

## Hypergranular $\gamma\delta$ T-Cell Lymphoma in a Heifer

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### Abstract

Lymphoma with large granular lymphocyte (LGL) morphology in a heifer is described. The most noticeable macroscopic finding was neoplastic involvement of the skin, mucosae of the alimentary tract, urinary bladder, and uterus. Histologically, lymphoma cells with large numbers of fine eosinophilic granules predominated in the neoplastic tissue, and the granules were positive for perforin. Surface CD3, CD5 or WC1 was expressed by some neoplastic cells. In addition to the epitheliotropism that is a feature of ordinary  $\gamma\delta$  T-cell lymphoma, the neoplastic cells showed angiodestruction and erythrophagocytosis. These phenomena were thought to result from marked activation of neoplastic  $\gamma\delta$  T-cells. The present lymphoma was distinct from the previously reported bovine NK-like T-cell lymphoma in its tissue distribution, cytomorphology, cytochemistry, and immunophenotype. Since this lymphoma was regarded as a cytologic variant of  $\gamma\delta$  T-cell lymphoma, the term hypergranular  $\gamma\delta$  T-cell lymphoma seemed to be more appropriate than  $\gamma\delta$  T-cell LGL lymphoma.

**Discipline:** Animal health

**Additional key words:** angiodestructive growth pattern, large granular lymphocyte lymphoma, perforin, WC1

### Introduction

Human T-cell large granular lymphocyte (LGL) leukemia is a heterogeneous disorder characterized by a persistent increase in the number of peripheral blood LGLs with fine or coarse azurophilic granules<sup>1</sup>. The granules contain a number of proteins that play a role in cytolysis such as perforin and granzyme B<sup>1</sup>.  $\alpha\beta$  T-cell receptors (TCRs) are expressed in most cases, and those with  $\gamma\delta$  TCRs are very rare<sup>1,8</sup>. A feline LGL lymphoma expressing surface CD3 and perforin was thought to be of T-cell origin<sup>3</sup>. Although perforin-like activity was not observed in 6 cases of feline LGL neoplasm, 2 cases expressed  $\alpha\beta$  TCRs<sup>2</sup>.

In cattle,  $\gamma\delta$  T-cell lymphoma is a neoplasm characterized by positive reactivity for WC1 and perforin, and epitheliotropism is seen in most cases<sup>4,7,11</sup>. Natural killer (NK)-like T-cell lymphoma has been reported in a calf, and many neoplastic cells had eosinophilic cytoplasmic granules that were of variable size and positive for perforin<sup>5</sup>. Here we report a case of hypergranular  $\gamma\delta$  T-cell

lymphoma in a heifer that did not resemble the human counterpart at all and was considered to be a cytologic variant of  $\gamma\delta$  T-cell lymphoma.

### Materials and methods

#### 1. Animal

A 16-month-old Holstein heifer was examined because of a 3-day history of anorexia. Clinical examination revealed emaciation, bilateral exophthalmos with epiphora, and hyperthermia (41°C). Despite antimicrobial treatment, the heifer's condition worsened with progressive exophthalmos, and it was euthanized one week after the first examination.

At necropsy, there were several tumor masses up to 5 cm in diameter on the vulva, perineum and perianal area. On cut section, they were homogeneous, white and fleshy. The skin of the udder showed diffuse swelling due to neoplastic involvement of the dermis and subcutis. The ocular muscles were almost completely replaced by white neoplastic tissue, and the glossal muscles were severely affected. There were sparsely distributed nodules up to

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1.5 cm in diameter in the obliquus internus abdominis and gluteal muscles. Similar nodules were also detected in some other skeletal muscles, and appeared to be generalized in distribution.

There were multiple tumor masses, 5 to 10 cm in diameter, on the mucosal surface of the rumen. Similar but smaller ones were detected in the reticulum and omasum, and the wall of the abomasum was diffusely thickened. Various sized tumor masses were scattered in the jejunum and ileum, and the majority were on the mucosal surface. Scattered tumor masses were found on the serosa of the colon and rectum. Nodules 1 to 2 cm in diameter were occasionally seen in the liver.

