Case Report

Spontaneous Myeloblastic Leukemia in a Young SD Rat

Takeo Shimo, Junichi Katayama, Akemi Saito, Yasuji Aoki, Eiichi Okezaki, and Osamu Nagata

Research Department, Research & Development Division, Hokuriku Seiyaku Co., Ltd., 37-1-1, Inokuchi, Katsuyama, Fukui 911-8555, Japan

Abstract: A hematopoietic neoplasm was found in a 14-week-old male Sprague-Dawley (SD) rat. Blood smear examination revealed the existence of numerous blastic cells with large, round or oval nuclei, and scant pale cytoplasm containing no granules. At autopsy, the liver and spleen were markedly enlarged and the right femur showed swelling and hemorrhage. Histopathologically, neoplastic cells infiltrated diffusely in the bone marrow, liver, and spleen, and destroyed their normal architectures. In the bone marrow neoplastic cells invaded into the surrounding tissues, while in the spleen they proliferated from the red pulp. Immunohistochemically, neoplastic cells were positive for lysozyme, while they were negative for hemoglobin, ED-1, OX-7, OX-8, and OX-12. Based on these findings, the present neoplasm was diagnosed as myeloblastic leukemia. (J Toxicol Pathol 2000; 13: 123~126)

Key words: Hematopoietic neoplasm, Myeloblastic leukemia, Immunohistochemistry, Lysozyme, SD rat

Myeloblastic leukemia is a myeloproliferative neoplasm originated from neoplastic blastemal cells. Generally, spontaneous hematopoietic neoplasms in SD rats have been classified into erythroleukemia, granulocytic or myelogenous leukemia and large granular lymphocyte (LGL) leukemia^{1,2}. To our knowledge, there is only a single report concerning spontaneous erythroleukemia in this rat strain³, and the incidence of LGL leukemia is also exceedingly low¹, in contrast to the most commonly occurring tumor in F344 rats^{4,5}. As for these hematopoietic neoplasms, there are some reports concerning granulocytic leukemia^{1,2,6,7}, but little has been known about such case reports as myeloblastic leukemia. In the present report, we describe morphological, immunohistochemical, and ultrastructural features of spontaneous myeloblastic leukemia in a young SD rat.

The present neoplasm was found in a male Crj:CD (SD) rat (Charles River Japan Inc., Shiga), one of 20 controls for each sex in a 13-week toxicity study. The rat was individually housed in a plastic cage with white flakes as bedding in an air-conditioned room (room temperature, $24 \pm 2^\circ\text{C}$; relative humidity, $60 \pm 10\%$; lighting cycle, 12 light/12 dark). Pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo) and tap water were available *ad libitum*.

The animal exhibited paralysis of the right hind limb, weakness, and anemia after week 8 of the study, and was sacrificed at week 9 of the study (14 weeks of age). Blood sample was taken from the abdominal aorta under ether anesthesia for hematology including blood smear morphology (May-Giemsa staining), and then the animal was subjected to a full autopsy. Main organs including the liver and spleen were weighed. A wide variety of tissues were fixed in

10% neutral buffered formalin, embedded in paraffin, sectioned at $4-5 \mu\text{m}$, and stained with hematoxylin and eosin (H-E) for microscopic examination. In addition, immunohistochemical staining was performed for lysozyme, ED-1, hemoglobin, OX-7, OX-8, and OX-12 using the liver and spleen tissue. For OX-7, OX-8, and OX-12 staining, before reactions with primary antibodies, 0.1% solution of trypsin and calcium chloride at pH 7.8 was applied at 37°C for 30 min after deparaffinization. Endogenous peroxidase was inactivated using 3% H_2O_2 and non-specific proteins were blocked with normal goat serum. These sections were then incubated with the following primary antibodies overnight at 4°C : monoclonal antibodies against lysozyme (DAKO, Glostrup, Denmark), ED-1 (BMA Biomedicals Ltd., Switzerland), hemoglobin (Cappel Lab, West Chester, PA, USA), OX-7 (anti-thy-1), OX-8 (anti-suppressor/cytotoxic T cells, anti-LGLs), and OX-12 (anti-IgG F [ab']₂, Harlan Sera-lab, Sussex, England) at a dilution of 1/300, 1/500, 1/100, 1/300, 1/1,000, and 1/300, respectively. Immunolocalization was detected using the avidin-biotin peroxidase complex method (DAKO) with 3,3'-diaminobenzidine as the chromogen and hematoxylin for counterstaining. Electron microscopic examination was performed on the liver tissue fixed in 10% neutral buffered formalin. Small pieces of liver tissues were fixed in 1% osmic acid in 0.1 M phosphate buffer and embedding in Epon 812. Tissue samples were cut with an ultramicrotome and stained with uranyl acetate and lead citrate for ultrastructural investigation using a JEM-1200 EX II electron microscope (JEOL, Tokyo, Japan).

