Mast Cell Sarcoma with Megakaryocytic Differentiation in a Calf

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ABSTRACT. A case of mast cell sarcoma in a 5-month-old Holstein female calf is described. Macroscopically, enlargement of the spleen, lymph nodes, tonsils and kidneys was noted, and there were tumor masses in the neck region and on the pleura and peritoneum. The pericardium and uterine and ureter walls were also involved by tumor. Most neoplastic cells had eosinophilic granules, which were metachromatic and positive for naphthol AS-D chloroacetate esterase and tryptase, whereas smaller numbers of cells were positive for factor VIII-related antigen, a marker of megakaryocytes. Some of the predominant type of these tumor cells were found within the epithelia of the lungs, tonsils, gastrointestinal tract, liver, ureters, urinary bladder and uterus. Their normal counterparts were considered to be globule leukocytes.

KEY WORDS: cattle, epitheliotropism, globule leukocyte, mast cell sarcoma.


Mast cell tumors in dogs are the most frequent malignant or potentially malignant tumor of the skin [7]. In humans, systemic mastocytosis is a disease caused by a neoplastic proliferation of mast cells. Most cases have the skin lesions of urticaria pigmentosa, and the majority of these have a relatively benign course [2]. In contrast, cases without skin lesions have a worse prognosis and may progress to transformation into mast cell leukemia or, more commonly, acute myeloblastic leukemia [9]. Highly malignant mast cell disorders such as mast cell leukemia and mast cell sarcoma are cytologically distinct from indolent systemic mastocytosis [2], and are not always associated with the mastocytosis [3].

In cattle, mastocytoma is a benign or low malignancy neoplasm of mast cell origin, occurring chiefly in the skin [8]. Although multiple skin lesions or metastases in visceral organs are not infrequent, cutaneous mastocytomas are characterized by rare or absent mitotic figures [8]. Leukemias of the mast cell lineage [19] or with both mastocytic and megakaryocytic differentiation [10] have been observed in cattle. Here, we report, in a calf, a mast cell sarcoma that possessed mast cell features such as metachromasia and tryptase positivity, but also factor VIII-related antigen expression in a minority of cells.

A 5-month-old Holstein female calf exhibited enlargement of the superficial lymph nodes, emaciation, growth retardation, rough hair coat and a rectal temperature of 40.5°C. The next day, hematological values were as follows: hematocrit, 17%; white blood cell count, 8,560 cells/µl with 2% neutrophils, 43% lymphocytes, 1% monocytes and 54% atypical cells with nuclear irregularity and/or coarse azurophilic granules in the cytoplasm. Antibodies to bovine leukemia virus (BLV) were not detected by the agar gel immunodiffusion test. A clinical diagnosis of calf-form leukemia was made, and euthanasia was performed seven days after the initial examination.

At necropsy, the superficial lymph nodes were highly to moderately enlarged, and were grayish white and homogeneous on cut section. The palatine tonsils were also enlarged. In the neck region, subcutaneous and paratracheal tumor masses up to 3 cm in diameter were sparsely distributed; the latter were not adherent to the cervical thymic lobes. The heart showed pallor throughout the pericardium. There were several tumor masses on the parietal and visceral pleurae, the largest being 10 × 5 × 2 cm. The spleen was greatly enlarged with uniform brown cut surfaces. There were two tumor masses measuring 14 × 4 × 1 and 12 × 5 × 3 cm on the liver, and several smaller ones on the ruminal serosa. Prominent enlargement of the mesenteric lymph nodes was observed with other abdominal nodes less severely affected. The kidneys were slightly enlarged, and the ureter walls were thickened. The uterine wall revealed diffuse thickening.

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (HE), Giemsa, toluidine blue (pH 4.1) and naphthol AS-D chloroacetate esterase (CAE). Selected sections were dewaxed and labeled by the streptavidin-biotin-peroxidase complex (SBC) method. The primary reagents employed were rabbit polyclonal antibodies to factor VIII-related antigen (BioGenex Laboratories, San Ramon, CA, U.S.A.), CD3 (Dako A/S, Glostrup, Denmark) and hemoglobin (Lipshaw Immunon, Pittsburgh, PA, U.S.A.). Additionally, mouse monoclonal antibodies to CD79a, HM57 (Dako A/S), myeloid/histiocyte antigen, MAC387 (Dako A/S), CD68, EBM11 (Dako A/S), macrophage, HAM56 (Dako Corporation, Carpinteria, CA, U.S.A.) and tryptase,
AA-1 (Lab Vision, Fremont, CA, U.S.A.) were also used. Subsequent procedures were carried out by means of an immunoperoxidase labeling system (Nichirei, Tokyo, Japan). Small pieces of formalin-fixed tissues were post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined by electron microscopy (EM).

Histologically, macroscopically visible lesions of the pleura were composed of neoplastic tissues, which extended into the lung parenchyma in the visceral pleura. In the alimentary system, there were neoplastic infiltrates chiefly in the gastrointestinal serosa, hepatic capsule, greater omentum and mesentry. Relatively large numbers of neoplastic cells were seen in the lamina propria of the forestomachs, ileum and cecum. Although there was only slight neoplastic infiltration into the hepatic parenchyma, some large bile ducts were heavily infiltrated. The spleen, thymus and highly enlarged lymph nodes were nearly completely replaced by malignant tissues, and many neoplastic cells were present in the palatine tonsils. There were large numbers of normal hematopoietic cells in the bone marrow, and tumor cells were admixed with them. Neoplastic involvement was severe in the uterus, ureters, urinary bladder serosa and heart, and interstitial neoplastic infiltrates were observed throughout the renal cortex. Epitheliotropic neoplastic mast cells were frequently observed in the tonsils, bronchial epithelium, intestine, bile ducts and ureters (Fig. 1a), but were rare in the stomach, urinary bladder and endometrial glands (Fig. 1b).

