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Immunohistochemical Characterization of Five Types of Lymphoid Neoplasms in Calves

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Abstract

Five types of lymphoid neoplasms in calves are described. Four cases with clinicopathologic features of the "calf form of lymphoma" were diagnosed as precursor B or T lymphoblastic leukemia. These neoplasms were positive for terminal deoxynucleotidyl transferase (TdT), a marker for immature lymphocytes. The fifth case (thymic B cell lymphoma) was also TdT positive, but was characterized by massive neoplastic involvement of the thymus. The sixth case involved bovine leukemia virus (BLV). As in the majority of previously reported BLV-associated cases, the neoplastic cells expressed CD5, and atypical giant cells were detected. Instead of enzootic bovine leukosis, in which at least two immunophenotypically distinct entities are included, a diagnosis of BLV-associated pleomorphic B-1 B cell lymphoma was made. The last case, involving an epitheliotropic γδ T cell lymphoma, was characterized by epitheliotropism in the gastrointestinal tract and WC1 expression. Since several histologic types of lymphoid neoplasm occur in calves, the term "calf form of lymphoma" should be abandoned for accurate diagnosis.

Discipline: Animal health
Additional key words: bovine leukemia virus, leukemia, lymphoma

Introduction

Enzootic bovine leukosis is a contagious disease of cattle caused by bovine leukemia virus (BLV)[22], and is a notifiable infectious disease in Japan (Domestic Animal Infectious Diseases Control Law). This disease must be differentiated from other lymphoid neoplasms for proper reporting. Diagnosis is based chiefly on the age of affected animals, sites of tumor formation, and BLV infection[22]. However, as in human lymphomas[7], histologic and immunophenotypic analyses are needed in order to make a correct diagnosis in cattle.

The "calf form of lymphoma" is characterized by generalized lymph node enlargement, leukemia, and neoplastic involvement of the bone marrow and spleen[22]. It usually develops in calves up to 6 months of age, but cases with the same features may occur in adult cattle[12]. In humans, precursor B cell and T cell neoplasms have features similar to those of the calf form[4,5]. Among the human B cell neoplasms, precursor B lymphoblastic leukemia is primarily a disease of children—75% of cases occur in children under 6 years of age—whereas precursor B lymphoblastic lymphoma tends to favor older individuals[4]. Precursor T cell neoplasms, in contrast, are more common in adolescents than in younger children[5]. Not only these lymphoid neoplasms but also other types may be observed in childhood[8].

The calf form, too, is divisible into B and T cell types[3,11]. The B cell type is CD5 negative and is of B-2 B cell origin[26]. Other lymphoid neoplasms such as natural killer (NK)-like T cell lymphoma and precursor B-1 B cell lymphoma have been reported in calves[17,24]. The thymic form of lymphoma characteristically occurs in year-

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lings of the beef breeds[22] and is of T cell derivation[25]. However, familial thymic lymphomas that developed in the young female offspring of one bull had some B cell markers and terminal deoxynucleotidyl transferase (TdT) and did not have T cell markers or CD5[9]. γδ T cell lymphoma is also seen in relatively young cattle[14,16,19], although it was also reported in a 14-year-old cow infected with BLV[1]. In this paper, we describe seven cases of lymphoid neoplasms in calves that were divided into five distinct categories (precursor B lymphoblastic leukemia, thymic B cell lymphoma, BLV-associated pleomorphic B cell lymphoma, precursor T lymphoblastic leukemia, and γδ T cell lymphoma), on the basis of histologic and immunophenotypic characteristics.

Materials and methods

1. Animals and history

We examined one female Japanese Black calf, one male crossbred calf, and five female Holstein calves. The clinical and macroscopic findings are presented in Table 1. The distribution of gross lesions in cases 1, 2, 5, and 6 was reminiscent of the calf form of lymphoma[22]. Neoplastic involvement of the thymus and mucosal surface of the alimentary tract was characteristic of cases 3 and 7, respectively. In case 4, multiple neoplastic lesions were present within the body cavity, and BLV infection was demonstrated by agar gel immunodiffusion test[15]. On the basis of these findings, a presumptive diagnosis of the adult form of lymphoma was made[1].

