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C A S E   R E P O R T

Morphological characterisation of malignant histiocytosis in a cat

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Malignant histiocytosis (MH) is a progressive systemic neoplastic proliferation of morphologically atypical histiocytes, well characterised in humans and dogs but only recently identified in the cat. In all species, liver, lung, lymph nodes, spleen and bone marrow are infiltrated by atypical histiocytes, and the disease is rapidly fatal. The purpose of this study was to describe the clinical, histological, immunohistochemical and ultrastructural findings of MH in a cat, together with the diagnostic work-up and a list of differential diagnoses. Clinical evaluation included a complete blood-cell count, serum biochemistry, urinalysis, serology and ultrasound examination. The cat had clinical signs of depression, thinness, dehydration, pale mucous membranes and tachycardia. Abdominal ultrasonography revealed generalised splenomegaly and hepatomegaly. Necroscopy showed whitish nodules, randomly scattered throughout the parenchyma in the spleen and liver. The periportal lymph nodes were greatly enlarged and the cut surface was uniformly greyish-white and translucent. Histological examination revealed pleomorphic proliferation of large round tumour cells, with numerous phagocytic vacuoles containing erytrocytes, leukocytes and haemosiderin. By immunohistochemistry, positivity for lysozyme and α1-antitrypsin and a scattered positivity for Mac 387 were observed. Ultrastructural features of tumour cells included cytoplasmic lipid droplets, lysosomes and phagolysosomes. MH in the cat needs to be differentiated from diffuse granulomatous disease, non-Hodgkin's lymphoma and Hodgkin's-like disease. The morphological features of the tumour cells, combined with immunohistochemical and ultrastructural observation, are consistent with a diagnosis of MH in the cat. (Folia Morphol 2008; 67: 299–303)

Key words: malignant histiocytosis, cat, lysozyme, α1-antitrypsin, Mac 387, ultrastructure

INTRODUCTION

Malignant histiocytosis (MH) is a progressive systemic neoplastic proliferation of morphologically atypical histiocytes [15, 17]. The disease, originally described in man as histiocytic medullary reticulosis and later termed malignant histiocytosis, is clinically characterised by fever, malaise, weight loss, generalised lymphadenopathy and hepatosplenomegaly [21, 22]. Anaemia is commonly found, and it is sometimes associated with leukopenia and thrombocytopenia. Histologically, the neoplastic histiocytes are found as a diffuse, non-cohesive proliferation in the lymph nodal medullary zones, splenic red pulp and hepatic sinusoids, lung, and bone marrow [21, 22].

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Most cases of MH have been reported in man [21, 22] and in dogs, particularly in the Bernese Mountain breed [10, 13, 17]. MH is uncommon in cats, and its documentation is limited to a few case reports [2, 3, 5, 6, 8, 19, 20]. Here we describe the clinical, histopathological, immunohistochemical and ultrastructural features of malignant histiocytosis in a cat, together with the diagnostic work-up and a list of differential diagnoses.

CASE REPORT

A ten-year-old spayed female domestic shorthair cat weighing 4 kg was examined for an 8-month history of listlessness, inappetence, progressive weight loss, marked lethargy, anorexia and vomiting. Physical examination revealed depression, thinness, approximately 8% dehydration, pale mucous membranes and tachycardia (205 beats/min). The rectal temperature was 38.6°C. A normocytic hypochromic regenerative anaemia (RBC: 2.6×10^{12}/L, reference interval 5.5–8.5×10^{12}/L; haematocrit: 13%, reference interval 30–45%; MCHC: 30%, reference interval 31–35%; MCV: 49 μ^3, reference interval 40–55 μ^3; reticulocytes: 4%, reference interval 0.2–16%) was revealed by haematological examination. A leukogram and thrombogram were normal. Serum biochemical abnormalities included mild hyperglycaemia (164 mg/dL; reference interval 70––110 mg/dL) and an increase in activity of alanine aminotransferase (60 UI/L; normal value < 50 UI/L). Urinalysis was normal. Serum tests with an ELISA assay (SNAP combo plus, IDEXX, Laboratories, Italia) for feline immunodeficiency virus, feline infectious peritonitis virus antibodies and feline leukaemia virus antigen were negative. Direct Coomb's test was negative and numerous blood smears failed to reveal *Mycoplasma haemofelis,* even if the absence of these organisms on a blood smear does not exclude the possibility of infection. In fact, this method not only lacks sensitivity but is also not adequately specific because the morphology of *M. haemofelis* can easily be confused with other micro-organisms such as rickettsia. Abdominal ultrasonography revealed splenomegaly and hepatomegaly with hyperechogenicity and moderate hepatic venous congestion. Response to supportive treatment was poor and the cat died one week later.

