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Nodal Pleomorphic B Cell Lymphoma at the Earliest Stage of Tumor Development in a Cow

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Abstract

Pleomorphic B cell lymphoma was detected in a small number of lymph nodes in an 8-year-old Holstein cow with persistent lymphocytosis associated with bovine leukemia virus (BLV). Necropsy showed enlargement of the left superficial inguinal and right deep inguinal lymph nodes. Large numbers of BLV copies were detected by real-time polymerase chain reaction analysis in the enlarged nodes, whereas little BLV was found in normal-sized lymph nodes. Histologically, the enlarged lymph nodes were occupied by diffusely growing pleomorphic cells. In a few other lymph nodes, in contrast, extensive areas of neoplastic mantle cells showing a transition to more malignant pleomorphic cells were observed. These virological and histological findings indicate that large numbers of viral copies may be closely related to transformation from neoplastic mantle cells to pleomorphic lymphoma cells. The current study supports the view that nodal pleomorphic B cell lymphoma is of mantle cell origin.

Discipline: Animal health
Additional key words: Bovine leukemia virus, lymphoma in situ, mantle zone, neoplastic transformation, persistent lymphocytosis

Introduction

B-1 B cells constitute a unique subset of B cells comprising about 5% of all B cells in humans and mice, but they are the major population of B cells in rabbits. The cells express the surface protein CD5 and play a role in defending body cavities (Murphy et al. 2008). In human B cell lymphomas, the majority are CD5-negative, but chronic lymphocytic leukemia and mantle cell lymphoma are characterized by CD5 positivity, with cyclin D1 characteristically expressed in the latter (Feller & Diebold 2004). Most cases of the former are postulated to correspond to antigen-experienced B cells (Chiorazzi et al. 2005), and may differentiate into cells with cytoplasmic immunoglobulin (cIg) (lymphoplasmacytoid lymphoma) (Lennert & Feller 1992). By contrast, most cases of the latter are postulated to be derived from follicular mantle cells corresponding to naive pre-germinal center B cells, and are usually negative for cIg (Feller & Diebold 2004).

In cattle, CD5-positive B cells constitute up to 30% of the adult peripheral blood lymphocyte population (Naessens & Williams 1992). CD5 is expressed in most peripheral B cell lymphomas, such as pleomorphic B cell lymphoma associated with bovine leukemia virus (BLV) (Iwama et al. 2013), cutaneous B-1 cell lymphoma (Anjiki et al. 2009), lymphoplasmacytoid lymphoma (Honda et al. 2009), and immunoblastic lymphoma (Murayama et al. 2011). Cases of BLV-associated pleomorphic lymphoma with large abdominal tumor masses are considered to originate from B-1 cells localized in body cavities (Abe et al. 2007). At earlier stages of enzootic leukosis, tumor cells in peripheral blood are thought to accumulate in the marginal sinus area and to subsequently proliferate and infiltrate into follicles (Koguchi et al. 1996). On the other hand, histological transition from

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mantle cell lymphoma to pleomorphic B cell lymphoma has been observed, suggesting that mantle cells are the normal counterpart of nodal pleomorphic lymphoma (Hagiwara et al. 2018). The current study reports a case of nodal pleomorphic B cell lymphoma, in which only a few lymph nodes were replaced by diffusely growing pleomorphic cells and had a high number of BLV copies. Its precursor lesions, which were observed in some other lymph nodes with lower numbers of copies and which consisted of extensively proliferating mantle cells, support the view that the postulated normal counterpart of nodal pleomorphic lymphoma is a peripheral B cell of the mantle zone.

Materials and methods

1. Animal

Antibodies to BLV were detected by enzyme-linked immunosorbent assay using a commercially available kit (JNC Inc., Tokyo, Japan) in a 6-year-old Holstein cow (white blood cell count [WBC], 23,300/μl with 85% lymphocytes). At the age of 8 years, this cow was euthanized because of persistent lymphocytosis (WBC, 21,000/μl with 78% lymphocytes).

2. Molecular examination

DNA obtained from blood and various lymphatic and nonlymphatic tissues was analyzed by real-time polymerase chain reaction (PCR) using a Cycleave PCR BLV detection kit (Takara Bio, Shiga, Japan) (Kanno et al. 2014).