2. Histologic and immunohistochemical examinations

Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (HE) and Giemsa. Selected sections were labeled by the streptavidin–biotin–peroxidase complex (SBC) method. As primary antibodies we used rabbit polyclonal antibodies to CD3 and TdT (Dako, Glostrup, Denmark) and to CD5 (Lab Vision, Fremont, CA, USA); and mouse monoclonal antibodies to CD79a (Dako) and WC1-N3 (Veterinary Medical Research and Development, Pullman, WA, USA). Antigen retrieval was performed by enzymatic digestion with pepsin at 37°C for 25 min (CD3) or microwave heating in 0.01 M citrate buffer (pH 6.0) at 90°C for 9 min (CD5, TdT, CD79a, WC1). Subsequent procedures were carried out using Histofine SAB-PO (M) and SAB-PO (R) kits (Nichirei, Tokyo, Japan). Antibodies were detected by incubation with 3,3′-diaminobenzidine tetrahydrochloride solution. Sections were counterstained with hematoxylin for microscopic examination. To ascertain the specificity of antibodies, we performed immunohistochemistry on normal bovine tissues as well.

Results

1. Histologic findings

The degree of neoplastic invasion in each organ is presented in Table 2. The bone marrow could be examined in three cases, and was replaced by neoplastic tissue in case 2 (Fig. 1). The spleen was completely packed with neoplastic cells in cases 1, 2, 5, and 6 (leukemia cases), but the architecture remained in the other cases. There were a great number of intravascular leukemia cells in the lungs in case 1. Although mild or no neoplastic invasion was detected in the lungs in cases 2, 4, 5, and 6, appreciable numbers of neoplastic cells were present in pulmonary alveolar capillaries (Fig. 2). Neoplastic involvement of the thymus was observed in cases 2, 3, and 5, and the architecture was preserved in cases 2 and 5 (leukemia cases). In case 3, however, it was completely obliterated by lymphoma cells, and sclerotic stroma was present (Fig. 3). In contrast to case 4, in which there were lymphoma cell accumulations on the gastrointestinal serosae, case 7 showed involvement of the lamina propria and submucosa of the abomasum, jejunum, and ileum (Fig. 4). Neoplastic cell accumulations were detected in the small intestinal submucosa in case 1, and in the auricular epicardium in case 6.

2. Cytologic findings

The cytologic features are summarized in Table 3. The neoplastic cells were relatively uniform in morphology in cases 1, 2, 3, 5, and 6 (neoplasms of immature lymphocytes) (Fig. 3), and were more pleomorphic in the other two cases. In case 4, the tumor cell size was variable among neoplastic lesions or among areas in a single lesion. Atypical giant cells showing varied morphology were sparsely distributed, and the most characteristic and specific ones contained bizarre nuclei with irregular contours and prominent nucleoli (Fig. 5). Atypical giant cells were absent in case 7.

3. Immunohistochemical findings

The immunohistochemical results and diagnoses are shown in Table 4. Cases 1, 2, 3, 5, and 6 (precursor lymphoblastic leukemia/lymphoma) expressed TdT (Fig. 6) and either CD79a or CD3 (Figs. 7, 8). The neoplastic cells in cases 4 and 7 expressed CD5 (Fig. 9) and WC1 (Fig. 10), respectively.

