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Spontaneous extracutaneous systemic mastocytosis in a cynomolgus macaque (*Macaca fascicularis*)

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Abstract

A uniform cell population of proliferating mast cells with poor cytoplasmic granularity and few eosinophilic infiltrates was observed in hepatic portal tracts and the cecal submucosa of an adult male cynomolgus macaque (*Macaca fascicularis*) that was part of a drug safety assessment toxicity study. The proliferating mast cells were positive for Giemsa and toluidine blue staining and had strong immunoreactivity for mast cell tryptase and CD68. Considering size, morphology, immunoreactivity and distribution of the lesions a benign proliferative disorder of connective tissue mast cells was diagnosed comparable to that seen in indolent systemic mastocytosis in humans, but lacking associated skin lesions. The finding of visceral mast cell proliferation has to be included in the spontaneous background pathology spectrum of cynomolgus macaques used in toxicological studies.

Key words: cecum, cynomolgus macaque, liver, mast cell proliferation, mast cell specific tryptase, visceral mastocytosis

Introduction

Mast cell disease, or mastocytosis, is defined as an increase in tissue mast cells that may involve several organ systems with manifestations ranging from isolated, benign proliferations to systemic, aggressive disorders (Horny et al., 2007). Proliferative mast cell disorders are exceedingly rare in nonhuman primates. Only three cases with a cutaneous manifestation have been described including the neck (baboon), the mid-ventral thorax and the thigh (rhesus macaque) (Colgin and Moeller, 1996; Jones et al., 1974; Seibold and Wolf, 1973). Visceral mast cell neoplasia has not been reported in nonhuman primates to date. This report describes the occurrence of extracutaneous mastocytosis in the liver and cecum of a 38-months-old male cynomolgus macaque (*Macaca fascicularis*) resembling indolent systemic mastocytosis in humans, but lacking the common involvement of the skin.

Materials and methods

The 38-months-old male cynomolgus macaque involved in a drug safety assessment toxicity study and was humanely euthanized (overdose of sodium pentobarbital) at the end of the study without showing any remarkable clinical signs. The animal was group-housed in a climate-controlled room with a minimum of 10 air changes/hour. The temperature and relative humidity ranged between 19 to 25 °C and 40 to 70%, respectively. Artificial lighting was controlled automatically to give a cycle of 12 hours light and 12 hours dark. The cages

were provided with wooden chips and tools for environmental enrichment, such as movable stainless steel mirrors, colored plastic tools, and colored plastic balls. A commercial pellet diet for nonhuman primates was offered once daily (Ssniff P10, Ssniff Spezialdiäten GmbH, Soest, Germany), and the animal received fresh fruits and bread twice weekly. Tap water was provided *ad libitum* via an automatic watering system.

A complete necropsy of the animal was performed at scheduled terminal kill. A wide spectrum of organs was fixed in 10 % neutral buffered formalin for at least 24 hours. Tissue samples were automatically paraffin-embedded, sectioned at 5 µm and stained with haematoxylin and eosin (H&E). Liver and cecum samples were also stained with PAS (periodic acid Schiff) reaction, toluidine blue and Giemsa stain.

Immunohistochemical investigations were carried out on paraffin-embedded sections of the liver using the PAP (peroxidase-anti-peroxidase) method and the chromogen DAB (diaminobenzidine). The primary antibodies used for immunohistochemistry included anti-mast cell specific tryptase-antibody (monoclonal mouse anti-human mast cell specific tryptase, clone AA1, DakoCytomation GmbH, Hamburg, Germany) at a dilution of 1:200, anti-chromogranin A-antibody (monoclonal mouse anti-human chromogranin A, clone DAK-A3, DakoCytomation GmbH, Hamburg, Germany) at a dilution of 1:200, anti-synaptophysin-antibody (polyclonal rabbit anti-human synaptophysin, DakoCytomation GmbH, Hamburg, Germany) at a dilution of 1:100, and anti-neuron specific enolase (NSE)-antibody (monoclonal mouse anti-human neuron specific enolase, clone BBS/NC/VI-H14, DakoCytomation GmbH, Hamburg, Germany) at a dilution of 1:200. Immunohistochemical staining with anti-CD68-antibody (monoclonal mouse anti-human CD68, clone KP1, DakoCytomation GmbH, Hamburg, Germany) was performed at a dilution of 1:50 using the APAAP (alkaline phosphatase-anti-alkaline phosphatase) method and the chromogen fuchsin. Antibody diluent with background reducing components (DakoCytomation GmbH, Hamburg, Germany) was used as a negative control for all primary antibodies. Spleen and lymph node of a cynomolgus macaque served as positive controls for the anti-CD2 and anti-CD68-antibodies. Cynomolgus adrenal gland was used as the positive control for the anti-chromogranin A, anti-synaptophysin, and anti-NSE-antibodies, and skin of a cynomolgus macaque was included as the positive control for the anti-mast cell specific tryptase-antibody staining. The slides were counterstained with Mayer's hemalaun (Merck, Darmstadt, Germany).

