MONOCYTIC LEUKEMIA IN CATTLE

I. CYTOLOGICAL OBSERVATIONS OF TWO CASES

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It is said that leukemic neoplasia in cattle has been distributed in wide areas of northeastern Europe, especially after World War II, and in some localities of the United States in recent years. Under these circumstances, special attention has been paid by many workers in these countries to the essential condition of the disease.

According to Bendixen(1) the cases of this disease have been classified largely into two type, an enzootic and a sporadic, for practical purposes. One type has been given the term "leukosis enzootica bovis" and manifests itself in two forms, a subclinical and a clinical. The other type consists of cases of sporadic occurrence and has been called juvenile leukosis or skin leukosis. Juvenile leukosis has been divided by Theilen et al. into two forms, calf and thymic, in the United States.

In Japan, the disease appears to show a sporadic occurrence. Consequently, the reported cases have been very few in this country. These cases have been duly reported, but they bear only brief descriptions limited to those of case-reporting form(3, 13, 17, 19, 22, 28).

It is felt strange, however, that leukemic neoplasia has occurred not infrequently among cattle in this district of Iwate Prefecture. More than twenty cases of the disease have been encountered in this laboratory during the past decade.

The materials collected from these cases were used primarily for cytological, gross-pathological, and histopathological investigation. As a result, a number of noticeable findings have been obtained by the present authors. Especially, it was confirmed that monocytic leukemia should be recognized as a different type of leukemia in cattle.

No descriptions of monocytic leukemia in cattle have been hitherto found in the literature in the knowledge of the authors. Accordingly, it is intended first to clarify the evidence of the occurrence of the leukemic type by describing in detail the cytopathological observations of the peripheral blood and viscera.

The present report principally concerns the cytopathology of monocytic leukemia in two cases.

MATERIALS AND METHODS

The materials examined were collected from two dairy cows, which are shown in the table below.

<table>
<thead>
<tr>
<th>Case number</th>
<th>Protocol number</th>
<th>Breed</th>
<th>Age in years</th>
<th>Date of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>246</td>
<td>Holstein cross-bred</td>
<td>9</td>
<td>Sep. 11, 1959</td>
</tr>
<tr>
<td>No. 2</td>
<td>257</td>
<td>Holstein cross-bred</td>
<td>3</td>
<td>Feb. 13, 1960</td>
</tr>
</tbody>
</table>

At ante-mortem examination, leukocyte and erythrocyte counts, and observation of blood smears were conducted. Bone marrow smears were also examined in one of the cows.

Blood was drawn from the jugular vein (about 3 ml) and placed on a glass slide. Blood smears were prepared immediately on clean-wiped slides and fire-dried. Total leukocyte and erythrocyte counts were done in each hemocytometer simultaneously. Most of the smears were subjected to May-Giemsa staining for morphological observation of leukemic cells and differential counts of nucleated cells. Others were used for the peroxidase reaction by Armitage's method.

In addition, supravit stained with neutral red (NR) and Janus green (JG), examination of phagocytic affinity to carbon particles, and observation under the phase contrast microscope were carried out with fresh blood from one of the cows.

For supravit stained with NR and JG, the following mixture was prepared beforehand: 40~50 drops of saturated solution of JG in absolute alcohol were added to 40 ml of 0.01% solution of NR in absolute alcohol. This mixture was spread evenly on a clean-wiped slide and allowed to evaporate, the excessive dye solution being cast away. Thus, many slides with a dye-film on one side were prepared. One drop of fresh blood was taken under a coverslip, which was immediately placed on the dye-film of the slide and sealed with melted solid paraffin to prevent the blood from evaporation. The preparations were put in an incubator for 5~10 minutes and examined microscopically at room temperature.

The examination of phagocytosis to carbon particles was conducted in accordance with the method of Sugiyama. The observation with the phase microscope was based upon the method devised by Oshima\textsuperscript{10}.

At autopsy, smears of visceral organs, such as the spleen, liver, and kidney, were also prepared and subjected to May-Giemsa staining and peroxidase reaction for morphological examination.

