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Canine Peripheral Nerve Sheath Tumor with Eosinophilic Cytoplasmic Globules

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Abstract. A 13-year-old male Shetland Sheepdog had a subcutaneous tumor in the left brachium. The tumor was removed and recurred several times at 5, 13, 16, 22, and 31 months after the initial presentation. Histologically, the removed nodules from the fourth resection were composed of neoplastic proliferation of round to fusiform cells, which possessed eosinophilic globules in their cytoplasm. The globules were periodic acid–Schiff positive and diastase resistant. Positive reactions for acid phosphatase were observed in the cytoplasm of the tumor cells. Ultrastructurally, these globules consisted of membrane-bound, dense structures containing dense granules, lucent vacuoles, and homogeneous materials. The recurrent tumors removed at the fifth resection consisted of spindle cell proliferation arranged in interlacing fascicles with wavy nuclei and containing a small number of cells with cytoplasmic globules. The tumor cells were immunoreactive to vimentin, S-100 protein, myelin basic protein, and neuron-specific enolase. The tumor was diagnosed as a peripheral nerve sheath tumor with eosinophilic cytoplasmic globules. These findings are unique for the histogenesis of granular cell tumors.

Key words: Dogs; eosinophilic globules; granular cell tumor; peripheral nerve sheath tumors; schwannoma.

In domestic animals, the terms benign or malignant schwannoma have been used for tumors derived from the peripheral nerve sheath. The term malignant peripheral nerve sheath tumor currently is preferred for spindle-cell sarcomas arising from the nerve sheath in humans.[4,7] Schwannomas in veterinary medicine have been reported in several animals and commonly occurs in dogs and cattle.[1,6,14] Histologically, schwannomas are characterized by an arrangement of sweeping fascicles of spindle cells. Schwannomas exhibit the variable morphology referred to as Antoni A and Antoni B patterns.[4,14] Antoni A areas are composed of compact spindle cells arranging in interlacing fascicles. Antoni B areas are far less orderly and less cellular, and the spindled or oval cells are arranged loosely. Further, some human histologic variants such as schwannoma with rhabdomyoblastic differentiation,[18] pseudoglandular schwannoma,[17] and epithelioid schwannoma,[8] have been reported. Here, we describe a canine peripheral nerve sheath tumor consisting of neoplastic cells with eosinophilic globules in their cytoplasm, giving an appearance of granular cell tumor. To our knowledge, such a histologic variant has not been reported in the dog.

A 13-year-old male Shetland Sheepdog suffered from edematous swelling in the left brachium and was referred to a private veterinary hospital on 6 November 1990. Soft tissue neoplasm was suspected. The first surgical resection was conducted 5 months after the initial presentation. Afterwards, the neoplasm recurred and was surgically resected again 13, 16, 22, and 31 months after the initial presentation. Multiple spheroid nodules up to 10 cm in diameter developed at the edematous subcutis. Continuity between the tumor masses and the normal brachial nerve fibers was not observed. Hemorrhagic and necrotic foci were noted on the cut surface. Biopsy samples obtained at the fourth and fifth resections were presented for pathologic examinations. The X-ray examination at the fourth resection revealed an abnormal nodule in the posterior lobe of the lung. The dog died 40 months after the first presentation. Necropsy was declined by the owner.

The removed tumors were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin-embedded histologic sections were cut on the microtome at 4 μm and routinely prepared and stained with hematoxylin and eosin (HE), periodic acid–Schiff (PAS) with or without diastase digestion, phosphotungstic acid hematoxylin (PTAH), reticulin silver impregnation, Masson trichrome, and elastica von Gieson stains. Frozen sections were made from the formalin-fixed specimen and stained with Sudan black. Enzyme histochemistry for acid phosphatase (Barka-Anderson's method, pH 5.0) was applied to the frozen sections. Immunohistochemical evaluation was carried out using rabbit polyclonal antibodies for myoglobin (Seikagaku Corp., Japan), keratin (Seikagaku Corp.), glial fibrillary acidic protein (GFAP, Dako, Denmark), S-100 protein (Dako, Denmark), myelin basic protein (MBP, Dako, Denmark), and α-1-antichymotrypsin (Dako, Denmark) and mouse monoclonal antibodies for desmin (Dako, Denmark) and vimentin (Dako, Denmark) with the avidin–biotin peroxidase technique. For electron microscopy, formalin-fixed samples were cut into 1-mm cubes, postfixed in osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined in a transmission electron microscopy (Hitachi H-600, Japan).

Histologically, the nodules removed at the fourth resection consisted of round to fusiform cells arranged loosely in a sheet; these cells had round, hyperchromatic nuclei (Figs. 1, 2). The neoplasm did not show any particular arrangement, but pseudorosette formations consisting of neoplastic cells proliferating around the blood vessels were occasionally seen. Well-developed dense reticulin networks were present around the individual tumor cells (Fig. 3). There were moderate numbers of collagen fibers in tumor tissues. There were approximately two to four mitotic figures per high power field (40×).

