Case Report

Pulmonary Histiocytic Sarcoma in Two Aged Dogs of a Beagle Colony

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Abstract: Pulmonary masses were observed in two aged Beagles maintained for a long period without any treatment. In the histopathology in both cases, the poorly demarked masses were composed of neoplastic cells proliferating in a sheet-like fashion within the alveolar lumen. These cells showed moderate to marked pleomorphism and atypia and had abundant eosinophilic to foamy cytoplasm. Phagocytosis and multinucleated giant cells were occasionally observed. Similar lesions with pulmonary infiltrates were not noted in any of the observed tissues in both cases. In the immunohistochemistry, the neoplastic cells were positive for vimentin, lysozyme and alpha-1-antitrypsin, but not for cytokeratin or lymphocytic markers such as CD3 or CD79a. The tumor cells also showed lectin binding affinities such as concanavalin A and ricinus communis agglutinin-1. These findings indicated that these neoplastic cells were derived from a histiocytic lineage. Therefore, we diagnosed them as pulmonary histiocytic sarcomas. (*J Toxicol Pathol* 2006; **19**: 143–146)

Key words: histiocytic sarcoma, lung, dog

Canine malignant proliferative histiocytic disorders have been suggested to be derived from macrophages or dendritic cells and have two proliferative patterns, disseminated and localized, as they do in humans^{1,3}. Disseminated histiocytic sarcomas are comprised of multiple lesions and are defined as lesions equivalent to malignant histiocytosis when histiocytic proliferations arise as the primary multicentric malignancy^{1,10}. The primary sites for disseminated histiocytic sarcoma are the lungs, in addition to the spleen, liver, subcutis, bone marrow and lymph nodes¹. Most localized histiocytic sarcomas have been suggested to occur in cutaneous and subcutaneous sites, but are rarely observed in the lungs¹. Pulmonary histiocytic sarcomas are not readily apparent in routine microscopy and are difficult to distinguish from large cell carcinomas due to their morphological characteristics^{2,8}. Histiocytic sarcomas have been reported to generally affect aged dogs and, in particular, such specific breeds as Bernese Mountain Dogs, Rottweilers and retrievers, although some cases have been observed in other breeds^{1,7}. Here, we describe the morphological features of pulmonary histiocytic sarcomas in two aged Beagle dogs that were maintained for an extended

period, without undergoing any toxicity studies, in order to collect clinical and pathological background data in a Beagle colony.

A colony of Beagle dogs, including the cases in this report, was maintained for a long period under proper breeding conditions at our facility. The care and treatment guidelines were approved by the Ethics Review Committee for Animal Experimentation of Sankyo Co., Ltd. An 11-year-old male Beagle dog (Case 1) and a 15-year-old male Beagle dog (Case 2) underwent euthanasia after exhibiting anorexia and becoming inactive. In the hematology and blood chemistry while still living, Case 1 showed hypoglycemia. In Case 2, anemia and high liver enzyme activities such as serum aspartate aminotransferase and alanine aminotransferase were observed.

In the gross pathology, a distinct whitish nodule, approximately 10 mm in diameter, was observed in one lobe of the lung in Case 1, but there were no similar lesions in the other organs. The lungs, liver, brain, heart, kidney, thyroid, prostate and pancreas were collected. In Case 2, a distinct nodule, approximately 70 × 30 × 20 mm, was observed in the left caudal lobe of the lung, but there were no similar lesions in the other organs. The lungs, liver, kidney, prostate, urinary bladder and gallbladder were collected. All samples were fixed in neutral buffered 10% formalin solution and embedded in paraffin by routine procedures.

Each paraffin-embedded section of 2 or 3 μ m thickness was stained with hematoxylin-eosin (HE). Immunohistochemistry was performed using the Dako

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Envision system (Dako Japan, Kyoto, Japan). All antibodies were purchased from Dako Japan. Each lung section was incubated with rabbit antisera against lysozyme (prediluted), alpha-1-antitrypsin (prediluted) and with mouse monoclonal antibodies against vimentin (clone V9, prediluted), cytokeratin (clone MNF116, prediluted), CD3 (clone

F7.2.38, 1:25) and CD79a (clone HM57, 1:25). Lectin histochemistry was performed by the streptoavidin-biotin complex method (Dako Japan). Lung sections were incubated with biotinylated lectins, including concanavalin A (ConA, 1:400) and ricinus communis agglutinin (RCA-1, 1:400), which were provided with the lectin screening kit

(Vector Laboratories, Burlingame, CA). The reaction products were visualized with 3, 3'-diaminobenzidine tetrahydrochloride.

In the histopathological examination of Case 1, poorly demarcated neoplastic infiltrates composed of pleomorphic round or polygonal cells in a sheet-like fashion replaced the lung parenchyma (Fig. 1). Alveolar septa were well preserved within the cellular foci. The tumor cells had ample eosinophilic to foamy cytoplasm that often showed histiocyte-like structures such as autophagic vacuolation. These cells showed moderate anisokaryosis and were often formed of binucleated or multinucleated cells with occasional mitotic figures. The lesions contained small necrotic foci and a slight infiltration of leukocytes, mainly lymphocytes. There were no neoplastic lesions, except for an insulinoma in the pancreas, in any of the examined tissues.

