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Clinical course and pathologic study of retrobulbar histiocytic sarcoma in a central bearded dragon (*Pogona vitticeps*)

Mitsuhiro IKEDA[1)], Hirotaka KONDO[1)]*, Yuka EHARA[2)], Kazuo GOTO[2)], Hisashi SHIBUYA[1)]

[1)]Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Nihon University, Kanagawa, Japan
[2)]Bell Animal Hospital, Gifu, Japan

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ABSTRACT. A central bearded dragon (*Pogona vitticeps*) presented with periorbital swelling and exophthalmos. A retrobulbar mass was detected, and enucleation with the mass was performed. Histologically, the mass was composed of a dense sheet and interlacing bundles of round to polygonal to short spindle-shaped cells with occasional bizarre mononuclear and multinucleated giant cells. Immunohistochemically, the neoplastic cells had various degrees of membranous and/or cytoplasmic granular reactivity to anti-ionized calcium-binding adapter molecule 1 and anti-CD204 antibodies. Ultrastructurally, the neoplastic cells had irregular nuclei and abundant cytoplasm with membrane-bound electron-dense lysosomes and endoplasmic reticula. These findings were consistent with a histiocytic sarcoma. The present study provided a detailed description of retrobulbar histiocytic sarcoma for the first time in a central bearded dragon.

KEYWORDS: central bearded dragon, histiocytic sarcoma, immunohistochemistry, *Pogona vitticeps*, transmission electron microscopy

The central bearded dragon (*Pogona vitticeps*) is an omnivorous lizard belonging to the family Agamidae and one of the most popular reptilian pets [15]. According to one retrospective study, bearded dragons (*Pogona* spp.) have a statistically significant higher proportion of various tumors than other lizards, and squamous cell carcinoma predominates, followed by spindle cell sarcoma and cholangiocellular carcinoma [8]. Histiocytic sarcomas have been reported in a common garter snake, boa constrictor, green anole (*Anolis carolinensis*), and bull snake [9, 16]. In addition, eight snakes and one chelonian with histiocytic sarcomas have been listed in one review article [4]. However, no information about the clinical course and pathological findings of histiocytic sarcoma other than diagnosis has been documented in these reports. Herein, the clinical course and gross, histopathological, immunohistochemical, and ultrastructural features of histiocytic sarcoma in a central bearded dragon are described for the first time.

The animal was an estimated 1-year-and-8-month-old, male, central bearded dragon weighting 214 g. It was presented to an animal hospital with periorbital swelling and exophthalmos involving the right eye and was treated with antibiotics and anti-inflammatory drugs for one and a half months, but symptoms gradually worsened (Fig. 1A). Ultrasonography showed a retrobulbar mass occupying the right orbit with compression of the eye to the outside. Although the mass was removed surgically with enucleation, the soft tissue surrounding the right optic nerve remained due to severe hemorrhage during the surgery. Grossly, the retrobulbar mass measured 3 × 1.5 × 1 cm and was firm, white to tan, and tightly adhered to adjacent sclera and the optic nerve (Fig. 1B). The mass and eye were fixed in 10% neutral-buffered formalin and submitted to the Laboratory of Veterinary Pathology at Nihon University for histopathological examination. After trimming, the tissue sample was routinely processed, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin. Immunohistochemistry was performed on sections using routine methods. After deparaffinization, antigen retrieval was achieved by a high-pressure steam sterilizer at 121°C for 10 min using target retrieval solution (Dako North America, Inc., Carpinteria, CA, USA). The primary antibodies used were anti-ionized calcium-binding adapter molecule 1 rabbit polyclonal antibody (Iba-1, dilution 1:250; FUJIFILM Wako Pure Chemical Corp., Osaka, Japan), anti-CD204 antibody (dilution 1:50, TRANS GENIC Inc., Kobe, Japan) and anti-human leukocyte antigen-DR mouse monoclonal antibody (HLA-DR, dilution 1:50; Dako Denmark A/S, Glostrup, Denmark) with overnight incubation at 4°C. Subsequently, the sections were dropped into Histofine simple cysteine MAX-PO (MULT1) (Nichirei Biosciences Inc., Tokyo, Japan) at room temperature for 30 min. Labeling was visualized with 3,3′-diaminobenzidine (DAB Tablet; FUJIFILM Wako Pure Chemical Corp.), and sections were counterstained

