

Immunohistochemical Study of Lymphomas of Abdominal Cavity Origin in Two Cows with Bovine Leukemia Virus

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Abstract

Lymphoid neoplasms of abdominal cavity origin were found in two cows infected with bovine leukemia virus (BLV). Case 1 was a 3-year-old Japanese Black cow affected with a B-1 B cell lymphoma. Case 2 was a 14-year-old Japanese Black cow that developed a $\gamma\delta$ T cell lymphoma. Immunohistochemically, CD79a-positive lymphoma cells were observed in case 1, whereas CD3-, WC1- or perforin-positive ones were seen in case 2. Despite the expression of CD5 in both lymphomas, the reactivity was surface or cytoplasmic in case 1 and surface membrane in case 2. Erythrophagia by tumor cells of both cases was considered to be due to the fact that their normal counterparts were more primitive forms than conventional lymphocytes.

Discipline: Animal health

Additional key words: B-1 B cell, CD5, $\gamma\delta$ T cell

Introduction

Innate-like lymphocytes are a type of lymphocyte that contribute to rapid responses to infection by acting early but which use a limited set of antigen-receptor gene segments to make immunoglobulins and T-cell receptors⁵. Three main classes of innate-like lymphocytes are B-1 cells, epithelial $\gamma\delta$ T cells and natural killer T cells, and B-1 cells and epithelial $\gamma\delta$ T cells defend the body cavity and body surfaces, respectively⁵.

Adult-form lymphoma, which is associated with bovine leukemia virus (BLV) infection, is immunohistochemically dividable into three types; B-1a, B-1b and B-2¹⁴. $\gamma\delta$ T cell lymphomas were found in BLV-negative cattle, and could be classified into several histologic subtypes^{7,11}. Although adult-form lymphoma is generalized in tissue distribution^{12,14}, $\gamma\delta$ T cell lymphoma has a tendency to affect the skin and mucous membranes^{7,10,11}. In this paper, we describe two cases of lymphoma of innate-

like lymphocyte origin (B-1 B cell lymphoma and $\gamma\delta$ T cell lymphoma) that were thought to have arisen from the abdominal cavity.

Materials and methods

Case 1 was a 3-year-old Japanese Black cow with a 2-month history of enlarging tumor masses in the pelvic area. In addition to dysuria with arching of the back from two days earlier, anorexia and colic appeared. Because of poor prognosis, the cow was euthanized the next day.

Case 2 was a 14-year-old Japanese Black cow. The cow showed anorexia and emaciation, and became unable to stand. The condition did not improve, and euthanasia was performed 6 days after onset of the clinical signs.

For the diagnosis of BLV infection, agar gel immunodiffusion⁸ and polymerase chain reaction³ were employed for case 1 and case 2, respectively, and both animals were found positive.

Tissues were fixed in 10% buffered formalin, embed-

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ded in paraffin, cut at 4 μ m and stained with hematoxylin and eosin (HE) and Giemsa. For immunohistochemical staining, the avidin-biotin-peroxidase complex (ABC) method was applied to paraffin sections. The primary antibodies used were rabbit polyclonal antibodies to CD3 (Dako, Glostrup, Denmark) and CD5 (Lab Vision, Fremont, CA, USA), and mouse monoclonal antibodies to CD79a (Dako), WC1-N3 (Veterinary Medical Research and Development, Pullman, WA, USA), macrophages (HAM56) (Dako), and perforin (Sumitomo Electric Industries, Osaka, Japan). The sections were pretreated by enzymatic digestion with pepsin (CD3, perforin) or microwave heating (CD5, CD79a, WC1-N3, HAM56). Subsequent procedures were performed using kits (Nichirei, Tokyo, Japan).

Results

Case 1

At necropsy, multiple tumor masses up to 30 cm in diameter were present on the peritoneum. The cut surface was white, with a soft friable area of necrosis in the center in larger masses.

Histologically, tumor cells proliferated diffusely in the intra-abdominal tumor tissues. The muscularis and subserosa of the urinary bladder were heavily infiltrated by tumor cells, and groups of tumor cells were present in lymphatic channels of the lamina propria. However, epitheliotropic tumor cells were absent. There were no neoplastic lesions in the liver, spleen, kidneys, heart, lungs, or mesenteric and hepatic lymph nodes.

The tumor cells varied in diameter from 5 to 17 μ m. Small to medium-sized cells predominated in some regions (Fig. 1A), and larger cells in others (Fig. 1B). Atypical large cells, which were characterized by vesicular nuclei, irregular nuclear contours or prominent nucleoli, were at times seen throughout the lesions (Figs. 1A & 1B). Occasional cells showed erythrophagocytosis (Fig. 1C). Immunohistochemical results are presented in Table 1. The neoplastic cells immunostained for cytoplasmic CD79a and surface or cytoplasmic CD5 (Fig. 1D), but not for CD3, WC1 or HAM56.

