Pathology of Lymphosarcoma in Sheep Induced with Bovine Leukemia Virus

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SUMMARY

Sixty-nine sheep were infected with bovine leukemia virus from bovine lymphosarcoma materials. Twenty-four developed lymphosarcoma and died from 13 to 66 (average, 29) months later. Circulating lymphocytes were increased to leukemia levels (70,000 to 403,000/cu mm blood) in only eight sheep within 2 to 3 months of death. Various lymph nodes and visceral organs including heart, abomasum, uterus, kidneys, and urinary tract were commonly affected as in cattle with the adult form of lymphosarcoma. In one sheep the skin was involved. The liver was involved in only one case. This was in contrast to more frequent involvement reported in literature for naturally occurring lymphosarcoma. The neoplasms in experimental sheep are regarded as a mixture of reticulum or histiocytic cells and lymphoid cells with transitional forms supported by a usually sparse and diffuse fibroplasia and a web of silver-staining reticulin fibers.

INTRODUCTION

The experimental production of lymphosarcoma in sheep with bovine lymphosarcoma material was first reported in 1969 by Wittman and Urbanbeck (25) and was apparently inspired (26) by observations of Enke (6, 7) in East Germany. In 1961, Enke et al. (7) reported the development of “lymphatic leukemia” in 45 of 500 to 600 sheep in the years 1959 to 1961. A 1961 study (18) of 8055 cattle in Sweden indicated a highly significant occurrence of lymphosarcoma in herds where fresh bovine blood containing Piroplasma had been used for more than 7 years for immunization of cattle against piroplasmosis. This report may have led Enke to suspect a similar source of infection in sheep observed by him, since in 1964 (6) he recalled that, 2 to 3 years previous to the 1959 to 1961 leukemia outbreak, the sheep had received 2 injections of Piroplasma-infected blood of a calf that came from a small herd of cattle affected with bovine leukemia.

The oncogenicity of BLV for sheep has been confirmed (Refs. 12, 17, and 26; M. Van der Maaten and J. M. Miller, personal communication). The infectivity of BLV for goats has been reported (9, 21). A C-type virus has been demonstrated in PHA-stimulated cultures of lymphocytes from affected sheep in a flock with enzootic lymphatic leukemia (19). The serum from these sheep, as well as from sheep and goats inoculated with leukemic material from affected sheep, had precipitin antibody to ether-resistant BLV antigen prepared in the United States and ether-treated antigen prepared from cultures of the C-type virus cultured from a leukotic sheep (20). It is therefore possible that this enzootic lymphatic leukemia outbreak in Germany may have been due to BLV.

The characteristic of naturally occurring lymphosarcoma (malignant lymphoma) in sheep has been recently studied in New Zealand and compared with reports in the literature constituting a total of 157 cases (11). These cases can be compared with the lesions of lymphosarcoma in 24 sheep induced with BLV.

