JARQ 45 (2), 225 – 231 (2011) http://www.jircas.affrc.go.jp

Cytologic and Immunophenotypic Investigation of Lymphohematopoietic Neoplasms in Cattle

Shugo MURAYAMA[1], Kenshi SATO[2], Tsutomu IKEHATA[3], Yoshihiro WADA[4], Yoshiharu ISHIKAWA[5] and Koichi KADOTA[5]*

[1] Chuetsu Livestock Hygiene Service Center (Uonuma, Niigata 949–7413, Japan)
[2] Kamikawa Livestock Hygiene Service Center (Asahikawa, Hokkaido 071–8154, Japan)
[3] Tokachi Livestock Hygiene Service Center (Obihiro, Hokkaido 089–1182, Japan)
[4] Abashiri Livestock Hygiene Service Center (Kitami, Hokkaido 090–0008, Japan)
[5] Hokkaido Research Station, National Institute of Animal Health, National Agriculture and Food Research Organization (Sapporo, Hokkaido 062–0045, Japan)

Abstract

Ten cases of bovine lymphohematopoietic neoplasms were investigated histologically, histochemically, and immunohistochemically, and were classified into eight histologic types on the basis of the origin and morphology of tumor cells. Case 1, a precursor B lymphoblastic leukemia, was positive for CD79a and terminal deoxynucleotidyl transferase. Case 2, a thymic B cell lymphoma, was also positive for these markers, but there were cytologic differences between the two cases. Cases 3-5 were diagnosed as pleomorphic B cell lymphomas, which were characterized by cytologic pleomorphism and expression of CD79a and CD5, and were etiologically associated with bovine leukosis virus (BLV). A case of diffuse large B cell lymphoma of the cerebrum (case 6) also showed a positive result for CD79a and CD5. However, the lymphoma was composed of a homogeneous population of large neoplastic cells, and was considered to be unrelated to BLV. The other B cell cases were categorized into immunoblastic (case 7) and lymphoplasmacytic (case 8) lymphomas, in which immunoglobulin-producing lymphoma cells were observed. In a cutaneous γδ T cell lymphoma (case 9), the neoplastic cells cytologically resembled those in case 6, but expressed CD3 and WC1. In case 10, an acute basophilic leukemia, some leukemia cells had intracytoplasmic granules that were metachromatic and tryptase positive but negative for naphthol AS-D chloroacetate esterase. Bovine lymphohematopoietic malignancies are classifiable into discrete histologic types according to immunophenotype. The classification is more scientific than the traditional one, the latter being based on the age of affected animals and/or the site of tumor formation.

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

Introduction

Most reports of lymphohematopoietic neoplasms in cattle are associated with enzootic bovine leukosis, which is a contagious disease of cattle caused by bovine leukosis virus (BLV)[14] and is a notifiable infectious disease in Japan (Domestic Animal Infectious Diseases Control Law). Although the disease must be differentiated from other lymphohematopoietic neoplasms for proper reporting, diagnosis is based on the age of affected animals, sites of tumor formation, and BLV infection[14]. T cell malignancies such as precursor T lymphoblastic leukemia[7] and γδ T cell lymphoma[1] have been reported in BLV-infected cattle, and were thought to have no relation to the virus[5]. Sporadic lymphoid neoplasms are divisible into calf, thymic, and cutaneous forms, but more than one disease entity is included in each form[2,6,7,12,16,17,19].

Taking into account the large population of normal hematopoietic cells in the bone marrow, a fair number of myeloid leukemias must occur in cattle. Reports of these, however, are far fewer than might be anticipated. This is presumably caused by the fact that acute myeloid leukemias are confused with lymphoblastic leukemias[7]. In this paper, we describe 10 cases of bovine lymphohematopoietic neoplasms in cattle that were compared cytologically

*Corresponding author: e-mail kkadota@affrc.go.jp
Received 11 March 2010; accepted 21 July 2010.

and immunophenotypically and were divided into eight histologic types.

