Acute Myeloid Leukemia with Mastocytic and Megakaryocytic Differentiation in a Calf

Tsutomu IKEHATA¹⁾, Yoshihiro WADA²⁾, Yoshiharu ISHIKAWA³⁾ and Koichi KADOTA^{3)*}

¹⁾Tokachi Livestock Hygiene Service Center, 59–6 Kisen, Kawanishi, Obihiro 089–1182, ²⁾Abashiri Livestock Hygiene Service Center, 323–5 Taisyō, Kitami 090–0008 and ³⁾Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo 062–0045, Japan

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ABSTRACT. A diagnosis of acute myeloid leukemia was made in a 10-month-old Holstein female calf. The leukemia was macroscopically characterized by great enlargement of the spleen and moderate enlargement of some lymph nodes. Histochemical and immunohistochemical examination disclosed the presence of neoplastic cells either containing metachromatic and tryptase-positive granules or expressing factor VIII-related antigen. The granules, which were positive for naphthol AS-D chloroacetate esterase and did not have particulate contents, were distinct from those of basophilic leukemia cells. This leukemia was thought to be derived from a common myeloid progenitor capable of giving rise to megakaryocyte-erythrocyte progenitors and granulocyte-monocyte progenitors with the ability to differentiate into mast cells.

KEY WORDS: cattle, factor VIII-related antigen, myeloid leukemia, tryptase.

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In humans, acute myelomonocytic leukemia is an acute leukemia characterized by the proliferation of both neutrophil and monocyte precursors, and is considered to be derived from a hematopoietic precursor cell with potential to differentiate into neutrophil and monocytic lineages [3]. In some cases, myeloid and monocytoid characteristics are simultaneously present in single leukemia cells [7]. Although uncommon, acute leukemias showing differentiation into both neutrophil and mastocytic or megakaryocytic lineages occur in humans [1, 10]. These two types of bilineal leukemia may originate from a multipotent myeloid stem cell capable of giving rise to granulocytic, mastocytic and megakaryocytic lineages [4, 5, 10].

In animals, acute myelomonocytic leukemia has been described chiefly in dogs and horses [9, 11], and there is one documented case in a 2-month-old calf [16]. Nearly all reports of mast cell and megakaryocytic leukemias are of canine or feline cases, but a case of bovine mast cell leukemia has been recorded [13]. This paper reports a case of acute leukemia with both mastocytic and megakaryocytic differentiation in a 10-month-old calf, the leukemia cells being characterized by intracytoplasmic metachromatic granules or positive immunolabeling for factor VIII-related antigen.

A 10-month-old Holstein female calf showed rhinorrhea, lacrimation and a rectal temperature of 40.0°C, and became unable to stand 5 days later. Because of poor prognosis, the animal was euthanatized. Before euthanasia, hematocrit was 19% and white blood cell (WBC) count was 10,750 cells/ μ l. On blood smears, there were 5% neutrophils and 95% atypical cells of various sizes, and intracytoplasmic

basophilic or azurophilic granules were occasionally seen in the latter. Many erythroblasts were admixed with these cells. At necropsy, the spleen was greatly enlarged (65 $\times$ 23 cm), with bulging and homogeneous cut surfaces. The subiliac, sacral and ischial lymph nodes were moderately enlarged. Mild pleural and pericardial effusion was yellowish brown and clear. No other abnormalities were detected.

Tissue samples from the spleen, left subiliac lymph node, liver, kidney, lungs, heart, intestine, and brain were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μ m, 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 antibodies used were rabbit polyclonal antibodies to factor VIII-related antigen (BioGenex Laboratories, San Ramon, CA, U.S.A.), CD3 (Dako, Glostrup, Denmark), CD5 (Lab Vision, Fremont, CA, U.S.A.) and hemoglobin (Lipshaw Immunon, Pittsburgh, PA, U.S.A.). Also used were mouse monoclonal antibodies to CD79a (Dako), myeloid/histiocyte antigen, MAC387 (Dako), CD68, EBM11 (Dako), macrophage, HAM56 (Dako Corporation, Carpinteria, CA, U.S.A.), and tryptase, AA-1 (Lab Vision). Subsequent procedures were performed 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) (JEM-1010, JEOL, Tokyo, Japan).