Discussion

In humans, TdT is a marker for immature lymphoid

Table 1. Clinical data and gross pathology

Case	Breed	Sex	Age	Clinical findings	Gross pathology
1	J	F	4 months	Generalized lymph node enlargement and fever. WBC, 247,000/μl. BLV negative	Neoplastic involvement of all of the lymph nodes examined, bone marrow, spleen, liver, and kidneys. A small white focus on the small intestinal mucosa
2	F1	M	9 days	Enlargement of the superficial lymph nodes at birth. WBC, 9,600/μl, 3 days later. Euthanized because of fever. BLV negative	Neoplastic involvement of almost all lymph nodes. Severe enlargement of the spleen and liver. Multiple white foci in the kidneys
3	H	F	216 days	Anorexia and depression. A large tumor mass on the left cervix and brisket. Tumor masses on rectal examination. WBC, 9,400/μl. BLV negative. Euthanized 5 days later because of dyspnea and inability to stand	A 46 × 25 cm thymic tumor mass on the left cervix and brisket. Smaller masses in the muscular tissues of the shoulder and buttock, mediastinum, and mammary gland. Enlargement of the abdominal and thoracic lymph nodes and spleen
4	H	F	278 days	Rectal temperature of 40.3°C and tachypnea. Enlargement of some abdominal lymph nodes on rectal examination 5 days later. Inability to stand 2 days later. Euthanized 11 days after the onset of clinical signs because of positivity for BLV and leukemia. WBC, 22,500/μl, with 25% atypical lymphocytes	Multiple tumor masses on the parietal peritoneum and pleura and on the serosae of the omasum, abomasum, and intestine. Severe neoplastic involvement of the renal capsule, but no neoplastic lesions in the kidneys. Grayish white lesions in the myocardium. Slight enlargement of the spleen with no neoplastic lesions on cut section
5	H	F	3 months	Generalized lymph node enlargement. BLV positive	Neoplastic involvement of all of the lymph nodes examined, liver, and kidneys
6	H	F	41 days	Anorexia, depression and staggering gait. Euthanized 2 weeks later because of inability to stand. WBC, 9,800/μl. BLV negative	Enlargement of the mandibular, superfical cervical, bronchial, and mesenteric lymph nodes. Small white foci on the auricles. Enlargement of the spleen
7	H	F	130 days	Anorexia with a slight fever. Killed 2 months after the onset of clinical signs because of staggering gait, enlargement of the superficial lymph nodes, and leukemia. WBC, 23,000/μl, with 98% lymphocytes	Generalized lymphadenopathy. Neoplastic lesions on the mucosae of the abomasum, jejunum, and ileum. Severe enlargement of the liver, with an accentuated lobular pattern. White neoplastic foci of varied size in the kidneys

J: Japanese Black, H: Holstein, F1: J × H.

Table 2. Degree of neoplastic invasion

Case	LN	BM	Sp	Li	Ki	Lu	Others
1	+++	NE	+++	+++	++	++	Small intestinal submocosa (+)
2	+++	+++	+++	++	+	–	Thymus (++)
3	+++	–	+	–	–	NE	Thymus (+++)
4	+++/++/+	–	+	–	–	–	Gastrointestinal serosae, mesentery, omentum (+++)
5	+++	NE	+++	+++	+	+	Thymus (++)
6	+++	NE	+++	++	–	+	Auricular epicardium (+)
7	+++	NE	++	+++	NE	NE	Intestine (+++), abomasum (+)

LN: Lymph node, BM: Bone marrow, Sp: Spleen, Li: Liver, Ki: Kidney, Lu: Lung.
+++: Heavy, ++: Moderate, +: Slight, –: No invasion, NE: Not examined.

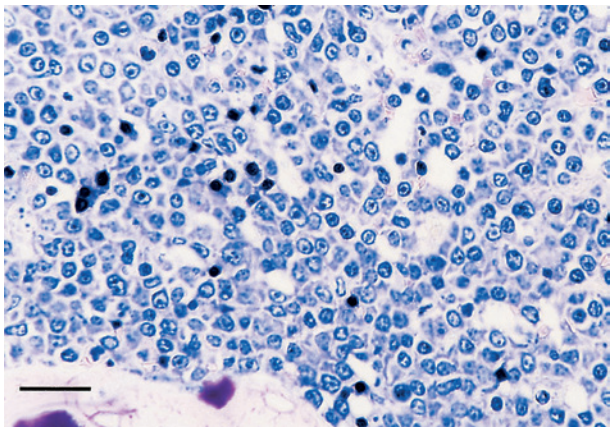

Fig. 1. Case 2, bone marrow
Except for some erythroblasts, the component cells are neoplastic. Giemsa. Bar = 25 µm.

