A Pathologic Study on Initial Lesions of Enzootic Bovine Leukosis

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Abstract. Thirteen cattle which were suffering from infection with bovine leukemia virus or persistent lymphocytosis, showing very slight or hardly detectable lesions were subjected to pathologic examination for initial lesions of enzootic bovine leukosis. Careful macroscopic observation revealed lesions swollen up and whitish in color on the cut surface of some of the lymph nodes. These lesions were confirmed by histologic examination as those of marked follicular hyperplasia accompanied with atypical blastic cells in the sinuses and paracortical area of the lymph nodes. Peyer's patches were extended and thickened due to follicular hyperplasia in the lymphatic tissue. Whitish spotted lesions on the gastric mucosa were also noted. Accumulation of atypical cells was noted microscopically in the upper layer of the propria of the stomach. From the findings obtained, it was stressed that the leukotic lesions initiated mainly in the lymph nodes. The significance of occasional infiltration of macrophages, as well as eosinophils, in the initial lesions was discussed.

Enzootic bovine leukemia (EBL) is a neoplastic proliferative disease of the lymphoreticular tissue due to infection with bovine leukemia virus (BLV). Many reports have been published on the pathomorphology of EBL. In their review of literature Loppnow et al. [20] pointed out that lesions had frequently been observed in the lymph nodes, heart, abomasum, and uterus. Herzog [13] and Beyer and Urbanek [7] examined natural cases of EBL and noticed follicular hyperplasia of the lymph nodes and spleen, as well as formation followed by proliferation of prolymphocytes in the extrafollicular region at the beginning of the disease. Ueberschär [36] reported that lesions were located in the extrafollicular region of the lymph nodes at the beginning of the disease and then extended along the sinuses.

It seems that no concrete agreement may be present among previous workers on the initial changes of the lymph nodes. Some workers [8, 9, 20, 26] recognized the accumulation of neoplastic cells in the cortex and medullary cords of the lymph nodes and some others [10, 17, 21, 30] in the medullary and intermediate sinuses initially. Besides, several workers [10, 15, 31, 33] pointed out that the initial lesions of EBL were seen in the atrial and abomasal wall.

BLV was recognized, however, as an etiologic agent of EBL after the papers cited above had been published. It has not been referred to in any previous report. The purpose of the present study is to investigate the lesions of animals infected with BLV which are not in the so-called tumorous stage as yet, and to clarify the pathogenesis of the disease.

Materials and Methods

Thirteen animals were selected from among those
Table 1. Hematologic and virologic findings of animals examined

| Group | Animal No.
<table>
<thead>
<tr>
<th>Breed</th>
<th>Age</th>
<th>RBC#4</th>
<th>WBC#6</th>
<th>Differential count of WBC#6</th>
<th>Absolute No. of MNC/cm³</th>
<th>Absolute No. of AtMC/cm³</th>
<th>BLV#8</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 (1207) HF</td>
<td>3</td>
<td>638</td>
<td>18.5</td>
<td>3.5</td>
<td>11.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 (1226) JS</td>
<td>6</td>
<td>670</td>
<td>25.0</td>
<td>10.5</td>
<td>37.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>3 (1062) HF</td>
<td>Ad</td>
<td>700</td>
<td>17.0</td>
<td>12.5</td>
<td>22.0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>4 (1208) //</td>
<td>1.5</td>
<td>634</td>
<td>18.2</td>
<td>21.5</td>
<td>26.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 (1158) //</td>
<td>2.5</td>
<td>660</td>
<td>32.0</td>
<td>10.0</td>
<td>27.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6 (1215) //</td>
<td>3</td>
<td>380</td>
<td>21.0</td>
<td>0.5</td>
<td>36.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>7 (1306) JS</td>
<td>//</td>
<td>879</td>
<td>28.5</td>
<td>11.0</td>
<td>25.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8 (1078) HF</td>
<td>4</td>
<td>590</td>
<td>23.1</td>
<td>8.2</td>
<td>8.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9 (1077) //</td>
<td>//</td>
<td>497</td>
<td>35.0</td>
<td>15.2</td>
<td>26.4</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>10 (1237) //</td>
<td>//</td>
<td>525</td>
<td>12.0</td>
<td>8.0</td>
<td>9.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11 (1080) //</td>
<td>//</td>
<td>500</td>
<td>23.0</td>
<td>5.0</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12 (1252) //</td>
<td>9</td>
<td>425</td>
<td>10.1</td>
<td>0</td>
<td>62.5</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>13 (1194) JB</td>
<td>6</td>
<td>700</td>
<td>9.5</td>
<td>6.5</td>
<td>59.5</td>
<td>0</td>
</tr>
</tbody>
</table>

