Cytology of B Cell Lymphomas in Cattle Infected with Bovine Leukosis Virus

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Abstract
Lymphoid neoplasms in 17 cattle having tested positive for bovine leukemia virus (BLV) were investigated by histology and immunohistochemistry. In 16 cases, the neoplastic cells were characterized by a marked variation in cell size, atypia in large cells, nuclear irregularity in smaller cells, and nucleolar prominence in cells with scant cytoplasm. These cytological features were used to specifically define pleomorphic lymphomas. In addition to lymphoid cells, immunoblastoid and plasmacytoid cells containing cytoplasmic immunoglobulin G were observed in the remaining case. Since a similar case was recorded as lymphoplasmacytoid lymphoma in a BLV-negative cow, this polymorphic case may not be related to the virus. Most lymphoma cases associated with BLV are classified as pleomorphic, and neoplasms termed enzootic leukemia should be diagnosed based on tumor cell morphology.

Discipline: Animal health
Additional key words: enzootic bovine leukemia, pleomorphism, polymorphism

Introduction
Enzootic bovine leukemia or adult form leukemia, which is presumed attributable to bovine leukemia virus (BLV), presents as a B cell lymphoma and is most frequently seen in animals between three and eight years of age¹²,¹⁶, though it may also arise in calves⁹. Taking into account the facts that only a small percentage of BLV-infected animals develop lymphoid neoplasms¹⁶ and that T cell neoplasms were observed in BLV-infected cattle, a relatively large population of diagnosed enzootic leukemia may be truly sporadic¹³.

Numerous bovine lymphoid neoplasms were classified using the National Cancer Institute Working Formulation for human lymphomas. Via this classification system the cleaved variant of the diffuse large cell type has been shown to constitute 38% of enzootic lymphomas versus 14% of sporadic lymphomas⁸⁰. Yin et al.²¹ examined 47 cases of enzootic and sporadic leukemia, which were divided into four histological types: lymphocytic; well-differentiated prolymphocytic; poorly-differentiated prolymphocytic; and histicytic. These classifications are based on the cell size and/or nuclear cleavage and fail to distinguish between BLV-positive and negative cases. In contrast, some cases of BLV-positive lymphoma, which were diagnosed as pleomorphic B cell lymphoma based on pleomorphism and atypia⁹,¹¹, were cytologically distinct from other lymphoid malignancies¹²,⁶-¹⁰,¹³,¹⁴,¹⁷,¹⁸.

In this paper, we describe the histology and immunohistochemistry of B cell lymphomas in 17 cows having tested positive for BLV by nested polymerase chain reaction (PCR). All, except one case of lymphoplasmacytoid lymphoma, were diagnosed as pleomorphic, and neoplasms termed enzootic leukemia should be diagnosed based on tumor cell morphology.

Materials and methods

1. Animals and gross pathology
In abattoir surveys, 17 cases of lymphoma were found in female cattle aged between two and 14 years. The youngest animal was 24 months old (case 17) and the second youngest was 29 months old (case 3). All cases, except 5 (Japanese Black) and 17 (F1 hybrid), were Hol-
stein cattle. Bilateral exophthalmos and the enlargement of some superficial lymph nodes were detected in case 11, and gait disturbance and tachypnea in case 17. The other animals appeared healthy. Cases 1-16 were macroscopically characterized by lymph node enlargement, intra-thoracic or intra-abdominal tumor masses, or tumor formation in the heart, uterus or skeletal muscles. The spleen and liver were not enlarged. In addition to enlargement of the thoracic and abdominal lymph nodes, the spleen and liver were severely swollen in case 17.

2. Nested PCR analysis of BLV

DNA was obtained from blood (cases 1, 2, 5), tissues (cases 3, 4, 10, 13, 16) or both (cases 6-9, 11, 12, 14, 15, 17). As blood samples, buffy coat was utilized in case 17, and peripheral blood leukocytes were collected by centrifugation after lysing erythrocytes by ammonium chloride treatment in the other cases. DNA was extracted from these samples using InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacture’s instructions. Nested PCR was carried out to amplify a 444 bp fragment of the env gene by using a method described previously.

3. Histological and immunohistochemical examinations

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Selected sections were dewaxed and labeled by the streptavidin-biotin-peroxidase complex (SAB) method. The primary antibodies used were: rabbit polyclonal antibodies to immunoglobulin M (IgM) (μ chain specific) and IgA (α chain specific) (1:1600; Bethyl Laboratories, Montgomery, TX, USA), to κ light chain and λ light chain (1:1600; Bethyl), and to CD3 (1:50; Dako A/S, Glostrup, Denmark) and CD5 (prediluted; Lab Vision, Fremont, CA, USA): a sheep polyclonal antibody to IgG (γ chain specific) (1:1600; Bethyl); and a mouse monoclonal antibody to CD79a, HM57 (1:25; Dako A/S). Antigen retrieval was performed by enzymatic digestion with pepsin at 37°C for 25 min (IgM, IgG, IgA, CD3) or microwave heating in 0.01 M citrate buffer (pH 6.0) at 90°C for 9 min (CD5, CD79a). Subsequent procedures were carried out via an immunoperoxidase labeling system (Nichirei, Tokyo, Japan).

Results

1. PCR analysis findings

All animals were found to be positive for BLV by PCR testing.