Materials and methods

1. Animals and history

We examined nine Holstein female cattle and one Holstein-Japanese Black crossbred heifer (case 5). The clinical and macroscopic findings are presented in Table 1. Except in cases 6 and 10, an agar gel immunodiffusion test[8] was performed for the demonstration of antibodies to BLV, and positive reactivity was detected in cases 3-5. The animal died in case 5, and the others were euthanized because of poor prognosis. According to the traditional classification[14], the cases were tentatively diagnosed as calf (case 1), thymic (case 2), adult (cases 3-5), cutaneous (case 9), and unclassifiable (cases 6-8, 10) types.

2. Histologic and immunohistochemical examinations

Tissues were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (HE) and Giemsa. Selected sec-

Table 1. Clinical data and gross pathology

Case	Age	Clinical findings	Gross pathology
1	70 days	Tachypnea and fever (40°C). Enlargement of the superficial lymph nodes. WBC, 71,000/μl. Ht, 17%	Severe enlargement of the superficial, thoracic and abdominal lymph nodes, and liver. Grayish white lesions in the kidneys and heart. Enlargement of the thymus. Homogeneous grayish white tissue in the bone marrow. Thickening of the ileal wall
2	20 months	Anorexia and depression. A large tumor mass in the neck region. Distention of the left abdomen. Tumor masses on rectal examination. WBC, 22,700/μl, with 90% lymphocytes. Ht, 34%	Severe enlargement of the cervical and thoracic thymus, with compression of the trachea and esophagus. Multiple tumor masses on the pleural and peritoneal surfaces. Sparse distribution of white nodules in trunk skeletal muscles. A tumor nodule adherent to the dura mater
3	4 years	Posterior paresis and inability to stand. WBC, 8,500/μl, with 19% lymphocytes, 78% neutrophils, and 3% monocytes. Ht, 27%	Moderate enlargement of the pelvic lymph nodes. Sparsely distributed nodules on the abomasal serosa and severe enlargement of the abomasal lymph nodes. A tumor mass surrounding the dura mater of lumbosacral segments of the spinal cord. Slightly enlarged superficial lymph nodes
4	4 years	Enlargement of the subiliac lymph nodes. Tumor masses on rectal examination. WBC, 14,900/μl, with 83% lymphocytes. Ht, 29%.	Enlargement of the pelvic lymph nodes. Sparsely distributed white nodules on the uterine horns
5	19 months	Complete loss of appetite and inability to stand. Scattered skin swellings. Exophthalmos of the left eye. WBC, 89,400/μl	Subcutaneous and orbital tumor masses. Generalized lymph node enlargement
6	13 months	Blindness, circling and torticollis. Severe circling and ear droop 3 days later	A 4 cm diameter tumor mass, located on the left hemisphere and partially adherent to the dura matter. Two epidural tumor nodules near the olfactory bulb
7	28 months	Anorexia. Enlargement of the superficial cervial lymph nodes. Tumor masses on rectal examination. WBC, 99,000/μl. Ht, 20%	Generalized lymph node enlargement, especially prominent in the superfical and pelvic nodes. Diffuse pallor of the myocardium
8	9 years	Severe enlargement of the parotid, superficial cervical, and subiliac lymph nodes. Tumor masses on rectal examination. WBC, 9,600/μl	Necropsy examination was not performed. The right subiliac lymph node was 20×7 cm, with homogeneous grayish white and raised cut surfaces
9	17 months	Generalized distribution of cutaneous plaques and nodules. Enlargement of the superficial lymph nodes. WBC, 8,600/μl, with 59% lymphocytes	Multiple milky white nodules of various sizes chiefly in the subcutis. Severe enlargement of the superficial cervical, subiliac, supramammary, and medial iliac lymph nodes
10	21 months	Exophthalmos of the left eye	Severe enlargement of the medial iliac and subiliac lymph nodes. A white nodule in the orbital fatty tissue