Multiple nodules, 0.5 to 1 cm in diameter, were present on the mucosal surface of the urinary bladder. Almost all parts of the ureteric wall were thickened. Frequently, the mucosal surfaces of the oviduct, uterus, vagina, and vestibulum were elevated by neoplastic proliferations up to 5 cm in diameter. Some smaller ones were present on the serosal surfaces. Most parts of both ovaries were replaced by neoplastic tissue.

The superficial, abdominal and thoracic lymph nodes were enlarged to varying degrees. The capsule of hepatic lymph nodes was infiltrated with neoplastic tissue, which extended into the adjacent pancreatic tissue. The left kidney, spleen and lung had single neoplastic nodules. Numerous neoplastic foci were present throughout the heart. Several tumor nodules up to 1 cm in diameter were adherent to the dura matter.

## 2. Histology, immunohistochemistry and electron microscopy

Tissue samples were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 4  $\mu\text{m}$ , and stained with hematoxylin and eosin (HE), Giemsa, phosphotungstic acid hematoxylin (PTAH) and naphthol AS-D chloroacetate esterase (CAE). Selected paraffin sections were dewaxed and labeled by the avidin-biotin-peroxidase complex (ABC) method. The primary antibodies used were rabbit polyclonal antibodies to CD3 $\epsilon$  (Dako, Glostrup, Denmark) and CD5 (Lab Vision, Fremont, CA, USA), and mouse monoclonal antibodies to CD79a (Dako), CD57 (BioGenex Laboratories, San Ramon, CA, USA) and WC1-N3 (Veterinary Medical Research and Development, Pullman, WA, USA). Subsequent procedures were carried out by means of an immunoperoxidase labeling system (Nichirei, Tokyo, Japan). Small pieces from formalin-fixed tissues 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

Histologically, there were dense diffuse growths of lymphoma cells in all of the nodular or massive lesions examined, but an angiocentric or angiodestructive growth pattern was found sporadically (Fig. 1A). The most outstanding finding was epitheliotropism, and intraepithelial tumor cells could be ascertained in the skin, rumen (Fig. 1B), reticulum, omasum, abomasum, jejunum, ileum, urinary bladder, uterus, and bronchiolar epithelium. Various degrees of neoplastic invasion were observed in the lymph nodes, and there were neoplastic infiltrates in the paracortical zones of partially involved lymph nodes.