MATERIALS AND METHODS

There were 24 cases of experimentally produced lymphosarcoma among 69 sheep inoculated with infective bovine lymphosarcoma materials (9, 17), Sheep V29, V31, V37, V40, V41, V42, and V44 received i.p. BLV, verified by EM, in a 2-day culture of PHA-stimulated lymphocytes from a 2.5-year-old cow (Case 3133) that died with a naturally occurring lymphosarcoma (17). The circulating lymphocytes had been 180,000 to 263,000/cu mm and were harvested and stored at −60° in 15% dimethyl sulfoxide with 20% fetal calf serum prior to culture. Sheep D50 received 440 × 10⁶ cells in a suspension of lymphoid tumor from a parotid lymph node of a 6-month-old calf (Case 3134) that died with generalized lymphosarcoma (17). Sheep A6 and A8 received i.p. BLV, verified by EM, in a culture of lymphocytes mixed from Sheep V33 and V34 that had been experimentally infected with BLV from Case 3133 (9). Sheep A18, A22, A25, A55, A57, and A61 received i.p. BLV, verified by EM, in a culture of lymphocytes from either Bovine 344 that had been experimentally infected with BLV from a naturally occurring case of lymphosarcoma or Bovine 521 experimentally infected with BLV from Case 3133 (9). Sheep A28 and A29 received the same BLV material as Sheep A18, except that the material had been frozen for 14 days at −60° (9). Sheep A42, A44, and A45 received buffy-coat suspension from Sheep A42 or...
V37 at a time when their lymphocytes would produce BLV, verified by EM, after a short-term culture stimulated by PHA (9). Sheep B6, B7, and B8 received i.p. BLV, verified by EM, in a short-term culture of lymphocytes from either Cow 344, which had been experimentally infected with BLV, or Cow H1915, which had been naturally infected with BLV. The infected material was mixed with milk from a cow with no precipitin antibody to BLV in a pasteurization experiment. 1 The sheep had been inoculated as 1-day- to 2-month-old lambs. They died or were killed when moribund with tumors from 13 to 66 (average, 29) months later. The experimentally inoculated sheep came from 2 flocks. One was a commercial herd with no reported occurrence of lymphosarcoma, and 11 uninoculated control lambs from this flock were normal (clinical examination, and negative for BLV precipitins) at 2 years of age. The other flock of 300 to 500 adult sheep for the past 25 years has been maintained by the College of Agriculture with veterinary supervision, and no known cases of lymphosarcoma have occurred. Most of the sheep inoculated with BLV in this report came from this flock, as well as 20 control lambs not inoculated and monitored by culture for BLV and precipitin tests for BLV antibody for 4 years. The 20 control sheep have remained negative. Cultures of circulating lymphocytes for demonstration of BLV, tests for precipitins to BLV, and counts of blood lymphocytes were done at various intervals (9). Lymphocytes were obtained from blood by a silicone method, cultured in a suspension with PHA and fetal calf serum for 2 to 3 days, and then pelleted for examination by EM for BLV. A double diffusion agar gel method was used for detecting presence but not titer of precipitins to a BLV antigen. Gross morphological changes were noted at necropsy, and tissues were fixed in buffered 10% formaldehyde for subsequent histological staining with hematoxylin-eosin, Van Gieson, Masson's trichrome, May-Grunwald-Giesma, and Bielschowsky silver stains.

**RESULTS**

A marked increase to leukemic levels (70,000 to 403,000/cu mm) of circulating lymphocytes was noted in 8 sheep (V31, V40, V42, A6, A22, A28, B6, and B8) 16 to 30 months after inoculation and within 2 to 3 months of death. The increase sometimes was rapid, developing in a few days. In 3 other sheep (V29, D50, and A45), the number of circulating lymphocytes had been within a normal range until a terminal drop to 1,150 to 2,000/cu mm of blood. Essentially, normal to slightly elevated numbers (5,000 to 12,000/cu mm of blood) of lymphocytes were noted during the experimental life of 14 sheep. In 3 of these sheep (V41, A18, and A61), a lymphocyte count of 15,000 was recorded once.

Lymphocytes from all 24 sheep produced leukemia virus on culture, some as early as 3 to 4 months after inoculation. Their sera contained precipitating antibody to BLV after inoculation that was evident at the time of or soon after BLV was manifest by culture of lymphocytes.

The neoplastic lymphoid cells varied in morphology from a primitive type with large vesicular nuclei (Fig. 4) to somewhat smaller more mature types (Fig. 9) with relatively more cytoplasm and with nuclei that contained more chromatin. The primitive characteristic of the lymphoid tumor cells was evident in the many nucleoli and fine chromatin pattern seen with imprint preparations (Fig. 16). In some tumors, there were also reticulum cells similar to but larger than the primitive type of lymphoid cells. In many of the tumors, there was such a mixture of cell types that the tumor could be called a histiolympocytic lymphosarcoma (10).

Both lymph nodes and visceral organs were involved, grossly or microscopically, with lymphoid neoplasia in all the experimentally inoculated sheep (Table 1). Sheep A29 may have had visceral organs affected but postmortem changes precluded their histological examination. In 3 other animals (V29, A55, and A61), lymphoid tumor infiltration of the liver, spleen, or heart was slight to moderate.

Lymph nodes most commonly affected and those usually examined were mediastinal, prescapular, prefemoral, mesenteric, and iliac. The extent of lymphoid tumor in these lymph nodes is reflected by their enlargement from 2 to 10 times their normal size (Table 2). The mesenteric lymph nodes were frequently involved (Fig. 3), but their size was difficult to measure with accuracy. Both of the paired lymph nodes were frequently but not always affected.

The heart was involved in 20 of 23 cases examined (Figs. 1 and 2). There was infiltration of lymphoid tumor in the ventricles in 16 cases and of the auricles in 14 cases. The right auricle only was affected in 2 cases and in combination with the ventricles in 8 other instances. In 3 cases, the left auricle was affected and not the right auricle. The lesions in the heart varied from gray-white streaks of lymphoid tumor to sheet-like irregular infiltrations.

The uterus was often affected with infiltration of the muscle as well as mucosa (Fig. 1).

