PATHOLOGICAL STUDIES ON SWINE LEUKOSIS
FINE STRUCTURE OF RETICULUM CELL SARCOMA AND LYMPHOSARCOMA

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Swine leukemia is the most common neoplasm in swine\textsuperscript{17,22,26}. Histological studies on the disease have been reported by many investigators. Many papers have been published on lymphosarcomas\textsuperscript{1-6,9-11,15,17,19-22,25,29}. Another terms, such as lymphocyto\textsuperscript{11}, lymphoblastoma\textsuperscript{2,15}, stem cell leukaemia\textsuperscript{11}, reticulosarcoma\textsuperscript{1} and Hodgkin's disease\textsuperscript{7,12,27}, were used according to the pathological characteristics of the respective neoplasms in the lymphatic tissue. As for leukemic diseases other than those of the lymphatic tissue, myeloid-cell, plasma-cell, and erythroid leukemia have also been reported by some investigators\textsuperscript{1,5,18}.

The present report concerns the fine structure of sarcoma cells in slaughtered cases of reticulum cell sarcoma and lymphosarcoma.

MATERIALS AND METHODS

Two cases examined were obtained in routine meat inspection at the Morioka Municipal Abattoir. Ultrathin sections for electron microscopy were made from materials taken by a method employed in the authors' laboratory\textsuperscript{14} within 30 minutes after slaughter. Sections 1 micron and 400 A thick were made for differentiation and orientation. A Hitachi HU 125E electron microscope with an accelerating voltage of 75 KV was used. In order to compare the fine structures, the lymphatic cells of the tonsils and the prefemoral and bronchial lymph nodes of normal pigs were studied.

For cytological observation, supravital staining was carried out. Tissue fragments were scraped from sarcoma tissues by a surgical knife before fixation, and suspended in a staining solution, which was a mixture of one drop each of 0.1% Janus green solution in physiological saline solution and 0.2% neutral red solution in physiological saline solution. One drop of the suspension on a slide glass was covered with a coverslip and observed under a phase contrast microscope after incubation at 37°C for 15 minutes. Stamp smears from sarcoma tissues were also observed after MAY-GIErMSA staining.

For histological examination, materials were collected from various parts of the whole body and fixed in 10% formalin solution. Paraffin sections were made and stained with hematoxylin and eosin.

RESULTS

Case 1.
Case history and macroscopic findings.

The case was a Landrace sow 8 months old weighing about 100 kg. It was slaughtered on January 6, 1969. At necropsy, the right parotid lymph node was about a baby's head in size. On the cut surface, the node was composed of soft and whitish sarcoma tissue. A dark reddish necrotic lesion, about a pigeon egg in size, was observed in the central part of the cut surface. The thymus was enlarged to 5 by 5 by 15 cm in size and became stiff, containing sarcoma tissue. The sternal lymph node of goose-egg size was observed adhering to the pleura with fibrous tissue.

Light microscopic findings.

In supravital staining, sarcoma cells were round or oval in shape and 10 to 14 μ in diameter. They possessed a spherical nucleus about 6 μ in diameter containing one or two nucleoli. The nucleus was of horseshoe shape occasionally. Mitochondria were coarse and numerous, scattered all over the cytoplasm. Several neutral-red granules were recognized in the cytoplasm. They formed no rosette-like clusters.

On stamp smears, sarcoma cells were round or oval in shape and 10 to 14 μ in diameter. The nucleus was 7 to 10 μ in diameter and round or oval in shape. It showed a concave appearance occasionally. One or two nucleoli were observed in the nucleus. The chromatin net was fine and slightly stained. The cytoplasm was stained faintly and basophilic, and the membrane was obscure.

Histologically, the parotid and sternal lymph nodes lost their characteristic structure due to the proliferation of sarcoma cells. The cells were atypical in shape and possessed an obscure cytoplasmic outline which was stained faintly with eosin. The nucleus was irregular in shape and rich in chromatin. In the thymus, the proliferation of sarcoma cells and residual Hassal's bodies were noticed.

Electron microscopic findings.