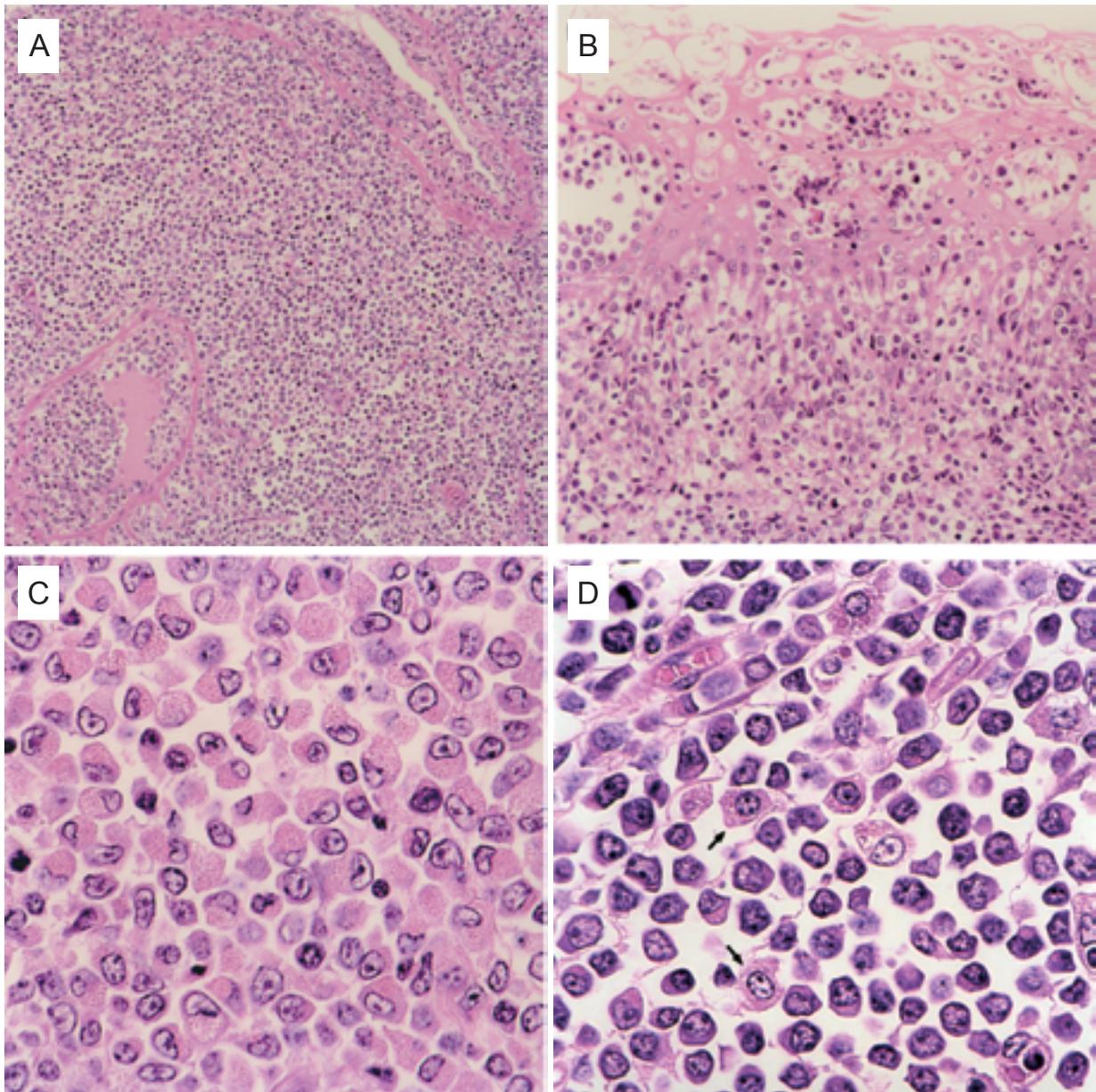
The neoplastic cells were 6 to 15  $\mu\text{m}$  in diameter, with a predominance of larger cells. The nuclei were vesicular and round, ovoid or irregular, with inconspicuous to medium-sized nucleoli. The cytoplasm was abundant, and many neoplastic cells contained fine eosinophilic granules (Fig. 1C). In certain places, however, agranular lymphoma cells proliferated preponderantly or exclusively (Fig. 1D). The granules stained red with Giemsa, and were positive for PTAH but not for CAE. A fair number of tumor cells showed erythrophagocytosis. Mitotic figures were plentiful.

Immunohistochemically, occasional neoplastic cells stained positively for surface CD3, CD5 or WC1. No tumor cells showed positive reactivity for CD57 and CD79a. The intracytoplasmic granules were weakly positive for perforin (Fig. 2A). Cytokeratin staining, for which epithelial cells stained positively, highlighted the epitheliotropism of the neoplastic cells (Fig. 2B).

Ultrastructurally, the most characteristic feature of the lymphoma cells was the presence of electron-dense granules in the cytoplasm (Fig. 3), though agranular cells were rarely seen. The granules varied between 0.1–0.8  $\mu\text{m}$  in diameter. The granule size tended to be larger in cells with abundant granules. There were moderate quantities of rough endoplasmic reticulum (RER) in cells with many granules, whereas cells with few or no granules were characterized by the paucity of organelles. Most nuclei were irregular in contour, having abundant euchromatin.