*Correspondence to: Kondo H: kondo.hirotaka@nihon-u.ac.jp, Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan

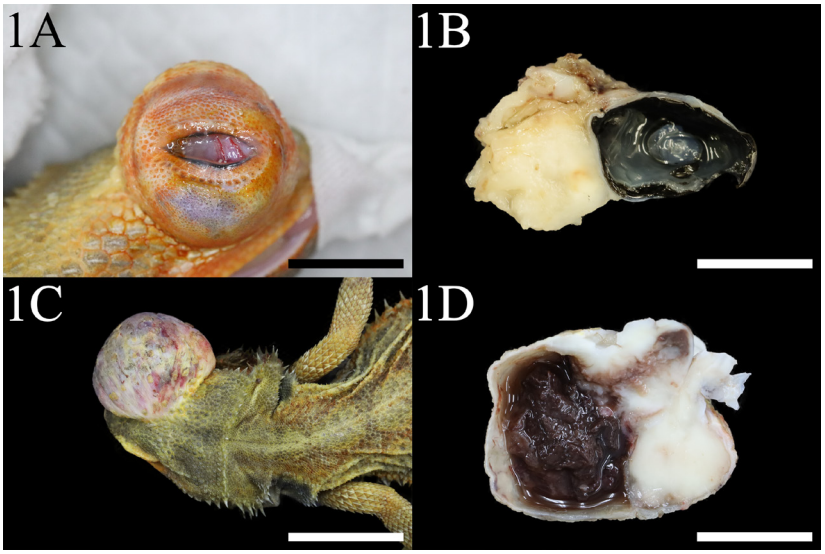

Fig. 1. Gross findings of the mass in a central bearded dragon (*Pogona vitticeps*). (**A**) Marked periorbital swelling and exophthalmos of the right eye. Bar=0.6 cm. (**B**) Cross-section of the retrobulbar mass and right eye. The mass is white to tan and firm. Bar=1.2 cm. (**C**) The recurrent mass protrudes from the right orbit. Bar=4.2 cm. (**D**) Cross-section of the recurrent mass is tan and solid with necrosis. Bar=1.8 cm.

with Mayer's hematoxylin. Positive immunohistochemical controls consisting of normal spleen from another central bearded dragon were included. Additionally, a formalin-fixed, tiny part of the mass was divided into 1-mm cubes, transferred to 4% glutaraldehyde, and examined by transmission electron microscopy.

Histologically, the retrobulbar mass was a non-encapsulated, poorly-demarcated, highly cellular neoplasm. The neoplasm comprised a dense sheet of round to polygonal cells and interlacing bundles of short spindle-shaped cells (Fig. 2A–B). The neoplastic cells had indistinct cell borders with moderate amounts of eosinophilic foamy cytoplasm. Nuclei were variably sized, round, with coarsely stippled chromatin and prominent nucleoli. Anisocytosis and anisokaryosis were remarkable, and a total of 15 mitoses were seen in 2.37 mm^2. Large, bizarre mononuclear cells and multinucleate giant cells were scattered throughout the mass, and rare phagocytosis of cellular debris or erythrocytes was present (Fig. 2A). Although the neoplastic cells showed mild invasion into adjacent sclera, there was no evidence of invasion of the neoplastic cells into the intraocular tissues and optic nerve. The neoplastic cells extended to the tissue margins on the sections examined.

Immunohistochemically, 80–90% of the neoplastic cells had moderate to intense, membranous and/or cytoplasmic granular reactivity to anti-Iba-1 antibody, and fewer cells were weakly positive for anti-CD204 antibody (Fig. 2C–D). The neoplastic cells were negative for anti-HLA-DR antibody. Adequate positivity to anti-Iba-1, anti-CD204, and anti-HLA-DR antibodies was observed in control tissues.

Ultrastructurally, the neoplastic cells had large, irregular, oval-to-polygonal nuclei with abundant cytoplasm. In the cytoplasm, there were moderate numbers of organelles especially including electron-dense lysosomes surrounded by a limiting membrane, endoplasmic reticula, and mitochondria (Fig. 2E). The histopathological, immunohistochemical, and ultrastructural findings were consistent with histiocytic sarcoma.

