

Immunohistochemical Study on Cutaneous B Cell Lymphoma in Two Cows

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Abstract

Two cases of cutaneous B cell lymphoma in Holstein cows aged 7 and 9 years are described. The animals, which were not infected with bovine leukemia virus, had neoplastic lesions not only in the skin but also in the abdominal cavity. The neoplastic cells were positive for surface CD79a and CD5 but not for terminal deoxynucleotidyl transferase (TdT), which is a marker for precursor T and B cells. The lymphomas, which were considered to be of mature B-1 B cell derivation, might be an adult counterpart of precursor B-1 B cell lymphoma in calves, because the calf lymphoma is similar to the current lymphomas in tissue distribution but expresses TdT as well as CD79a and CD5.

Discipline: Animal health

Additional key words: B-1 B cell lymphoma, CD5, nonepidermotropic, terminal deoxynucleotidyl transferase

Introduction

In cattle, cutaneous lymphoma is dividable roughly into T or B cell lineage⁶. The former includes at least 2 types of epidermotropic T cell lymphoma: CD4- or CD8-positive lymphoma resembling mycosis fungoides and WC1-positive $\gamma\delta$ T cell lymphoma^{3,5,6,9}. The B cell type, which is presumably nonepidermotropic, is a rare disease of young animals unrelated to bovine leukemia virus (BLV)⁷. Although 3 cases of cutaneous lymphoma were demonstrated to be of B cell derivation by CD79a immunostaining, no reference was made to epidermotropism or BLV infection⁶. In this paper, we report 2 cases of cutaneous lymphoma that were characterized by expression of CD79a and CD5.

Materials and methods

1. Animals

Clinical and macroscopic findings are presented in Table 1. Both cases were Holstein cows. Macroscopic features common to both cases were tumor formation in the skin and abdominal cavity.

2. Histology and immunohistochemistry

Tissues were fixed in 10% buffered formalin, embedded in paraffin, cut at 4 μ m, and stained with hematoxylin and eosin (HE). For immunohistochemical staining, the avidin-biotin-peroxidase complex (ABC) method was employed for paraffin sections. The primary antibodies used were a mouse monoclonal antibody to CD79a (Dako, Glostrup, Denmark), and rabbit polyclonal antibodies to CD3 ϵ (Dako), CD5 (Lab Vision, Fremont, CA, USA), and terminal deoxynucleotidyl transferase (TdT) (Dako). The sections were pretreated by enzymatic digestion with pepsin (CD3 ϵ) or microwave heating (CD79a, CD5, TdT). Subsequent procedures were carried out using kits (Nichirei, Tokyo, Japan).

Results

Histologically, in case 1, diffuse neoplastic growths were seen in the lesions detected macroscopically. Neoplastic involvement of the epidermis could not be confirmed because the superficial zone of the skin was not collected for fixation. The neoplastic tissue in the serosa of the omasum extended into the lamina propria, but the epithelium was intact. In the tumor masses of the small

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Table 1. Clinical data and gross pathology

Case	Age	Clinical findings	Gross pathology
1	9 y	Anorexia. Multiple tumor masses on the skin. Wheezing owing to compression of the trachea by cervical masses. Tumor masses on rectal examination. WBC, 17,000/ μ l. BLV negative. Killed because of rapid enlargement of the masses.	Multiple tumor masses in the subcutis and/or musculature of the head, neck, shoulders, trunk, and buttocks. Enlargement of the superficial and abdominal lymph nodes. A large tumor mass, 35 x 15 x 10 cm, on the serosa of the omasum, and 3 smaller ones on the serosa of the small intestine. Tumor nodules on the epicardium and endocardium. A fetus of 6 months.
2	7 y	Anorexia and cutaneous tumor masses before 5th calving. BLV negative. Killed 3 weeks after calving because of cutaneous and intra-abdominal tumor masses.	Generalized distribution of tumor masses in the subcutis and/or musculature, with larger ones on the hip, chest and udder. A 40 cm diameter tumor mass adherent to the omasum, duodenum and pancreas. Tumor nodules in the adipose capsule.

intestine, the serosa and tunica muscularis were replaced by neoplastic tissue, and the tunica submucosa was considerably invaded by lymphoma cells. Sparse neoplastic infiltrates were observed in the splenic red pulp.

Neoplastic cells were 3.5 to 12 μ m in diameter. Although variously sized cells intermingled, some areas consisted exclusively of cells of similar size (Figs. 1A & 1B). Nuclei were round to oval with finely stippled chromatin and small to medium-sized nucleoli, but irregular nuclear or binuclear profiles were sometimes seen. Cytoplasm was scant, and erythrophagia by tumor cells was rare (Fig. 1C). Mitotic figures were numerous, with rates of 10 to 31 per high-power field (HPF).