RESULTS

1. Case 1
   a. Clinical history

   The cow was kept in the southern region of Iwate Prefecture. She had been sterile, despite repeated artificial insemination, since she calved in January, 1959. Her daily milk yield was approximately 15 l at that time. On August 10th, 1959, she began to lose condition and has sometimes presented dyspnea since then. At the end of August, she developed the signs of heart disorder and her daily milk yield decreased to 6~8 l. On September 8th, the temperature stood at 40°C, while the pulse was 102 and the respiration rate 70. The milk yield dropped to 2 l on the following day. Finally, she died on September 10th immediately after she was transported to Morioka for further examination.

   b. Blood picture

   The results of blood examination conducted by a veterinarian were as follows.

   \begin{tabular}{|c|c|c|}
   \hline
   Date & Erythrocyte count (per cmm) & Leukocyte count (per cmm) \\
   \hline
   6/IX '59 & 4.00 millions & 300,000 \\
   7/IX '59 & 3.49 millions & 421,500 \\
   \hline
   
   \end{tabular}

   As indicated above, the leukocyte count showed a striking increase, while the
erythrocyte count showed a considerable decrease.

In blood smear stained with May-Giemsa stain, mononuclear cells appeared almost exclusively in close contact with one another. Other leukocytes, such as neutrophils and lymphocytes, were only sparsely seen among these cells. The nuclei of the mononuclear cells displayed polymorphism, having various smooth indentations. They showed fine lacy structures of chromatin. Usually, a few nucleoli were also seen in the nucleus. The mononuclear cells generally possessed poor cytoplasm, showing some processes and fluctuations at their peripheries. Their cytoplasm stained grayish blue.

It seemed that there were some differences in figure among nuclear lobations with the progress in the course of disease. Namely, in blood smears prepared on September 7th, the cells showed generally 2~3 nuclear lobations, which presented a rod-, horseshoe-, kidney-, or heart-shaped appearance. On September 10th, or the day before death, the cells displayed atypical figures of nuclei. Most of them had several or 10 odd nuclear lobations, which exhibited a plum-flower or violet-like appearance. Rarely, some of these cells had an ingested erythrocyte or vacuoles within their cytoplasm.

From the findings mentioned above, these mononuclear cells were regarded as cells of the monocyte series. They measured 13~15μ in diameter when they were monoblastic cells, and 16~20μ when they were promonocytic cells. Mitosis, direct and indirect, was frequently seen in them, giving the appearance of active proliferation. Some figures of these cells are shown in Figs. 1, 8, and 11.

![Fig. 1. Various Figures of Leukemic Cells in Blood Smears (Case 1)](image)

Monoblastic cells on the most upper line.

No noteworthy findings were made on the erythrocyte series, except the occurrence of polychromasia.
The differential leukocyte count was as follows.

<table>
<thead>
<tr>
<th>Monocyte series</th>
<th>95.4</th>
<th>97.8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile type</td>
<td>2.4</td>
<td>1.2%</td>
</tr>
<tr>
<td>Mature type</td>
<td>2.4</td>
<td>1.2%</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>1.0</td>
<td>1.0%</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>1.0</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

Approximately 0.5% of the leukemic cells showed direct and indirect mitosis.

C. Findings in visceral smears

In smears prepared from the liver and spleen, most of the cells had various nuclear indentations. Thus, they manifested the features of the monocytic series. Some of these cells, however, presented a trend to disintegrate, with their obscure cytoplasm. Peroxidase reaction was entirely negative in these cells.

2. Case 2

a. Clinical history

The cow was kept in the vicinity of Morioka. She began to sweat in the middle of December, 1959, and show anorexia, depression, and dyspnea at the beginning of January, 1960. According to a veterinarian who examined her on January 27th, her temperature stood at 41.3°C and a marked cardiac disturbance was noted at that stage. The leukocyte count reached 200,000 per cmm on February 2nd. With a diagnosis of leukemia, she was transported to Iwate University for detailed examination. She fell into collapse on February 10th and died finally three days later.

b. Hematological findings

The blood cell counts prior to death were as follows.

<table>
<thead>
<tr>
<th>Erythrocyte</th>
<th>2.03 millions (per cmm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte</td>
<td>200,000 (per cmm)</td>
</tr>
</tbody>
</table>

(1) Findings in blood smears

As in the previous case, mononuclear cells were noted in close contact with one another in blood smears. Most of these cells presented the typical appearance of monocytes (Figs. 3, 4, and 12).

Although the mononuclear cells largely resembled those of the previous case in morphology, the leukemic cells seemed larger in this case. They measured 12~18μ (mostly 14~15μ) in diameter when they were monoblastic cells, and 15~27μ (mostly 20~22μ) when they were promonocytic cells.