#1: All were female, except animal No. 7 (1306).
#3: Ad, Adult.
#4: RBC, Red blood cell count, × 10⁴/cm³.
#5: WBC, White blood cell count, × 10⁴/cm³.
#7: MNC, Mononuclear cell.
#8: +, Positive serum BLV gp-antibody but electron microscopy not done for BLV particles.
+++, Positive serum BLV gp-antibody with BLV particles detected. NE, Not examined.

affected with EBL and autopsied during a 5-year period from 1975 to 1979. They were sero-positive for immunodiffusion (ID) test of BLV antibodies and/or proved to be leukemic as examined with the hematologic diagnostic key of the European Community (EC) as the criterion. Although an atypical mononuclear cell (AtMC) [24] was recognized in blood smears prepared from most of the animals, macroscopic lesions were hardly or indistinctly observed at autopsy. The mononuclear cell (MNC) count was 3,250-20,160/cm³, and the AtMC count 0-3,040/cm³ in the 13 animals. Ten animals were affected with lymphocytosis and the remaining three showed a normal range of MNC count with the AtMC count of 145-1,414/cm³ (Table 1). All the animals were killed by bleeding for autopsy after brief clinical and routine hematological examinations during an observation period of several days.

Stamp smears, paraffin sections, and ultrathin sections were prepared from various organs of the whole body, mostly lymph nodes and hemolymph nodes. They were subjected to May-Giemsa staining, hematoxylin and eosin staining, and uranyl acetate and lead citrate staining [24], respectively.

Buffy-coat cell cultures were used for detection of BLV by electron microscopy. Serum antibody was examined by the ID test with BLV gp-antigen prepared from a fetal lamb kidney cell line infected with BLV [24].

**Results**

The animals examined were divided into 3 groups, 1 to 3, according to the lymphoproliferative progression of lesions (Table 2).

A. Lesions of lymphatic tissues

Obvious tumorous lesions were observed only in group 3, but the enlargement of lymph nodes was noted at variable degree. Irregular-shaped whitish swelling was evi-
Table 2. Lesions in lymphatic tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal No.</th>
<th>I</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular hyperplasia of Spleen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tonsils</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Peyer's patches</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hemolymph nodes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Lymph nodes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Atypical blastic cell infiltration in Lymph nodes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Eosinophil infiltration in Lymph nodes</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>Plasma-cellular infiltration in Lymph nodes</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

-, None. ±, Very slight. +, Slight. ++, Moderate. ++++, Marked. +++, Neoplastic.

ident in the cortical area of the cut surface in the lymph node. Similar hyperplastic lesions were detected in such lymphatic tissues as hemolymph nodes, spleen, and Peyer’s patches.

Group 1 (Nos. 1–3): In each animal a few atypical lymphocytes were seen in several lymph nodes.

Such lymph nodes as the prescapular, subiliac, parotid, bronchial, hepatic, gastric and mesenteric were slightly enlarged in this group. Some of them were irregular-shaped and partially swollen on the cut surface of the cortical area. Histologically, follicles with a small or medium-sized germinal center increased in number. Reticulum cells were hyperplastic. Lymphoid or lymphoblastoid cells with a nucleus of atypical form and basophilic cytoplasm were occasionally recognized in the medullary sinus. Large lymphoblastoid cells were accumulated in the paracortical area of the hepatic lymph node in one animal. Plasma cells proliferated occasionally in the medullary cords. Eosinophils infiltrated noticeably in the medulla, trabeculae, hilus, and capsule.