3. Histological and immunohistochemical examinations

Tissues from various organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Representative sections were selected and stained for immunohistochemistry using the streptavidin-biotin immunoperoxidase (SAB) method with a commercially available Histofine kit (Nichirei, Tokyo, Japan). The primary antibodies utilized were rabbit polyclonals to human CD20 (prediluted; Spring Bioscience, Pleasanton, USA), human κ light chain, human λ light chain (prediluted; Fitzgerald, Acton, USA), human CD5 (1:400; Pierce Biotechnology, Rockford, USA), human CD3 (1:50; Dako A/S, Glostrup, Denmark), human cyclin D1 (prediluted; Lab Vision, Fremont, USA) and human Ki-67 (1:200; Abcam, Cambridge, UK), and mouse monoclonals to human CD79a, HM57 (1:25; Dako A/S) and proliferating cell nuclear antigen (PCNA), PC10 (prediluted; BioGenex, San Ramon, USA). Antigen retrieval was by enzymatic digestion with 0.05% pepsin at 37°C for 25 minutes (CD3), or microwave heating in 10 mM citrate buffer, pH 6.0 at 90°C for 9 minutes (CD5, cyclin D1, Ki-67, CD79a, PCNA).

Results

1. Gross pathology

The left superficial inguinal and right deep inguinal lymph nodes were enlarged, and the former was hemorrhagic and necrotic. The right superficial cervical lymph node was slightly enlarged. No abnormalities were found in the other organs examined.

2. Molecular findings

The number of BLV copies in lymphohematopoietic tissues is presented in Table 1, and was 3,698, 95 and 20 copies/100 ng DNA in peripheral white blood cells, lungs, and liver, respectively. At the age of 6 years, the BLV copy number was 27,530 copies/100 ng DNA in peripheral white blood cells.

3. Histological and immunohistochemical findings

On histological examination, the left superficial inguinal lymph node was almost entirely replaced by diffusely growing pleomorphic cells (Fig. 1A), accompanied by widespread necrosis and hemorrhage. The right deep inguinal lymph node was also severely affected, but lymphatic tissues with proliferation of neoplastic mantle cells were still present. The right axillary lymph node was moderately affected by pleomorphic cells. In the lymph node of the left paralumbar fossa and the left and right superficial cervical lymph nodes, lymphoid follicles replaced by neoplastic mantle cells with varying pleomorphism tended to fuse with each other to form extensive sheet-like lesions (Fig. 1B). In addition, transition from neoplastic mantle cells to pleomorphic cells was observed in or near marginal sinuses (Figs. 1B, 1C). Similar proliferation of neoplastic mantle cells was observed in the ruminal, right superficial inguinal, and renal lymph nodes, but the pleomorphism of mantle cells was mild and no discrete transition to pleomorphic cells was detected. Mantle zones were expanded in some lymphatic follicles of the left subiliac, right mandibular, jejunal and popliteal lymph nodes. The frequencies of proliferating mantle cells and malignant pleomorphic cells are listed in Table 1.

Cytologically, diffusely growing pleomorphic cells, which predominated in a few lymph nodes, markedly varied in size and had round, oval or irregular nuclei with slightly condensed chromatin and sometimes prominent

nucleoli. Many of the cells had relatively narrow cytoplasmic bands (Fig. 1A). In the lymph node of the left paralumbar fossa and the superficial cervical lymph nodes, neoplastic mantle cells effacing the structure of lymphoid follicles were medium in size and characterized by round to elongated nuclei, faintly condensed chromatin, inconspicuous nucleoli and abundant cytoplasm (Fig. 1D). In deeper sites of mantle cell lesions, the predominant cells tended to be moderate in size with round or ovoid nuclei with slightly condensed chromatin and small- to medium-sized nucleoli and scanty cytoplasm, but larger cells with medium-sized nucleoli and abundant cytoplasm were admixed with them (Fig. 1E). Mitotic figures were seen occasionally in lesions of diffuse pleomorphic B cell lymphoma, and occasionally or rarely in areas of neoplastic mantle cells. Immunohistochemically, diffusely proliferating pleomorphic cells and neoplastic mantle cells expressed CD20 (Fig. 2A) and CD79a, and rarely expressed CD5 and cyclin D1 (data not shown). The former cells stained intensely for PCNA (Fig. 2B) and Ki-67 (data not shown), whereas the majority of the latter stained more weakly. CD3 and immunoglobulin light chains were negative in both tumor cell types.