As in case 1, gross lesions consisted of sheets of neoplastic cells in case 2. In the skin, a large number of neoplastic cells were present in the musculature and subcutis, but the epidermis was not affected (Fig. 1D). In the abdominal cavity, neoplastic cells invaded the wall of the omasum and extended into the lamina propria, but there was no invasion into the epithelium (Fig. 1E).

Neoplastic cells varied in size from 3.5 to 10 μ m in diameter. Small to medium-sized cells predominated, but some areas consisted chiefly of larger cells. The cells had cytologic features similar to those in case 1. There were numerous mitotic figures (22-45/HPF).

Immunohistochemically, most neoplastic cells in cases 1 and 2 stained for surface CD79a (Fig. 2A) and surface CD5 (Fig. 2B). There were no neoplastic cells reactive to the other markers. In the omasum, intraepithelial lymphocytes were CD3 ϵ positive (Fig. 2C), whereas CD79a-positive neoplastic cells were found in the lamina propria (Fig. 2D).

Discussion

The classification of primary cutaneous B cell lymphomas in humans includes 5 histologic groups: follicle

center cell lymphoma, immunocytoma/marginal zone B cell lymphoma, large B cell lymphomas of the legs, intravascular large B cell lymphoma, and plasmacytoma². Among them, follicle center cell lymphoma is the most common type². This lymphoma, consisting of centrocytes and centroblasts, is cytologically more similar to the current cases than the other groups of lymphoma, but is devoid of CD5 expression². The present lymphomas, showing CD5 positivity and having neoplastic lesions in the skin and abdominal cavity, resembled a B-1 B cell lymphoma in a newborn calf⁸. However, the present lymphomas lacked TdT, which is a marker of precursor T and B lymphoblasts, and was considered to be a neoplasm of mature B-1 B cells and an adult counterpart neoplasm of calf lymphoma with TdT expression⁸.

In human lymphoid neoplasms, the pattern of CD3 ϵ staining is usually cytoplasmic, but surface membrane staining is rarely seen¹. Since, unlike T cells, natural killer (NK) cells can express cytoplasmic CD3 alone, bovine large granular lymphocyte lymphoma exhibiting surface CD3 ϵ was judged to be of T cell lineage and not to be of NK cell lineage⁴. Considering the fact that cytoplasmic staining is characteristic for CD79a¹, expression of surface CD79a in the present study seemed to be an unusual finding. As in CD3 staining in T cell neoplasms⁴, this pattern of staining might be helpful in classifying B cell neoplasms in cattle, though its significance is still unknown.

Nonepitheliotropic cutaneous lymphoma in cattle is said to be a rare disease of young animals unassociated with BLV⁷. In a recent report, in contrast, 3 cows affected with cutaneous B cell lymphoma ranged from 4 to 5 years in age⁶. Because these reports were lacking in histologic or immunohistochemical findings, we could not determine whether the previously reported cutaneous lymphomas came into the same category as the present cases. In case 2, neither the epidermis nor the omasal epithelium was infiltrated by lymphoma cells. This lymphoma was