2. Histological findings

In all cases, histological analysis demonstrated that neoplasms were observed mainly in the macroscopically visible lesions. No neoplastic tissues were seen in the spleen and hepatic parenchyma of cases 1-14. In cases 15 and 16, there were a number of intrasinusoidal or intravascular neoplastic cells in the liver, and sparsely or relatively densely distributed neoplastic cells in the spleen. In case 17, the splenic architecture was effaced by neoplastic cell infiltration, and numerous neoplastic cells were present in the lumens of alveolar capillaries and larger blood vessels, as well as in hepatic sinusoids and portal triads.

In cases 1-16, the neoplastic cells were highly variable in cell size among the affected organs examined (Figs. 1, 2), among areas in a single neoplastic tissue, or in a single microscope field (Fig. 3). Atypical large cells, which contained highly irregular nuclei and occasionally prominent nucleoli, were readily discernible in most cases (Figs. 1, 3) but considerably rare in others (Fig. 4). We often detected prominent nucleoli in neoplastic cells with scant cytoplasm (Fig. 4). In areas consisting of relatively small or variously sized neoplastic cells, smaller cells frequently or sometimes possessed irregularly contoured nuclei (Fig. 5). These four cytological features were observed in all pleomorphic cases. In case 17, the neoplastic tissues comprised lymphoid, immunoblastoid and plasmacytoid cells (Fig. 6), which had round, oval or slightly irregular nuclei with inconspicuous nucleoli. The cytoplasm was abundant in the latter two.

3. Immunohistochemical findings

The neoplastic cells in cases 1-16 expressed CD79a and CD5 (Fig. 7), though CD5-positive cells varied in number between the different cases. The other cell markers tested were absent. In case 17, the neoplastic cells were CD79a and CD5 positive, and we observed the presence of cytoplasmic IgG (γ and λ chains) in plasmacytoid and immunoblastoid cells (Fig. 8).

Discussion

In previous reports of enzootic bovine leukosis, the cytological classifications were primarily based on tumor cell size, which can cause difficulties in classifying enzootic bovine leukosis from other leukoses. Similar to the reports for pleomorphic B cell lymphoma, in this study the main cytological findings from cases 1-16 were pleomorphism and atypia, which are not so prominent in other histological types of lymphoid neoplasms. Such features were considered characteristic of the majority of lymphoma cases truly associated with BLV. Relatively similar features are observed in human and simian
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retrovirus-associated T cell lymphomas\textsuperscript{4,13} but not in B cell lymphomas listed in classifications of human lymphoid neoplasms\textsuperscript{11}.

The nuclear shape was an important criterion for classifying bovine lymphomas according to the National Cancer Institute Working Formulation\textsuperscript{20}. Nuclear cleavage is characteristic of human follicle center cell lymphoma, and also visible in the normal counterpart cells\textsuperscript{11}. In the present pleomorphic cases, the nuclear irregularity was highly marked, presenting a bizarre appearance. Such irregularity resembles that of highly malignant sarcomas or carcinomas\textsuperscript{5}, and does not exist in normal lymphocytes.

In an immunohistochemical study, 16 of 33 enzootic leukosis cases were CD5 negative\textsuperscript{21}. However, in our study CD5 was expressed on neoplastic cells from cases 1-16, although the number of CD5-positive neoplastic cells varied from case to case. This suggests that the CD5 expression tends to be lost or greatly diminished during neoplastic transformation. Since similar phenomena were observed for other lymphocyte markers in bovine lymphoid neoplasms\textsuperscript{8,13}, it is possible that cases with few positive cells are mistakenly judged negative.

Lymphoid malignancies in the terminal stage are frequently characterized by widespread distribution of neoplastic lesions and the existence of many neoplastic cells in blood, and share similar macroscopical features. In the current study, most of the animals appeared healthy, and were thought to be at relatively early stages of tumor development. In cases 1-14, there were no neoplastic tissues in the parenchyma of the spleen and liver. Cases 15 and 16 were considered to be somewhat more advanced, because the spleen was moderately affected. Although clinical abnormalities were noted in case 17, the severe involvement of the spleen, liver and peripheral blood and the absence of extranodal tumor masses were reminiscent of a lymphoma with a marked tendency to become leukemic\textsuperscript{6}.

There were no Ig-producing lymphoma cells in cases 1-16, which is an important feature of pleomorphic B cell lymphoma\textsuperscript{13}. In case 17, conversely, immunoblastoid and plasmacytoid cells showed IgG production. Similar cells were observed in lymphoplasmacytoid and lymphoplasmacytic lymphomas in BLV-negative cattle\textsuperscript{6,13}. As in the former, CD5 was expressed on neoplastic cells in case 17, and a diagnosis of lymphoplasmacytoid lymphoma was made\textsuperscript{6}. This case was not considered to be one of enzootic leukosis, in which the presence of surface Ig was demonstrated but not that of cytoplasmic Ig\textsuperscript{19}. As most lymphoid neoplasms in cattle can be classified into discrete histological types based on scientific evidence\textsuperscript{8,13}, the ambiguous typology, in which only four forms of leukosis are listed based on the animal’s age, sites of tumor formation and/or BLV infection\textsuperscript{12,16} should not be used in histopathological studies.

References