The mononuclear cells had nuclei which showed various shapes looking like horseshoe, heart, butterfly, calabash, and doughnut. Rarely, a seven-lobed nucleus was seen. Figures of direct and indirect mitosis were also present. The cytoplasm was rather wide and presented indentations or processes at its peripheries, which were stained dark. Frequently, 1~2 or 10 odd vacuoles were also contained in the cytoplasm. Azure granules were rather obscure. They were detected very rarely in the cytoplasm, forming groups of several granules. Furthermore, all the leukemic cells gave a negative peroxidase reaction (Fig. 5).

In the erythrocyte series, anisocytosis was noted and polychromatic cells were also present sparsely.
The differential leukocyte count was as follows.

<table>
<thead>
<tr>
<th>Date</th>
<th>Monocyte series</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4/II '60</td>
<td>5/II '60</td>
<td>6/II '60</td>
<td></td>
</tr>
<tr>
<td>Juvenile type</td>
<td>96.2</td>
<td>95.1</td>
<td>95.2</td>
<td>98.8%</td>
</tr>
<tr>
<td>Mature type</td>
<td>2.9%</td>
<td>4.0%</td>
<td>3.6%</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>—</td>
<td>0.6%</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.9%</td>
<td>0.2%</td>
<td>0.8%</td>
<td></td>
</tr>
<tr>
<td>Plasma cell</td>
<td>—</td>
<td>0.1%</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Approximately 0.6% of the leukemic cells showed mitosis.

(2) Findings in bone marrow smears

Most of the nucleate cells were regarded as of the monocyte series. It seemed that the other nucleated cells had almost undergone the pressure and degenerating processes due to the proliferation of the leukemic cells. Some of the monocytes also showed a trend to disintegrate, with naked nuclei. At a glance, those cells presenting a satisfactory figure seemed to belong to the monocyte series.

![Fig. 2. Leukemic Cells in Bone-Marrow Smears (Case 2)](image)

The monocytes in the bone marrow smear almost resembled those in the blood smear in morphology, except that many of them possessed abundant cytoplasm. They showed indentations and processes at the peripheries of their cytoplasm, staining dark in these portions. Furthermore, their nuclei revealed polymorphism as seen in the blood smear and had fine lacy chromatin (Figs. 2, 6, and 13). Nucleoli were generally obscure in many of them. Figures of direct and indirect mitosis were observed in 0.6% of the leukemic cells.

The differential count of nucleate cells was as follows.

<table>
<thead>
<tr>
<th>Monocyte series</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile type</td>
<td>96.2</td>
</tr>
<tr>
<td>Mature type</td>
<td>1.9%</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>1.5%</td>
</tr>
<tr>
<td>Endothelium</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

Naked nuclei were seen in approximately 20 per cent of the nucleate cells other than those mentioned above. Though it was impossible to determine the exact origin
of the naked nuclei, most of them were regarded as those of cells of monocyte series from their morphological and staining features.

(3) Supravital staining with NR and JG

Supravital staining of the blood with NR and JG showed the typical figures of neutral-red rosettes in the cytoplasm of almost all the nucleated cells. Neutral-red vacuoles were seen as granules of almost the same size in a group or, rarely, in two groups. The granules varied in number, ranging from 10~15 to 40~50. They formed a dense mass when they were 40~50 in number. Some of these granules showed a tendency to become somewhat larger with the lapse of time. They did not, however, grow so large as in histiocytes. Besides, granules were seen distributed throughout the cytoplasm in mature monocytes which possessed abundant cytoplasm. There were a number of JG granules presenting dotted or rod-like figures. They usually gathered around the rosettes. They also formed clumps far from the rosettes (Figs. 9 and 10).

(4) Phagocytic affinity to carbon particles

Phagocytic action upon carbon particles was generally absent in the present leukemic cells. It was recognized in some leukemic cells observed in preparations which had been kept in the incubator for many hours. Carbon particles were scattered in the cytoplasm. They were almost the same in size, but varied in number with individual cells, the count ranging approximately from 5 to 40.

(5) Observations under the phase microscope

In the leukemic cells, the cytoplasm seemed to be very narrow and deficient in viscosity. Most of the cells having distinct nuclear indentations presented many slender processes at their peripheries. The surfaces of the nuclei showed mild undulations without marked irregularity, and mostly indistinct outlines (Figs. 15 and 16). Nucleoli were rarely seen, as dark spots, in the center of the nucleus (Fig. 20). Occasionally, several vacuoles, large or small, were recognized in the cytoplasm (Fig. 17). Most of the leukemic cells were approximately twice as large as the erythrocytes.