tions were labeled by the streptavidin–biotin–peroxidase complex (SBC) method. As primary antibodies we used rabbit polyclonal antibodies to immunoglobulin M (IgM) (μ chain specific) and IgA (α chain specific) (Bethyl laboratories, Montgomery, TX, USA), to κ light chain and λ light chain (BioGenex Laboratories, San Ramon, CA, USA), to CD3 (Dako A/S, Glostrup, Denmark) and terminal deoxynucleotidyl transferase (TdT) (Dako Corporation, Carpinteria, CA, USA), and to CD5 (Lab Vision, Fremont, CA, USA); goat polyclonal antibody to IgG (γ chain specific) (Bethyl); and mouse monoclonal antibodies to CD79a (Dako A/S) and cytokeratin (clone: MNF116) (Dako Corporation), to tryptase (Lab Vision), and to 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 (heavy chains, CD3) or microwave heating in 0.01 M citrate buffer (pH 6.0) at 90°C for 9 min (TdT, CD5, CD79a, cytokeratin, WC1). Subsequent procedures were performed using Histofine SAB-PO (R), SAB-PO (G), and SAB-PO (M) kits (Nichirei, Tokyo, Japan). Antibodies were detected by incubation with 3,3′-diaminobenzidine tetrahydrochloride solution. Sections were counterstained with hematoxylin for microscopic observation. To ascertain the specificity of antibodies, immunohistochemistry on bovine normal tissues were carried out as well.

3. Ultrastructural examination

Small pieces of formalin-fixed tissues in case 7 were post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined by electron microscopy (EM).

Results

1. Histologic findings

Diffuse neoplastic growths were detected in the macroscopically visible lesions in all cases. There were great numbers of leukemia cells in the lumens of alveolar capillaries in case 1. In spite of severe neoplastic invasion into the thymus, the thymic architecture survived in case 1, and it was obliterated and completely replaced by massive neoplastic tissue with stromal fibrosis in case 2. The neoplastic cells in cases 1 and 2 were large to moderate in size with slightly irregular nuclei. In case 1, the chromatin was moderately clumped, and medium-sized nucleoli predominated (Fig. 1). In case 2, in contrast, the chromatin was finely dispersed, and the nucleoli were inconspicuous (Fig. 2).

Nearly all neoplastic cells in case 3 were large or very large with highly irregular nuclear profiles and frequently with prominent nucleoli (Fig. 3). Large neoplastic cells predominated in cases 4 and 5, but areas of smaller neoplastic cells were detected. Nuclear irregularity and prominent nucleoli were observed irrespective of tumor cell size (Figs. 4 and 5), and atypical giant cells were detected. The chromatin was moderately clumped in cases 3-5, and coarser than in the other seven cases. Most neoplastic cells in case 6 were large, and medium-sized cells were admixed with them. The cells contained nuclei the contours of which were often mildly irregular, and the size of nucleoli and the degree of chromatin condensation were moderate (Fig. 6).

The neoplastic tissue in case 7 was characterized by large neoplastic cells with round nuclei, prominent, solitary, and centrally located nucleoli, finely stippled chromatin, and abundant cytoplasm (Fig. 7), but neoplastic cells with two or three nucleoli or smaller cells were also seen. Rarely, neoplastic giant cells with single or multiple nuclei were present. In case 8, the neoplastic tissue consisted chiefly of lymphocytoid and plasmacytoid cells, occasionally with larger immunoblastoid cells (Figs. 8 and 9). The plasmacytoid cells sometimes showed a cartwheel pattern of heterochromatin, but tended to have more scant cytoplasm than normal plasma cells.

In the epitheliotropic lesions in case 9, hair follicle epithelia were heavily infiltrated by neoplastic cells, whereas the epidermis was less heavily affected. The lymphoma cells were large with rounded nuclei with medium-sized nucleoli and moderately clumped chromatin, and the cytoplasm was relatively abundant (Fig. 10). In case 10, the lymph nodes examined were completely displaced by neoplastic tissue, in which appreciable numbers of normal erythroblasts were intermingled. The neoplastic cells were mostly large with round or oval nuclei having medium-sized or small nucleoli and slightly condensed chromatin. Some neoplastic cells contained fine eosinophilic granules (Fig. 11), which stained purple or blue with Giemsa (Fig. 12) and metachromatically with toluidine blue.

2. Immunohistochemical findings

The immunohistochemical results and diagnoses are shown in Table 2. Cases 1 and 2 expressed CD79a and TdT (Fig. 13). Unlike in case 2, cytokeratin-positive reticular structures remained throughout the neoplastic tissue in case 1. In cases 3-6, the lymphoma cells displayed positive reactivity for CD79a and CD5. Although absent in these cases, cytoplasmic Ig (cIg) was detected in cases 7 (Fig. 14) and 8. There were CD3- or WC1-positive neoplastic cells in case 9 (Fig. 15), and tryptase-positive ones in case 10 (Fig. 16).