Although the animal had remained in good condition after the surgery, the mass gradually recurred approximately one month after the surgery. Radiographs showed osteolysis of the adjacent skull and right nasal cavity. The owner elected euthanasia (approximately three months after the surgery) due to severe anorexia and emaciation and difficulty in management of the mass. A cosmetic necropsy was performed at Nihon University. Grossly, the soft, broad-based, raised mass protruded from the right orbit and measured 4.2 × 4.0 × 2.3 cm (Fig. 1C). The cross-section of the mass was tan and solid, with a cystic space containing dark brown necrotic tissue and cloudy fluid (Fig. 1D). The mass had similar histopathological and immunohistochemical features to the surgically removed mass. The only significant lesion observed by histopathology included hepatocellular vacuolar changes consistent with lipidosis. Grossly and histopathologically, there was no evidence of metastatic lesions of the neoplasm, leading to the final diagnosis of a localized histiocytic sarcoma.

Neoplasia is relatively common in reptiles, and the reported prevalence of neoplasms in agamids, to which the central bearded dragon belongs, is 31.8% in retrospective articles [4, 8]. On the other hand, a previous retrospective study showed the prevalence of neoplasia in agamids during a 9-year period was 8.6%, and, therefore, the proportion of neoplasm is increasing [4, 8]. Although most reptile species examined are of uncertain age, 38 of 52 (73%) lizards for which an age was provided were under the age of 10 years [5]. Of tumors, soft tissue sarcoma and lymphoma are the most common neoplasms in lizards [4]. Lymphoma is the most commonly reported hematopoietic tumor, but other hematopoietic neoplasms such as histiocytic disorders are less common than lymphoma [1, 4, 9]. Although histiocytic sarcoma is sometimes diagnosed in small mammals such as dogs and four-toed hedgehogs, it appears that it is very rare in reptiles [9, 11]. Histiocytic sarcoma is a malignant neoplasm originating from histiocytes, which

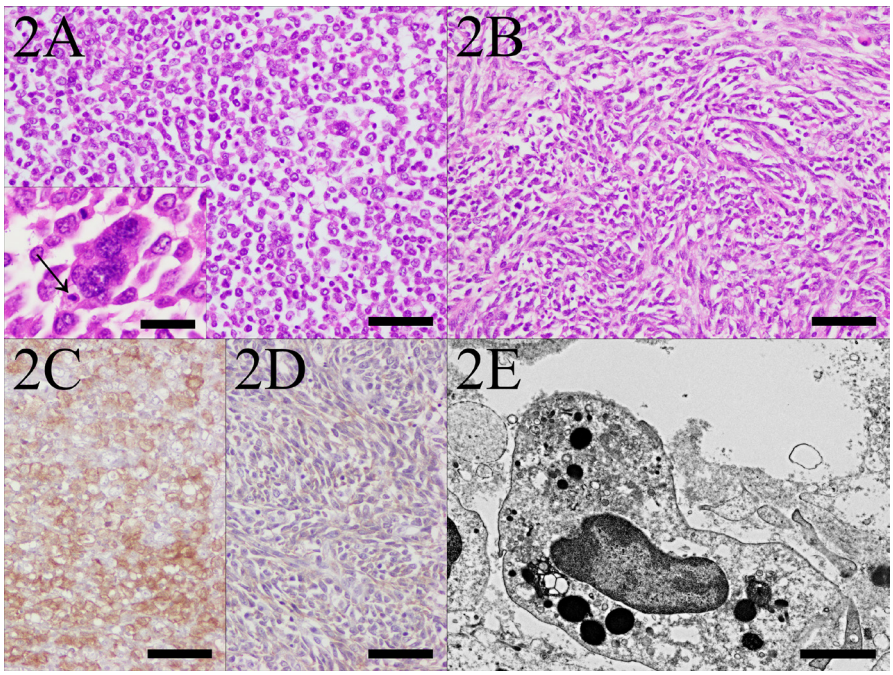

Fig. 2. Histological, immunohistochemical, and ultrastructural findings of the retrobulbar, histiocytic sarcoma in a central bearded dragon (*Pogona vitticeps*). (**A**) The mass is composed of a dense sheet of round to polygonal cells. Hematoxylin and eosin stain. Bar=50 µm. Inset: Phagocytosis of cellular debris by a bizarre multinucleated giant cell (arrow). Hematoxylin and eosin stain. Bar=20 µm. (**B**) Some areas of the mass consists of short spindle-shaped cells arranged in interlacing bundles. Hematoxylin and eosin stain. Bar=50 µm. (**C**) More than 80% of neoplastic cells are positive for anti-Iba-1 antibody. Immunohistochemical stain. Counterstained with Mayer's hematoxylin. Bar=50 µm. (**D**) The neoplastic cells are positive for anti-CD204 antibody. Immunohistochemical stain. Counterstained with Mayer's hematoxylin. Bar=50 µm. (**E**) Ultrastructurally, the neoplastic cells have irregular nuclei, with moderate numbers of lysosomes, endoplasmic reticula, and mitochondria in the cytoplasm. Transmission electron microscopy. Bar=2 µm.

