NOTE *Pathology*

Immature T cell neoplasms in three young cattle

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ABSTRACT. Immature T cell neoplasms in three young Holstein cattle with neoplastic involvement of the thymus are described. Case 1, with a precursor T lymphoblastic leukemia (calf form of leukosis), was an 86-day-old female calf. The leukemia was characterized by replacement of the bone marrow and spleen by leukemia cells, but preservation of epithelial frameworks throughout the thymus. The other two neoplasms were thymic γδ T cell lymphomas, which were observed in a 246-day-old steer (case 2) and a 16-month-old heifer (case 3). Histological examination revealed obliteration of the normal thymic architecture and stromal fibrosis, with the spleen and liver far less severely affected than in case 1. There were cytological differences bewteen the tumors in case 1 and cases 2 and 3. Additionally, WC1 and CD8 were expressed only in the latter. Thus, the leukemia and these lymphomas should be regarded as independent disease entities on the basis of histological and immunohistochemical characteristics.

KEY WORDS: cattle, juvenile leukosis, lymphoblastic leukemia, thymic γδ T cell lymphoma

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In the World Health Organization (WHO) classification of human lymphoid neoplasms, precursor T lymphoblastic leukemia and precursor T lymphoblastic lymphoma (thymic T cell lymphoma) are considered the same disease, differing in the extent of bone marrow infiltration [4]. However, there are immunophenotypic differences between them [14], which suggests that most cases of leukemia derive from a T cell progenitor in the bone marrow, while the normal counterparts of the lymphoma are thymocytes [17].

In cattle, immature lymphoid neoplasms are divisible into several types based on the immunophenotype and tissue distribution of neoplastic lesions [5–7, 19, 20], with nearly all cases expressing terminal deoxynucleotidyl transferase (TdT), a marker for immature lymphocytes. Thymic lymphoma is characterized by formation of large tumor masses with stromal fibrosis in the mediastinum [16]. These lymphomas are generally of immature T cell derivation, but neoplasms with immature B cell phenotypes have been reported as well [2, 6]. There are cytological differences between thymic B cell lymphoma and precursor B lymphoblastic leukemia [7]. Here, we report two types of immature T cell neoplasm that were also distinct by histology and immunophenotype.

Three Holstein cattle with neoplastic involvement of the thymus were investigated. The clinical and macroscopic findings are presented in Table 1. The clinical diagnoses were calf form of leukosis in case 1 and thymic form of leukosis in cases 2 and 3. Antibodies to bovine leukosis virus (BLV), as detected by the agar gel immunodiffusion test, were present in case 1 and its mother, but not in case 3. BLV was not examined in case 2.

Tissues from various organs were collected for histological evaluation, but unlike in case 1, the bone marrow was not sampled in cases 2 and 3. They were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (HE) and Giemsa. Immunohistochemistry was carried out by the streptavidin-biotin complex/horseradish peroxidase (SAB) method on histological sections using Histofine SAB kits (Nichirei, Tokyo, Japan). The primary antibodies were rabbit polyclonal antibodies to human CD3 (Dako A/S, Glostrup, Denmark), human CD5 (Pierce Biotechnology, Rockford, IL, U.S.A.) and bovine TdT (Dako Corp., Carpinteria, CA, U.S.A.), and mouse monoclonal antibodies to bovine CD8 (CC63, AbD Serotec, Oxford, U.K.), human CD79a (HM57, Dako A/S), WC1 (CC101, AbD Serotec), expressed on γδ T cell receptor-positive lymphocyte subpopulations [18] and cytokeratin (CK) (MNF116, Dako Corp.). For comparison, normal thymic tissues from a 7-month-old female Holstein calf were also examined in the same manner.

In all cases, histological analysis demonstrated that neoplasms were observed chiefly in the macroscopically visible lesions, and enlarged lymph nodes were nearly completely occupied by neoplastic tissues. In case 1, the sternal bone marrow was heavily infiltrated by leukemia cells, but smaller numbers of hematopoietic cells were admixed with them. Except for some residual lymphatic follicles, the spleen was displaced by neoplastic tissue, and solid, cohesive sheets of leukemia cells predominated. The thymic tissue was almost entirely replaced by neoplastic cells, but it was not rare to see Hassall's corpuscles or myoid cells. Severe neoplastic infil-

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Table 1. Clinical data and gross pathology