At post-mortem examination all cavities contained a yellowish-green serosanguineous fluid. Significant gross lesions were evident in the spleen, which was enlarged, firm and contained multiple whitish nodules from 0.5 to 4.0 mm in diameter,
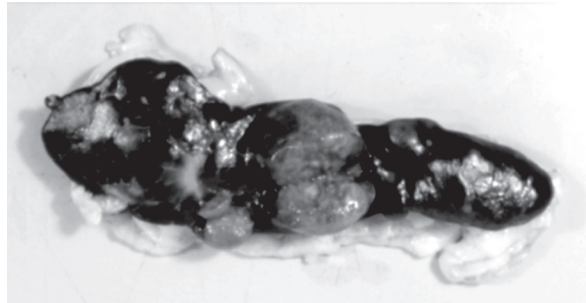

Figure 1. Spleen: multiple, whitish nodules, randomly scattered throughout the parenchyma.

randomly scattered throughout the parenchyma (Fig. 1). Similar nodules were observed in the liver. The periportal lymph nodes were greatly enlarged and the cut surface was uniformly greyish-white and translucent.

Tissue samples of all organs were fixed in 10% neutral buffered formalin, embedded in paraffin wax and stained with haematoxylin and eosin, Periodic Acid-Schiff (PAS), Gomori's methenamine silver and the Gram and Ziehl-Nielsen techniques to reveal the presence of pathogens.

Immunohistochemical staining was performed on sections from the collected samples using a commercial kit (Dako LSAB K680, Burlingame, CA, USA). The paraffin sections were dewaxed, rehydrated and blocked for endogenous peroxidase activity by treatment for 20 min with 0.3% H_2O_2 and in absolute methanol for 30 min. The sections were incubated overnight in a humidified chamber at 4°C with well-established monoclonal antibodies against Myeloid/ /Histiocyte antigen (clone Mac 387), CD 79 — B cell (clone HM 57), CD 3 — T cell, lysozyme (Muramidase), α1-antitrypsin and anti-vimentin (clone V9), obtained from DAKO (Burlingame, CA, USA). All the antibodies were well characterised and regularly used in the veterinary laboratories. Briefly, the primary antibodies were all diluted 1 in 300 and applied overnight at 4°C, and the reaction was developed with 3,3-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) and counterstained with haematoxylin. Controls for staining specificity were omission of the primary antibody, its replacement with non-immune sera or use of an irrelevant antibody. The intensity of labelling in each specimen was scored as: 0, absent; +, weak; ++, moderate; +++, strong.

For electron transmission microscopy (TEM) the samples were fixed in 2.5% glutaraldehyde, post-
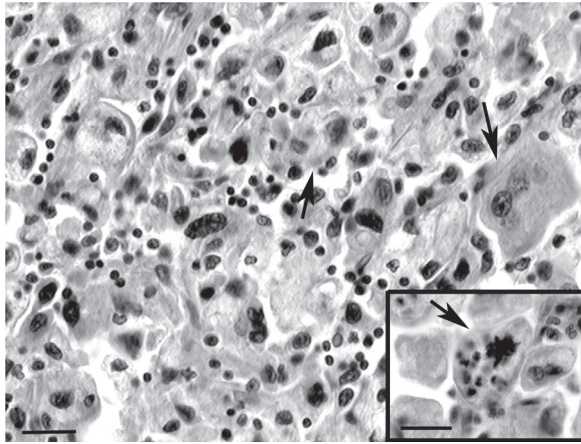
Figure 2. Spleen: pleomorphic malignant histiocytes mixed with some lymphocytes; HE ×250. Insert: atypical mitosis in neoplastic cells which contain phagocytised leucocytes. HE ×500.
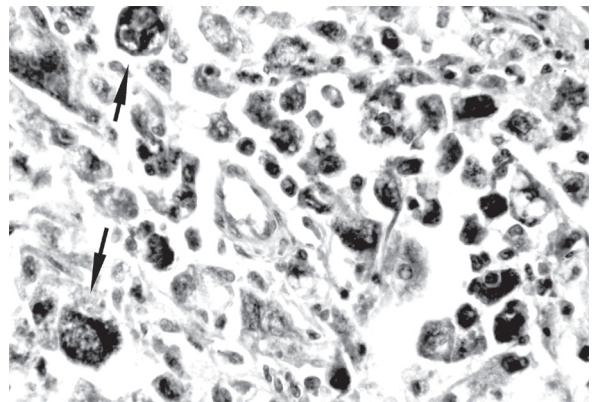
Figure 3. Lysozyme immunoreactivity in tumour cells (arrows). Streptavidin-Biotin-Method ×500.

