Case Report

Spontaneous granulocytic leukemia in a NOD/Shi-*scid* IL-2Rγ^null mouse

Hirotoshi Akane[1], Sumiko Okuda[1], Yasuaki Oishi[1], Atsuko Ichikawa[1], and Hajime Tabata[1*]

[1] CMIC Pharma Science Co., Ltd., 10221 Kobuchisawa-cho, Hokuto-shi, Yamanashi 408-0044, Japan

Abstract: Here, we report a case of spontaneous granulocytic leukemia in a 51-week-old male NOD/Shi-*scid* IL-2Rγ^null (NOG) mouse. The mouse showed progressive anemia and rough respiratory movement. Macroscopically, the spleen was discolored and enlarged. Histologically, the bone marrow of the sternum and femur was highly cellular and almost exclusively filled with neoplastic cells. The nuclei of neoplastic cells were large, oval to slightly irregular in shape, and a small number of cells had kidney- or ring-shaped nuclei. Neoplastic cells extensively infiltrated the organs, and the spleen and liver were prominently involved. Immunohistochemically, a large population of neoplastic cells in the red pulp of the spleen and sinusoid of the liver was positive for myeloperoxidase. Based on the histological features, this case was diagnosed with granulocytic leukemia. This novel information on spontaneous tumors may be helpful for the appropriate use of this mouse strain in further research. (DOI: 10.1293/tox.2020-0092; J Toxicol Pathol 2021; 34: 241–244)

Key words: granulocytic leukemia, NOG mouse, spontaneous tumor

Spontaneous granulocytic leukemia is rarely reported in several strains of mice, although chemical agents and ionizing radiation may increase the incidence in some mouse strains[1,2]. NOD/Shi-*scid* IL-2Rγ^null (NOG) mice are severely immune-deficient animals, and present defects in T-, B-, and natural killer cells, and poorly functioning macrophages and dendritic cells by the knockout of IL-2 receptor γ-chain (IL2Rγc) into the NOD-*scid* strain[3]. This mouse strain is used for non-clinical tests, such as the tumorigenicity test, to assess the quality and safety of biological products for regenerative medicine using human-derived cell/tissue, because of the markedly better engraftment of xenogeneic cell[4–6]. Although the immunodeficient state and its implications for xenografts have been well studied, little information is available on spontaneous tumors occurring in this mouse strain, such as thymic lymphoma, mammary gland adenoma/adenocarcinoma, bronchiolo-alveolar carcinoma, rhabdomyosarcoma, and teratoma[7]. Here, we report a case of granulocytic leukemia in a 51-week-old male NOG mouse.

The animal was obtained from In-Vivo Science Inc. (Kanagawa, Japan) at the age of 6 weeks and was kept untreated. The animal was individually housed in a plastic cage with an individually ventilated cage system (Maxi-Miser Caging System; Oriental Giken Inc., Tokyo, Japan) within environmentally controlled barrier system room, which were maintained at a temperature of 22 ± 2°C with a humidity of 50 ± 20% and a 12-h light/dark cycle. The mouse was fed an autoclaved pelleted diet (CRF-1; Charles River Japan, Inc., Tokyo, Japan) with autoclaved tap water *ad libitum*. The animal was handled according to the protocol approved by the Institutional Animal Care and Use Committee of CMIC Pharma Science Co., Ltd., which is accredited by AAALAC International.

This mouse showed clinical signs of weakening, such as anemia, rough respiratory movement, and body weight reduction at between 49 and 51 weeks of age. The animal was euthanized at 51 weeks of age. At necropsy, the spleen was discolored and enlarged. No obvious abnormal gross findings were observed in the other organs. The thymus and lymph node were atrophic, similar to those of normal NOG mice.

All organs and tissue samples were routinely collected, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at approximately 3 μm. For histopathological examination, the sections were stained with hematoxylin and eosin (HE). Sections of the spleen and liver were immunohistochemically stained for rabbit anti-human myeloperoxidase (MPO) polyclonal antibody (Dako Japan, Tokyo, Japan) as a myeloid cell marker, rabbit anti-human CD3 monoclonal antibody (Nichirei Biosciences Inc., Tokyo, Japan) as a T cell marker, and rabbit anti-human Pax-5 monoclonal antibody (Abcam Inc., Cambridge, UK) as a B cell marker[8]. Sections were incubated with the antibodies at room temperature for 1 h, and immunodetection was carried out using a Histofine® MOUSESTAIN KIT (Nichirei Biosciences Inc.) with 3,3′-diaminobenzidine/H_2O_2 as the chro-

mogen. Immunostained sections were then counterstained with hematoxylin.