Marked follicular hyperplasia was recognized in the enlarged hemolymph nodes examined. Follicles with a large germinal center increased in number and contained many cells with a mitotic figure. Slight eosinophilic infiltration was observed in the spleen.

Peyer’s patches were swollen up and tangible through the serosa of the intestine. There were large follicles and cellular proliferation in the perifollicular area. Follicular hyperplasia was recognized in the tonsils.

Group 2 (Nos. 4–12): In each animal a moderate number of atypical lymphocytes were seen in most of the lymph nodes.

Most of the lymph nodes were swollen slightly to a size of thumb tip to hen’s egg. The smallest node weighed 40 g and the largest, or the left supramammary lymph node of No. 4 170 g. On the cut surface of the lymph node, the cortex was extended in width and swollen irregular-shaped lesions of from rice grain to red bean size were noted (Fig. 1).

Histological examination revealed moderate (Nos. 6, 8–10, and 12) or marked (Nos. 4, 5, 7, and 11) follicular hyperplasia in the lymph node, where follicles increased in
number and size (Fig. 2). Mitotic cells and macrophages were noted at the enlarged germinal center. Many plasma cells were detected in the medullary cord of the lymph nodes, except those in No. 5. Slight to marked eosinophilic cell infiltration was noted in the medulla, hilus, capsule, and trabeculae. Sinus endothelial cells increased in size and number, and many of them were liberated. Comparatively large irregular-shaped reticulum cells and lymphoblastoid cells were observed in the sinus, where many atypical plasmablastoid cells were noted, especially in No. 12 (Fig. 3).

Many hemolymph nodes were also enlarged to the size of thumb tip, presenting a granular appearance on the cut surface. Follicular hyperplasia was noted histologically. It was accompanied with splenomegaly in the spleen. Slight lymphocytic infiltration was observed in some parts of the splenic red pulp. Tonsillar lymph follicles were enlarged with many mitotic cells. Peyer’s patches were also hyperplastic.

Group 3 (No. 13): This animal exhibited neoplastic proliferation in the medullary sinuses of the lymph and hemolymph nodes.

The left prescapular lymph node weighed 115 g and measured 24x13 cm. The internal iliac lymph nodes were as large as hen’s eggs. Many of the other lymph nodes were also enlarged. The original structure was obscure on the swollen cut surface in most of the organs. Histological examination disclosed infiltrating neoplastic cells accompanied with a considerable number of macrophages in the dilated sinuses in most of the lymph nodes. The medullary cords, as well as the cortical area, were comparatively narrow. Neoplastic cells were irregular in contour and variable in size. The rim of cytoplasm was rather narrow. The nuclei were round, polymorphic, indented or lobulated, containing a fine chromatin network and one or two nucleoli.

Many of the hemolymph nodes were spherical and as large as the tip of the little finger to that of the thumb. The cut surface showed a marble-like pattern. Histologically, neoplastic cells proliferated in the sinuses.

B. Other findings

No macroscopic lesions were detected in the cardiac atrium. Histologically, slight lymphocytic and plasma cellular infiltration was noted in the myocardium or atrial epicardium in Nos. 2 and 3. Whitish granular lesions of rice grain size were disseminated on the mucosal surface of the abomasum in all the three groups (Fig. 4). They were confirmed as focal accumulations of polymorphic lymphoid cells in the upper layer of the mucosal propria (Fig. 5).

In addition to the lymphoproliferative lesions, eosinophilic cell infiltration was noted in many animals of each group. It was prominent in various organs and tissues of Nos. 7–9 and 11, especially of No. 8.

Electron microscopy revealed lymphocytes, lymphoblasts, immature plasma cells, and atypical cells in the lymph sinuses of the animals of groups 1 and 2. The neoplastic cells in No. 13 of group 3 presented characteristics of reticular cell type [11, 24, 57] (Fig. 6). They were polymorphic and had numerous cytoplasmic projections.