Discussion

In cattle, pleomorphic B cell lymphoma is characterized by cytological pleomorphism and atypia, and is associated with BLV infection (Murayama et al. 2011, Iwama et al. 2013, Hagiwara et al. 2014). In the present case, similar cytological characteristics were observed in the left superficial and right deep inguinal lymph nodes, and a diagnosis of pleomorphic B cell lymphoma was made. In some other lymph nodes, lymphoid follicles had become greatly enlarged and were frequently fused with each other to form large solid sheets, composed of mantle cells with various degrees of pleomorphism. In spite of such a growth pattern, the majority were weakly positive for cell proliferation markers (PCNA and Ki-67), and were reminiscent of cells in a precancerous condition. However, their immediate transition to diffusely growing large pleomorphic cells in marginal sinuses implies that these mantle cell lesions should be regarded as a “lymphoma in situ.” In humans, cyclin D1-positive B cells are found in the mantle zones of reactive lymphoid follicles. This condition has been designated “in situ mantle cell lymphoma,” and is considered a very early stage of mantle cell lymphoma or even a preneoplastic state (Hsu et al. 2014).

The current case and cases of mantle cell lymphoma (Hagiwara et al. 2018), in which histological progression to pleomorphic B cell lymphoma appeared to occur not only toward the outside but also within mantle cell lesions, support the view that BLV-associated nodal

Table 1. Number of BLV copies and frequency of pleomorphic cells and mantle cells

Lymph nodes or other organs	BLV copies/100 ng DNA	Pleomorphic cells	Proliferating mantle cells
Right deep inguinal	5,994	+++	+
Left superficial inguinal	4,620	+++	–
Right axillary	1,179	++	+
Of the left paralumbar fossa	471	+	+++
Right superficial cervical	267	+	+++
Left superficial cervical	240	+	+++
Ruminal	476	–	++
Right superficial inguinal	464	–	++
Renal	297	–	++
Left subiliac	134	–	+
Right mandibular	87	–	+
Jejunal	63	–	+
Popliteal	52	–	+
Spleen	245	–	–
Palatine tonsil	61	–	–
Thymus	3	–	–
Sternal bone marrow	3	–	–

+++: always or usually present; ++: frequently present; +: occasionally present; –: rarely present or absent.