Results

Gross examination of the cynomolgus monkey did not yield any pathological findings. At histological examination, the liver and the cecum contained accumulations of uniform, densely packed, round to polygonal

cells (approximately 15-18 μm) with distinct cell borders, central round to ovoid, finely stippled nuclei and abundant faintly basophilic, partly finely granular cytoplasm. In the liver, these cell infiltrates were mainly located in the portal regions (Figure 1). A solitary island of similar cells was present in the submucosa of the cecum close to the ileocecal valve (Figure 2). The cell nests were well-demarcated, unencapsulated and contained a scarce fibrovascular stroma as well as few diffusely scattered mature eosinophils (Figure 3). Mitotic figures were not present in both locations. Histologically, the bone marrow was unremarkable. Special stains (toluidine blue and Giemsa stain) revealed the presence of blue to violet metachromatic granules within the cytoplasm of approximately 30 % of the round cells (Figure 4). Via immunohistochemistry, strong immunoreactivity for mast cell specific tryptase and CD68 was present in 90 respectively 100 % of the proliferating mast cells (Figures 5,6). Cells were negative for synaptophysin, chromogranin A and PAS reaction, whereas 80 % of the cells had weak immunoreactivity for neuron specific enolase. On the basis of the histological and immunohistochemical results, and with regard to the World Health Organization (WHO) classification of mastocytosis in humans, the diagnosis of an extracutaneous systemic mastocytosis was made.⁹

Discussion

In nonhuman primates, mast cell disease is a rare disease entity. So far, mast cell tumors have only been described as solitary masses in the skin of two rhesus macaques (*Macaca mulatta*) and a baboon (*Papio* sp.) showing a comparable histopathological pattern as well-differentiated canine cutaneous mast cell tumors.^{2,6,8} However, at least in one rhesus macaque, the tumor was regarded as benign on the basis of its well-differentiated appearance, the lack of mitoses and the absence of local recurrence or systemic disease.

In the present case of the cynomolgus macaque, the mast cells in the hepatic portal tracts and the cecal submucosa presented as a uniform cell population with poor cytoplasmic granularity and few eosinophilic infiltrates, which was positive for Giemsa and toluidine blue staining and showed strong immunoreactivity for mast cell tryptase and CD68 confirming the mast cell origin of the cells (Hauswirth et al., 2006; Horny et al., 2007; Horny and Valent, 2002). The weak immunoreactivity for NSE remains unclear, but as the cells were negative for the classical neuroendocrine cell marker chromogranin A, a neuroendocrine cell proliferation could be excluded. The adjacent hepatic/cecal tissue was unremarkable. Secondary changes due to mast cell degranulation such as gastroduodenal ulceration could not be observed. A leukaemic process could be excluded as histopathological examination of the bone marrow did not reveal increased mast cell numbers.

Considering size, morphology, immunoreactivity and distribution of the lesions a benign proliferative disorder of connective tissue mast cells is suspected comparable to that seen in indolent systemic mastocytosis in humans, but lacking associated skin lesions. Indolent systemic mastocytosis is a benign proliferative disorder and represents the most common form of systemic mast cell disease in humans. Infiltrates of well-differentiated mast cell are most often found in the spleen, the lymph nodes and the liver, and there usually is involvement of the skin (Valent et al., 2001). In mastocytosis with liver involvement, mast cells are predominantly aggregated in the portal tracts, but are also loosely scattered throughout the parenchyma and have to be distinguished from reactive mast cell hyperplasia (e.g. in liver fibrosis) (Benyon, 1999). In over 90 % of patients with systemic mastocytosis, the pathogenesis involves a clonal proliferation of the *c-KIT* protooncogene product, which is a transmembrane type-III tyrosine kinase receptor for stem cell factor (SCF), a cytokine involved in multiple signalling pathways including proliferation, migration, and differentiation of mast cells. Point mutations of *c-KIT* result in ligand-independent *KIT* activation and tumor development. However, *c-KIT* mutations are not specific for mastocytosis and are also reported in other neoplastic diseases (seminoma, myeloid leukaemia) (Horny et al., 2007; Pignon, 1993).

Because of the uniformity of the mast cells and the absence of mitotic figures and invasive growth, it is assumed that the mast cell proliferation in the cynomolgus macaque had a benign biological behaviour without impairment of clinical health as described for human indolent systemic mastocytosis. However, due to the poor granularity of the proliferating cells and the multifocal distribution, an early malignant metastasizing process with an unidentified primary tumor, maybe of mucosal mast cell origin, can not completely be excluded. Unfortunately, preparation of additional intestinal segments or tissue sections to evaluate the presence of a primary tumor, reveal other organ manifestations of mast cell accumulations, or determine pathogenetic pathways of the proliferative disease was not possible due to GLP (good laboratory practice) regulations imposed on this study.

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Legend of figures

Figure 1. Liver, cynomolgus macaque. Island of uniform, densely packed, round to polygonal cells with faintly basophilic, finely granular cytoplasm in a hepatic portal tract. Arrows indicate the presence of bile ducts. H&E, Scale bar = 100 μm .

Figure 2. Cecum, cynomolgus macaque. Focal accumulation of similar cells in the cecal submucosa in close association with parts of the gut-associated lymphoid tissue (GALT) of the ileocecal valve. H&E, Scale bar = 200 μm .

Figure 3. Liver, cynomolgus macaque. The uniform round to polygonal cells have distinct cell borders, a size of approximately 15-18 μm , central round to ovoid nuclei and abundant faintly basophilic, partly finely granular cytoplasm and contained few collagenous streamers as well as diffusely scattered mature eosinophils (arrows). H&E, Scale bar = 50 μm .

Figure 4. Liver, cynomolgus macaque. At least some of the cells revealed distinct metachromatic granules stain. Giemsa stain, Scale bar = 50 μm .

Figure 5. Liver, cynomolgus macaque. Almost all cells showed strong immunoreactivity for mast cell specific tryptase. Immunohistochemistry, PAP-DAB, Scale bar = 50 μm .

Figure 6. Liver, cynomolgus macaque. All mast cells showed strong immunoreactivity for CD68. Immunohistochemistry, APAAP-fuchsin, Scale bar = 100 μm .