Detection of BLV and serum antibodies to this virus was performed in 10 animals with positive results shown in Table 1.

Discussion

The animals examined were infected with BLV and/or showed persistent lymphocytosis, except one, No. 13, in which neoplastic proliferation was recognized. Persistent lymphocytosis and the appearance of AtMC in the peripheral blood may be a representation of lymphoreticular hyper-
plasia or neoplastic transformation in the internal organs.

Follicular hyperplasia observed in the present studies would explain the phenomenon of peripheral persistent lymphocytosis as described by previous authors [5, 6, 12, 13]. The distribution of affected lymph nodes was restricted in group 1. Almost all the lymph nodes were affected in group 2. When obvious neoplastic cell proliferation occurred in these organs as observed in group 3, the lymph follicles were already static. Neoplastic cell-like large atypical lymphoblastoid and polymorphic histiocytic or reticular cells were observed in the medullary sinus of the lymph nodes with follicular hyperplasia. Similar findings were reported by Dungworth et al. [10] on the early lesions of EBL detected.

In some of the present sections of the lymph nodes, accumulations of large lymphoblastoid cells were occasionally observed in the sinuses and paracortical area. Some previous authors [7, 20, 30, 36] reported extrafollicular proliferation of distinguishable neoplastic cells in the lymph nodes. Theoretically, two theories of histogenesis may be considered in the extrafollicular proliferation of transformed cells. One of them is that these cells may have resulted from accelerated lymphatic proliferation in situ. The other is that the cell may have been derived from metastatic migration in nature. Marked follicular hyperplasia may result in cellular transformation, as well as migration into the paracortical area and the sinus, where proliferation may take place in such manner as observed in animal No. 13. Similar changes were recognized as early lesions of the disease by several authors [7, 10, 21].

Enlargement in size and increase in number of follicles were observed in the hemo-lymph nodes, as noted in the lymph nodes. Many mitotic cells, as well as macrophages, were seen at the germinal center. There were neoplastic cells in the sinus of animal No. 13 of group 3. Some workers [8] pointed out that neoplastic lesions were uncommon in the hemolymph nodes of leukotic cattle, but many authors [10, 14, 19, 33] reported the frequent occurrence of these lesions. On the other hand, Ueberschär [36] expressed his opinion that the reaction of germinal lymphatic tissues might result in the transformation of lymphatic and/or reticulo-histiocytic cells.

Asserting that the lesions of the atrial wall, abomasum and uterus, Stöber [31] attached much importance to the lymphocytic accumulation in these tissues. Similar assertion was made by Bederke et al. [2, 3], Järplid [15] and Beyer [4] studied the pathogenesis of the heart lesion in the disease and noticed lymphocytic and blastoid cell accumulation in the subepicardium of the right atrium. In the present animals, similar cell accumulation was frequently observed, but no cellular lesions were confirmed as initial ones of the disease.

The leukotic lesion of the disease was frequently observed in the abomasum, as well as in the heart [18, 20, 37]. Gastric lesions were usually located in the submucosa and subserosa in tumorous leukotic animals. The present authors [24] previously reported animals with neoplastic lesions in the upper layer of both propria and submucosa separately. In the present examination, a lesion of accumulation of polymorphic mononuclear cells was found in the upper layer of the propria. It may possibly be a lesion preceding tumorous changes in the abomasum.

Electron microscopic observation was done on neoplastic cells of bovine leukosis by not a few investigators [11, 34, 35]. The neoplastic cells of animal No. 13 were of
reticular cell type in the classification made by some previous authors [11, 24, 37]. It was pointed out that differentiation between neoplastic and normal cells was not easy and that major difference consisted in the quantity of organelles [34]. In the present examination, it was rather difficult in animal No. 13 to differentiate atypical lymphoblastoid or histiocytic cells morphologically from neoplastic cells.