In Case 2, an irregular and poorly demarcated tumor infiltrate accompanied diffuse necrotic foci. Tumor cells showed more atypical and pleomorphic structures than the neoplastic cells of Case 1, although they were fairly similar to those of Case 1. These cells showed marked anisokaryosis and nuclear atypia (Fig. 2). Phagocytosis and bizarre multinucleated giant cells were frequently observed in the tumor cells (Fig. 2). Mitotic figures were also observed more frequently than in Case 1. Peribronchial vascular invasion was occasionally noted. Reactive leukocytic cells, mainly lymphocytes, were scattered in the infiltrate. Similar lesions with pulmonary infiltrates were not noted in any of the observed tissues, although hepatocellular adenoma was observed in the liver.

In the immunohistochemical and lectin histochemical examinations, the tumor cells in both cases were negative for cytokeratin (Fig. 3), although the residual alveolar epitheliums were strongly positive. In contrast, intense reactivity for vimentin antibody was observed in tumor cells of both cases. Lysozyme was strongly positive in the small round neoplastic cells and neutrophils, and weak and patchy in the large or multinucleated neoplastic cells (Fig. 4). The infiltrates in both cases showed moderate and diffuse positive for alpha-1-antitrypsin (Fig. 5). Neither case

showed immunoreactivity for CD3 or CD79a. In both cases, lectin histochemistry for ConA demonstrated that a high affinity for binding lectin was observed in almost all of the infiltrates, as well as in the bronchial epitheliums. The tumor cells in both cases were also positive for RCA-1 (Fig. 6), although the affinity for binding lectin was observed to be weaker for RCA-1 than for ConA.

Based on the above-mentioned findings, the diagnosis for these cases was pulmonary histiocytic sarcoma. The proliferation patterns of the infiltrates in these cases were similar in many ways to the large cell carcinomas in canine lungs described in the *WHO International Histopathological Classification of Tumors in Domestic Animals*². Large cell carcinoma is characterized by the filling of alveolar spaces by large and round-to-polyhedral neoplastic cells with occasional foamy cytoplasm. In these cases, the infiltrates showed phagocytic structures, lectin binding affinities for ConA⁵ and RCA-1⁹, and immunoreactivity for lysozyme and alpha-1-antitrypsin, but not for cytokeratin. Moreover, these cells expressed CD18, which is suggested to be one of the most useful criteria for identifying a histiocytic origin (this data was kindly provided by Dr. P.F. Moore and V.K. Affolter)¹. These findings indicated that the tumors were derived from a histiocytic lineage.

Granulomatous inflammation and lymphomatoid granulomatosis must be considered as the differential diagnosis in these cases. In Case 2, the lesion accompanied inflammatory changes, which consisted mainly of the proliferation of histiocyte-like cells as well as diffuse necrotic foci with scattered leukocytes. The histiocyte-like cells showed not only a monotonous proliferative pattern but also marked cellular atypia and pleomorphism, moderate to high mitotic activities and vascular invasions. Thus, the proliferative cells in this case were considered as an element of the neoplasm rather than as a reactive macrophage. Lymphomatoid granulomatosis is composed of pleomorphic round cells with occasional multinucleated giant cells and shows angiocentric or peribronchiolar proliferations^{2,6}. The anaplastic cells in lymphomatoid granulomatosis are suggested to be of lymphoid origin and have no evidence of histiocytic lineage. However, no obvious localizations of the

Fig. 1. Case 1: The infiltrates are composed of pleomorphic histiocytic cells in a sheet-like fashion. Alveolar septa are well preserved. HE. Bar = 80 μ m.

Fig. 2. Case 2: Tumor cells show marked cellular pleomorphism and atypia. Phagocytosis and bizarre multinucleated giant cells with abundant eosinophilic cytoplasm are exhibited. HE. Bar = 40 μ m.

Fig. 3. Case 1: Cytokeratin (clone MNF116) is negative for neoplastic cells, although residual alveolar epitheliums are positive. Dako Envision Polymer method. Bar = 50 μ m.

Fig. 4. Case 2: Lysozyme immunoreactivity in pulmonary histiocytic sarcoma. Stain intensity varies in accordance with the neoplastic morphology. Dako Envision Polymer method. Bar = 50 μ m.

Fig. 5. Case 2: The infiltrates are uniformly positive for alpha-1-antitrypsin. Dako Envision Polymer method. Bar = 50 μ m.

Fig. 6. Case 1: Lectin ricinus communis agglutinin-1-positive neoplastic cells. Biotinylated lectin/streptavidin biotin complex method. Bar = 50 μ m.

infiltrates were observed in these cases. Moreover, the results of histiocytic markers such as lysozyme, alpha-1-antitrypsin and binding lectins were positive in the tumor cells of both cases, but lymphocytic markers such as CD3 or CD79a were not, which was useful for the exclusion of lymphomatoid granulomatosis from the differential diagnosis for these cases.

In a previous study concerning the incidence of primary spontaneous lung neoplasms in controlled Beagle colonies⁴, pulmonary histiocytic sarcomas were not observed in 40 neoplasms of 398 dogs. Since almost all of the malignant neoplasms in the previous report⁴ were pulmonary carcinomas, the evidence suggests that pulmonary histiocytic sarcomas are rare neoplasms in Beagles.

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