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Spontaneous primary B cell lymphoblastic lymphoma of the spleen of a 24-month-old male C57BL/6 mouse

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Abstract

Spontaneous lymphoid neoplasms are common among aged C57BL/6 mice, predominantly manifesting as T cell lymphomas. However, primary B cell lymphoblastic lymphomas are rare and poorly characterized, especially in laboratory animals. This case report describes a primary B cell lymphoblastic lymphoma in the spleen of a 24-month-old male C57BL/6 mouse. Gross examination revealed an enlarged spleen with a homogenous, white, firm mass replacing the normal parenchyma. Histopathological analysis revealed that the splenic architecture was diffusely replaced by neoplastic lymphoid cells with high mitotic activity. Immunohistochemical staining confirmed the B cell origin of the neoplasm, demonstrating strong CD20 positivity and CD3 negativity. This case underscores the importance of detailed pathological evaluation in aged laboratory mice, especially to distinguish spontaneous lesions from treatment-induced effects in experimental studies. These findings contribute to the understanding of lymphoid neoplasms in laboratory animals, as well as provide valuable reference data for pre-clinical research in toxicology and oncology.

Introduction

Spontaneous neoplasms are frequently observed in aged laboratory mice; these are documented to establish baseline data for control groups in experimental studies. Lymphoid neoplasms are among the most common malignancies in C57BL/6 mice. While both B and T cell lineages are observed, recent reports suggest a predominance of B cell lymphomas, particularly in aged animals.[1,2] However, certain sub-types, such as primary B cell lymphoblastic lymphomas, remain under-characterized and warrant detailed investigation. Lymphoblastic lymphomas are characterized by the proliferation of immature lymphoid cells (*i.e.*, lymphoblasts), which can affect both B and T cell lineages.[3,4] These neoplasms typically present as mass lesions in primary lymphoid organs, such as the spleen, thymus, and lymph nodes.[5,6] Lymphoblastic lymphoma is more frequently reported in younger laboratory mice and juvenile domestic animals, but this case report highlights its occurrence in an aged C57BL/6 mouse; thus, expanding our understanding of its occurrence.[7,8] Case reports of lymphoblastic lymphoma in laboratory animals are relatively rare, but these provide essential insight into the process of spontaneous disease.[9,10] While most cases in laboratory mice involve T cell lymphomas, cases of B cell lymphoblastic lymphomas have been documented sporadically, mostly in aged animals.[11] In domestic animals, particularly young dogs, lymphoblastic lymphomas are associated with aggressive behavior and poor prognosis.[12,13]

This report comprehensively describes the gross, histopathological, and immunohistochemical findings of a primary B cell lymphoblastic lymphoma in the spleen of a 24-month-old C57BL/6 mouse. These findings can serve as valuable baseline data for future research.

Case Description

The male C57BL/6 mouse was obtained from Koatech Technology Corporation (Pyeongtaek, Korea). This mouse was included in a national project by the Laboratory Animal Resources Bank, aiming to collect and archive pathological data from aged, untreated control animals for reference use in biomedical research. No treatment, experimental manipulation, or

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genetic modification was applied during the animal's lifetime. After 1 week of acclimatization, the mouse was housed as a part of a control group under monitored temperature, humidity, and light parameters (24.00 ± 2.00 ˚C, 50.00 ± 20.00% relative humidity, and 12/12 hr light/dark cycle, with lights on at 07:00), as well as *ad libitum* access to food and water. The treatment and experimental protocols were approved by the Institutional Animal Care and Use Committee of the Preclinical Research Center of the K-MEDI hub (Approval Number: KMEDI-23110901-01), following the relevant guidelines. At 24 months of age, the animal weighed 42.66 g. During routine necropsy, the spleen exhibited distinct gross morphological abnormalities. The head of the spleen was normal in size and color, but the remaining portions were markedly enlarged, measuring 23.00 × 7.00 × 6.00 mm (Fig. 1A) and exhibiting a uniform white discoloration (Fig. 1B). The affected regions of the spleen had irregular borders, and the normal splenic parenchyma was replaced with homogenous, white, firm tissue.