Table 1. Immunohistochemistry of lymphoma cells

	Case 1	Case 2
CD3	–	+
WC1	–	+
CD5	+	+
CD79a	+	–
HAM56	–	–

+: positive, –: negative.

Case 2

At necropsy, great numbers of grayish white tumor masses, which were often fused, were present on the parietal peritoneum near the lumbar vertebrae. Similar but smaller ones were found on the parietal pleura near the thoracic vertebrae. Tumor nodules 2 or 3 cm in diameter were sparsely distributed on the great omentum and mesenterium. The mediastinal, bronchial and hepatic lymph nodes were enlarged.

The neoplastic tissues in the body cavities were composed of diffuse neoplastic growths, and variously sized areas of hemorrhage and/or necrosis were often observed. The architecture of the enlarged lymph nodes examined was completely effaced by tumor cells. No neoplastic lesions were seen in the liver, spleen, kidneys, heart, or lungs.

The tumor cells measured 4 to 15 μ m in diameter. Most areas consisted of medium-sized cells (Fig. 2A), which were admixed with larger cells in some areas (Fig. 2B). There was more frequent erythrophagia than in case 1 (Fig. 2C). The neoplastic cells frequently exhibited cell membrane immunostaining for CD5 (Fig. 2D). Similar numbers of CD3-positive lymphoma cells were detected, but the majority showed cytoplasmic staining. In parts, WC1-positive or perforin-positive lymphoma cells were ascertained. Neither CD79a-positive nor HAM56-positive neoplastic cells occurred.

Discussion

Yin et al.¹⁴ divided adult-form lymphomas into B-1a, B-1b and B-2 cell types. The first type is characterized by CD5 positivity. B-1, or CD5-positive, B cells are a minority subset of B cells comprising about 5% of all B cells in mice and humans, and are present in large numbers in the peritoneal and pleural cavity fluids⁶. This suggests that CD5-positive adult-form lymphoma may arise from the body cavities. The present BLV-associated lymphoma (case 1), which was CD5 positive and had neoplastic lesions within the abdominal cavity, was considered to be a case supporting this view. In the CD5-positive cases reported by Yin et al.¹⁴, there were no cases suggestive of abdominal cavity origin.

The majority of bovine WC1-positive $\gamma\delta$ T cell lymphomas affect the skin or mucous membranes, and show epitheliotropism^{7,10,11}. This corresponds to the fact that WC1-positive $\gamma\delta$ T cells are found in high numbers in the tissues and seem to defend the body surfaces^{5,13}. In the lymphoma in case 2, some neoplastic cells expressed WC1, and neoplastic lesions were present chiefly within the abdominal cavity. The lymphoma was thought to be of intra-abdominal $\gamma\delta$ T cell origin⁷. Like B-1 cells, $\gamma\delta$ T

