An Immunohistochemical and Ultrastructural Study of Thymic Lymphoma in a Steer

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Abstract
A thymic lymphoma was found in a 14-month-old Holstein steer. Use of antisera applicable to paraffin-embedded sections showed that the tumor cells were of T-cell lineage. Although the cells were characterized histologically by the presence of irregular nuclei, electron microscopic examination disclosed that they were cleaved. Although nuclear convolution and cleavage are important in the subtyping of some human lymphomas, these characteristics may not be related to the immunophenotype of the tumor cells bovine lymphomas.

Introduction
Nuclear convolution which is a cytological marker for human T-cell malignancies may be present in human thymic lymphomas. Valli et al. divided canine, feline and bovine lymphoid tumors into cleaved and non-cleaved types, but complex indentations of tumor cell nuclei producing serpentine profiles were recognized ultrastructurally in a canine cutaneous lymphoma. In addition, the presence of nuclei showing a convoluted or cloverleaf-like conformation was verified by electron microscopy in canine and swine thymic lymphomas. There is no convincing evidence that nuclear convolution occurs in bovine lymphomas including the thymic form. Here we describe a case of bovine thymic lymphoma, with emphasis placed on the ultrastructural observation of the shape of the nucleus in the tumor cells.

Materials and methods
Tissues were fixed in 10% neutral buffered formalin and processed according to standard methods for paraffin embedding. Sections were stained with hematoxylin and eosin (HE). Additionally, selected sections were stained by the avidin-biotin-peroxidase complex immunoperoxidase technique (ABC). The following monoclonal antibodies were used: anti-human CD79a (HM57) (Dako, Denmark) and anti-major histocompatibility complex class II (MHC II) (H42A) (VMRD, USA). Anti-human CD3 (Dako) was also utilized. An immunoperoxidase staining kit (BioGenex Laboratories, USA) was used in the subsequent processes. For electron microscopy, small pieces from formalin-fixed tissues were post-fixed in 1% osmium tetroxide, embedded in epoxy resin, stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (TEM).

Results
1) Clinical and macroscopic observations
A 14-month-old Holstein steer was anorexic and emaciated, with a swollen brisket, enlarged lymph nodes in the neck region, severe jugular vein distention, and edema in the intermandibular space. The animal's temperature was 40.7°C rectally, the pulse was 104 per minute, and the respiration was 44 per minute. Hematological values were as follows: hematocrit, 34%; white blood cell count, 9,900 cells/µL; red blood cell count, 6,390,000 cells/µL. Atypical lymphoid cells were oc-
sionally observed on smears of the peripheral blood. Antibodies to bovine leukemia virus were not detected by the agar gel immunodiffusion test. Since the animal was considered to have a poor prognosis, it was euthanized. At necropsy, there was a massive enlargement of the thymus. The retropharyngeal, cervical, axillary and bronchomediastinal lymph nodes were markedly enlarged, and the largest measured more than 20 cm in the greatest dimension.

2) Light microscopy

The thymus and affected lymph nodes were almost entirely replaced by neoplastic tissue, and hemorrhages and necrotic foci were observed. There was a moderate infiltration of neoplastic cells in the heart, and a slight one in the liver and kidneys. The neoplastic cells were medium to large in size, and many cells showed irregular nuclear contours (Fig. 1). The nucleoli were mediumsized or inconspicuous, and the chromatin was finely clumped. Mitotic figures were abundant.

3) Immunohistochemistry

In the thymic tissue, the neoplastic cells gave a positive reaction for CD3 (Fig. 2), but not for CD79a and MHCII.

4) Electron microscopy

Most of the tumor cells showed nuclear irregularity of varying degree. Some nuclei were slightly irregular and others were cleaved (Fig. 3), though a few cells contained more irregular nuclei (Figs. 4, 5). The cells had poorly to slightly developed organelles, which had a tendency to localize in one portion of the cytoplasm. Occasional cells contained several dense bodies near the Golgi complex (Fig. 6), but compact accumulation of the bodies was seldom observed.

Discussion

In a bovine thymic lymphoma, the neoplastic lymphocytes were considered to be of thymic T-cell origin based on the immunostaining of formalin-fixed, paraffin-embedded tissue sections using a rabbit anti-human T-cell, CD3 polyclonal antibody[6]. This antibody has been used for the identification of T-cell lymphomas in other animals including dogs, cats[7], pigs[8] and a monkey[9]. On the other hand, MHCII and CD79a were useful B-cell markers, respectively, in bovine and equine lymphomas[10,11,12] and in various animal species[13,14,15,16]. The present case where a reaction with CD3 alone was observed could be categorized as a T-cell lymphoma.

The presence of clustered dense bodies, which have been identified as lysosomes, was reported to be an ultrastructural characteristic of T-cell neoplasms in humans[17], pigs[18] and a monkey[19]. Similar bodies have been described in bovine cutaneous lymphomas derived from T-cells[20,21], and were also observed in our case though the majority of them was not as compactly arranged.

In humans, follicular center cells sometimes display cleaved nuclei, which are considered to be the most important cytological finding in B-cell lymphomas, whereas nuclear convolution is characteristic of T-cell lymphomas[22]. Thrall et al.[23] demonstrated the presence of nuclear convolution in a canine cutaneous lymphoma resembling Sézary syndrome in man. In bovine cutaneous lymphomas, by contrast, the tumor cells contained round, oval or cleaved nuclei[24,25]. In a canine mediastinal lymphoma, the cells showed cloverleaf-like, convoluted nuclei[26]. Likewise, convolution or multilobation was observed in swine thymic T-cell lymphomas[27]. Although Parodi et al.[28] reported that the convoluted form of tumor cells was confirmed by electron microscopy in bovine thymic lymphomas, the nuclei showed only a slight irregularity in the electron micrograph. In our case, slightly irregular or cleaved nuclei were...
detected. A few nuclei appeared to be more irregular, but were partially indented in contrast to convoluted nuclei with extensive folding. Such an irregularity could be interpreted as a variation of cleavage. Conversely, nuclear convolution was reported to be present in cases of bovine B-cell lymphoma, although no ultrastructural examination was performed.

Lymphoproliferative diseases resembling mycosis fungoides (cutaneous T-cell lymphomas) are uncommon in dogs, and nuclear indentation or lobulation is generally less conspicuous than in humans and is not usually detected by light microscopy. Vernau et al. classified bovine lymphoid neoplasms observed by light microscopy based on the tumor cell size and nuclear shape, and presented high power photomicrographs depicting nuclear convolution or cleavage. The tissues, however, seemed inadequately preserved for cytological observation, because the tumor cells showed indistinct cell boundaries with or without pyknotic nuclei. In the subsequent study, Vernau et al. reported the absence of correlation between the immunophenotype and cell type or nuclear morphology in bovine B-cell lymphomas. The identification of nuclear profiles may be difficult in routine paraffin-embedded sections, and there is no convincing ultrastructural evidence of nuclear convolution in bovine T-cell lymphomas.

References