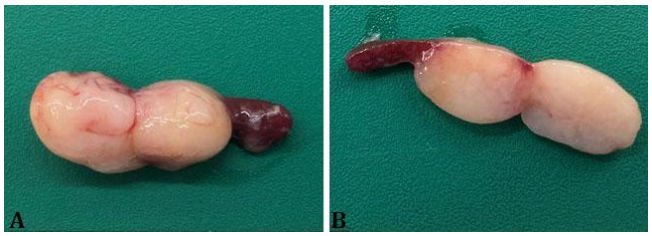

Fig. 1. Gross appearance of spleen. **A)** The head (dorsal region) appears normal, whereas the remaining portion is enlarged with white coloration; **B)** Longitudinal section of the spleen.

The tissues within the mass and of other organs were fixed with 10.00% neutral buffered formalin, except for the testes and eyeballs, being preserved in 10.00% neutral buffered formalin and then fixed with Davison's fixative (HFX1071; Gangdong Group Co., Eumseong, South Korea). The fixed tissues were prepared with a tissue processor (Thermo Fisher Scientific Inc., Runcorn, UK). The paraffin-embedded tissue blocks were prepared into 4.00-μm sections, mounted on glass slides, and then stained with Hematoxylin and Eosin using an autostainer (Dako Coverstainer; Agilent, Santa Clara, USA). Histochemical staining and Masson's trichrome staining were also performed. To characterize the lymphoid population, immunostaining was performed with the following primary antibodies: CD3 (Abcam, Cambridge, UK) and CD20 (Abcam). Negative controls were performed on normal splenic tissue sections by omitting the primary antibodies (CD3 and CD20). These controls showed no staining, thereby confirming the specificity of the immunohistochemical reactions.

Histopathological examination revealed diffuse effacement of the normal splenic architecture by sheets of neoplastic lymphoid cells (Fig. 2A). These cells were small to medium in size, approximately 10.00 - 12.00 μm in diameter, with scant deeply basophilic cytoplasm and indistinct cell borders. The nuclei were round to oval, containing finely dispersed chromatin and 1-2 prominent eosinophilic nucleoli (Fig. 2B). Occasional apoptotic bodies and a high mitotic index (10 mitoses *per* high-power field) were observed, indicating active proliferation. The gross appearance of coalescing white nodules was found to correspond histologically to an extensive expansion of neoplastic lymphoid cells originating from the white pulp, which progressively replaced the surrounding red pulp. This diffuse infiltration resulted in complete architectural effacement, eliminating the normal demarcation between white and red pulp regions. There was no evidence of stromal fibrosis or infiltration by other cell types, such as neutrophils or macrophages. No other infiltration of neoplastic cells was found in the liver, lymph nodes, or bone marrow. Immunohistochemical staining revealed that the neoplastic cells were strongly positive for CD20 and negative for CD3, respectively confirming their B cell origin and ruling out a T cell lineage (Fig. 3).

Based on the aforementioned pathologic analyses, the mass was diagnosed as a primary B cell origin lymphoblastic lymphoma of the spleen. According to the Bethesda classification for mouse lymphoid neoplasms, these cytological features, including uniform lymphoblast-like morphology, high mitotic activity, and diffuse architectural replacement, are consistent with a diagnosis of B-lymphoblastic lymphoma.

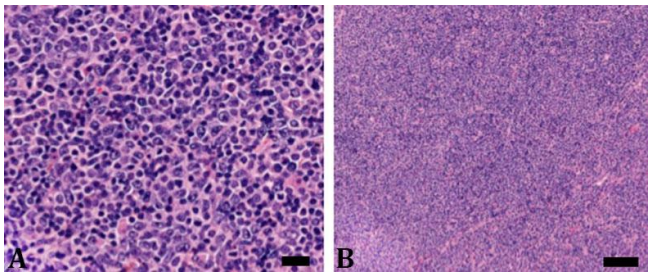

Fig. 2. Histopathological examination of the splenic mass. **A)** Normal splenic architecture was replaced with neoplastic cells, (Scale bar: 100 μm); **B)** Neoplastic cells were small to medium in size with scant cytoplasm and many mitotic figures, (Scale bar = 20.00 μm).