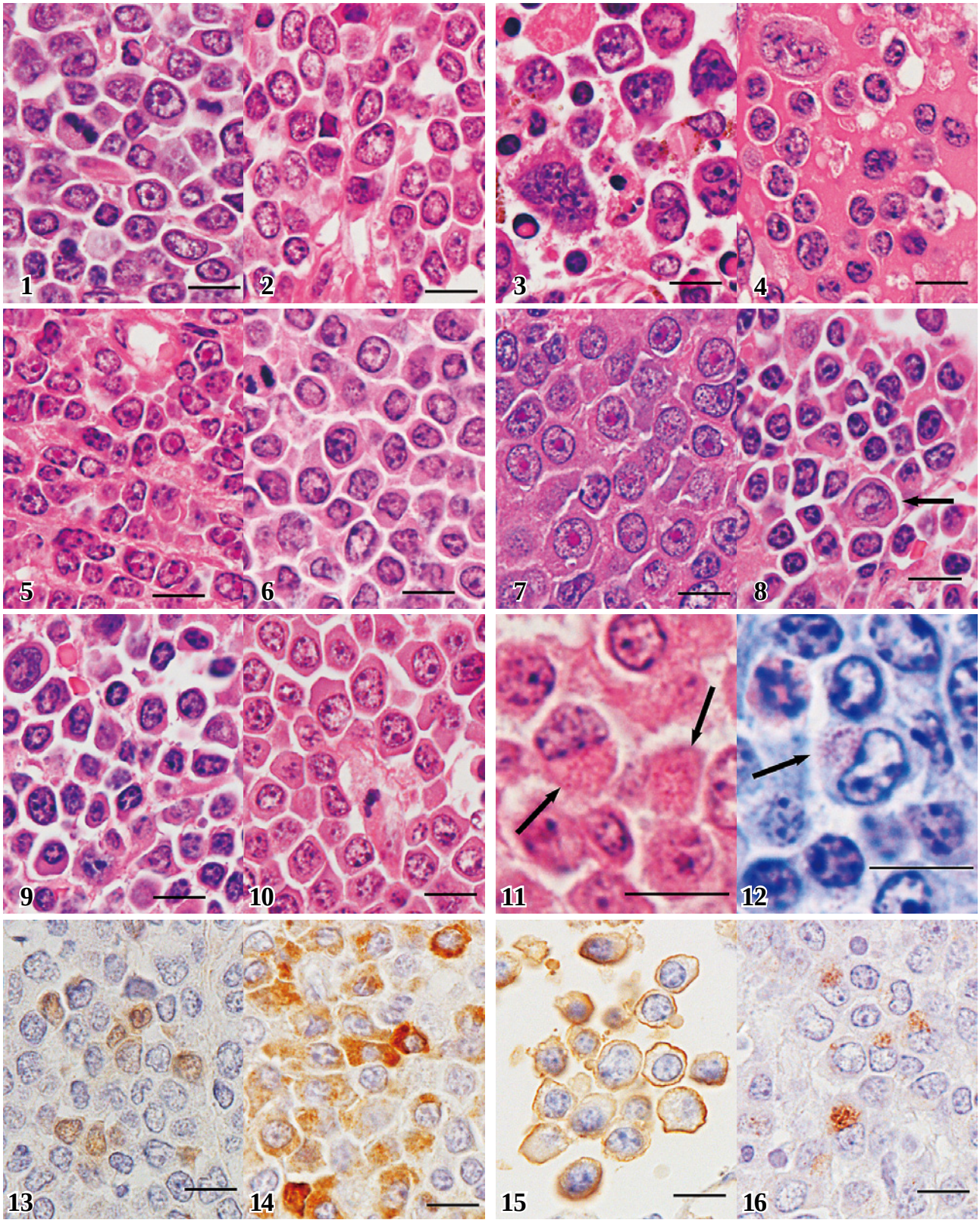

Fig. 1. Case 1, thymus
Relatively large nucleoli are observed in some leukemia cells. HE. Bar = 10 µm.