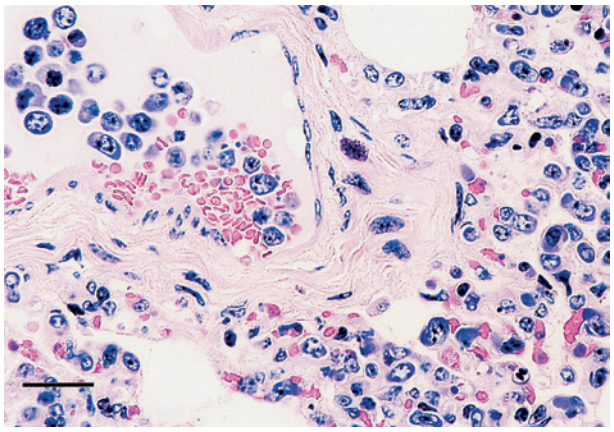

Fig. 2. Case 6, lung
A large number of neoplastic cells are present within alveolar capillaries and a venule (upper left). Giemsa. Bar = 25 µm.

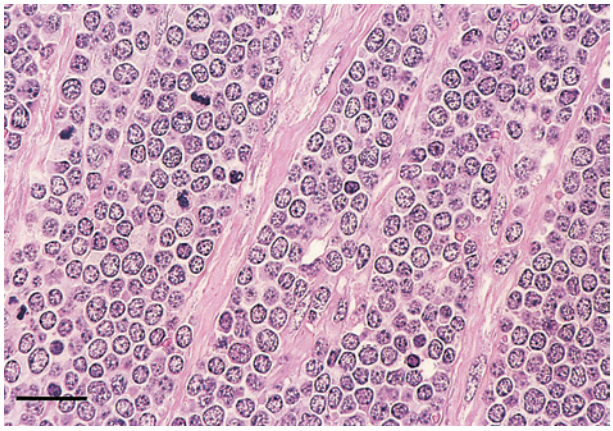

Fig. 3. Case 3, mediastinal tumor
The tumor tissue, which is composed of lymphoma cells uniform in size and shape, is traversed by several sclerotic bands. HE. Bar = 25 µm.

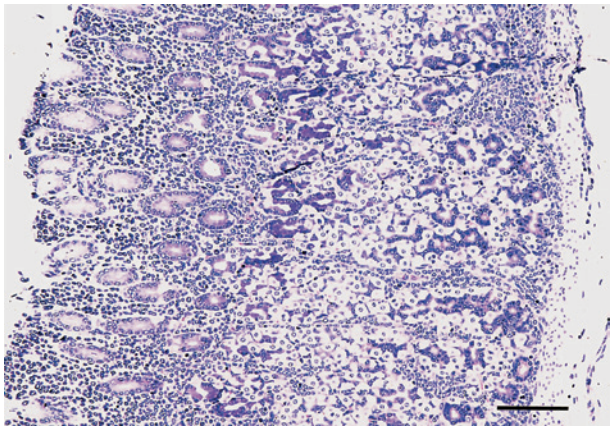

Fig. 4. Case 7, abomasum
Neoplastic cells are located chiefly in the superficial part of the lamina propria (left). Giemsa. Bar = 100 µm.

Table 3. Cytologic characteristics

Case	Nuclei	Nucleoli	Chromatin	Mitoses	Others
1	Round, oval, or slightly irregular	Inconspicuous	Finely dispersed or clumped. Vesicular in some larger cells	13-28/HPF	
2	Round, oval, or slightly irregular	Vary from small to prominent	Finely stippled or clumped	23-37/HPF	
3	Round to oval	Inconspicuous	Finely stippled	12-18/HPF	Uniform in size and shape
4	Round, oval, or irregular	Vary from small to prominent	Finely stippled or clumped. Vesicular in some larger cells	22-31/HPF	Atypical giant cells
5	Round, oval, or irregular	Small to medium-sized	Finely or moderately clumped	16-23/HPF	
6	Round, oval, or slightly irregular	Small to medium-sized	Finely or moderately clumped	20-36/HPF	
7	Round, oval, or irregular	Small to medium-sized	Finely or moderately clumped	11-18/HPF	

HPF: High-power field, +++: Heavy, ++: Moderate, +: Slight, –: No invasion.