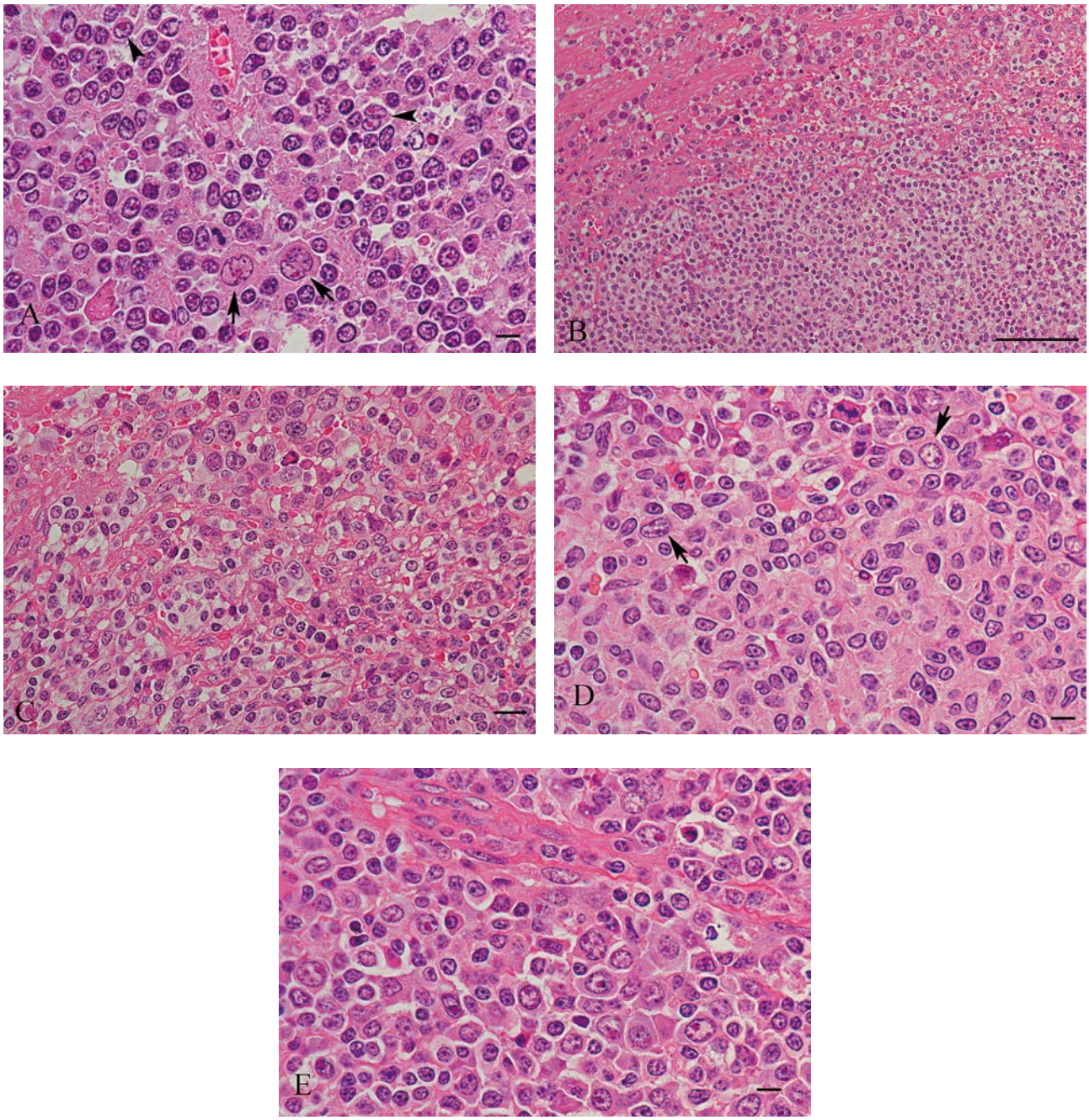

Fig. 1. Histology

A: A few large atypical lymphoma cells (arrows) are observed in an area composed exclusively of smaller cells. Arrowheads indicate lymphoma cells with prominent nucleoli and relatively scant cytoplasm. Left superficial inguinal lymph node, HE, Bar = 5 μm. B: This mantle cell lesion is located in the site of pre-existing follicles, and larger pleomorphic cells are also seen in the marginal sinus and capsule (top). Right superficial cervical lymph node, HE, Bar = 50 μm. C: Neoplastic mantle cells appear to be transforming into pleomorphic cells (top) in the marginal sinus. Right superficial cervical lymph node, HE, Bar = 10 μm. D: In a sheet-like growth having replaced cortical lymphatic follicles, most neoplastic mantle cells have relatively homogeneous cytological characteristics, but a few large cells (arrows) are visible. Right superficial cervical lymph node, HE, Bar = 5 μm. E: In this medullary part of a sheet-like growth, neoplastic mantle cells are more variable in cell and nuclear size than those in Fig. 1D. Right superficial cervical lymph node, HE, Bar = 5 μm.

pleomorphic B cell lymphoma is derived from mantle cells. As in human mantle cell lymphoma (Feller & Diebold 2004), it is highly probable that focal or sheet-like growth patterns, which are suggestive of a close relationship with mantle cells, are abolished at advanced stages of tumor development. However, an admixture of neoplastic mantle cells and more pleomorphic cells may result in a marked variation in tumor cell size in single tissue sections (Iwama et al. 2013), which can be a helpful index for distinguishing pleomorphic lymphoma from other histological types of lymphoma (Kadota & Ishikawa 2017a, 2017b). Mantle cell lymphoma is rarely seen in cattle, so neoplastic mantle cells may immediately shift to a pleomorphic cell state without showing typical growth patterns in most nodal lymphomas associated with BLV. By contrast, neoplastic mantle cells are considered to less easily transform to pleomorphic cells in mantle cell lymphomas (Hagiwara et al. 2018).