Most tumor cells had spheroid eosinophilic globules of various sizes in the cytoplasm (Fig. 2). The globules were

PAS positive and diastase resistant and stained deeply blue with PTAH, lightly yellow with elastica von Gieson stain, and argyrophilic with silver impregnation. Positive reactions for acid phosphatase were observed in the cytoplasm of the tumor cells (Fig. 4). Ultrastructurally, these globules consisted of membrane-bound, dense bodies 3 μm in average diameter that contained dense granules, electron-lucent vacuoles, and homogeneous materials (Fig. 5).

The last surgery was performed 9 months after the fourth resection. Histologically, the obtained samples were composed mostly of fusiform cells arranged in a storiform pattern, and their nuclei had a wavy or buckled shape (Fig. 6). In some parts, round or short fusiform cells showed rosettelike alignment of nuclei. Approximately 5% of the tumor cells possessed cytoplasmic eosinophilic globules, compared with 60% of cells in the earlier biopsy; these cells were seen throughout tumor tissues.

Immunohistochemically, tumor cells reacted moderately to strongly for vimentin, S-100 protein (Fig. 7), NSE, and MBP (Fig. 8), but were negative for keratin, α-1-antichymotrypsin, GFAP, myoglobin, and desmin. Similar immunostaining patterns were seen in neoplastic cells with cytoplasmic eosinophilic globules.

Immunohistochemically, antibodies against S-100 protein, MBP, and NSE have been widely used to suggest a neural origin of cell types.[4] In the present case, tumor cells were reactive for S-100 protein, MBP, NSE, and vimentin, suggesting a neuroectodermal lineage. Positive reactions with antibodies for S-100 protein and MBP have been also reported in 50–90% and 40%, respectively, of human malignant peripheral nerve sheath tumors.[4] The negative reactions to keratin, desmin, and myoglobin suggested no close relationship to epithelial or muscle cells. The interlacing fascicle and pseudorosette arrangements, which were seen mainly in the fifth resection, have been described in Schwann-cell-derived tumors. Based on these histologic and immunohistochemical findings, the present tumor was considered to be originated from the peripheral nerve sheath.

Tumor cells with cytoplasmic eosinophilic globules appeared in the fourth resection samples and in the fifth resection samples, but in decreased number. The globule-possessing round cells showed immunoreactions similar to those of the spindle cells in fascicules, suggesting the morphologic similarity between round and spindle cell forms. Histological features of repeated recurrences or metastatic lesions often differ from those of primary tumors in human malignant fibrous histiocytoma[3] and liposarcomas,[13] and such variability also commonly occurs in recurrent tumors in veterinary medicine.

The globule-possessing round cells are different from infiltrating macrophages engaged in erythrophagocytosis. The round cells were negative with α-1-antichymotrypsin antibody, which has been used for detection of macrophages.[4] Further, ingested erythrocytes did not react to PAS, and no erythrocyte-like structures were detected by electron microscopy. Electron microscopic findings indicate lysosomal origin of the globules; apparently they are autophagosomal in nature. Acid phosphatase is a marker enzyme for lysosomes. Enhanced expression of acid phosphatase was found in the globule-possessing cells. These positive reactions further suggest an autophagosomal origin for the eosinophilic globules.

Presence of PAS-positive, diastase-resistant granules is characteristic of granular cell tumors.[4,5,9] These granules consisted of membrane-bound autophagic vacuoles containing cellular debris such as degraded mitochondria, myelin figures, and fragmented rough endoplasmic reticulum.[4] Granular cell tumors have been rarely reported in dogs,[1,2,10,12] horses,[1,11,15] and cats.[16] In dogs, the tongue is the most common site.[1] In horses, granular cell tumors have been reported mostly in the lung.[1] Rarely, they also occur in the brain,[2,10] heart,[12] and lymph nodes.[2] Myoblasts, Schwann cells, and undifferentiated mesenchymal cells have been proposed as the cells of origin for granular

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Fig. 1. Peripheral nerve sheath tumor; dog. Fourth resection sample. Note loosely packed round cells. HE. Bar = 50 μm.

Fig. 2. Peripheral nerve sheath tumor; dog. Note round cells with eosinophilic cytoplasmic globules (arrows) of various sizes. HE. Bar = 20 μm.

Fig. 3. Peripheral nerve sheath tumor; dog. Well-developed reticulin fibers are around the tumor cells. Reticulin silver impregnation. Bar = 40 μm.