Histologically, the splenic architecture was completely obliterated by neoplastic cells, though normal-appearing erythroblasts were sparsely distributed. There were great numbers of neoplastic cells in the subiliac lymph node, and

* CORRESPONDENCE TO: KADOTA, K., Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo 062–0045, Japan.
e-mail: kkadota@affrc.go.jp

Fig. 1. Histology of neoplastic cells. (A) Subiliac lymph node. Megakaryocytoid cells have broad to relatively scant cytoplasm (short arrows). Purple intracytoplasmic granules are visible in mastocytoid cells (long arrows). Giemsa. Bar=10 μ m. (B) Subiliac lymph node. There are weakly red granules in the cytoplasm of some mastocytoid cells. One neutrophil has stained intensely (arrow). CAE. Bar=10 μ m. (C) Subiliac lymph node. A mastocytoid cell containing purple or blue granules in the cytoplasm. Toluidine blue. Bar=10 μ m.

Fig. 2. Immunohistochemistry of leukemia cells. (A) Subiliac lymph node. Neoplastic cells of the megakaryocytic series, which react positively for factor VIII-related antigen, exhibiting a marked variation in cell size. SBC. Bar=20 μ m. (B) Subiliac lymph node. A large neoplastic mast cell with tryptase-positive granules is visible. An adjacent normal mast cell (arrow) has a smaller nucleus and a cytoplasm filled with many granules. SBC. Bar=10 μ m.

residual lymphatic tissues were detected in places. In the lungs, many neoplastic cells were present in the lumina of alveolar capillaries, and a few were perivascularly in the stroma. There were neoplastic cell accumulations in hepatic portal triads and smaller numbers of neoplastic cells in sinusoidal or vascular lumina. Stromal neoplastic infiltration was mild in the kidneys and minimal in the myocardium.

The neoplastic cells were divisible into blastoid, mastocytoid, and megakaryocytoid cells (Fig. 1a). The most predominant were blastoid cells, which measured 5.5–13.5 μ m in diameter. The nuclei were round, oval, or slightly irregular, with slightly to moderately clumped chromatin and small to medium-sized nucleoli. The cytoplasm was small to moderate in amount, and was basophilic or amphophilic.

However, some blastoid cells contained a clear area in the cytoplasm, which caused peripheral displacement of the nuclei, and were interpreted to have rhabdoid features. Although cytologically similar to blastoid cells, the mastocytoid cells contained granules in the cytoplasm, which were intensely eosinophilic in HE-stained sections, purple or rarely blue with Giemsa (Fig. 1a), and positive for CAE (Fig. 1b). Smaller numbers of mastocytoid cells exhibited metachromasia with toluidine blue (Fig. 1c). There was considerable variability in granule size from one cell to another, though it was similar in any one cell. Phagocytosis by blastoid or mastocytoid cells was rare. In megakaryocytoid cells (up to 25 μm in diameter), the cytoplasm was abundant, eosinophilic, and finely granular, though some cells with a large nucleus showed a high nucleocytoplasmic ratio (Fig. 1a). The nuclei were mostly round or oval, and occasionally multiple. Larger megakaryocytoid cells engulfing erythrocytes and/or neoplastic cells appeared to show emperipolesis. Mitotic figures were plentiful (17–30/high power magnification field).

Immunohistochemically, megakaryocytoid cells of varied sizes were labeled for factor VIII-related antigen (Fig. 2a). The simultaneous presence of the antigen and CAE activity in single neoplastic cells was not found in serial sections. Tryptase positivity was confirmed in some mastocytoid cells (Fig. 2b). No neoplastic cells expressed markers of the lymphocyte, neutrophil, histiocyte or erythrocyte lineages.

Ultrastructurally, the neoplastic cells, which had poorly to slightly developed organelles, were divided into three types chiefly based on features of intracytoplasmic granules. Mastocytoid cells had an accumulation of fair numbers of round to oval granules (Fig. 3a). The majority were electron dense and homogeneous. Less dense granules had a finely granular appearance, but distinct particles were absent. Megakaryocytoid cells were characterized by round to elongated rod-shaped, electron dense homogeneous granules. In addition, there were demarcation membranes, glycogen particles and decreased numbers of ribosomes in the most differentiated cells (Fig. 3b). Electron dense granules were also seen in most of the blastoid cells, but they were smaller in size and number than in the other types of cells. An aggregate of intermediate filaments was detected in some blastoid cells (Fig. 3c), and rarely in mastocytoid cells.