cells of B, T, or NK cell lineage, and is expressed in lymphoblastic neoplasms of those lineages[7]. The presence of TdT has been demonstrated in swine lymphoid neoplasms such as thymic T cell lymphoma[13] and precursor B lymphoblastic lymphoma[10], and in bovine lymphoid neoplasms, including thymic B cell lymphoma[9] and precursor B-1 B cell lymphoma[24]. Cases 1, 2, 5, and 6 were characterized by TdT expression, many intravascular neoplastic cells, complete replacement of normal splenic architecture by neoplastic cells and absence of extranodal tumor masses, and the calves were diagnosed as having precursor B or T lymphoblastic leukemia, according to the classification of human lymphoid neoplasms[4,5]. The white blood cell (WBC) counts in cases 2 and 6 were similar to that in case 3, and were lower than those in cases 4 and 7. This implies that WBC counts are not high in early-stage lymphoblastic leukemia. In cases 3, 4, and 7, several extranodal tumor masses were present, and the spleen was far less severely affected than in the leukemia cases. On the basis of these findings, we classified the disease as lymphoma. Because the WBC count of a lymphoma may be high at an advanced stage of tumor development, histologic and immunophenotypic findings are indispensable for discriminating between a true leukemia and a lymphoma with many WBCs in the blood.

Although lymphomas with a large mediastinal mass are usually derived from thymocytes[25], those of B cell origin have been reported in young cattle[9]. The thymic lymphoma in case 3 with positivity for CD79a and TdT and negativity for CD5 was immunophenotypically similar to previously reported cases[9]. We thought it to be of immature thymic B-2 cell origin[24,26], and not to correspond to human mediastinal large B cell lymphoma, which is a mature B cell neoplasm[7]. This view was supported by the fact that B cell progenitors of each stage are present in the mouse thymus and resemble those in the

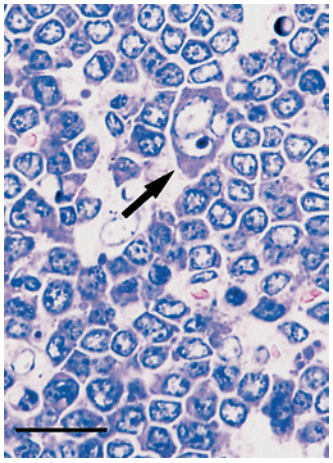

Fig. 5. Case 4, mesentery
In addition to large lymphoma cells, an atypical giant cell with a vesicular nucleus and a prominent nucleolus (arrow) is visible in this field. Giemsa. Bar = 20 µm.

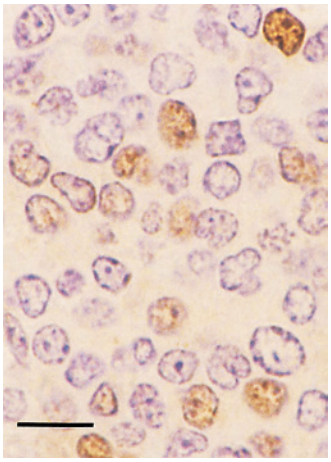

Fig. 6. Case 1, small intestine
Several leukemia cells show nuclear positivity for TdT. SBC. Bar = 10 µm.

Table 4. Immunohistochemistry and histologic diagnosis

Case	CD79a	CD5	CD3	WC1	TdT	Diagnosis
1	++	–	–	–	+	Precursor B lymphoblastic leukemia
2	++	–	–	–	+	Precursor B lymphoblastic leukemia
3	++	–	–	–	+	Thymic B cell lymphoma
4	++	++	–	–	–	BLV-associated pleomorphic B cell lymphoma
5	–	++	++	–	+	Precursor T lymphoblastic leukemia
6	–	++	++	–	+	Precursor T lymphoblastic leukemia
7	–	+	++	+	–	Epitheliotropic γδ T cell lymphoma

++: Mostly or frequently positive, +: Occasionally or rarely positive, –: Negative.

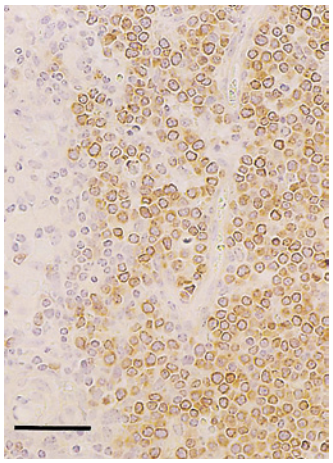

Fig. 7. Case 2, thymus
The cortex is replaced by CD79a-positive lymphoid cells, but the medulla remains (left). SBC. Bar = 50 µm.