Sarcoma tissues were filled up with polymorphous cells ranging from 10 to 14 μ in diameter. Two phases, proliferous and functional, were detected in the cells of sarcoma tissues.

Proliferous phase: The nucleus was spherical or oval in shape. Distribution of chromatin was coarse. Condensation of chromatin in the nuclear envelope was recognized. DNA granules were distributed scatteringly. One or two nucleoli were frequently observed. Golgi complex was prominent. Rough-surfaced endoplasmic reticulum (Er) was distributed around the nucleus. Ribosomes were scattered diffusely. Mitochondria were oval in shape or elongated and 1 to 2 μ in long diameter. They were scattered around the nucleus (Fig. 1). Some of them were frequently clustered around the nucleus.

Functional phase: Most of the cells in this phase were polymorphous in shape. The nuclear envelope possessed an irregular concave surface. A nuclear pocket was formed inside the envelope occasionally. Chromatin distribution was coarse. The nucleus possessed one or two nucleoli occasionally. DNA granules were numerous and scattered in the nucleus. Pinocytotic vesicles were recognized on the cell membrane occasionally. Mitochondria were distributed dispersedly or eccentrically around the nucleus. They were elongated and 1 to 2 μ and occasionally 3 μ in diameter. Golgi complex was prominent, and Ers were numerous and distributed extensively (Fig. 2).

Neoplastic cells of both phases were scattered intricately in the sarcoma tissue. Moreover, there were intermediate-type cells of the two phases (Fig. 3), and a small number of phagocytic-phase cells with some phagosomes and many organelles. Sarcoma cells were frequently recognized in blood capillaries in the tumor tissue (Fig. 4).

The case was diagnosed as reticulum cell sarcoma on the basis of the findings mentioned above.
Case 2.
Case history and macroscopic findings.
The case was a female Landrace 6 months old weighing about 80 kg. It was slaughtered due to emaciation on July 28, 1970.

The suprapharyngeal lymph node was as large as a goose-egg. On the cut surface, the characteristic structure of the node was no more seen. Focal hemorrhage was recognized at the center of the node. A rosary-like arrangement of sarcoma nodules as large as pigeon-eggs in size was observed along the jugular vein. The bronchial lymph node was so enlarged that it was of hen's egg size. A row of bead-like nodules was recognized in the mediastinal lymph nodes of pigeon-egg size. One of the sternal lymph nodes was 20 by 10 by 5 cm in size and weighed 360 g. It adhered to the pleura. The gastric lymph node was enlarged to be 2 cm in diameter.

Sarcoma was also recognized in the pericardium, which was thickened and adhered to the pleura.

Light microscopic findings.
When observed under the phase contrast microscope, sarcoma cells were 10 to 13 μ in diameter. In them, the nucleus was spherical in shape and circumscribed with a narrow rim of cytoplasm. One to four nucleoli were present in the nucleus. The cytoplasm contained numerous fine mitochondria and a few fine granules stained with neutral red.

In stamp smears of the lesions, sarcoma cells were spherical and 8 to 10 μ in diameter, containing a nucleus with dense chromatin. One to four nucleoli were present in the nucleus. Each cell had a narrow rim of basophilic cytoplasm. Another type of cells with a fine chromatin network and 3 or 4 nucleoli in the nucleus was observed frequently.

Histologically, the prefemoral, hepatic, splenic, mesenteric, and prescapular lymph nodes revealed proliferation of sarcoma cells in medullary cords. The suprapharyngeal, cervical, bronchial, mediastinal, and gastric lymph nodes lost thire characteristic structure and were filled with sarcoma cells. The neoplastic cells were polymorphous. In most of them, nucleus was spherical in shape and the nuclear envelope was concave occasionally. The nucleus was encircled by a narrow rim of basophilic cytoplasm. Sarcoma cells were also noticed in the bone marrow of the sternum and ribs, in the sinusoid of the liver, in the interstitial tissue and the tissues around blood vessels of the lungs, and in the adipose tissue of the pericardium.

Electron microscopic findings.
Sarcoma tissues were composed of polymorphous cells showing several appearance (Fig. 5). They were divided into the following two types of cells.