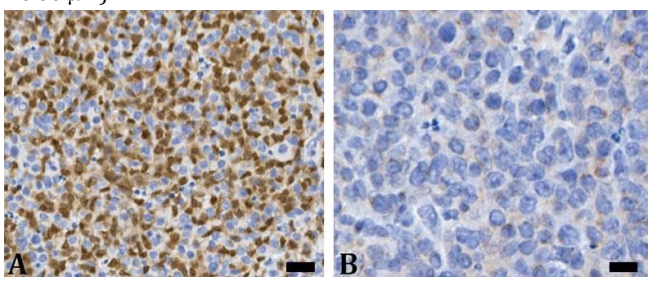

Fig. 3. Immunohistochemical analysis of the neoplastic cells. **A)** The CD20 antibody was strongly positive, suggesting a B cell origin, (Scale bar: 10.00 μm); **B)** The CD3 antibody was negative, ruling out a T cell origin, (Scale bar = 20.00 μm).

Discussion

Lymphoid neoplasms are a well-documented spontaneous pathology in aged C57BL/6 mice, with B cell lymphomas, particularly of follicular type, being commonly observed in the spleen and mesenteric lymph nodes.[8] The present case is a rare occurrence of primary B cell lymphoblastic lymphoma, as evidenced by the strong CD20 positivity and absence of CD3 expression. This case is notable because lymphoblastic lymphomas of B cell origin are uncommon in both laboratory and domestic animals.[14,15] Lymphoblastic lymphomas are aggressive neoplasms typically characterized by the proliferation of immature lymphocytes.[16] Interestingly, the present case was observed in an aged mouse but showed a localized presentation with no evidence of systemic involvement, which may reflect a biologically less aggressive behavior. In this case, although the neoplastic cells exhibited high mitotic activity, the lesion was confined to the spleen without systemic involvement. This may suggest that the tumor was detected at an early stage, that the C57BL/6 strain has unique tumor biology influencing disease progression, or that this represents a relatively indolent variant of B cell lymphoblastic lymphoma. In domestic animals, particularly dogs, B cell lymphoblastic lymphomas often manifest as a multi-centric disease, involving the spleen, lymph nodes, and bone marrow. These neoplasms are highly proliferative and herald a poor prognosis due to their aggressiveness.[17] In contrast, the indolent appearance of the neoplasm in this mouse highlights the variability in disease presentation and progression, despite having the same tumor classification. This case underscores the importance of comprehensive baseline data regarding spontaneous neoplasms in aged laboratory mice, being especially helpful for distinguishing treatment-induced effects from background lesions in pre-clinical studies. These findings also emphasize the need for advanced diagnostic techniques, such as immune-histochemistry, to accurately classify lymphoid neoplasms. The CD20, although is not the most commonly used B cell marker in murine pathology, was utilized in this case due to its proven immunoreactivity in mouse tissue and its strong expression in late pre-B to mature B cell stages. While markers, such as CD45R (B220), paired box 5 (PAX5), and CD19, are typically preferred for precise B cell phenotyping, they were not available at the time of analysis. Future cases will benefit from the inclusion of a broader panel of B cell markers for more accurate sub-classification. Differential diagnosis was also considered. Marginal zone and follicular lymphomas generally retain partial splenic architecture and exhibit nodular or follicular patterns, whereas in the present case the normal splenic architecture was completely effaced by uniform lymphoblast-like cells. Moreover, myeloid leukemias with lymphoid morphology are typically accompanied by systemic involvement or evidence of granulocytic/ monocytic differentiation, which were absent in this case. These findings support the diagnosis of a primary B cell lymphoblastic lymphoma. Since B cell lymphoblastic lymphomas in C57BL/6 mice are rare, this case can be particularly valuable among researchers studying the pathogenesis of lymphoid tumors and their implications in experimental and comparative oncology.[18,19] The documentation of this case expands our existing knowledge base of lymphoblastic lymphomas in laboratory animals. Aside from enhancing our understanding of spontaneous pathologies, such reports also provide critical insights for interpreting experimental results in toxicology and oncology research. Although histopathological examination did not reveal infiltration in the liver, lymph nodes, or bone marrow, additional analyses, such as bone marrow cytology and peripheral blood evaluation, were not performed. This represents a limitation of the present case, and future reports would benefit from including these methods to more conclusively exclude leukemic transformation or multi-centric disease.

This case report describes a primary B cell lymphoblastic lymphoma in the spleen of a 24-month-old male C57BL/6 mouse. The gross and histopathological findings, alongside the immunohistochemical analysis, provide valuable reference data for spontaneous lymphoid neoplasms in aging laboratory mice. This data can build on our understanding of background pathologies in pre-clinical studies.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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