are interstitial dendritic cells or bone marrow macrophages in a broad sense [12]. Spindle cell sarcomas such as leiomyosarcoma, rhabdomyosarcoma and undifferentiated pleomorphic sarcoma (formerly malignant fibrous histiocytoma or anaplastic sarcoma with giant cells), and lymphoid neoplasm are the primary differential diagnoses of histiocytic sarcoma. Previously, a high-grade anaplastic sarcoma that resembled a histiocytic sarcoma was reported in a 5-year-old, male central bearded dragon [17]. The authors showed that the anaplastic sarcoma was composed of sheets of spindloid to polygonal cells with occasional multinucleated giant cells [17]. When histiocytic sarcoma is presumed, immunohistochemical staining is helpful to differentiate histiocytic sarcomas from other lesions. Iba-1, HLA-DR, CD204, CD163, and CD18 are known examples for confirmation of histiocytic lineage by immunohistochemistry [12]. In the present case, expression of Iba-1 and CD204 was observed in the neoplastic cells; for this reason, other tumors were ruled out. In addition, ultrastructural findings were helpful to ruled out spindle cell sarcomas because dense bodies of leiomyosarcoma and striation of rhabdomyosarcoma were lacked. It has been shown previously that macrophages and microglial cells of reptilian cells were immunoreactive to Iba-1 in the veiled chameleon (*Chamaeleo calyptratus*), central bearded dragon, alligator (*Alligator mississippiensis*), panther chameleon (*Furcifer pardalis*), and the boa constrictor (*Boa constrictor constrictor*) [2, 3, 7]. The present study supports the usefulness of assessing Iba-1 and CD204 expression in reptile tissues.

Ultrastructurally, in histiocytic sarcomas, the neoplastic cells are non-cohesive and lack intercellular junctions [13]. Of the neoplastic cells, mononuclear histiocytes have irregular nuclei, lipid droplets, numerous lysosomes, and short profiles of rough endoplasmic reticula, and multinucleated histiocytes are similar to cytoplasmic features in addition to numerous secondary lysosomes [13]. Generally, Langerhans cells have Birbeck's granules in the cytoplasm, but they were absent in the present case. The ultrastructural features of reptilian neoplastic cells have only been reported in gastric neuroendocrine carcinoma, peripheral nerve sheath tumors, plasma cell tumors, mast cell tumors, liposarcoma, and odontogenic neoplasm [9]. Thus, the present study is an important addition to the morphology of neoplasia in reptile.

In the present case, the animal presented with periocular swelling and exophthalmos of the right eye. Periocular swelling is generally caused by trauma and/or infections secondary to exophthalmos. Bacterial infections of the eyes, eyelids, and adjacent tissues are common in certain lizards, and ocular swelling is observed in infections involving the retrobulbar space [14]. A non-neoplastic lesion has been reported as an orbital varix, probably caused by trauma, with one eye swelling [10]. Eyelids and periocular tissues are the sites of predilection for squamous cell carcinoma in central bearded dragons [9]. Of the special senses, periocular glands commonly give rise to neoplasms in reptiles, especially iguanas and chameleons [9]. In addition, swelling of the eyelids is observed in hypovitaminosis, with abundant keratin accumulated in the conjunctival sac [6]. When non-specific ocular lesions are noticed, both

neoplastic and non-neoplastic lesions should be considered.

In conclusion, the present study showed for the first time in a central bearded dragon: 1) the clinical course of a localized form of histiocytic sarcoma; 2) the histopathological and ultrastructural characteristics; and 3) the usefulness of assessing Iba-1 and CD204 expression in reptile tissues. Although histiocytic sarcomas are rare in reptiles, they should be included in the differential diagnosis when the neoplastic cells are pleomorphic.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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