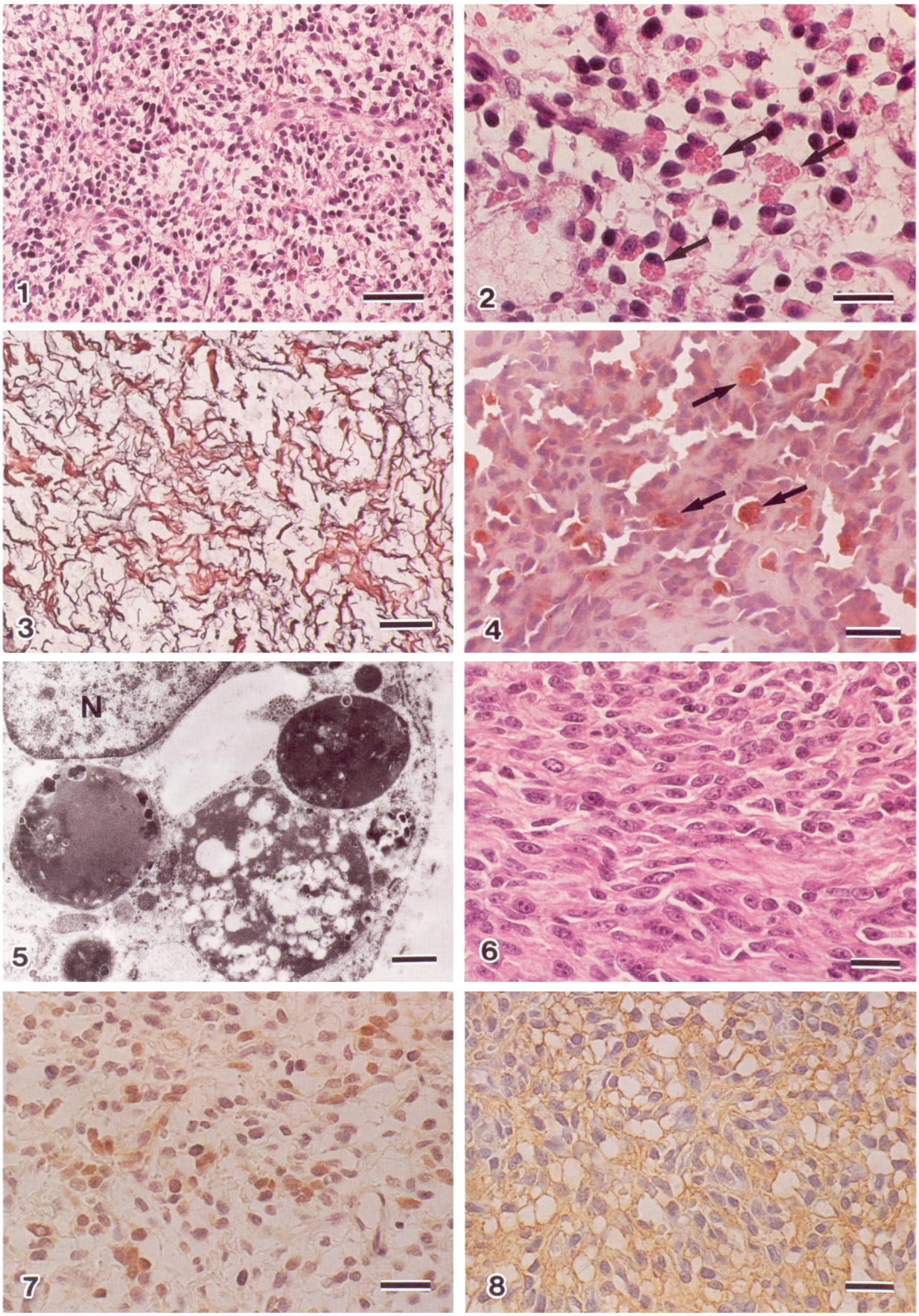
Fig. 4. Peripheral nerve sheath tumor; dog. Note acid phosphatase activity in the cytoplasm of the tumor cells (arrows). Barka-Anderson's method for acid phosphatase. Bar = 40 μm.

Fig. 5. Peripheral nerve sheath tumor; dog. Close examination of the fine structures of the eosinophlic globules reveals membrane-bound dense bodies containing the dense granules, lucent vacuoles, and homogeneous material. N-nucleus. Bar = 1 μm.

Fig. 6. Peripheral nerve sheath tumor; dog. Fifth resection sample. The tumor consists of spindle cell proliferation arranged in an interlacing fascicle with wavy nuclei. HE. Bar = 20 μm.

Fig. 7. Peripheral nerve sheath tumor; dog. The cytoplasm and nucleus of many tumor cells are positive for S-100 protein. Immunohistochemistry, hematoxylin counterstain. Bar = 20 μm.

Fig. 8. Peripheral nerve sheath tumor; dog. Immunohistochemistry. Positive reactions for myelin basic protein are observed in the cytoplasm of all neoplastic cells. Immunohistochemistry, hematoxylin counterstain. Bar = 20 μm.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cell tumors, but the precise histogenesis remains undetermined. However, recent immunohistochemical studies on human granular cell tumors appear to support the neural derivation.[4] The present canine peripheral nerve sheath tumor with cells having cytoplasmic eosinophilic globules might help shed light on the histogenesis of the granular cell tumor.

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