Fig. 2. Case 2, mediastinal tumor
The neoplastic cells have inconspicuous nucleoli and finely dispersed chromain. Bar = 10 µm.

Fig. 3. Case 3, lymph node
Highly atypical large neoplastic cells with bizarre nuclei are present in the lymphatic sinus. Bar = 10 µm.

3. Ultrastructural findings

In case 8, the lymphocytoid cells contained few organelles, whereas the rough endoplasmic reticulum was moderately to slightly developed in the plasmacytoid cells (Fig. 17).

Discussion

There were cytologic differences between cases 1 and 2, though the same immunophenotype was observed. The neoplastic cells in the former were characterized by moderate clumping of chromatin and medium-sized nucleoli, and similar cytologic features have been seen in precursor T lymphoblastic leukemia[7]. On the other hand, the lymphoma cells in the latter, which had nuclei with inconspicuous nucleoli and finely dispersed chromain, resembled those in precursor B-1 B cell lymphoma[16] or in immature γδ T cell lymphoma (unpublished data). Despite neoplastic involvement of the thymus in case 1, the thymic architecture was maintained, and this is charac-

Table 2. Immunohistochemistry and histological diagnosis

Case	CD79a	λ	κ	μ	γ	α	CD5	CD3	WC1	TdT	Diagnosis
1	++	–	–	–	–	–	–	–	–	+	Precursor B lymphoblastic leukemia
2	++	–	–	–	–	–	–	–	–	+	Thymic B cell lymphoma
3-5	++	–	–	–	–	–	+	–	–	–	Pleomorphic B cell lymphoma
6	++	–	–	–	–	–	+	–	–	–	Diffuse large B cell lymphoma
7	++	++	–	+	+	–	+	–	–	–	Immunoblastic lymphoma
8	++	++	–	–	++	–	–	–	–	–	Lymphoplasmacytic lymphoma
9	–	–	–	–	–	–	+	++	+	–	Cutaneous γδ T cell lymphoma
10	–	–	–	–	–	–	–	–	–	–	Acute basophilic leukemia

++ : mostly or frequently positive, + : occasionally or rarely positive, – : negative.

←

Fig. 4. Case 4, medial iliac lymph node
Nuclear atypia is observed not only in a large neoplastic cell (upper left) but also in smaller ones. Bar = 10 μm.

Fig. 5. Case 5, tumor adherent to the jejunal serosa
The neoplastic cells possess disproportionally large nucleoli, compared with relatively small cell size and scant cytoplasm. Bar = 10 μm.

Fig. 6. Case 6, cerebral tumor
The neoplastic cells appear homogeneous and less atypical than those in cases 3-5. Bar = 10 μm.

Fig. 7. Case 7, supramammary lymph node
The large neoplastic cells are characterized by rounded nuclei with a large central nucleolus and large amounts of cytoplasm. Bar = 10 μm.

Fig. 8. Case 8, subiliac lymph node
This area consists chiefly of plasmacytoid cells, but a typical immunoblastoid cell is visible (arrow). Bar = 10 μm.

Fig. 9. Case 8, subiliac lymph node
The neoplastic cells showing polymorphism vary in cell size and nuclear size. Bar = 10 μm.

Fig. 10. Case 9, superficial cervical lymph node
Medium-sized nucleoli and fair amounts of cytoplasm are characteristic of the neoplastic γδ T cells. Bar = 10 μm.

Fig. 11. Case 10, superficial cervical lymph node
There are minute eosinophilic granules in the cytoplasm of leukemia cells (arrows). Bar = 10 μm.

Fig. 12. Case 10, superficial cervical lymph node
Intracytoplasmic granules in a leukemia cell (arrow) are more readily detectable than in HE-stained sections. A neutrophil is also visible (upper left). Bar = 10 μm.

Fig. 13. Case 2, mediastinal tumor
The nuclei of some lymphoma cells have a positive reaction for TdT. Bar = 10 μm.

Fig. 14. Case 7, supramammary lymph node
Most neoplastic cells exhibit a positive reaction for the λ light chain in the cytoplasm. Bar = 10 μm.

Fig. 15. Case 9, superficial cervical lymph node
The neoplastic cells express WC1 in the cell membrane. Bar = 10 μm.