Histopathologically, the bone marrow of the sternum and femur was highly cellular and almost exclusively filled with neoplastic cells (Fig. 1A). The fat tissue was completely displaced. There were occasional cellular foci consisting of erythroid and megakaryocytic elements in the marrow, which were deemed to be remnants of normal hematopoiesis. Most neoplastic cells showed a blastic appearance, characterized by large, oval to slightly irregular shaped nuclei, and with a reticular chromatin containing one or more prominent nucleoli. There was a small number of cells with kidney- or ring-shaped nuclei and a few cells with lobed nuclei were also present (Fig. 1B). Granules in the cytoplasm of neoplastic cells were not obvious in the sections stained with HE. Numerous mitotic figures were found in the marrow, and occasional apoptotic bodies were also observed. Neoplastic cells markedly invaded the periosteum and the surrounding skeletal muscle. There was extensive infiltration of neoplastic cells into other organs and tissues, and the spleen and liver were prominently involved. In the spleen, neoplastic cells were observed throughout the red pulp (Fig. 2). Capsular infiltration of neoplastic cells was evident. A few developing erythroid series and megakaryocytes were observed. Megakaryocytes containing one or some myeloid cells in the cytoplasm, suggesting emperipolesis, were occasionally observed. In the liver, neoplastic cells infiltrated the Glisson's capsule and hepatic sinusoids diffusely (Fig. 3). Neoplastic cells with kidney- or ring-shaped nuclei were also evident in some blood vessels. Some hepatocytes in the periportal area, with prominent neoplastic cell infiltration, showed degeneration or necrosis, whereas the normal structure of the hepatic lobule remained intact. Neoplastic cell infiltration was also found in other organs, including the alveolar wall and perivascular region of the lung, interstitium in the kidney, sinusoid in the adrenal glands, and mucosa and submucosa in the gastric tract (stomach and intestine). Immunohistochemically, a large population of neoplastic cells in the red pulp of the spleen (Fig. 4) and in the sinusoid of the liver was positive for MPO. CD3 and Pax-5 positive cells were not detected. The results of CD3 and Pax-5 immunostaining were similar to those of the spleen of normal NOG mice, which were histologically hypoplastic and showed no CD3 or Pax-5 positive cells.

Immunohistochemical investigation revealed that the neoplastic cells originated from the myeloid cell lineages. In some of the involved areas, especially the spleen and liver, a small number of cells had kidney-, ring-, or lobed shapes indicative of differentiating myeloid series. These findings suggest that neoplastic cells are derived from immature granulocytes. The case of severe myelopoiesis may be evident by the presence of all types of developing myeloid series, in contrast to the dominance of immature cells in leukemia. In addition, progressive anemia is a common clinical sign of granulocytic leukemia, and increases in erythroid and megakaryocytic cells are poor[1]. The minor population of granulocytes with lobed nuclei in the affected area without obvious inflammation indicates the findings of the present case to be neoplastic, and not reactive myelopoiesis.

In granulocytic leukemia, the bone marrow may be replaced by immature and mature myeloid cells. In a previous report on other strains of mice, these neoplastic cells diffusely infiltrated the liver, spleen, lymph nodes, etc.[2], and these cells in the spleen and liver were mainly located in the red pulp and around the Glisson's capsule[1]. Thus, the leukemic cell distribution in the present case was identical to that in the previous case.