The abomasum was involved (Figs. 11 and 17) in 12 of 19 cases in which it was specifically examined. In 1 animal (D50), lymphoid tumor caused erosion of the wall with perforation and peritonitis.

The wall of the urinary bladder was grossly involved with lymphoid tumor, and frequently the ureters had tumor infiltration in the submucosa. The seminal vesicles of the 1 intact male sheep, B7, had lymphoid tumor growth.

The kidneys were not often severely affected with lymphoid tumor which was usually localized in nodular masses (Fig. 1) or in the capsule. In 4 instances, tumor growth in the ureters had led to moderate hydronephrosis.

In only 1 instance (V31) did lymphoid tumor cause the spleen to be slightly enlarged (15 x 12 x 5 cm; 375 g). In 1 other case (V29), there were scattered focal masses of splenic lymphosarcoma. In 3 other cases, there were lymphoid tumor cells in the pulp without splenomegaly.

In 2 cases, there was peribronchial lymphoid tumor and in 3 other cases tumor was localized on the pleura.

Tumor was rare on the intestine (Fig. 17), being localized on the peritoneal surface in 3 cases and encircling the duodenum in another.

The livers were either normal or fatty in appearance. By histological examination, except for lymphoid tumor cells in the vascular bed, there was moderate tumor infiltration in the portal triad areas in only 1 animal (B8).

Bone marrow for histological study was obtained in only 4
Table 1
Involvement of organs and lymph nodes in 24 sheep with lymphosarcoma induced by BLV and the percentage of frequency compared with naturally occurring lymphosarcoma as reported by Johnstone (11) and Anderson and Jarrett (1)

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Ref. 11 68 16 74 48 47 47 37 50 68
Ref. 1 29 16 29 32 5 5 3 13

<sup>a</sup> - examined and not involved; ±, slight involvement; +, involved.

Lymphosarcoma in Sheep with BLV
cases. The rib, femoral, and sternal marrow in 1 animal (V31) had areas of lymphoid tumor infiltration.

Ovaries were normal in the 13 cases examined. In 1 instance the adrenal was affected. In 2 cases, lymphoid tumor was found in the musculature of the diaphragm.

In 1 case (B8), diffuse lymphoid tumor growth was replacing much of the mammary gland tissue (Fig. 10). The pancreas contained scattered 1- to 5-mm tumor nodules in Sheep A25. There was a large lymphoid tumor of the parotid salivary gland in Sheep D50 (Figs. 5 and 6).

Sheep V40 had extensive lymphoid tumors in the skin (Figs. 14 and 15) in addition to many affected lymph nodes and visceral organs.

Hemal nodes, usually adjacent to the hilus of affected lymph nodes, were also infiltrated with tumor. The areas occupied by thymic tissue had lymphoid tumor growth in 11 of 20 cases. In most instances, tumor was infiltrating between fat cells and sometimes mixed with small, dark-staining thymocytes. In 2 cases (V42 and A42), a fibroblastic proliferation (Fig. 13) was marked and displaced much of the lymphoid tumor.

The association of marked fibroblastic lymphosarcoma was noted in 3 sheep. An irregularly shaped pelvic tumor mass involved the iliac lymph nodes of Sheep A22 and adjacent tissues. Most of the tumor was lymphosarcoma with scattered fibroblasts but in some areas the fibroblasts predominated. Sheep A6 had a similar pelvic tumor mass in which the fibroblastic cells were more densely arranged (Fig. 12); in addition, there was a mixed lymphoid and fibroblastic tissue involving the abdominal wall, omentum, and abdominum. Sheep V42 had a 16- x 10- x 10-cm tumor in the upper cervical region (Fig. 19) and a 9- x 8- x 5.5-cm mass at the thoracic inlet, both composed of a mixture of lymphoid and fibroblastic cells (Fig. 13). A lymphoid tumor mass involving the wall of the rumen had areas of fibroblastic cells in Sheep D50.

Sheep V44 died with tumor 66 months after inoculation and had extensive involvement of lymph nodes as well as the esophagus, abomasum, mesentery, retrobulbar tissue, and body wall (Figs. 17, 18, and 20). Tumor also infiltrated the body wall of Sheep A25.

DISCUSSION

Lymphosarcoma as experimentally produced in sheep with BLV can best be compared with the series of naturally occurring cases described by Johnstone (11) and Anderson and Jarrett (1). A major difference from the presently described cases seems to be the more frequent involvement of the liver and spleen in the naturally occurring cases and in 5 of 9 experimental cases reported by Urbanek et al. (24). There was more frequent involvement of the uterus, heart, and abomasum in experimentally produced lymphosarcoma (Table 1). Johnstone (11) found tumor in 74% of the lymph nodes of the iliac, 68% of the mediastinal, 63% of the mesenteric, and 63% of the cervical regions, which is comparable to that found in the experimental sheep.