In the case reported here, two lineages of neoplastic cells were observed. Cells of the megakaryocytic lineage were characterized by positivity for factor VIII-related antigen [2], whereas some others contained metachromatic or tryptase-positive granules. Since CAE activity has been observed in a bovine cutaneous mastocytoma but not in a bovine acute basophilic leukemia [12], the neoplastic cells with CAE-positive metachromatic granules were considered to belong to the mast cell lineage. This view was supported by ultrastructural findings: Takahashi *et al.* [12] observed particles in granules of electron density in basophilic leukemia. In contrast, there were no distinct particles in the current case. Although the bone marrow was not examined

Fig. 3. Ultrastructure of neoplastic cells. (A) Subiliac lymph node. A mastocytoid cell contains electron dense granules. EM. Bar=1 μm . (B) Subiliac lymph node. There are granules of varied size and shape in the cytoplasm of a neoplastic megakaryocyte. Arrows indicate demarcation membranes. EM. Bar=1 μm . (C) Subiliac lymph node. Blastoid cells are characterized by the paucity of cytoplasmic organelles. An accumulation of intermediate filaments are shown by arrows. EM. Bar=2 μm .

histologically, the absence of tumor masses, the preponderance of atypical cells on the blood smears, and the presence of many blast cells within pulmonary alveolar capillaries indicated that this case was an acute leukemia [6, 8, 12]. On the basis of these findings, the final diagnosis of acute myeloid leukemia with mastocytic and megakaryocytic differentiation was made. The relatively low WBC count may imply that the calf was not in the final stage of leukemia [6].

In human myelomegakaryoblastic and myelomastocytic leukemias, the leukemia cells are bilineal [1, 10], while myelomonocytic leukemia is bilineal or biphenotypic [3, 7]. In a calf myelomonocytic leukemia, the majority of the atypical cells were reported to have cytochemical features of both monocytic and myeloid cell lines [16]. In the current case, the simultaneous existence of CAE-positive granules and factor VIII-related antigen in individual leukemia cells could not be ascertained by examination of serial sections. Since an acute bilineal leukemia may evolve into a biphenotypic leukemia in humans [3], it is probable that a similar phenomenon occurs in advanced stages of bovine leukemias showing divergent differentiation.

In humans, acute myeloblastic leukemia is more prevalent than acute megakaryoblastic and mast cell leukemias [3], and acute myelomegakaryoblastic and myelomastocytic leukemias are rare [1, 10]. A combination of mastocytic and megakaryocytic lineages in the differentiation of an acute leukemia may be exceedingly rare, even in humans. In addition to these three types of leukemia with characteristics of two cell lines, myelomonomegakaryocytic and erythromyelomegakaryocytic leukemias have been reported in humans [1]. The existence of these leukemias was considered to be evidence that the leukemic clone arises from a common myeloid progenitor capable of giving rise to megakaryocyte-erythrocyte progenitors (MEPs) and granulocytemonocyte progenitors (GMPs) with the ability to differentiate into mast cells [5]. In myelomonocytic, myelomastocytic and murine erythromegakaryocytic [14] leukemias, however, the clone may originate from a progenitor having more limited differentiation potential, such as GMP and MEP [4, 5].

In addition to the current case, acute basophilic leukemia [12], acute myelomonocytic leukemia [16], and acute erythremic myelosis (pure erythroid leukemia) [15] have been reported in calves. Considering the large population of the normal counterpart cells, reports of non-lymphocytic leukemias seem to be too few. Not only these leukemias, but also calf-form leukosis (acute lymphoblastic leukemia), are characterized by many neoplastic cells in the blood, severe enlargement of the spleen, involvement of the bone marrow, and paucity of extranodal tumor masses [6]. Presumably, it is not infrequent that the former are overlooked as the latter without detailed examination, and this is the cause of few reports of non-lymphocytic leukemias in calves.

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