Hematology revealed marked decreases in erythrocyte counts ($211 \times 10^4/\mu\text{l}$), hematocrit value (12.9%), and hemoglobin concentration (4.2 g/dl) as well as apparently increased leukocyte counts ($434 \times 10^2/\mu\text{l}$). In the blood smear, numerous abnormal cells with scant pale blue cytoplasm containing no granules and large, round or oval nuclei were seen (Fig. 1). In addition, a small number of cells with very few granules showing differentiation to premyelocytes and nucleated erythrocytes were also noted. Macroscopically,

Received: 25 January 2000, Accepted: 27 March 2000

Mailing Address: Takeo Shimo, Research Department, Research & Development Division, Hokuriku Seiyaku Co., Ltd., 37-1-1, Inokuchi, Katsuyama, Fukui 911-8555

FAX: 0779-88-8022

e-mail: takeo.shimo@hokuriku-seiyaku.co.jp

Fig. 7. Electron photomicrograph of a neoplastic blastemal cell. Note the nucleus is large, oval, with coarse chromatin along the nuclear membrane, and the cytoplasm contains abundant polyribosomes (Pr), a few mitochondria (M), and rough endoplasmic reticulums (rER). Bar = 1 μ m.

the liver (52 g) and spleen (4.5 g) were markedly enlarged. The right femur showed swelling and hemorrhage at the proximal region. The thymus appeared atrophic.

Histopathologically, diffuse infiltration of neoplastic cells in the bone marrow, liver, and spleen was a prominent feature. The femoral and sternal bone marrows were predominantly replaced by the neoplastic cells with large, round or oval nuclei containing one or more nucleoli and a scant rim of pale cytoplasm, which invaded into the surrounding skeletal muscle and adipose tissues, with hemorrhage (Fig. 2). In the liver, the normal architecture was badly disrupted by

diffuse proliferation of neoplastic cells, the remaining hepatocytes showing degeneration or necrosis (Fig. 3). In the spleen, the red pulp was completely replaced by neoplastic cells and most of the lymph follicles were obliterated (Fig. 4). In the lymph nodes (mesenteric and submandibular), neoplastic cells were limited to peripheral sinusoidal areas. Involvement in the kidney and lung was minor. In the above-mentioned tissues, mitotic figures were prominent and neoplastic cells were evident in blood vessels.

Immunohistochemically, neoplastic cells in the liver and spleen were positive for lysozyme (Fig. 5), whereas they were

- Fig. 1. Smear preparation of the peripheral blood. Note two neoplastic myeloblasts with round or oval nuclei and scant pale cytoplasm containing no granules, and three nucleated erythrocytes. May-Giemsa stain, $\times 1650$.
- Fig. 2. Myeloblastic leukemia (bone marrow). The marrow cavity is predominantly replaced by monotonous leukemic cells, and they invade into the adjacent skeletal muscle. H & E stain, $\times 200$.
- Fig. 3. Myeloblastic leukemia (liver). The normal architecture is destroyed by diffuse infiltration of leukemic cells, the remaining hepatocytes showing degenerative or necrotic changes. H & E stain, $\times 330$.
- Fig. 4. Myeloblastic leukemia (spleen). The red pulp is completely replaced by leukemic cells, and most of the white pulp also obliterated. H & E stain, $\times 165$.
- Fig. 5. Immunohistochemistry of leukemic cells in the liver. Note leukemic cells are positive for lysozyme. Lysozyme immunostain, $\times 660$.
- Fig. 6. Immunohistochemistry of leukemic cells in the spleen. Note leukemic cells are negative for OX-8, while some cells of the remaining lymph follicle (right edge) are positive. OX-8 immunostain, $\times 165$.

negative for hemoglobin, ED-1, OX-7, OX-8 (Fig. 6), and OX-12. Accordingly, the following hematopoietic neoplasms can be denied: for histiocytic sarcoma, derived from the mononuclear-phagocyte system, no reactivity for ED-1^{2,8}; for LGL leukemia, no reactivity for OX-8, a marker of LGLs^{1,2}; for lymphomas/lymphocytic leukemia, no reactivity for OX-7, OX-8, and OX-12; for erythroleukemia, no reactivity for hemoglobin.

Ultrastructurally, neoplastic cells were characterized by large, round to oval nuclei with prominent nucleoli and scant cytoplasm (Fig. 7). The nuclei had a chromatin network that was coarsely distributed along the nuclear membrane. The cytoplasm contained only a limited number of organelles such as rough endoplasmic reticulum and mitochondria except abundant free polyribosomes.