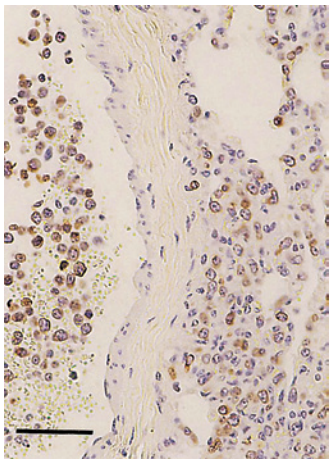

Fig. 8. Case 6, lung
CD3 is expressed in intravascular leukemia cells. SBC. Bar = 50 µm.

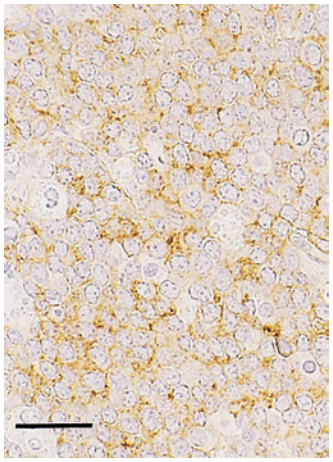

Fig. 9. Case 4, small intestinal serosa
Many lymphoma cells exhibit surface CD5. SBC. Bar = 25 µm.

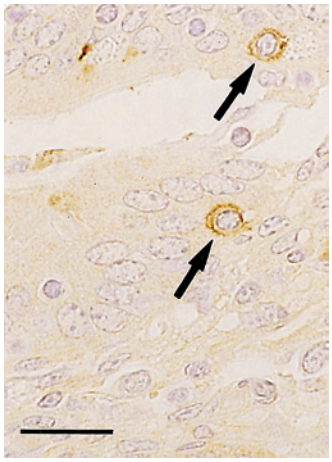

Fig. 10. Case 7, small intestine
Intraepithelial WC1-positive lymphoma cells are indicated by arrows. SBC. Bar = 20 µm.

bone marrow[2]. A similar case of precursor B lymphoblastic lymphoma, in which the neoplastic cells expressed TdT and some pan-B cell antigens and were negative for CD5, has been reported in a 19-year-old woman with a large mediastinal mass, with a proposed origin from precursors of normal thymic medullary B cells[18].

The calves in cases 4 and 5 were infected with BLV. Case 5 involved a CD3-positive T cell leukemia, and was judged to have no etiological relation to BLV[1,6]. In contrast, the tissue distribution, cellular pleomorphism, atypical giant cells with bizarre nuclei, and immunophenotype in case 4 supported the diagnosis of BLV-associated pleomorphic B-1 B cell lymphoma of abdominal cavity origin[1]. We have avoided the term "enzootic bovine leukosis" because this is applied to lymphomas of BLV-infected adult cattle, and it is highly probable that lymphoid neoplasms etiologically unassociated with BLV are included in the category[1].

Epitheliotropic γδ T cell lymphoma, which is characterized by WC1 expression and epitheliotropism in different epithelia, is a disorder specific to cattle[14,16,19]. Since the same features were observed in case 7, the calf was diagnosed as having this disorder. Although this type of lymphoma may resemble pleomorphic B-1 B cell lymphoma in tissue distribution, the latter is cytologically much more pleomorphic[1].

An NK-like T cell lymphoma and a precursor B-1 B cell lymphoma have been reported in other calves[17,24]. As shown in cases 1, 2, 5, and 6, the typical "calf form of lymphoma" involves neoplasms of precursor T or B cells.

In practice, however, various histologic types must be included in this category, when we consider the paucity of reports about other types of lymphoid neoplasms in calves. Moreover, it is probable that acute non-lymphocytic leukemias[21,23] are mistakenly classified into this category. Thus, the term "calf form of lymphoma" is used as a "catch-all" for calfhood neoplasms of the lymphoid and hemopoietic tissues for which immunohistochemistry and histochemistry are not performed. Likewise, two or more immunophenotypically distinct neoplasms are included in the adult[26], thymic[9,25], and cutaneous forms[14,20,27].

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