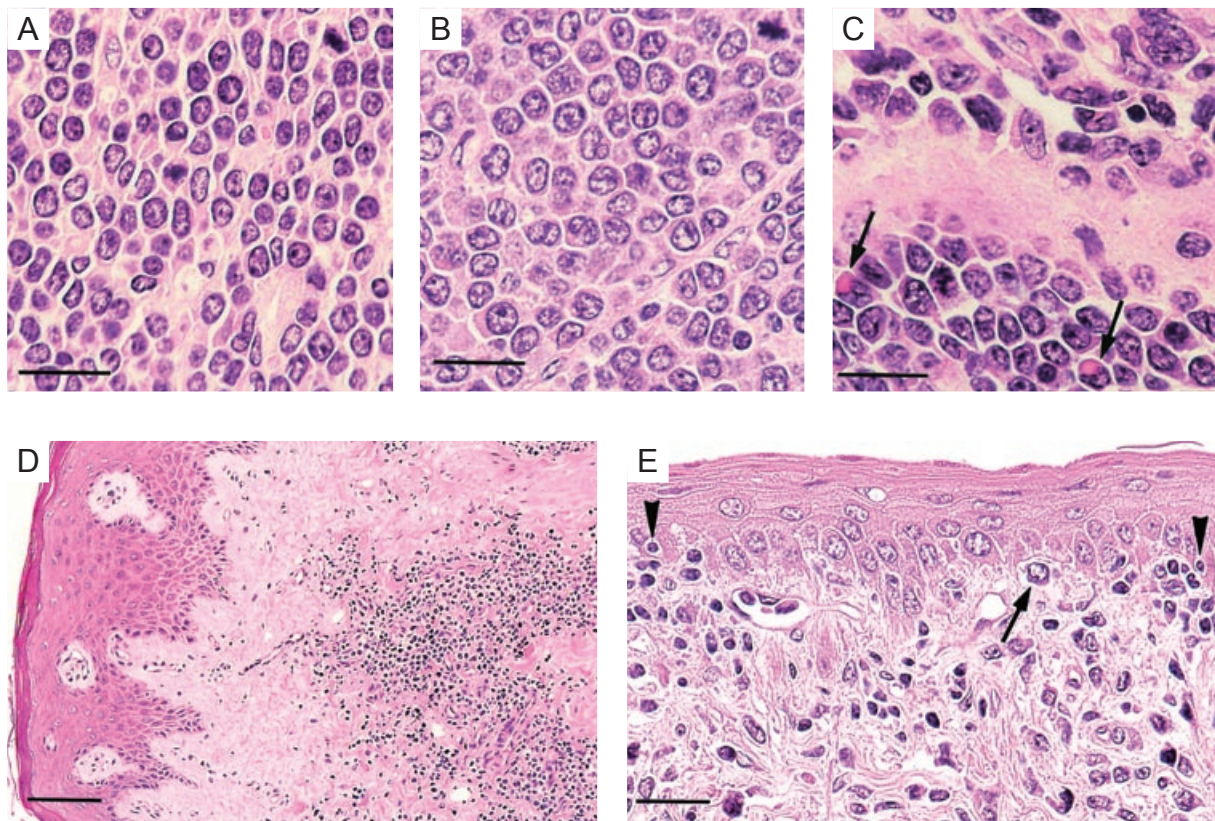


Fig. 1. Histology of neoplastic lesions

A: Case 1. Skeletal muscle. This area consists of medium-sized lymphoma cells. HE. Bar = 20 μ m. B: Case 1. Skeletal muscle. The neoplastic cells are larger than those in Fig. 1A, though other cytologic features are similar. HE. Bar = 20 μ m. C: Case 1. Skeletal muscle. There is a variation in tumor cell size. An atypical giant cell with an irregular nucleus is visible at the upper right corner, and smaller cells show erythrophagia (arrows). Bar = 20 μ m. D: Case 2. Skin. Neoplastic cells are located chiefly in the subcutis, and are not invasive into the epidermis (left). HE. Bar = 100 μ m. E: Case 2. Omasum. A lymphoma cell is detectable just beneath the epithelium (arrow). Smaller cells with heterochromatic nuclei appear to be normal lymphocytes, and some are present intraepidermally (arrowheads). HE. Bar = 25 μ m.

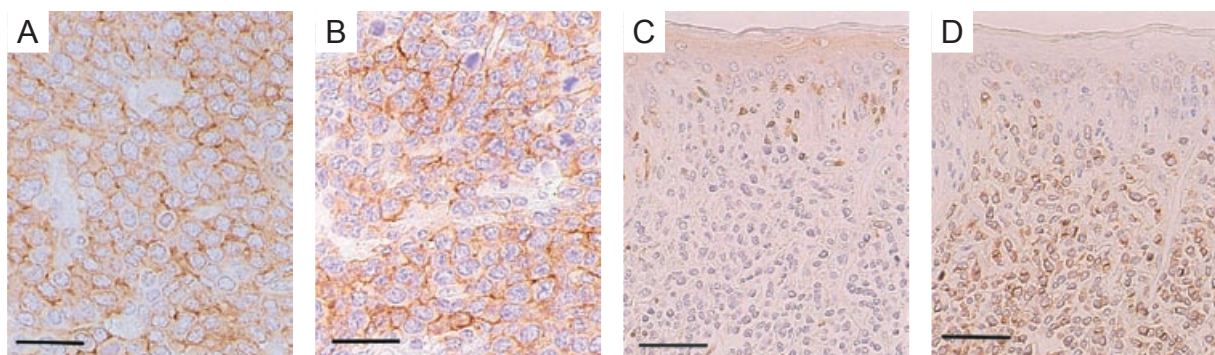


Fig. 2. Immunohistochemistry of neoplastic lesions

A: Case 2. Skeletal muscle. A large number of lymphoma cells express CD79a on the cell surface. ABC method. Bar = 25 μ m. B: Case 2. Skeletal muscle. As in CD79a staining, the cell membrane stains positively for CD5. ABC method. Bar = 25 μ m. C: Case 2. Omasum. CD3 ϵ -positive small lymphocytes are sparsely distributed under the epithelium. ABC method. Bar = 50 μ m. D: Case 2. Omasum. CD79a-positive neoplastic cells are located in a deeper area than that of normal T lymphocytes. ABC method. Bar = 50 μ m.

in marked contrast to $\gamma\delta$ T cell lymphoma with tropism for various types of epithelium such as the epidermis and gastrointestinal epithelia³. In conclusion, CD5-positive non-epidermotropic B cell lymphoma in adult cattle uninfected with BLV is a mature B-1 B-cell neoplasm, and there may be neoplastic lesions in the abdominal cavity.

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