Anderson et al. (2) proposed a classification system for lymphosarcoma in domestic animals with 3 main forms, namely, multicentric, thymic, and alimentary, and in addition a skin form. In their work, the alimentary form, involving intestine, mesenteric lymph nodes, and often liver, seemed to be a useful classification when applied for the disease in cats and dogs but less so for cattle, sheep, and pigs. Johnstone (11) classified his 22 cases and 134 other lymphosarcoma cases of sheep in 8 other reports as 97 multicentric, 25 alimentary, 14 thymic, and 20 skin. The classification of an alimentary form in sheep may be somewhat arbitrary since none of the experimentally produced cases would fit the criteria.

Involvement of the skin and thymus occurs in both naturally occurring and experimentally produced lymphosarcoma of sheep. This may simply be an extension of the lymphoid neoplasia and not distinct forms of the disease as in cattle. In bovine lymphosarcoma, skin lesions may regress (3, 15) or the animal later develops the multicentric form with involvement of visceral organs and lymph nodes (3, 14). The thymic form of lymphosarcoma in cattle is peculiar in that, in 6 cases examined thus far, their sera had no precipitin antibody to ether-resistant BLV antigen, and blood lymphocytes from 2 cases failed to produce BLV on short-term PHA-stimulated culture.

The fibroblastic reaction with lymphosarcoma in 5 experimental sheep has a counterpart in the bovine thymic form of lymphosarcoma where we have noted fibroblastic reaction in the thymus. Although the significance of this is not obvious, it would appear that BLV in lymphoid tumor can stimulate hyperplasia of fibroblasts as well as lymphoid cells. Serums from sheep with fibrosarcoma induced with feline sarcoma virus (23) were submitted by Theilen et al. (22) and found to be negative for antibody to the ether-resistant antigen of BLV.

Mammerickx et al. (12) illustrated a large tumor mass of lymphosarcoma involving the kidneys to the pelvis.

Table 2

Dimensions of lymph nodes frequently affected with lymphosarcoma in sheep inoculated with BLV

<table>
<thead>
<tr>
<th>Lymph Node</th>
<th>Dimensions (cm)</th>
<th>Av.</th>
<th>Range</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediastinal</td>
<td>9 x 3 x 3</td>
<td>17 x 3 x 1-5 x 2 x 2</td>
<td>2 x 1 x 0.5</td>
<td></td>
</tr>
<tr>
<td>Prescapular</td>
<td>5.9 x 4.1 x 3</td>
<td>18 x 12 x 6-3.2 x 2.5 x 2</td>
<td>3 x 1 x 2</td>
<td></td>
</tr>
<tr>
<td>Prefemoral</td>
<td>3.3 x 2 x 1.6</td>
<td>8 x 5 x 3-2.5 x 2 x 1.5</td>
<td>2 x 1 x 0.5</td>
<td></td>
</tr>
<tr>
<td>Supravaginal</td>
<td>3.4 x 2.2 x 1.8</td>
<td>6 x 2.5 x 2-2 x 1 x 1</td>
<td>1 x 1 x 0.5</td>
<td></td>
</tr>
<tr>
<td>Iliac</td>
<td>6.6 x 4 x 3</td>
<td>14 x 10 x 5-3.5 x 2.5 x 1.5</td>
<td>2 x 1 x 0.5</td>
<td></td>
</tr>
</tbody>
</table>

*a* In Sheep A44, the iliac nodes were involved in a larger tumor mass extending from the kidneys to the pelvis.

the abdominal wall that apparently grew from grafts at the cicatrix following a cesarean operation in a cow. He also illustrated s.c. lymphoid tumors in a sheep in which fatal lymphoid neoplasia developed after inoculation p.o. of blood from bovine “enzootic leucose” (12). Lymphosarcoma has developed in 8 sheep following inoculation p.o. of bovine blood and as early as 10 months (13). Lymphoid tumor growth s.c. was found in 4 of 24 cases of this series and was very large in 2 (Fig. 18). Such growth may follow trauma in which neoplastic lymphoid cells from the blood localize and establish the tumor. Attempts to produce such tumors by s.c. implants of fresh tumor from one sheep to another have failed but these would be heterologous and not autologous grafts.

