Case Report

Spontaneous Erythroid Leukemia in a 7-Week-Old Crl:CD (SD) Rat

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Abstract: A young male Crl:CD (SD) rat with erythroid leukemia that presented with emaciation, abdominal distension and a pale visible mucosal membrane was euthanized at 7 weeks of age. At necropsy, enlargement of liver, spleen and pancreatic lymph node was noted. Analysis of blood smear samples revealed many mono- or binucleated erythroblasts that had PAS-positive vacuoles in the cytoplasm. Histopathologically, neoplastic proliferation of atypical cells was observed in the hepatic sinusoids, splenic red pulp, bone marrow, pancreatic lymph node, kidney and lung. Neoplastic cells showed a round to spindle shape, and some neoplastic cells had deeply stained small nuclei and small cytoplasms and resembled erythroblasts. Immunohistochemically, many neoplastic cells were positive for hemoglobin. To our knowledge, this is the first report of erythroid leukemia in a rat of this age. The observed features were similar to those of pure erythroid leukemia in humans. (J Toxicol Pathol 2010; 23: 91–94)

Key words: Crl:CD (SD) rat, erythroid leukemia, spontaneous

Erythroid leukemia or erythroleukemia is a myeloproliferative disorder characterized by an excessive proliferation of erythrogenic cells1–4. It is known that erythroid leukemia can be induced in rats by administration of 7,8,12-trimethyl-benz[a]anthracene and nitrosoureas, but spontaneous erythroid leukemia is extremely rare in rats1. To our knowledge, only one case, involving a 16-week-old female Slc:SD rat, has been reported4. In this report, we describe the histological and immunohistochemical characteristics of a new case of spontaneous erythroid leukemia in a younger rat.

A 6-week-old male Crl:CD (SD) rat (Charles River Japan, Shiga, Japan) was individually housed in a plastic cage in an environmentally controlled room (room temperature, 23 ± 3°C; relative humidity, 30–60%; lighting cycle, 12 h light/12 h dark) and supplied with a pellet diet and tap water ad libitum during acclimatization to its new surroundings. It presented with emaciation, abdominal distention and a pale visible mucosal membrane and was sacrificed under anesthesia at 7 weeks of age. A peripheral blood sample was collected for a smear to determine cell morphology. Liver, spleen, pancreatic lymph node, adrenal glands, femoral bone marrow, heart, kidneys, lungs and testes were fixed in 10% neutral-buffered formalin and then paraffin-embedded. Paraffin sections were stained with hematoxylin and eosin (HE) for histological examination. For immunohistochemical examination, the liver and spleen sections were examined with rabbit anti-mouse hemoglobin antibody (Cappel Lab., OH, USA). Autoclave pretreatment was performed before reactions with primary antibody. Endogenous peroxidase was inactivated using 3% H2O2, and non-specific proteins were blocked with normal goat serum. Sections were then incubated with the primary antibody overnight at 4°C at a dilution of 1/1000. Immunolocalization was performed using the avidin-biotin peroxidase complex method (Dako Japan, Kyoto, Japan) with 3,3’-diaminobenzidine as the chromogen and counterstaining with hematoxylin. Smear preparations of peripheral blood were subjected to May-Giemsa staining and periodic acid-Schiff (PAS) reaction. Electron microscopy was performed on the liver and spleen tissues. Small pieces of tissue were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and 2% osmic acid and then embedded in Epoxy resin (Epok 812). Ultrathin sections cut on an ultramicrotome were stained with uranyl acetate and lead citrate and examined with a JEM-1010 electron microscope (JEOL, Tokyo, Japan).

At necropsy, severe enlargement of the liver, spleen and pancreatic lymph node was observed (Fig. 1). No gross lesions were observed in the thymus.

Blood smear preparations revealed numerous erythroblasts (average of 50% of total nucleated cells),
mainly orthochromatic erythroblasts. There were some abnormal erythroblasts that exhibited cytoplasmic vacuolation or that were binucleated (Fig. 2a). Vacuoles observed in the cytoplasms of erythroblasts were positive for the PAS reaction (Fig. 2b).

