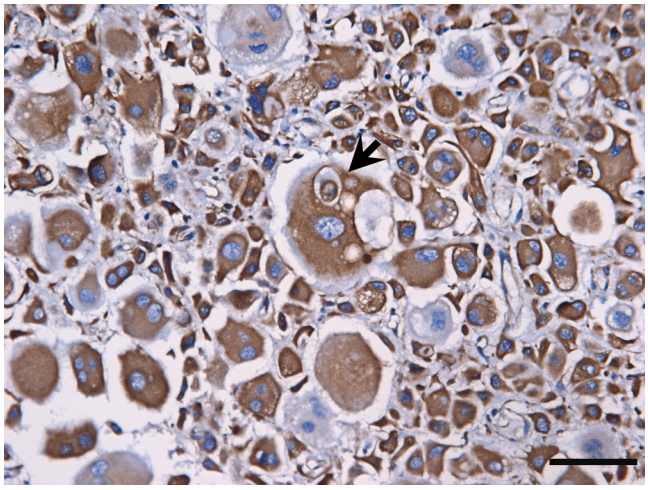

Fig. 3. Histiocytic sarcoma in a rabbit. Neoplastic cells express vimentin showing intense cytoplasmic immunoreactivity. The giant neoplastic cell shows phagocytosis (arrow). IHC. Bar=50 μm.

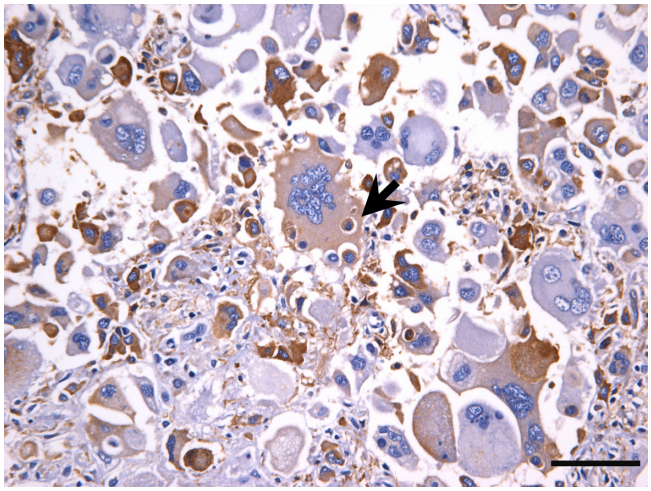

Fig. 4. Histiocytic sarcoma in a rabbit. Neoplastic cells express CD204 showing intense membranous and weak cytoplasmic immunoreactivity. The giant neoplastic cell shows phagocytosis (arrow). IHC. Bar=50 μm.

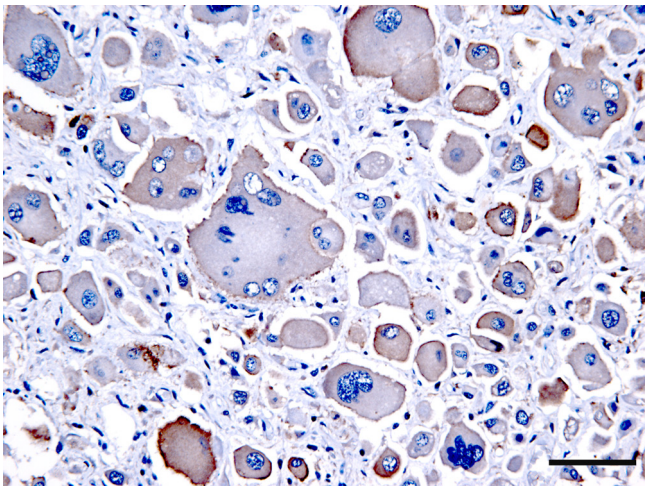

Fig. 5. Histiocytic sarcoma in a rabbit. Neoplastic cells express Iba-1 showing intense membranous immunoreactivity. IHC. Bar=50 μm.

HS tissues comprise sheets of large, pleomorphic neoplastic cells with marked cytological atypia characterized by multinucleated cells and high mitotic index [1, 10, 15]. A definitive diagnosis of HS requires the combination of immunohistochemical and morphological characterization. Histiocytic/monocytic markers in humans, dogs, ferrets and cats include CD204, CD163, human leukocyte antigen (HLA)-DR, myeloid/histiocyte antigen (Mac) 387, Iba-1 and CD68 [4, 7, 9, 13, 14]. Cells of an interstitial dendritic cell origin are CD1a+, CD4+, CD18+, CD90+, CD204−, Iba-1+ and E-cadherin+, whereas cells of a macrophage origin are CD1a+/−, CD18+, CD90+, CD204+, Iba-1+ and E-cadherin− [10, 15]. Neoplastic cells express CD204 in canine HS arising from various tissues including the spleen, lung, joint and brain [7, 13]. In canine HS with hemophagocytosis, CD204 and CD163 are expressed in neoplastic histiocytic cells [7]. On the other hand, neoplastic cells with phagocytosis exhibited immunoreactivity to CD204, Iba-1 or lysozyme in ferrets and cats [5, 14]. In the present case, neoplastic cells expressed vimentin, CD204, Iba-1 and lysozyme, but not CD163 and E-cadherin, suggesting that they were of macrophage origin. Therefore, CD204, Iba-1 and lysozyme may be useful markers to diagnose HS with hemophagocytosis in rabbits.

HS in rabbits is extremely rare, and only two cases, in which the primary masses occurred in the lung or skin with lymph node metastasis, have been reported [6, 8]. Although both neoplasms consisted of polygonal neoplastic cells and multinucleated neoplastic cells similar to the present case, hemophagocytosis and hemosiderin were not observed. In the present case, it is difficult to identify the primary location, because disseminated neoplastic masses were observed in various organs at the time of first visit.

In both dogs and cats, hemophagocytic HS frequently presents in the splenic red pulp and bone marrow, and it is an aggressive disease associated with a poor prognosis [3, 5, 10–12]. Canine hemophagocytic HS presents with distinct clinicopathological symptoms, including regenerative hemolytic anemia, thrombocytopenia, hypoalbuminemia and hypocholesterolemia [11, 12, 15]. However, HS with hemophagocytosis has not been previously reported in rabbits. In the present case, hemophagocytosis was often observed in all neoplastic tissues examined including the intrapleural cavity, spleen, liver, lung and kidney, suggesting that the anemia was due to hemophagocytosis of the neoplastic cells. However, we could not examine other hematopoietic organs, such as the bone marrow and lymph nodes.

In conclusion, to our knowledge, this is the first report of disseminated HS with hemophagocytosis in a domestic rabbit. The present case study provides insight into the diagnosis of rabbit HS and the pathological features of histiocytic neoplasms.

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