Fig. 16. Case 10, superficial cervical lymph node
Leukemia cells containing tryptase-positive cytoplasmic granules are evident in this field. Bar = 10 μm.

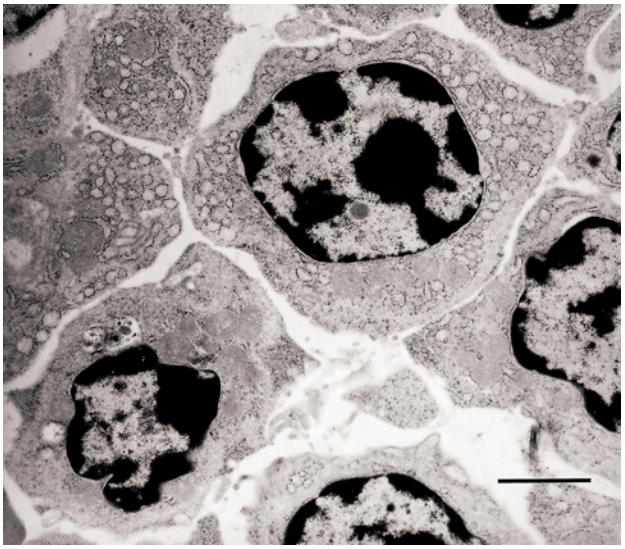

Fig. 17. Case 8, subiliac lymph node
There are moderate quantities of rough endoplasmic reticulum in plasmacytoid cells, with vesicular dilatation of cisternae, whereas a lymphocytoid cell contains a few organelles (lower left). Bar = 2 µm.

teristic of late-stage lymphoblastic leukemia[7]. Formation of large tumor masses with stromal fibrosis, which was observed in case 2, characterizes thymic B cell and T cell lymphomas[7,17].

Cytologic pleomorphism and atypia were confirmed in cases 3-5, and we judged that the cases were etiologically associated with BLV. In contrast, the neoplastic tissue in case 6 was composed of cells that were uniform in size and shape and similar to those in BLV-negative cutaneous B-1 B cell lymphoma[2]. Such cytologic features and the presence of localized relatively small intracranial lesions support the view that the lymphoma in case 6 is unassociated with BLV. As in previous reports[15,18], Ig production was not recognized in these B-1 B cell lymphomas.

In cases 7 and 8, Ig-producing neoplastic cells were detected. Since the neoplastic tissue in case 7 consisted mainly of cIg-positive large neoplastic cells, a diagnosis of immunoblastic lymphoma was made[5]. In case 8, the neoplastic cells were lymphocytoid, immunoblastoid, or plasmacytoid, and did not express CD5. The lymphoma diagnosed as lymphoplasmacytic lymphoma[5] was distinct from lymphoplasmacytoid lymphoma, characterized by CD5 expression and absence of the cartwheel distribution of heterochromatin[3]. As in the human counterpart, it is probable that transformation to immunoblastic lymphoma may occur in lymphoplasmacytoid lymphoma[3].

In normal tissues, B-1 B cells are in many ways analogous to intraepithelial γδ T cells[10]. Likewise, their malignant counterparts (cases 6 and 9) showed considerably similar cytomorphology, though the cells in case 9 had more widespread cytoplasm. Although it is difficult to distinguish between the neoplastic cells in case 9 and in ordinary cutaneous lymphomas with regression of cutaneous lesions[4], marked neoplastic invasion of hair follicles is a feature of γδ T cell lymphomas with cutaneous involvement [6,11].

There was neither CD3 nor CD79a expression in case 10. Unlike in neutrophilic myeloblastic leukemia[13], it was not easy to find intracytoplasmic granules in preparations stained with HE or Giemsa from this case. However, tryptase immunohistochemistry was very helpful in detecting neoplastic basophilic promyelocytes or myelocytes. Almost all acute myeloid leukemias in calves are mistakenly categorized into "calf form of leukosis"[7]. This is a "catch-all" category for calfhood neoplasms of the lymphoid and hemopoietic tissues[7], and should not be used in histopathology-based articles[5].