Case	Sex	Age	Clinical findings	Gross pathology
1	F	86 days	Enlargement of superficial lymph nodes. Swelling in the cervical area. WBC, 105,380/μl, with 100% atypical lymphocytes. Ht, 30%. Euthanized because of calf form of leukosis.	Great enlargement of the superficial, thoracic and abdominal lymph nodes. Enlargement of the spleen and cervical and thoracic thymus. Multiple tumor nodules on the ruminal serosa.
2	C	246 days	Swelling in the cervical area. WBC, 71,300/μl, with 86% lymphocytes. Euthanized because of thymic form of leukosis.	A 20 × 15 × 15 cm, hard tumor mass, located on the left side of the trachea, was continuous with the markedly enlarged cervical thymus. The thoracic thymus was also highly enlarged. Marked enlargement of the cervical, mediastinal, bronchial and most abdominal lymph nodes. Slight enlargement of the spleen. Multiple white foci in the myocardium. A white focus each in the liver and left kidney.
3	F	16 months	Swelling in the presternal region. Ruminal tympany. WBC, 59,400/μl, with 91% atypical lymphocytes. Euthanized because of thymic form of leukosis.	A large nodular tumor mass, located ventral to the trachea and colored pale yellow on cut section. The thoracic thymus was replaced by a nodular tumor mass. Enlargement of the superficial cervical, axillary, thoracic and some abdominal lymph nodes. A few white nodules on the hepatic capsule. Slight bulging of splenic cut surfaces.

F=female; C=castrated male; WBC=white blood cell count.

trates were observed in the liver, lungs, uterus and palatine tonsils, and moderate to mild infiltrates in the gastrointestinal tract, pancreas, kidneys and urinary bladder. Perivascular neoplastic infiltrates were noted in the other tissues examined, such as the heart, tongue, cerebrum, medulla oblongata and pituitary gland.

Cases 2 and 3 revealed histological findings similar to each other. The thymic tissue was completely replaced by neoplastic tissue, with localized (case 2) or widespread (case 3) stromal fibrosis. Although comprising many lymphoma cells, the splenic red pulp still had enough space for erythrocytes to collect. Except in the nodular accumulations of tumor cells, infiltration was very mild in the liver. Intravascular neoplastic cells were detected in the lungs.

The leukemia cells in case 1 were 5–12 μm in diameter, containing round, oval or variously irregular nuclei with finely clumped or dispersed chromatin. Medium-sized eosinophilic nucleoli were observed in a minority of cells, but were inconspicuous in most (Fig. 1A). The cells possessed small to moderate amounts of cytoplasm. In cases 2 and 3, the lymphoma cells measured 4–9 μm in diameter, frequently with cleaved nuclei. The nucleoli were inconspicuous in almost all cells, and the chromatin was finely dispersed (Fig. 1B). The cytoplasm was scant. Erythrophagocytosis by tumor cells was sometimes seen in case 2 and was rare in the other cases. Mitoses were frequently seen in all cases.

Immunophenotypes of tumor cells and histological diagnoses are shown in Table 2. Unlike in case 1, CD8- or WC1-positive neoplastic cells were present in cases 2 and 3 (Fig. 2A). The number of TdT-positive cells was larger in case 3 (Fig. 2B). Staining for CK and desmin indicated survival of normal epithelial reticular cells and myoid cells, respectively, in case 1, and residual epithelial frameworks were observed throughout the thymus (Fig. 2C). These cells were absent in case 3, and barely surviving frameworks were detected in small areas of the tumor tissue in case 2 (Fig. 2D). In the control animal, most thymocytes were positive for CD3, CD5 and CD8. WC1-positive thymocytes were frequently observed in the medulla, but rarely in the cortex. Conversely, almost all cortical thymocytes were TdT-positive, but there were few positive cells in the medulla.