## Discussion

In the case described here, many neoplastic cells that had numerous eosinophilic granules in the cytoplasm were reminiscent of mast cells or myelocytes, but did not show metachromasia and did not stain with peroxidase or CAE<sup>9,10</sup>. On the basis of the expression of CD3, CD5, WC1, and perforin by neoplastic cells as well as the abundance of cytoplasmic granules, a presumptive

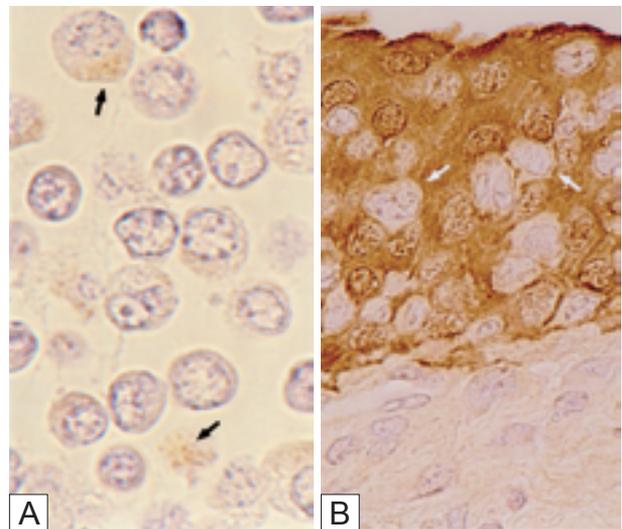


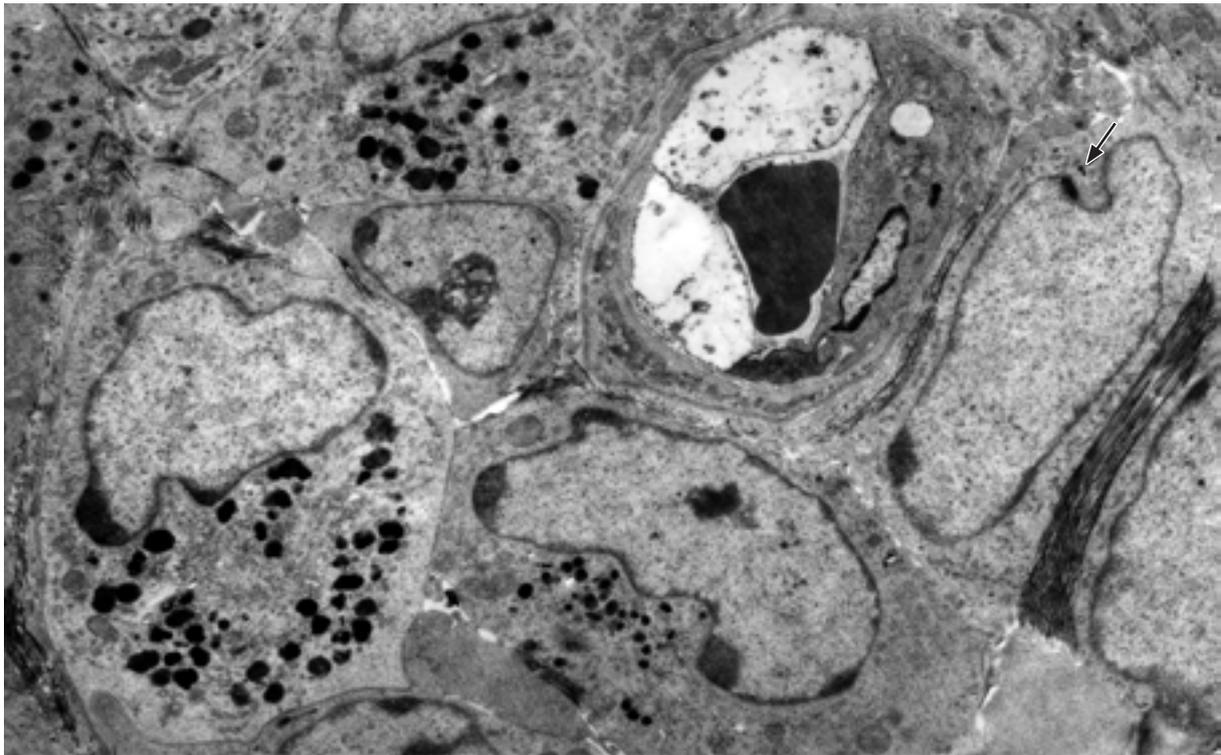
**Fig. 1. Histology of neoplastic lesions of the heifer**