In reports on the classification of bovine leukosis, there was no correlation between morphologic cell type and immunophenotype[15,18]. This is presumably due to the fact that the classification is based on cell size and/or nuclear cleavage. In BLV-associated pleomorphic lymphomas, the tumor cells vary in size from case to case and may be variable among areas in single neoplastic tissues. The nuclear cleavage is a feature of human follicular lymphoma[9] but not of BLV-associated lymphomas. Alternatively, sporadic lymphoid neoplasms may have been included in BLV-positive cases[1,7]. The current study revealed that lymhohematopoietic neoplasms in cattle are cytologically and immunophenotypically classifiable into discrete histologic types. In the traditional classification system, not based on scientific evidence, only four forms of leukosis are presented, and miscellaneous histologic types are included in each form[7]. Such ambiguous typology, which has persisted for a long time in the veterinary literature, is simple and readily usable, but is not suitable as histopathologic diagnoses.

References

1. Abe, Y. et al. (2007) Immunohistochemical study of lymphomas of abdominal cavity origin in two cows with bovine leukemia virus. *JARQ*, **41**, 153–156.
2. Anjiki, T. et al. (2009) Immunohistochemical study on cutaneous B cell lymphoma in two cows. *JARQ*, **43**, 33–36.
3. Honda, H., Ishikawa, Y. & Kadota, K. (2009) Lymphoplasmacytoid lymphoma in a cow. *J. Vet. Med. Sci.*, **71**, 673–676.
4. Ishino, S. et al. (1988) Histopathological observations on regression of skin lymphosarcoma in five cows. *J. Vet.*

Med. A, **35**, 578–585.
5. Kadota, K. (2010) Lymphoma. *In* Systemic Animal Pathology, 2nd ed., eds. Hayashi, T. et al., Buneido, Tokyo, 56–59 [In Japanese].
6. Kadota, K. et al. (2001) γδ T-cell lymphoma with tropism for various types of epithelium in a cow. *J. Comp. Pathol*., **124**, 308–312.
7. Kagawa, Y. et al. (2009) Immunohistochemical characterization of five types of lymphoid neoplasms in calves. *JARQ*, **43**, 239–245.
8. Kono, Y. et al. (1982) Changes in antibody titers in cattle infected clinically and subclinically with bovine leukemia virus. *Int. J. Cancer*, **30**, 655–657.
9. Lennert, K. & Feller, A. C. (1992) B-cell lymphomas. *In* Histopathology of Non-Hodgkin's Lymphomas, 2nd ed., Springer-Verlag, Berlin, 53–163.
10. Murphy, K., Travers, P. & Walport, M. (2007) Innate Immunity. *In* Janeway's Immunobiology, 7th ed., Garland Science, New York, 39–108.
11. Sato, K., Shibahara, T. & Kadota, K. (2002) γδ T-cell lymphoma in a cow. *Aust. Vet. J*., **80**, 705–707.
12. Schweizer, G., Hilbe, M. & Braun, U. (2003) Clinical, haematological, immunohistochemical and pathological findings in 10 cattle with cutaneous lymphoma. *Vet. Rec*., **153**, 525–528.
13. Takayama, H. et al. (1996) Acute myeloblastic leukaemia in a cow. *J. Comp. Pathol*., **115**, 95–101.
14. Theilen, G. H. & Madewell, B. R. (1987) Bovine. *In* Veterinary Cancer Medicine, 2nd ed., eds. Theilen, G. H. & Madewell, B. R., Lea & Febiger, Philadelphia, 408–430.
15. Vernau, W. et al. (1997) The immunophenotypic characterization on bovine lymphomas. *Vet. Pathol*., **34**, 222–225.
16. Yamamoto, S. et al. (2007) Precursor B-1 B cell lymphoma in a newborn calf. *J. Vet. Diagn. Invest*., **19**, 447–450.
17. Yamazaki, Y. et al. (2000) An immunohistochemical and ultrastructural study of thymic lymphoma in a steer. *JARQ*, **34**, 195–198.
18. Yin, S. A. et al. (2003) Relation between phenotype of tumor cells and clinicopathology in bovine leukosis. *J. Vet. Med. Sci*., **65**, 599–606.
19. Zwahlen, R. D., Tontis, A. & Schneider, A. (1987) Cutaneous lymphosarcoma of helper/inducer T-cell origin in a calf. *Vet. Pathol*., **24**, 504–508.