Histopathologically, neoplastic erythroblasts with small dark cytoplasms and round to oval nuclei were observed in cells in many organs (Fig. 3). In the spleen, neoplastic proliferation was observed mainly in the red pulp and resulted in atrophy of white pulp (Fig. 3a). Neoplastic cells showed a round to spindle shape, and some neoplastic cells had deeply stained small nuclei and small cytoplasms and resembled erythroblasts. Mitotic figures were frequently seen. In the liver, the same neoplastic cells observed in the spleen infiltrated into sinusoid and compressed hepatic cells (Fig. 3b). The number of round neoplastic cells was larger than that in the spleen. In the pancreatic lymph node, neoplastic cells replaced almost all the normal architecture. Almost all proliferating cells were round. In the femoral bone marrow, multifocal neoplastic proliferation was observed, and almost all neoplastic cells showed a spindle shape (Fig. 3c and d). In the lung, neoplastic cells were only observed in the blood vessels. In the kidney, round neoplastic cells were observed in blood vessels and capsules.

Immunohistochemically, about half of the neoplastic cells were positive for hemoglobin (Fig. 4a); most round cells showed positive reactions, but spindle-shaped neoplastic cells were negative for hemoglobin. No ED1-positive neoplastic cells were observed; only Kupffer cells were positive for ED1 (Fig. 4b).

In electron microscopy, it was revealed that neoplastic cells had round to oval nuclei with thick, coarse chromatin. In the cytoplasm, there were numerous free ribosomes, glycogen and a small number of organelles (Fig. 5a). This glycogen in the cytoplasm might be the PAS-positive vacuoles observed in the blood smear preparation. The spindle-shaped neoplastic cells had almost the same features of the nucleus and cytoplasm (Fig. 5b).

These findings were almost consistent with the features of erythroid leukemia reported previously in a rat⁴. However, one difference from previous reports was that there were numerous spindle-shaped neoplastic erythroblastic cells. This has not been reported in cases of

Fig. 1. Gross appearance of the abdominal cavity. Enlargement of the liver and spleen is noted.

Fig. 2. Smear preparation of peripheral blood. Numerous abnormal erythroblasts exhibit cytoplasmic vacuolation or are binucleated (a). The cytoplasmic vacuole is positive for the PAS reaction (b). a: May-Giemsa stain. b: PAS reaction. Bar=50 μm.
Fig. 3. Light micrographs of the spleen, liver and bone marrow cavity in the femur. Neoplastic proliferation of erythroblastic cells is observed in red pulp of the spleen (a). Neoplastic cells are observed in the sinusoid and compress hepatic cells (arrowhead) (b). In the bone marrow, monotonous and multifocal proliferation of neoplastic cells is observed (c), and almost all neoplastic cells are spindle shaped (d). Bar=50 μm.

Fig. 4. Immunohistochemical staining of the liver for hemoglobin and ED1. Many neoplastic cells are positive for hemoglobin (a). Neoplastic cells are negative for ED1; only Kupffer cells in the compressed hepatic tissue show a positive reaction (b). Bar=50 μm.
leukemia\(^1\)-\(^4\). Although, these spindle-shaped cells were negative for hemoglobin, they showed the same features as round neoplastic cells in the examination by electron microscopy; they might have been immature hematopoietic cells. It has reported that hematopoietic progenitor cells become spindle shaped\(^5\).

According to the World Health Organization classification (FAB), acute erythroid leukemia is divided into 2 groups: erythroleukemia and pure erythroid leukemia. Erythroleukemia is characterized by proliferation of erythroblastic cells and granulocytic cells. In contrast, pure erythroid leukemia is extremely rare and frequently associated with complex cytogenetic abnormalities. It involves a neoplastic proliferation of immature cells committed exclusively to the erythroid lineage with no evidence of a significant myeloblastic component\(^6\)-\(^8\). In the present case, we observed many features that resembled characteristics of pure erythroid leukemia.

A case of spontaneous erythroid leukemia in a rat of this age has not been previously reported. The observed features were similar to those of pure erythroid leukemia in humans.

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