Based on the morphology of cells, granulocytic leukemia in mice is classified into two types: the juvenile type, which is comparable to human acute myelogenous leuke-
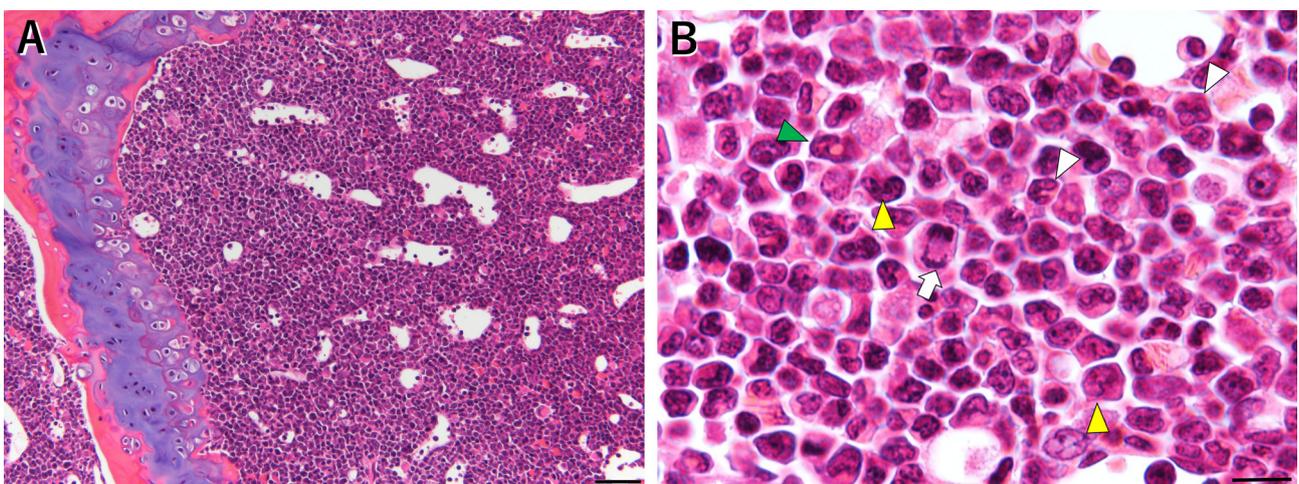

Fig. 1. Histological images of the bone marrow of the femur. A: Neoplastic cells diffusely proliferate, thereby replacing the bone marrow. B: The nuclei are large, oval to slightly irregular in shape. A small number of cells have kidney- (yellow arrowhead), ring- (green arrowhead), or lobe- (white arrowhead) shaped nuclei. Mitotic figure is indicated by white arrow. HE stain. Black bar=50 μm (A), 10 μm (B).

mia, and the mature type to human chronic myelogenous leukemia[1]. In the juvenile case, neoplastic cells in the bone marrow, spleen, and liver were dominated by poorly differentiated cells, whereas myeloid elements representing various degrees of maturation can be seen and the percentage of myeloblasts was low in some mature cases[2, 9]. In the present case, leukemic cells were dominated by an immature form with large, oval to slightly irregularly shaped nuclei. In addition, considering the clinical findings that general health deterioration, including progressive anemia and rough respiratory movement, developed acutely; therefore, the present case is assumed to be of the juvenile type.

In a previous publication on NOG mice, neoplastic lesions were generally uncommon; however, thymic lymphoma has been reported at 0.82% in the age group ranging from 16 to 40 weeks[7, 10]. To the best of our knowledge, spontaneous granulocytic leukemia has not been previously reported in NOG mice. In several strains of mice, including B6C3F1 and BALB/c, granulocytic leukemia rarely occurred[2, 11], and has not been reported in the case of these mice at 12 months of age or younger. The present case developed granulocytic leukemia at 51 weeks of age, which was a relatively young age. This novel information on spontaneous tumors may be helpful for the appropriate use of this mouse strain in further research.