The present lysozyme-immunoreactivity in neoplastic cells was the key finding for diagnosis. Lysozyme has been described to exist in hematopoietic neoplasms derived from granulocytes^{2,9-11} including myeloblasts⁸ and mononuclear-phagocyte system^{2,8}. Thus the cell origin of the present neoplasm is considered to be one of these cell components. The invasion of neoplastic cells into the surrounding tissues from the bone marrow, apparently resulted in paralysis of the right hind limb. The proliferative pattern in the spleen centered on the red pulp. These observations strongly suggest that the primary site of origin is the bone marrow. The cell origin is speculated to be myeloblasts since the present neoplasm was predominantly composed of poorly differentiated blastic cells, although a small number of cells showing differentiation to premyelocytes were seen. The assumption that the neoplasm derived from immature myelogenous cells can be supported by the fact that the lesion developed acutely and caused anemia⁷. Generally, myeloproliferative neoplasms have been diagnosed as granulocytic or myelogenous leukemia, and neoplastic cells usually represent a single immature or mature developmental stage, but in some cases various stage cells may exist^{1,2,6,7}. Therefore, such documentation suggests that the diagnosis of myeloproliferative neoplasms has been made without reference to the degree of cell maturity. Moreover, it has been reported that such hematopoietic neoplasms involve the liver, spleen, lymph nodes, bone marrow, kidney and so on^{1,2,7}, the distribution being in line with the present case. Experimentally, myeloblastic leukemia has been known to be induced when 1-butyl-1-nitrosamine (BNU) was given to Donryu rats¹², the morphology being in line with the present findings. Putting emphasis on the point that the present hematopoietic neoplasm is predominantly composed of monotonous blastemal cells which are likely to be originated from the bone marrow, myeloblastic leukemia is considered to be more appropriate for diagnosis.

In conclusion, taking the cell morphology and immunohistochemical characteristics into consideration, the present hematopoietic neoplasm was diagnosed as myeloblastic leukemia. This may be the first detailed case report of sponta-

neous myeloblastic leukemia in SD rats.

Acknowledgements: We wish to thank Dr. Shim-mo Hayaishi, Molecular Pharmacology Laboratories, Takeda Chemical Industries, Ltd. for his technical support on immunohistochemistry.

References

1. Frith CH. 8. Hematopoietic neoplasms in the mouse and rat including LGL lymphoma and nonlymphoid neoplasms. In: Pathology of Neoplasia and Preneoplasia in Rodents. Ban-nasch G (ed), Stuttgart, New York: Schattauer, 155-67, 1997.
2. Frith CH, Ward JM, and Chandra M. The morphology, immunohistochemistry, and incidence of hematopoietic neoplasms in mice and rats. *Toxicol Pathol* 1993; **21**: 206-18.
3. Nonoyama T, Hayashi S, Urano T, Yagami K, and Miyajima H. Spontaneous erythroleukemia in a 16-wk-old female Slc: SD rat. *Toxicol Pathol* 1993; **21**: 335-39.
4. Haseman JK, Arnold J, and Eustis SL. 35. Tumor Incidences in Fischer 344 Rats. In: Pathology of the Fischer Rat. Boorman GA, Eustis SL, Elwell MR, Montgomery CA, Jr, and Mackenzie WF (eds), New York: Academic Press, 555-64, 1990.
5. Haseman JK, Hailey JR, and Morris RW. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update. *Toxicol Pathol* 1998; **26**: 428-41.
6. Frith CH. Morphological classification and incidence of hematopoietic neoplasms in the Sprague-Dawley rat. *Toxicol Pathol* 1988; **16**: 451-57.
7. Gal F, Sugar J, and Csuka O. Granulocytic Leukemia, Rat. In: Monograph on Pathology of Laboratory Animals. Hematopoietic System. Jones TC, Ward JM, Mohr U, and Hunt RD (eds), New York: Springer-Verlag, 39-45, 1990.
8. Ogasawara H, Mitsumori K, Onodera H, Imazawa T, Shibutani M, Takahashi M, Ward JM, and Maekawa A. Spontaneous histiocytic sarcoma with possible origin from the bone marrow and lymph node in Donryu and F-344 rats. *Toxicol Pathol* 1993; **21**: 63-70.
9. Greenberger JS, Campos-Neto A, Parkman R, Moloney WC, Karpas A, Schlossman SF, and Rosenthal DS. Immunologic detection of intracellular and cell-surface lysozyme with human and experimental leukemic leukocytes. *Clin Immunol Immunopathol* 1977; **8**: 318-34.
10. Taylor CR. Other "hematopoietic cell" markers. In: Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist. Philadelphia, WB Saunders Company, 142-62, 1986.
11. Ward JM, Rehm S, and Reynolds CW. Tumours of the hematopoietic system. In: Pathology of Tumours in Laboratory Animals. Vol. 1: Tumours of the Rat, 2nd ed. Turusov V and Mohr U (eds), Lyon: International Agency for Research on Cancer, 625-65, 1990.
12. Odashima S, Ogiu T, Maekawa A, and Nakatate M. Leukemias induced by 1-alkyl-1-nitrosoureas in Donryu rats. *Gann Monograph on Cancer Research* 1975; **17**: 405-15.