The majority of the neoplastic cells were mastocytoid, and were 4 to 9 µm in diameter. The nuclei were round, oval or occasionally irregular, with small to medium-sized nucleoli and slightly condensed chromatin, and binuclear cells were rarely seen. The cytoplasm was abundant and contained eosinophilic granules, which tended to be larger in size and smaller in number in the affected mucosae (Fig. 1b and 1c). The granules stained purple and occasionally blue or red with Giemsa, and cells having granules with different colors were rare. Such a color variation was observed mainly in the mucosae (Fig. 1a). Many mastocytoid cells showed metachromasia in the granules, which were apt to be more bluish in larger ones (Fig. 1b). Almost all of the granules were positive for CAE. Megakaryocytoid cells were admixed with mastocytoid cells with smaller granules, and were nearly absent in the mucosae with many mastocytoid cells. The cells were 5 to 18 µm in diameter, having round or oval, clear vesicular nuclei with inconspicuous nucleoli. Larger cells tended to contain multiple nuclei. The cytoplasm was abundant, eosinophilic and finely granular. The mitotic count ranged from 8 to 14 mitotic figures per high-power field.

Immunohistochemically, mastocytoid cells were frequently positive for tryptase (Fig. 2a), whereas factor VIII-related antigen was expressed in the megakaryocytoid cells (Fig. 2b). The other markers examined were not expressed in the neoplastic cells.

Ultrastructurally, the mastocytoid cells had cytoplasmic granules and poorly to slightly developed rough endoplasmic reticulum. The former were round to ovoid and varied in size between cells (Fig. 3a). The majority were dense or moderately dense, but some were devoid of contents. Aggregates of intermediate filaments were rarely seen. Megakaryocytoid cells were characterized by round to rod-shaped granules, and demarcation membranes were observed in larger cells (Fig. 3b).

In the case described here, the presence of multiple tumor masses, no elevation of white blood cell count and cytological atypia and pleomorphism supported the diagnosis of sarcoma [11]. Histologically, most neoplastic cells had metachromatic and tryptase-positive granules that are characteristic of mast cells or basophils [15], whereas a minority of cells expressed factor VIII-related antigen, a marker for cells of the megakaryocytic lineage. The predominant cells also showed CAE activity in the granules, which is observed in mast cell tumors but not in acute basophilic leukemia [10, 15]. On the basis of these findings, a presumptive diagnosis of mast cell sarcoma with a megakaryocytic component was made.

Bovine cutaneous mastocytoma, which is composed of a monotonous population of neoplastic mast cells with oval nuclei, may show visceral metastasis, but mitotic figures are few or absent [8]. It is considered to be a benign or low malignancy neoplasm. In a less differentiated form with slight or no eosinophil infiltration, the cytoplasmic granules are smaller in number than in a well differentiated form and show very faint metachromasia, but do not vary in size in any form (unpublished data). In the current sarcoma, by contrast, the neoplastic mast cells showed a marked variation in granule size, distinct metachromasia, relatively large numbers of mitotic figures, and epitheliotropism. These findings indicate that mast cell sarcoma in cattle is a new disease entity but not a more malignant form of the mastocytoma. Likewise, mast cell sarcoma in humans is an extremely rare and aggressive type of mast cell disease, and the neoplastic cells differ significantly from mast cells in indolent mastocytosis [2].

A case of bovine myeloid leukemia with mastocytic and megakaryocytic differentiation was thought to be derived from a common myeloid progenitor [10], while the marked preponderance of mastocytoid cells in the present case suggested another cell differentiation mechanism. The zinc finger transcription factor GATA-1 requires direct physical interaction with the cofactor friend of GATA-1 (FOG-1) for its essential role in erythroid and megakaryocytic development. It seems unlikely that FOG-1-induced lineage switching of mast cell progenitors (MCPs) occurs physiologically in mice, but ectopic expression of FOG-1 in committed MCPs blocks cell maturation and redirects them into the erythroid, megakaryocytic, and granulocytic lineages [1]. A similar phenomenon, which is associated with MCPs but not with common myeloid progenitors [10], may have occurred in the current case, because megakaryocytoid cells were observed in areas composed of less differentiated mastocytoid cells and the bone marrow was not severely involved by neoplastic cells. Taking this view into account, the final diagnosis was mast cell sarcoma with megakaryocytic differentiation.

The neoplastic mast cells in the present sarcoma were cytologically similar to those in myeloid leukemia with mastocytic and megakaryocytic differentiation [10]. However, the size of metachromatic cytoplasmic granules tended
to be larger in the mucosae, and epitheliotropic neoplastic mast cells were observed in the lungs, tonsils, gastrointestinal tract, liver, ureters, urinary bladder and uterus. These findings imply that some neoplastic cells were at the stage of differentiation corresponding to intraepithelial mast cells (globule leukocytes) [5]. This sarcoma was therefore thought to be in a more differentiated stage than the leukemia composed chiefly of blast cells [10]. Globule leukocytes are mononuclear cells with metachromatic cytoplasmic granules, and are found in epithelium and connective tissues of the respiratory, digestive, urinary and reproductive systems [5]. The cells are believed to be mucosal mast cells, although their origin as a subpopulation of T lymphocytes has also been proposed [5]. Globule leukocytes were suggested to be of T cell or natural killer (NK) cell lineage in goats and cats [4, 12, 13]. Considering the existence of intraepithelial T lymphocytes with eosinophilic granules and their malignant counterparts including hypergranular γδ T cell lymphoma.
REFERENCES