Although histological diagnosis was not available, the number of BLV copies in cattle with enzootic leukosis was significantly higher than in BLV-positive healthy cattle (Somura et al. 2014). In the case described here, there were many neoplastic B cells in lymph nodes that were replaced by pleomorphic cells and also many neoplastic B cells in nodes composed mainly of neoplastic mantle cells. However, the viral copy number was greatly elevated at the stage of pleomorphic lymphoma. This may be due to multiple integrations of BLV proviral DNA in some more highly pleomorphic and atypical cells. In humans, some patients with adult T cell leukemia/lymphoma (ATL) who show multiple integrations of human T cell lymphotropic virus type 1 (HTLV-1) proviral DNA exhibit an extremely aggressive clinical course, whereas patients with the typical integration of a single provirus have an indolent clinical course (Shimamoto 1997). This suggests that the number of integrations per cell are correlated with the grade of malignancy of ATL.

Unlike in human ATL with HTLV-1, Southern blot analysis for monoclonal integration of BLV is possible only in some BLV-positive lymphomas (Jacobs et al. 1992). In the current case, neoplastic cell homing may have caused tumor cell growth in mantle zones of multiple lymph nodes. Alternatively or in addition to this, it is probable that the widespread distribution of less aggressive neoplastic mantle cells implies true multicentric carcinogenesis, and a marked elevation of BLV copy numbers in cattle with pleomorphic lymphoma may facilitate further development and transformation of tumors. Multicentric carcinogenesis and multiple integration of proviral DNA in more malignant cells may negatively influence the formation of monoclonal or oligoclonal bands in the Southern blot for monoclonality of BLV-associated lymphoma.

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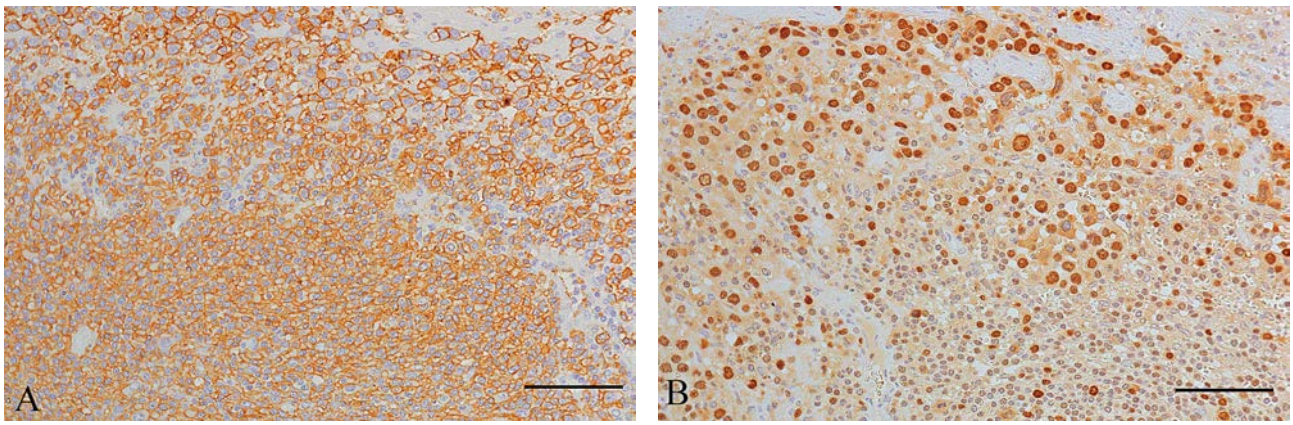

Fig. 2. Immunohistochemistry
A: Both neoplastic mantle cells and larger pleomorphic cells (top) are positive for CD20. Left superficial cervical lymph node, SAB, Bar = 50 μm. B: Large pleomorphic cells staining intensely for PCNA (upper left) in the marginal sinus and capsule, but with the majority of neoplastic mantle cells more weakly positive. Left superficial cervical lymph node, SAB, Bar = 50 μm.

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