Plasma-cell infiltration observed in the lymph nodes may be considered as morphologic expression of serum-antibody production. Macrophages were found in the lymph sinuses among the neoplastic cells of animal No. 13. Their functional activity is still obscure in this case. It seems that the macrophages may recognize antigenic neoplastic cells and play a role of transmitter of immunologic information in the initial lesion.

Peripheral eosinophilia, as well as eosinophilic cell infiltration, was observed in organs and tissues in all the animals examined, except Nos. 12 and 13. It was reported that eosinophilia appeared prior to clinical manifestation of the disease [23, 29, 38]. Seils [27, 28], put a great significance on eosinophilia in bovine leukemia and speculated that such immunologic phenomenon might be induced by hypersensitivity of the adrenal glands. Eosinophilic cell infiltration has frequently been observed in tissues in bovine leukemia [2, 3, 13, 30], human lymphadenosis, lymphoma, and myeloid leukemia [39], and Hodgkin’s disease [32]. It is also known that eosinophils are likely to appear prior to malignant changes and that complement may be related to the cell infiltration [1, 16, 25]. Miller and Olson [22] observed eosinophilia in biopsy materials in the regressed case of lymphosarcoma. In the present examination, the significance of eosinophilia was not elucidated, but this change may have had some immunologic effects. The interpretation of the initial lesions of EBL will be valuable for the clarification of the pathogenesis of this disease.

Acknowledgments. This work was supported in part by Grant-in-Aid for Scientific Research Fund No. 456212 of the Ministry of Education, Science and Culture, Japan.

References

INITIAL LESIONS OF ENZOOTIC BOVINE LEUKOSIS


地方性ウシ白血病に見られる初期病変に関する病理学的解析：大島寛一・佐藤繁・岡田幸助（岩手大学農学部家畜病理学講座）—ウシ白血病ウイルスに感染しているか、あるいは持続性リンパ球増多症を示し、かつ剖検において病巣がほとんど認められないか、またはきわめて軽微な13頭のウシについて病理学的検索を行った。精細な肉眼的検査により、不特定複数のリンパ節の側面において、類白色を帯び軽度に膨隆する領域が、主として皮質に認められた。組織学的には、この部位に一致して著明な嗜酸性増生と、洞あるいは副皮質領域における非定型的幼若大型細胞の出現が認められた。所見はその程度に従って5群に分けて記載された。バイエル板は隆起し、リンパ組織の増生が観察された。また第四胃粘膜に類白色類粒状膨隆部が散在し、鏡下で固有層上層における異型性の強い細胞の集簇巣として認められた。これらのことから、病巣が最初リンパ節に発現することが強調され、そこにしばしば出現する大食細胞や好酸球の意義について、若干の議論が加えられた。

Explanation of Figures

Fig. 1. Cut surface of the internal iliac lymph node of animal No. 4. Whitish lesions swollen up on the cut surface are seen in the cortical region. ×1.3.

Fig. 2. Marked follicular hyperplasia of the lymph node shown in Fig. 1. H-E staining, ×42.

Fig. 3. Large basophilic lymphoblastoid cells and irregular-shaped reticulum cells are numerous in the medullary sinus, and plasma cells in the medullary cord (upper left) in the popliteal lymph node of animal No. 12. H-E staining, ×429.

Fig. 4. Disseminated miliary and grain-sized lesions swollen up on the abomasal mucosa in animal No. 1. ×1.3.

Fig. 5. Mononuclear cell infiltration in the upper proprial layer of the abomasum in the lesion shown in Fig. 4. H-E staining, ×105.

Fig. 6. Electron micrograph of reticulum-cellular neoplastic cells observed in the medullary sinus of the superficial cervical lymph node of animal No. 13. The cells are polymorphic with hairy and villous projections. Numerous ribosomes and other organelles are recognized in the cytoplasm. Golgi apparatus is occasionally observed. Nuclei are polymorphic and indented, in which nuclear pockets as well as nucleoli are frequently observed. ×9,300.