Juvenile bovine leukosis has been classified into calf, intermediate and thymic forms on the basis of the distribution of neoplastic lesions [12]. Yin *et al.* [21] recognized no cytological or immunophenotypic differences between these forms and proposed to classify them into one category, i.e. juvenile T cell lymphoma. The current study, by contrast, does document distinct cytological differences between the precursor T lymphoblastic leukemia (calf form of leukosis of T cell lineage) and thymic T cell lymphomas. In addition, WC1 and CD8 were expressed in cases 2 and 3 but not in case 1, and this implies that the lymphoblastic leukemia and thymic lymphomas originated from a T cell progenitor of the bone marrow and a thymocyte, respectively. The same view has been presented in human cases, and analysis of the expression of CD8 and CD56 was helpful in dividing precursor T cell neoplasms into two entities [14, 17]. Although bone marrow histology could not be performed in cases 2 and 3, the number of neoplastic cells in the thymus, spleen and liver clearly differed from case 1. The severe destruction of the thymus in cases 2 and 3 was considered to be due to the fact that the lymphomas occurred primarily in this organ, whereas in case 1, multiple perivascular metastases may have allowed the preservation of CK-positive frameworks. It may be difficult to compare lymphoid neoplasms at different stages of tumor development, based on the size and tissue distribution of neoplastic lesions, while severe tumor growth in various organs and marked elevation of white blood cell count suggested that the present three cases were terminal. The animal's entire body including the thymus is affected in advanced T lymphoblastic, B lymphoblastic and myeloblastic leukemias in cattle [6, 15]. The previously reported intermediate form of juvenile leukosis with generalized distribution of neoplastic lesions [12] can be interpreted as an acute leukemia of unknown origin, because of the absence of immunohistochemistry.

Taking into account the large number of WC1- or CD8-positive lymphocytes in the normal thymic tissue of the control calf, it is highly probable that their malignant coun-

Table 2. Immunohistochemistry and histological diagnosis

Case	CD3	CD5	CD8	WC1	TdT	CD79a	Diagnosis
1	+++	+++	–	–	+	–	Precursor T lymphoblastic leukemia
2	+++	+++	+	+	+	–	Thymic γδ T cell lymphoma
3	+++	+	++	++	+++	–	Thymic γδ T cell lymphoma

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

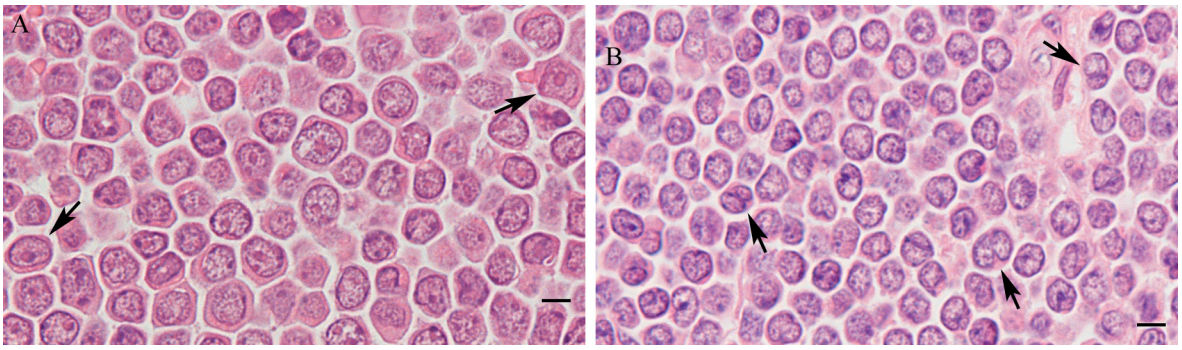

Fig. 1. Histology of neoplastic tissues and cells. (A) Case 1, thymus. Most leukemia cells have distinct cytoplasm. Arrows indicate leukemia cells with relatively prominent nucleoli. HE. Bar=5 μm. (B) Case 2, thymus. Lymphoma cells are characterized by inconspicuous nucleoli and scant cytoplasm. Cloven nuclei are shown by arrows. HE. Bar=5 μm.