(6) Findings in visceral smears

Visceral smears were prepared from the spleen, liver, lymph nodes, kidneys, omentum, and adipose tissue around the spinal cord of the lumbo-sacral portion. The cells in these smears manifested figures characteristic of the monocyte, except those in the process of disintegration and those in the preparations from the lymph nodes and omentum. Especially, the cells contained in the tissues around the spinal cord showed apparent figures of the monocyte (Fig. 7).

The cells in the visceral smears all gave negative peroxidase reactions. No monocytc cells were noted in smears prepared from the fluids of the thoracic and abdominal cavities.

DISCUSSION

From the present cytological observations, monocytic leukemia was confirmed to have occurred as one of the types of bovine leukemia in the two cases. Needless to say, this confirmation is based on the cytological characteristics of the leukemic cells observed.

The independency of the monocytic series has already been established on the basis of its idiomorphic properties. It is well known that the above conception owes its fundamentals primarily to the extensive and systematic studies by Amano~3,5~ in the field of human hematology.
In conformity to this conception, MURAI\textsuperscript{40} has previously made cytological observation of monocytes in the horse, using materials collected by spleen puncture, with special reference to equine infectious anemia. Similar studies have been conducted later by SAKAI et al.\textsuperscript{41} and KONNO\textsuperscript{42}. Especially, detailed description has been given by the latter author on the morphology of the monocyctic series, together with that of other cells. As a result, the morphology of the monocyctic series in the horse has already been clarified by the authors cited above.

On the other hand, detailed observations have been made on the morphological features of monocytes of cattle in the present leukemic cases. They indicate that the monocytes of this animal species resemble those of the horse in morphological properties. Particularly, it is apparent that the monocytes of both animal species give almost negative peroxidase reaction. It was considered to be presumably due to the specific condition of leukemic cells that the phagocytosing ability to carbon particles was generally inactive in these cells of the present cases.

At any rate, it may be said that the independency of the monocyctic series was particularly confirmed also in the animals of the present cases from the actual condition of leukemic cells. Furthermore, it is suggested that the monocytes possessed such nature that they exhibited active proliferation voluntarily in the circulating blood; that is, the leukemic cells showed such a striking increase in number in the blood as described before. Consequently, it is considered that great importance should be attached to the high leukemic nature as one of the characteristics of the hematological findings of monocyctic leukemia.

It seems that bovine leukosis has been considered as a neoplastic hyperplasia principally in the reticulo-histiocytic system\textsuperscript{14, 40}. Most of the cases, however, have been generally designated as lymphosarcoma\textsuperscript{3, 41, 42}, lymphoblastoma\textsuperscript{39}, lymphocytoma\textsuperscript{40}, lymphadenosis\textsuperscript{7, 43, 44}, or lymphatic leukosis\textsuperscript{37}. Thus, the majority of the bovine cases have been realized as of lymphatic nature, although some differences are recognized among interpretations of the histogenesis of the leukemic cells.

In view of the results of the present cytological examinations, it is particularly felt necessary to intensify the recognition of the cell property of the monocyctic series. For instance, it is pointed out by AMANO\textsuperscript{11} in his report that Rieder cells, which mean such cells as showing lobated nuclei in acute leukemia in man, should be regarded not as lymphatic cells but apparently as the cells in monocyctic leukemia, judging from their original figures. Furthermore, it is also described that the "Paramyeloblast" of NAEGELI, which is a larger myeloblast with a lobated nucleus, should have the meaning of the "monoblast or promonocyte" of AMANO. Accordingly, it may be said that special caution is necessary in the use of the terms Rieder cell, paramyeloblast, and paralymphoblast to designate the mononuclear cells which have lobated nuclei.

Although the cases examined here are very limited in number, it is believed that the results of the present cytological examination may have an important significance on the promotion of morphological studies on bovine leukosis.

**SUMMARY**

Hematological studies, as well as cytological observations on smear preparations of various tissues, were carried out in two cases of bovine leukemia. From these observations, the cases were identified as those of monocyctic leukemia.

The principal results were as follows.
1. In the two cases, a striking increase was presented in the leukocyte count, which was approximately 200,000 and 420,000 per cmm, respectively, at the end of the course of disease.