fixed in osmium tetroxide and embedded in low-viscosity Spurr resin. Ultrathin sections were cut, counterstained with 0.5% uranyl acetate and lead citrate and examined with a Zeiss 902 electron transmission microscope.

Significant histological lesions were observed in sections of the spleen, liver, lungs, periportal and mediastinal lymph nodes. Histological examination of the spleen revealed foci of pleomorphic giant histiocyte-like cells that varied widely in size (from 20 to 35 μm) and had an abundant fine granular structure in a foamy cytoplasm and fairly well-delineated cell margins. The cells had centrally-placed round nuclei of variable size or eccentrically-placed indented or folded nuclei, with a regular chromatin pattern and one or two generally large indistinct nucleoli. Individual cells were atypical and showed anisocytosis and anisokaryosis. There were much mitosis, and bizarre mitotic figures were readily seen (Fig. 2). Among these cells multinucleated giant cells were frequently present. Both multinucleated and mononucleated tumour cells showed phagocytosis of the erythrocytes and leucocytes (Fig. 2).

Bone marrow was cytologically evaluated. The cellularity was in the range of normal to slightly increased, with hyperplasia of the erythroid population (myeloid/erythroid ratio = 0.36). Atypical histiocytic cells (50%) as seen in the splenic mass were present in the bone marrow, and these were characterised by large cytoplasmic basophilia and prominent and multiple nucleoli. Many histiocytic cells were phagocytising myeloid and erythroid cells and platelets. Rare multinucleate giant cells were present. Furthermore we observed immature mye-

loid cells (4%), mature myeloid cells (8%), immature erythroid cells (10%), mature erythroid cells (20%), lymphocytes (5%) and megakaryocytes (3%).

Neoplastic histiocytes, morphologically similar to those seen in the spleen, were also observed in the liver, in the lungs and in the periportal and mediastinal lymph nodes.

PAS, Gomori's methenamine silver and the Gram and Ziehl-Nielsen stains did not show the presence of bacteria or fungi in the cytoplasm of the atypical cells.

By immunohistochemistry most tumour cells were positive for lysozyme, although the intensity of staining was greatly variable (Fig. 3); α1-antitrypsin immunoreactivity was less frequent and less intensely stained compared to lysozyme stain, while Mac 387 immunoreactivity was variable and only a few cells were strongly positive. Only a few neoplastic cells were positive for vimentin. No neoplastic cells were positive for CD 3 and CD 79 (the immunohistochemistry results are summarised in Table 1).

Ultrastructurally, the neoplastic cells were non-cohesive and showed ovoid or pleomorphic multi-segmented nuclei with dispersed chromatin. Nucleoli were usually large and sometimes multiple. Lipid droplets and numerous primary and secondary lysosomes were seen in many cells (Fig. 4). The ultrastructural features of the tumour cells were consistent with those of histiocytic cells [4, 7].