A: Porta hepatis. Two blood vessels are heavily invaded and destroyed by neoplastic cells. HE.  $\times 100$ . B: Rumen. Tumor cells are isolated or in groups within the epithelium. HE.  $\times 200$ . C: Jejunum. Almost all tumor cells have numerous acidophilic granules in the cytoplasm, which are responsible for displacement of the nuclei. HE.  $\times 600$ . D: Jejunum. Although this is the same lesion as in Fig. 1C, lymphoma cells with granules (arrows) are inconspicuous. HE.  $\times 600$ .

**Fig. 2. Immunohistochemistry of neoplastic lesions of the heifer**

A: Jejunum. Arrows indicate neoplastic cells that are weakly positive for perforin. ABC.  $\times 1,000$ . B: Urinary bladder. The transitional epithelium is clearly seen with cytokeratin staining, which highlights the prominent epitheliotropism of cytokeratin-negative lymphoma cells (arrows). ABC.  $\times 600$ .





**Fig. 3. Electron microscopy of tumor cells**

Uterus. Inhabiting the cytoplasm of lymphoma cells, a great number of electron-dense granules are concentrated to one side of the cells. One lymphoma cell with few granules (arrow) is also visible. EM.  $\times 6,000$ .

diagnosis of  $\gamma\delta$  T-cell LGL lymphoma was made<sup>1,4</sup>. In humans, the majority of T-cell LGL neoplasms are positive for  $\alpha\beta$  TCRs, and cases expressing  $\gamma\delta$  TCRs are rare<sup>1,8</sup>. Compared with human cases, the cytoplasmic granules were much more prominent in the current case, and were readily discernible in histologic sections. Moreover, this lymphoma was quite dissimilar clinicopathologically to the human counterpart, which is a chronic lymphoid leukemia and has an indolent clinical course<sup>1</sup>.

In the sinusoids of the normal rat liver, LGLs have numerous small cytoplasmic granules, whereas peripheral blood LGLs contain a few larger granules<sup>12</sup>. The hepatic LGLs are considered to be a specific population of highly activated or further differentiated LGLs<sup>12</sup>. With this in mind, the present lymphoma with neoplastic cells having numerous small granules was interpretable as a neoplasm of predominantly activated LGLs, and the hypergranular cells might be in the ultimate stage of  $\gamma\delta$  T-cell activation. This case was cytologically and cytochemically distinct from a bovine NK-like T-cell lymphoma whose component cells had CAE-positive granules of various sizes and numbers<sup>5</sup>. In addition, the lymphoma showed little tendency to form tumor masses and the liver was severely affected<sup>5</sup>.

Although the number of cytotoxic granules was much greater than in ordinary  $\gamma\delta$  T-cell lymphomas<sup>11</sup>, the present neoplasm was very similar to some of these lymphomas in its tissue distribution and epitheliotropism<sup>4,7</sup>. The neoplasm was regarded as their cytologic subtype, and was designated hypergranular  $\gamma\delta$  T-cell lymphoma. An angi-destructive growth pattern is characteristic of human NK-cell neoplasms, but focal vasodestructive proliferation may also be present in human  $\gamma\delta$  T-cell lymphomas<sup>13</sup>. The present neoplasm showed a similar growth pattern and considerable erythrophagocytosis. The former has not been seen in bovine  $\gamma\delta$  T-cell lymphomas, but slight erythrophagia was detected in a meningeal  $\gamma\delta$  T-cell lymphoma<sup>11</sup>. These phenomena in the present case may be due to marked activation of neoplastic  $\gamma\delta$  T-cells; abundant cytotoxic granules are presumably associated with destruction of blood vessels<sup>6</sup>.

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