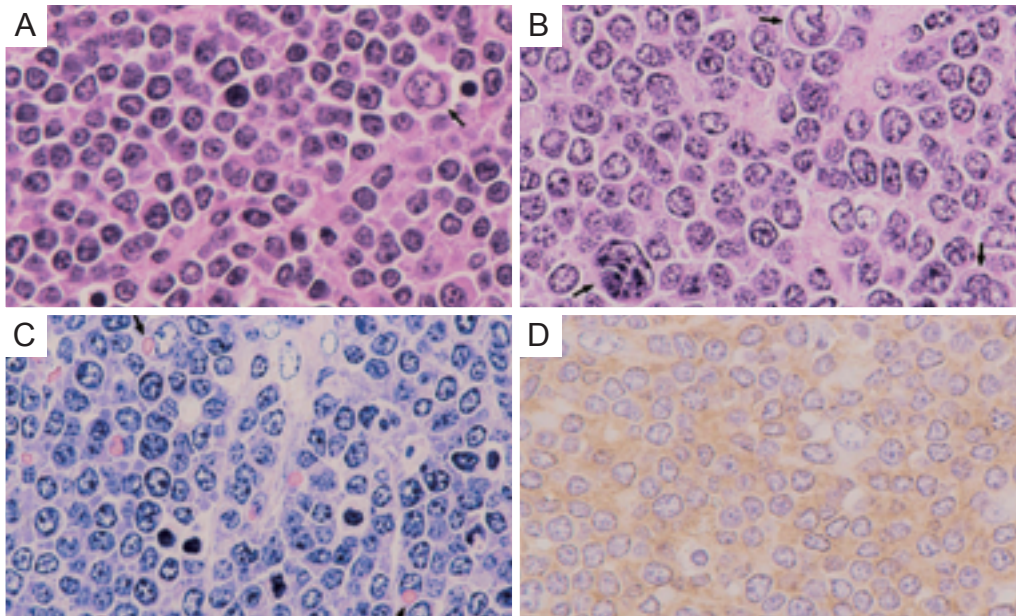


Fig. 1. Histology and immunohistochemistry in case 1

- A: Most tumor cells are small or intermediate in size and the nuclear chromatin is relatively densely clumped, but an atypical larger cell with a vesicular nucleus is visible (arrow). HE. $\times 500$.
- B: Atypical cells with bizarre nuclei (arrows) are admixed with medium-sized to large cells with moderately clumped chromatin. HE. $\times 500$.
- C: Neoplastic cells showing erythrophagocytosis (arrows) vary in size. Giemsa. $\times 500$.
- D: Nearly all tumor cells express CD5 in the cytoplasm. ABC. $\times 500$.

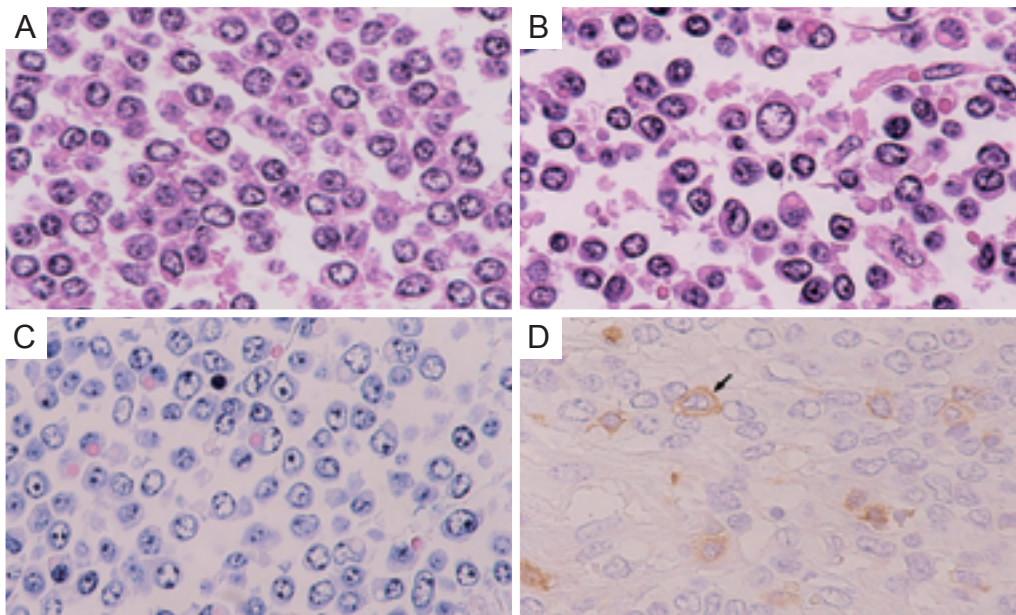


Fig. 2. Histology and immunohistochemistry in case 2

- A: In this area, there are medium-sized lymphoid cells which are fairly uniform in size and shape. HE. $\times 500$.
- B: Except for cell size, the lymphoma cells are morphologically similar. HE. $\times 500$.
- C: A fair number of erythrophagocytic tumor cells are visible. Giemsa. $\times 500$.
- D: Anti-CD5 shows positive staining of some lymphoma cells, and staining is on the surface membrane (arrow). ABC. $\times 500$.

lymphocytes may play a role in defending the body cavities in cattle.

In case 2, the 14-year-old cow was infected with BLV, but the lymphoma seemed to be etiologically unassociated with BLV because cattle with $\gamma\delta$ T cell lymphoma are usually BLV negative^{7,11}. If immunohistochemistry had not been performed, the lymphoma might have been mistakenly diagnosed as adult-form lymphoma of abdominal cavity origin. Compared with case 1, however, the lymphoma cells were considerably uniform in morphology, and atypical large cells were absent. Such cytologic differences may be helpful in arriving at a correct diagnosis; when a lymphoma showing the cytologic features described above is found in an adult cow with BLV, immunostaining for lymphocyte markers is needed. By this methodology, however, B cell lymphomas unrelated etiologically to BLV cannot be distinguished from those etiologically related to BLV in BLV-infected cattle.

As in the present lymphomas, erythrophagocytosis has been observed in a bovine natural killer-like T cell lymphoma⁹. The normal counterparts of these lymphomas are known as innate-like lymphocytes, and lie at the interface between innate and adaptive immunity^{4,5}. Innate-like lymphocytes are older than conventional ones in terms of evolutionary history, and stand at phylogenetic positions nearer to macrophages^{1,2,6}. These facts suggest that phagocytic activity may not be infrequent in neoplasms of evolutionarily older lymphocytes.

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