2. The nucleated cells in the blood were exclusively those of the monocytic series, which consisted mainly of the juvenile types. Neutrophilic leukocytes and lymphocytes were only sparsely seen among these leukemic cells.

3. All the monocytes in the blood and visceral organs gave negative peroxidase reactions.

4. Collected by bone marrow puncture, the cells were shown not infrequently in the process of disintegration. Most of the cells which remained intact were regarded as those of the monocytic series.

5. The characteristic figures of the monocyte were also confirmed in cells observed in smears prepared from various viscera and tissues, especially tissues around the spinal cord of the lumbo-sacral portion.

REFERENCES

牛の単球白血病について

I. 細胞学的観察2例

三浦定夫・大島寛一
岩手大学農学部獣医学科
（昭和41年10月8日受付）

従来、著者らは岩手県内に発生する牛白血病例について、細胞病理学的および病理組織学的研究を行なっている。その中で、発症後約1ヶ月の経過で死亡した乳牛2例について細胞学的観察を行なった。その結果、これらが単球白血病であることを確認した。

血液所見上、白血球数はそれぞれ約20万および42万を示し、赤血球数はこれに反して減少し、それぞれ200万および300万を示した。末梢血液のメイ-Gム染色的細胞標標は、濃密に並ぶ単核細胞群を示し、好中球およびリンパ球は、きわめてまれにしか認められなかった。これらの単核細胞は、過酸化物酵素反応が陰性で、核は、類円形を示すものから、各種各様の形を示すものなど、多岐多様である。

しかし、いずれも核はせん細であって、中には明瞭な核仁を示すものもある。またしばしば直接および間接分裂像が認められる。原形質は灰青色に染まり、広狭種々である。1例における骨髄液の塗抹標本では、同様の細胞が主体をなし、特に原形質の突起形成が著明に認められた。また、末梢血液に中性赤・マーヌス細胞系染色を行うと、所在細胞はほとんどすべて定型的中性赤血球を形成する。骨髄貯藏能も、一部の細胞に認められた。

なお、肺、肝、腎および骨髄管内増殖組織などのメイ-Gム染色標本においても、出現細胞はおよそ末梢血液中に見られたものと同様の形態および染色性を示し、単球の特徴を具えたものであった。
EXPLANATION OF PLATES

PLATE I

All the figures were observed at high-power magnification (×1,000).

Fig. 3. Blood smear (Case 2). Monocytes are seen in close contact with one another. May-Giemsa staining.

Fig. 4. Same as the above.

Fig. 5. Blood smear (Case 2). Monocytes have given negative peroxidase reaction. Armitage's method.

Fig. 6. Bone marrow smear (Case 2). Most of the cells are regarded as monocytes. Cytoplasmic processes are seen in one of the cells. May-Giemsa staining.

Fig. 7. Smear of proliferated tissue around the spinal cord of the lumbo-sacral portion (Case 2). The cells show figures characteristic of the monocyte. May-Giemsa staining.

Fig. 8. Blood smear. (Case 1) containing mitotic monocytes. Giemsa staining.

Fig. 9. So-called neutral-red-rosettes in leukemic cells in the blood (Case 2). All leukemic cells seem to show some shrinkage. Supravital staining with neutral red alone.

Fig. 10. Same blood as above. Neutral-red-rosettes and Janus-green granules in the leukemic cells. Supravital staining with neutral red and Janus green.

PLATE II

Leukemic cells in various smears. Mononuclear cells, consisting mainly of monocytes, are seen in close contact with one another. May-Giemsa staining. ×1,000.

Fig. 11. Blood smear (Case 1).

Fig. 12. Blood smear (Case 2).

Fig. 13. Bone marrow smear (Case 2).

Fig. 14. Smear of proliferated tissue around the spinal cord (Case 2).

PLATE III

Leukemic cells observed under the phase microscope. ×1,500.

Fig. 15. Cytoplasm very narrow and deficient in viscosity. Nuclear surfaces show mild undulations without marked irregularity.

Fig. 16. A cell (center) with an indented nucleus and many ciliary processes at the periphery of its cytoplasm.

Fig. 17. A cell with vacuoles in its cytoplasm.

Fig. 18. The same picture as above seen at a changed focus.

Fig. 19. Accumulation of leukemic cells.

Fig. 20. A cell (lower part) with a nucleolus as a dark spot.