Germinoblast-like cells: The cells were 10 to 13 μ in diameter. Their nuclei were round or oval in shape and had a wavy contour. Formation of a nuclear pocket was observed on the nuclear envelope. Chromatin was coarse, but condensed inside the nuclear envelope. One or two gross nucleoli were present usually. Numerous DNA granules were contained in the nucleus. Golgi complex was occasionally observed. A small quantity of rough-surfaced endoplasmic reticulum (Er) was noted. Round and small mitochondria were numerous. Most of them were less than one micron in diameter. Occasionally, they were elongated and about 2 μ in long diameter. Numerous ribosomes and polyribosomes were distributed. A massive clump of polyribosomes was frequently recognized in the cytoplasm (Figs. 5 and 6).

Lymphocyte-like cells: In general, these cells revealed higher electron density than the cells mentioned above. They were 10 to 12 μ in diameter. Invagination and nuclear
pocket formation were frequently recognized in the nuclear envelope. The nucleus contained coarse chromatin, DNA granules, and one or two nucleoli. Mitochondria gathered close to the concave portion of the nucleus. Numerous ribosomes and polyribosomes were observed (Fig. 7).

Moreover, several atypical cells were recognized. Some of them belonged to an intermediate type between the 2 types mentioned above. Some possessed an invaginated nucleus and numerous mitochondria (Fig. 8). Other cells contained invaginated nucleus and clumps of polyribosomes in the cytoplasm (Fig. 9). Phagosomes and a degenerated nucleus were seen in some of the tumor cells. From these findings, the case was diagnosed as lymphosarcoma.

**DISCUSSION**

Many reports had been published on the histopathology of swine leukemia. Engler15 investigated 30 cases of the disease encountered at an abattoir, which consisted of 27 cases of lymphosarcoma and one case each of myeloid, plasmacytic and erythroid leukemia. Bodtling5 recognized also one case each of lymphatic leukemia, reticulosarcoma, cosinophilic myelosis, and stem-cell leukemia among slaughtered pigs. Moreover, histological studies were made on many cases of lymphosarcoma22 to 27 and myelosis28. Forbus and Captan7, Hodgson22, and Savage and Isa27 proposed Hodgkin's disease-like disorders.

Among those who studied leukemia of domestic animals ultrastructurally, Fujimoto et al.20 investigated bovine leukemia. They classified the disease into three types, lymphoid, reticulum, and lymphoreticular cell types. Based on their findings, lymphosarcoma cells were classified into a lymphocytic and a lymphoblastic type. Lymphocytic sarcoma cells contained few organelles. On the other hand, lymphoblastic sarcoma cells had numerous ribosomes and other organelles. The cells of reticulum cell type were variable in size and structure. Most of them were immature or undifferentiated. They possessed more numerous organelles than lymphosarcoma cells. Knocke16, Uber-schar24, Sorenson and Theilen25, and Nazarian et al.22 studied bovine lymphosarcoma ultrastructurally. They observed formation of a nuclear pocket22, invagination of the nuclear envelope28,29, an increase in number of ribosomes28, and the presence of virus-like particles26,29 in sarcoma cells.

Hori13 investigated human cases of reticulum cell sarcoma ultrastructurally. He classified the neoplastic cells into five phase according to the degree of maturation, proliferous, functional, phagocytic, producing, and dark cell phases. On the other hand, Mori and Lennert22 reported the presence of three types of cells characterized by the kind of organelles detected in human cases of the disease.

In present study, reticulosarcoma cells could be divided into proliferous, functional, and phagocytic phases, according to the cytological classification by Hori13. The cell of the functional phase was immature and contained a small number of organelles, showing a similarity to the lymphocyte. The cell of the functional phase possessed more organelles than that of the proliferous phase. The cell of the phagocytic phase was characterized by the presence of phagosomes in the cytoplasm. Some cells were classified into intermediate types among those types. However, neither cell of the producing phase nor cell of the dark cell phase was recognized in the present case.

