Primary Splenic Lymphoma in a Horse

Tadashi TANIMOTO, Shin-ichiro YAMASAKI, and Yuji OHTSUKI*1
Chuo Meat Inspection Laboratory, Kochi Prefecture, 38-1 Ebinomaru, Kochi 780 and 1Department of Pathology, Kochi Medical School, Kochi, Oko, Nankoku 783, Japan
(Received 8 October 1993/Accepted 22 February 1994)

ABSTRACT. A well-demarcated solitary splenic mass (20 × 20 × 15 cm in size) containing hemorrhagic and necrotic foci was observed in a 4-year-old Thoroughbred stallion. Histologically, the mass consisted of lymphoma cells of the diffuse large non-cleaved type, with a high mitotic index and scattered macrophages that formed a starry sky pattern. The lymphoma cells revealed diffuse positivity for acid phosphatase and alpha naphthyl butyrate esterase, and were also positive for intracytoplasmic IgM on occasion, and mostly for proliferating cell nuclear antigen. Ultrastructural examination revealed moderately-developed rough endoplasmic reticulum sometimes with dilated cisternae. Thus, the diagnosis was a primary splenic lymphoma of B cell origin, but the exact reason for the absence of invasive growth or metastasis despite the high proliferative activity of this neoplasm was unclear. —KEY WORDS: horse, lymphoma, splenic neoplasm.


Little is known about primary splenic lymphoma in domestic animals [1, 8, 10]. We describe an equine case of primary splenic lymphoma that was investigated by histological, enzyme cytochemical, immunohistochemical, and ultrastructural techniques.

A 4-year-old Thoroughbred stallion race horse was slaughtered after fracturing the left foreleg. Until the time of the fracture, the animal had been apparently healthy.

Routine autopsy revealed a solitary mass, 20 × 20 × 15 cm in size, in the ventral part of the spleen (Fig. 1). The firm, well-demarcated mass was yellow-white in color and contained scattered foci of hemorrhage and necrosis. Besides the bone fracture, no gross abnormalities were observed in the other organs such as the regional lymph nodes, liver, and bone marrow.

Microscopical examination showed that the mass was well-demarcated but not encapsulated and compressed the adjacent splenic tissue. It consisted of diffuse proliferation of lymphoma cells (Fig. 2) associated with hemorrhagic and necrotic foci. The lymphoma cells were large in size. Their round to oval nuclei contained moderate amounts of margination of chromatin, had a thin nuclear membrane, and contained a few nucleoli (Fig. 3). These cells had moderate amounts of amphiphilic cytoplasm and the mitotic index was high (Fig. 3). According to the National Cancer Institute Working Formulation, these findings were compatible with diffuse large cell non-cleaved lymphoma [9]. Throughout the mass, scattered macrophages showing a starry sky pattern (Fig. 2) and fibromuscular stromal cells were observed. The red pulp of the adjacent spleen contained many siderophages. No abnormalities were seen either in any of the other organs or blood smear.

Enzyme cytochemical studies showed that the lymphoma cells were weakly positive for acid phosphatase (ACPase) and alpha naphthyl butyrate esterase (ANBE) in a diffuse granular pattern, but were negative for alkaline phosphatase (ALPase), beta glucuronidase (BG), and peroxidase (PO). Staining of fresh mass imprints was done using kits (BG kit from Sigma Chemical Co., U.S.A. and the others from Muto Pure Chemicals, Japan).

Immunohistochemical studies were performed with an avidin-biotin-peroxidase complex kit (HISTOFINE SAB-PO kit, Nichirei, Japan) using formalin-fixed, paraffin-embedded sections of the neoplasm, and antibodies to horse IgM (Bethyl Laboratories, U.S.A.) and proliferating cell nuclear antigen (PCNA, clone PC10, Dako, Japan). The specific reaction was visualized with diaminobenzidine solution containing NiCl2 to intensify the positivity. Lymphoma cells were positive for intracytoplasmic IgM on occasion (Fig. 4), and mostly for PCNA (Fig. 5).

Ultrastructural examination showed that the large lymphoma cells had moderate amounts of marginalized heterochromatin in a round to oval nucleus and poorly developed cytoplasmic organelles (Fig. 6), including some strands of rough endoplasmic reticulum (rER) with occasional dilation of cisternae, scattered polysomes, some large mitochondria, clustered coated vesicles, and some lipid droplets. No viral particles were seen.

In humans as well as horses, primary splenic lymphoma (PSL) is very rare, although secondary involvement is common [7, 8]. Human PSL usually affects the while pulp, grows diffusely and is generally of the small lymphocytic, large cell, or mixed cell type [7]. In contrast, large cell and immunoblastic lymphomas are believed to be the most common types of PSL in horses [10]. The present case also showed a simple diffuse growth pattern of the large cell type.

With regard to equine lymphoma, only a few immunohistochemical and ultrastructural studies are available besides the conventional clinical, anatomical, and histological data [2, 3, 8]. Therefore, we could not fully interpret our findings. Diffuse granular positivity for ACPase and ANBE is also a feature of B cell lymphoma in humans together with intracytoplasmic immunoglobulin positivity, strands of rER, and a non-cleaved nucleus containing margination of chromatin [4, 6]. On the basis of these findings, the present case was diagnosed as primary splenic lymphoma of the diffuse large non-cleaved type, B

* CORRESPONDENCE TO: OHTSUKI, Y., Department of Pathology, Kochi Medical School, Kochi, Oko, Nankoku, Kochi 783, Japan.
Fig. 1. Cut-surface of the well-demarcated large solitary mass (asterisks) at the ventral part of the spleen, revealing scattered hemorrhagic and necrotic foci. Bar = 10 cm.

Fig. 2. Diffusely proliferated lymphoma cells intermingling scattered macrophages in a starry sky pattern. May-Giemsa. × 150.

Fig. 3. Higher magnification of Fig. 2. The large-sized lymphoma cells possessing round to oval nuclei and moderate amounts of cytoplasm. Note many mitotic figures (arrows). May-Giemsa. × 470.

Fig. 4. Intracytoplasmic immunopositivity for IgM in some lymphoma cells. Avidin-biotin-peroxidase complex method with enhanced diaminobenzidine reaction. Without nuclear stain. × 300.

Fig. 5. Nuclear immunopositivity for proliferating cell nuclear antigen in most of lymphoma cells. Avidin-biotin-peroxidase complex method with enhanced diaminobenzidine reaction. Without nuclear stain. × 300.

Fig. 6. An electron micrograph showing a representative lymphoma cell with moderate amounts of margined heterochromatin and a prominent nucleolus in a round nucleus. The cytoplasm contained some strands of rough endoplasmic reticulum (rER), numerous polysomes, and several large mitochondria. × 5,300.
cell origin. Human PSL often metastasizes to the splenic lymph nodes but rarely to other organs, although metastases to the bone marrow, liver, and lymph nodes have been reported [5].

Interestingly, no invasive growth or metastasis was observed in the present case, although the lymphoma cells showed a high proliferative activity. Moreover, human PSL with only splenic involvement had a favorable prognosis [5], and the present case showed no significant clinical abnormalities despite the large solitary splenic mass.

ACKNOWLEDGEMENT. We wish to thank Dr. S. Ishino (Tohoku Branch Laboratory, National Institute of Animal Health) for his advice with the immunohistochemistry.

REFERENCES