DISCUSSION

A definitive diagnosis of MH has to be made histologically on the basis of the distribution of the neoplastic cells in the body and on the immuno-

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Table 1. The immunohistochemistry results

Antibodies	Percent age of positive neoplastic histiocytes (intensity of labelling)
Lysozyme (muramidase)	90% (+++)
α1-antitrypsin	50% (++)
Myeloid/histiocyte antigen (clone Mac 387)	40% (+)
Anti-vimentin (clone V9)	10% (+)
CD 3 — T cell	Negative (0)
CD 79 — B cell (clone HM 57)	Negative (0)
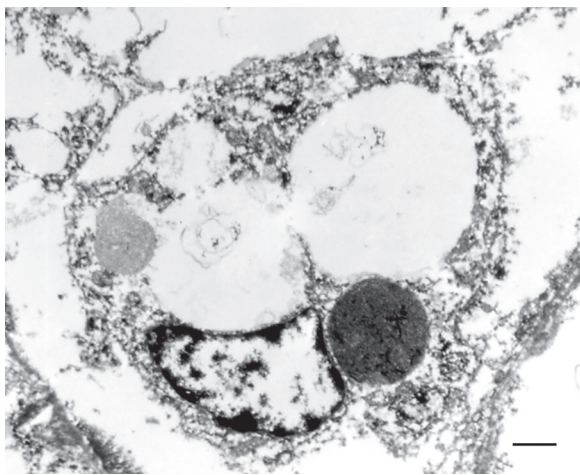
Figure 4. Neoplastic histiocyte with an expanse of cytoplasm, large lysosomes and an erythrophagosome. Electron microscopy (EM) ×14,500.

histochemical and ultrastructural findings. Malignant histiocytosis in the cat needs to be differentiated from diffuse granulomatous disease, non-Hodgkin's lymphoma and Hodgkin's-like disease.

The morphological appearance of the tumour cells observed in this study was consistent with a histiocytic lineage. The presence of large pleomorphic mononuclear cells and many multinucleated giant cells with abundant, often vacuolated, cytoplasm, as well as the observation that these cells also showed phagocytic activity, suggest a histiocytic lineage.

The histiocytic lineage of the tumour cells was inferred from the immunohistochemical demonstration of lysozyme and α1-antitrypsin and from the absence of immunoreactivity for CD 3 and CD 79, markers of T and B cells respectively. In humans and

dogs lysozyme and α1-antitrypsin are both well characterised immunohistochemical markers of mononuclear phagocyte differentiation [1, 11, 14], and lysozyme has been demonstrated in almost all reported cases of human, canine and feline MH [9, 12, 13, 16, 22, 23].

The lack of positivity for Mac 387 and for vimentin could be explained by a high degree of anaplasia achieved by neoplastic cells in the case described.

A further condition that should be considered in the list of differential diagnoses is diffuse granulomatous disease. This disorder is most often a consequence of viral and bacterial infections (including Mycobacterium tuberculosis and atypical mycobacteria) and is characterised by the presence of phagocytic histiocytes in the bone marrow, lymph nodes, liver and spleen. In MH the neoplastic histiocytic cells show marked atypia, whereas in the granulomatous disease the predominant cell type is a mature and actively phagocytic macrophage [1].

Non-Hodgkin's lymphoma, particularly of the T-lineage, may be misdiagnosed as MH when a histiocytic proliferation is associated with the condition, or if the neoplastic T cells exhibit haemophagocytosis [1]. Immunohistochemistry for CD 3 and Mac 387 is usually helpful in diagnosis of T-cell lymphoma. In the differential diagnosis we also considered Hodgkin's lymphoma (lymphocytes depletion subtype), because many Reed Sternberg-like large cells with the morphological hallmarks of polyploidy (double or multi-lobed nuclei) were observed. One case of Hodgkin's-like disease with the morphological hallmarks of Reed-Sternberg cells has been reported in cat [18], but unlike the Reed-Sternberg cells the neoplastic histiocytes in the present case did not show eosinophilic nucleoli and displayed a propensity for phagocytosis.

Furthermore, the electron microscopy findings confirmed the histiocytic lineage of the tumour cells. Cytoplasmic vacuoles, lipid droplets, and numerous lysosomes are well recognised features of the macrophages and have already been described by others in human MH [5, 16].

In conclusion, the morphological features of the tumour cells with the prevalence of cytologically bizarre, highly phagocytic, multinucleated giant cells, taken together with the immunohistochemical and ultrastructural results, represent significant features in enabling a diagnosis to be made of MH and in differentiating malignant histiocytosis from other conditions.

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