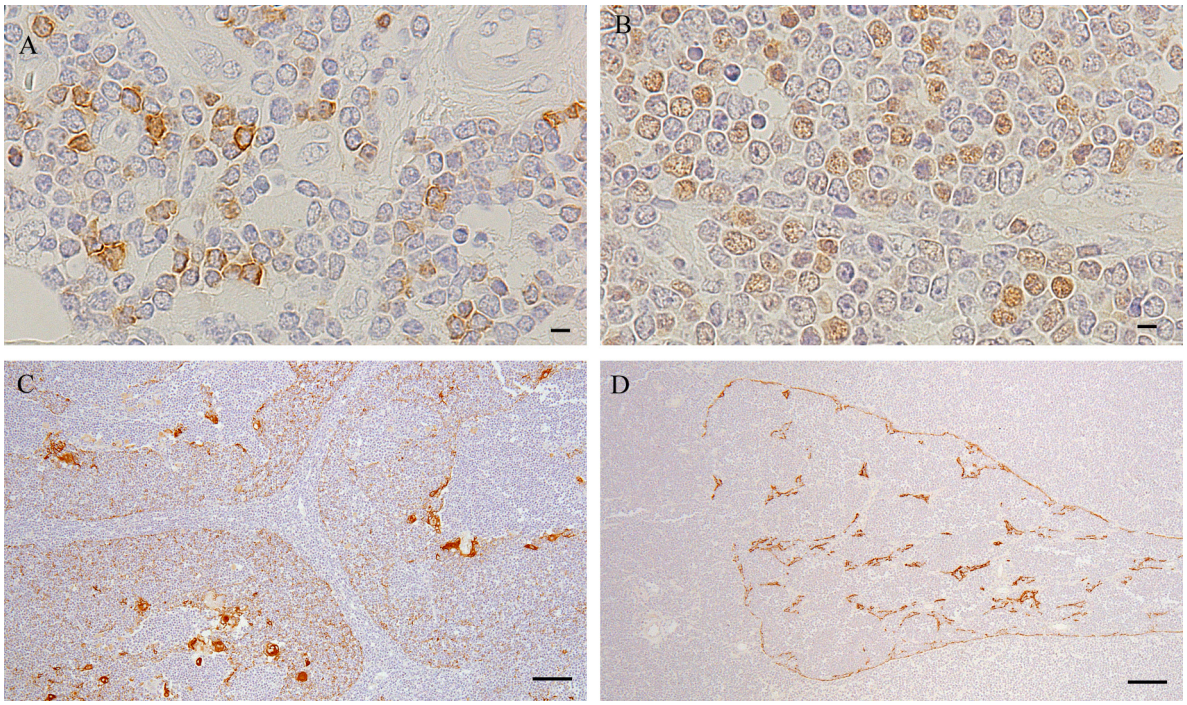

Fig. 2. Immunohistochemistry of neoplastic tissues and cells. (A) Case 3, thymus. Cell surface WC1 appears to be present on some lymphoma cells, although it is not easy to judge whether the staining is surface or cytoplasmic because of narrow cytoplasmic bands. SAB. Bar=5 μm. (B) Case 3, thymus. Many lymphoma cells show nuclear positivity for TdT. Bar=5 μm. (C) Case 1, thymus. Although leukemia cells have replaced normal thymocytes, CK-positive reticular tissues are preserved relatively well. SAB. Bar=100 μm. (D) Case 2, thymus. The thymic architecture is nearly completely effaced by lymphoma cells, but surviving epithelial frameworks are detectable by CK staining in this field. SAB. Bar=100 μm.

terparts also express these markers. As in a swine thymic lymphoma with WC1 expression [10], a final diagnosis of thymic γδ T cell lymphoma was made in cases 2 and 3. The preponderance of γδ T lymphocytes in cattle and swine results in more frequent occurrence of γδ T cell lymphomas than in humans [5, 10], which is evidence that there are species differences in lymphoid neoplasms.

As in cases 2 and 3, there is a report of WC1 expression by an immature cell variant of γδ T cell lymphoma in a fetal calf. Since the lymphoma was characterized by formation of tumor nodules in the skin and subserosal tissues and the cells had less irregular nuclear contours [5], this neoplasm was considered distinct from thymic γδ T cell lymphoma. The presence of two such histological types may suggest the existence of thymic and extrathymic differentiation pathways of γδ T lymphocytes in cattle, because each T cell tumor has a normal equivalent and retains many of the properties of the cell from which it develops [8]. Compared with case 3, smaller numbers of TdT-, WC1- or CD8-positive cells were observed in case 2, and stromal fibrosis was less prominent. Such a lymphoma may be at an intermediate stage of cell differentiation between case 1 and case 3, but was cytologically included in the category of thymic lymphoma.

BLV-associated pleomorphic B cell lymphoma may occur in calves [6], the youngest of which was 54 days old (unpublished data). This type of lymphoma has been observed in fetuses, but that could be interpreted as resulting from metastasis from the maternal lymphoma [3, 13]. Antibodies to BLV were detected in an 86-day-old calf (case 1) and are presumably maternal [11]. Judging from the previous reports [1, 6], the present T cell leukemia was not considered to be associated with BLV. Bovine lymphoid neoplasms are divisible into discrete histological types cytologically and immunophenotypically [7]. The diagnosis of enzootic leukosis must be made by cytology or histopathology [9]. However, the diagnosis is actually based on the animal's age and BLV infection [9, 16] and hence should be regarded as including cases arising in cattle with BLV, but etiologically not associated with this virus [3, 7].

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