Fujimoto et al.20 reported that the cells of reticulum cell type were polymorphous in shape and possessed a more prominent Golgi complex and more numerous SERs than the lymphosarcoma cells. These findings were identical with those on the present case.
It was also mentioned\(^8\) that it was fairly difficult to distinguish immature reticulum tumor cells from immature or undifferentiated lymphoid tumor cells. In case 1 of the present investigation, numerous immature sarcoma cells were observed. They revealed, however, characteristic structures of reticulum cells and could be differentiated from the lymphatic cells due to these structures. They were frequently recognized in blood capillaries of the tissues examined. The findings would indicate that the case studied was one of leukemic reticulum cell sarcoma.

In case 2 of the present investigation, the lymphosarcoma cells were more atypical than cells of the lymphatic series in normal lymph nodes. Some of them were morphologically similar to germinoblasts and others to lymphocytes. Moreover, undifferentiated lymphatic cells were frequently observed in this case. The formation of a nuclear pocket, invagination of the nuclear envelope, and appearance of numerous DNA granules in the nucleus were characteristic of the present case. An increase and cluster formation of ribosomes and polyribosomes and the occurrence of numerous mitochondria of small size were also prominent. Golgi complex was scarce. Such findings as an increase in number of mitochondria and the presence of massive clump of polyribosomes in the cytoplasm were a little different from those case of bovine lymphosarcoma\(^8,16,28,30\). They might be characteristic of swine lymphosarcoma cells.

**SUMMARY**

Ultrastructural studies were carried out on one case each of reticulum cell sarcoma and lymphosarcoma in swine.

Two phases, proliferous and functional, were observed in reticulum cell sarcoma. The cell of the proliferous phase contained a rather small number of organelles, and that of the functional phase possessed numerous organelles.

In the case of lymphosarcoma, there were germinoblast-like and lymphocyte-like cells in the neoplastic tissues. An increase in number of ribosomes and polyribosomes in the cytoplasm and the formation of a nuclear pocket were characteristic of both types of cells.

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豚白血病に関する病理学的研究
細膸肉腫およびリンパ腫の従微細構造について

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(昭和47年8月4日受付)

豚における細胞肉腫およびリンパ腫の各1例について、従微構造の検索を行なった。
細胞肉腫には、おもに2種類、すなわち増殖相および機能相の細胞が観察された。増殖相の細胞
は、少数の細胞内器を有していた。一方、機能
相の細胞は、多数の細胞内器を有していた。

リンパ腫では、Germinoblast 様およびリン
パ様細胞が見られた。これらの細胞では、細胞
質内における ribosome および polyribosome の
増加ならびに nuclear pocket の形成が特徴的で
あった。

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EXAMINATION OF PLATES

PLATE I

Fig. 1. Reticulum cells of proliferous phase. Golgi complex, Er and mitochondria are recognized. ×5,400.

Fig. 2. Reticulum cell of functional phase. Organelles are prominent. Pinocytotic vesicles are also recognized. ×10,700.

PLATE II

Fig. 3. Reticulum cell of the intermediate phase between proliferous and functional phases. Organelles are considerably numerous. ×5,500.

Fig. 4. Reticulum cell of functional phase in a blood capillary. Various organelles are recognized in the cell. ×4,900.

Fig. 5. Lymphosarcoma tissue. Germinoblast-like (G) and lymphocyte-like (L) cells are differentiated. ×5,400.

PLATE III

Fig. 6. Germinoblast-like lymphosarcoma cells. A cluster of polyribosomes (C) and a nuclear rocket (arrow) are seen. ×5,100.

Fig. 7. Lymphocyte-like lymphosarcoma cells. Mitochondria and vesicles are in the concave portion of the nucleus. Numerous polyribosomes filling up the cytoplasm and nuclear pocket (arrow) are observed. ×5,600.

Fig. 8. Atypical lymphosarcoma cell. An invaginated nuclear envelope and numerous mitochondria are present. ×3,000.

Fig. 9. Atypical lymphosarcoma cell. An invaginated nuclear envelope and clusters of polyribosomes are prominent. ×5,800.
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PLATE I