Disclosure of Potential Conflicts of Interest: All authors declare that there are no conflicts of interest.
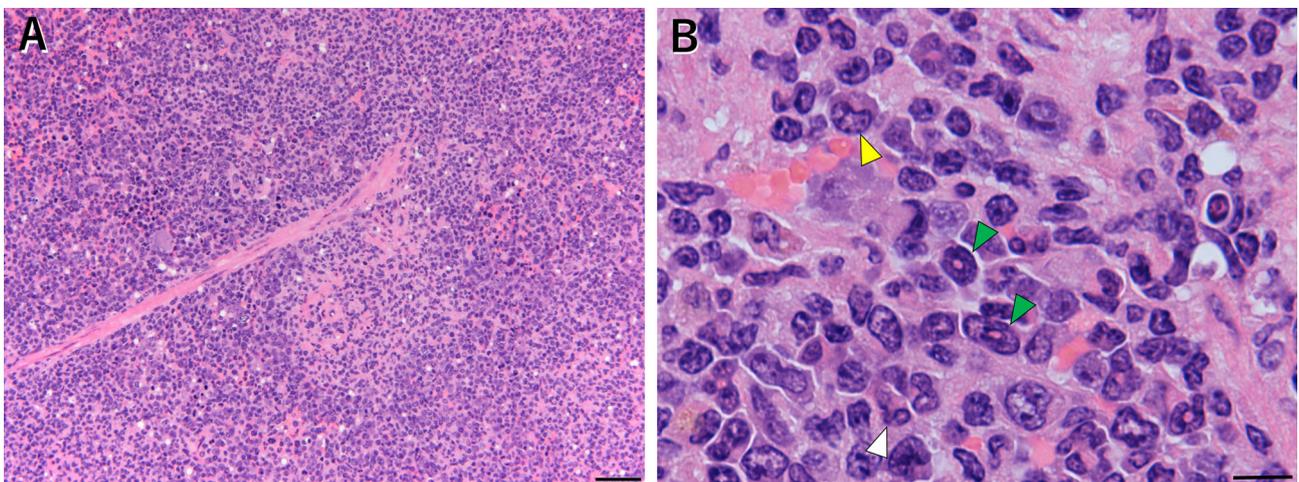
Fig. 2. Histological images of the spleen. A: Neoplastic cells are found throughout the red pulp. B: The nuclei are large, oval, slightly irregular, kidney- (yellow arrowhead), ring- (green arrowhead), or lobe- (white arrowhead) shaped nuclei. HE stain. Black bar=50 μm (A), 10 μm (B).
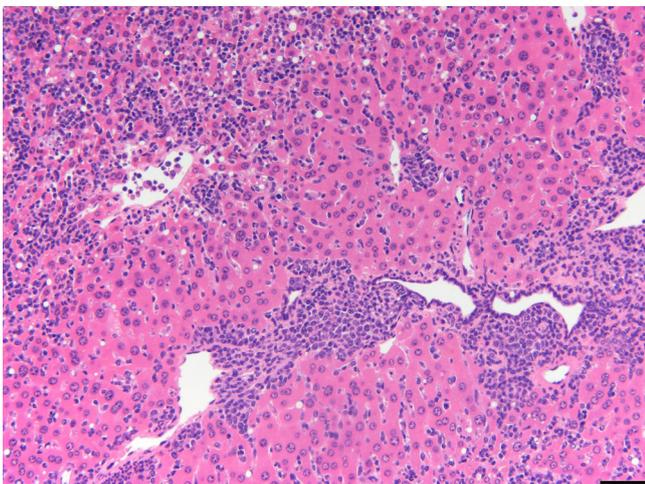
Fig. 3. Histological images of the liver. Neoplastic cells infiltrated the Glisson's capsule and the sinusoid. HE stain. Black bar=50 μm.
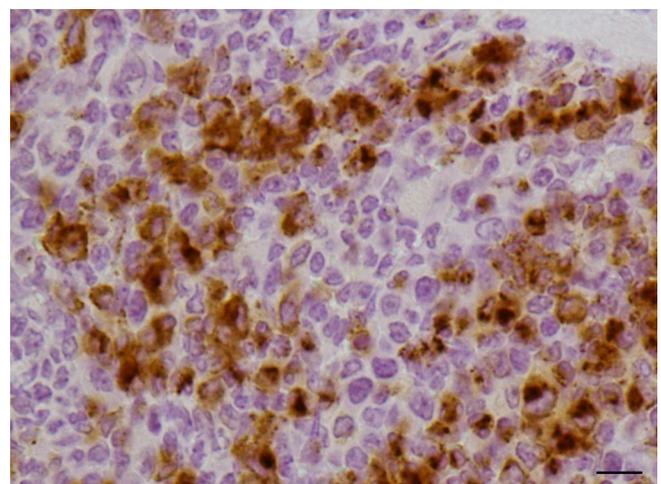
Fig. 4. Immunohistochemical staining of the spleen. A large population of the neoplastic cells in the red pulp is positive for myeloperoxidase. Immunohistochemical stain. Black bar=10 μm.

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