Nine sheep with experimentally induced lymphosarcoma died from 10 to 29 (average, 19.5) months after s.c. inoculation with bovine blood (24, 26). In 1 instance, Paulsen et al. (21) noted death with lymphosarcoma in a sheep in the beginning of the 5th year after s.c. injection of 40 ml of serum from a sheep that died with a very high leukemia level. In the present series, 1 sheep died with tumor 5.5 years after inoculation with tumor tissue from a juvenile form of bovine lymphosarcoma. The long latency may have been due to a small amount of infective material inoculated. By contrast, most of the sheep with lymphosarcoma found at slaughter are old animals according to the review of Bostock and Owen (5).

The similarity of lymphosarcoma involving certain lymph nodes, heart, abomasum, uterus, kidneys, and urinary tract in cattle (16) may be noted in this series in sheep. The same pattern of involvement was found (13) in lymphosarcoma produced in 8 of 33 sheep by inoculation p.o. of blood from a cow with lymphocytosis (leukemia). In addition, there was tumor behind the eye, in the spinal canal, and s.c. (13).

Considerable detail on the histopathology of bovine lymphosarcoma has been reported (4, 23). In 1 study (4), the classification by cell type in 6 previous reports was considered, and from this series of 36 cases a 7th classification was developed. Most (23 cases) were “retikulose” or “retikulosarkomatose” with small to large and polymorphous reticulum cells; 13 were lymphocytic or stem cell types with 8 designated as “lymphosarkomatose” (4). In the other study (23), 35 cases were divided into early, developing, and end stages of “lymphosarkomatose 10. lymphoreticulare sarkomatose 8, lymphosarkom 3, lymphatische leukose 10, and reticulosarkom 4.” For the present, at least, it seems sufficient to regard the neoplastic cells in the experimental sheep as a mixture of reticulum or histiocytic cells and lymphoid cells with varied transitional cells supported by a usually sparse and diffuse fibroplasia and a web of silver-staining reticulin fibers (Figs. 7 and 8). In bovine lymphosarcoma, there was also a mixture of lymphoid and reticulum cells and either might predominate (8). EM of bovine lymphosarcoma supported the essential information provided by light microscopy (8).

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Fig. 1. Diffuse lymphosarcoma involving left iliac lymph nodes, nodular tumors, kidney, diffuse in heart (right auricle and ventricle opened), left ovarian lymph node, and wall of both uterine horns which have been incised to show thickening caused by diffuse tumor (Sheep V41).

Fig. 2. Heart, Sheep A6, with lymphoid tumor cells infiltrating musculature and concentrated in subendothelial and epicardial areas. H & E, × 26.

Fig. 3. Enlarged mesenteric lymph node that weighed 2.2 kg and (left) mediastinal lymph node from Sheep B8.

Fig. 4. Lymphoid tumor cells in iliac lymph node of Sheep V41. Note relatively large vesicular nuclei. H & E, × 600.

Fig. 5. Tumor of parotid salivary gland Sheep D50. The enlarged parotid lymph node with intact but incised capsule may be noted to the right of the parotid tumor.

Fig. 6. Collecting ducts and collapsed acini of parotid salivary gland occur as wedge-shaped band being compressed by lymphoid tumor growth (Sheep D50). H & E, × 150.

Fig. 7. Lymphosarcoma in right auricle of heart of Sheep A6 shows concentration of reticulin fibers surrounding small groups of tumor cells. Bielschowsky silver, × 450.

Fig. 8. Lymphosarcoma in wall of rumen of Sheep V44 shows irregular distribution of reticulin fibers partially enclosing large groups of tumor cells. Bielschowsky silver, × 400.

Fig. 9. Portion of an iliac lymph node of Sheep A22 shows intact capsule with lymphosarcoma of the node and infiltration of tumor about fat cells outside in the hilus. H & E, × 60.

Fig. 10. Diffuse lymphoid tumor growth in mammary gland in Sheep B8. H & E, × 100.

Fig. 11. Lymphoid tumor in abomasum of Sheep A22. Note relative absence of tumor in mucosa from protective band of muscularis mucosa. H & E, × 60.

Fig. 12. Fibroblastosis in pelvic region mass involving also lymphoid tumor in iliac lymph nodes (Sheep A6). H & E, × 300.

Fig. 13. Fibroblastic cells with a few lymphoid cells in 9 x 5.5 x 8-cm tumor mass near thoracic aperture of Sheep V42. This animal also had irregular fibroblastic lymphoid tumor (16 x 10 x 10 cm) adjacent to suprathyroid lymph nodes diffusely enlarged with lymphoid tumor cells. H & E, × 800.

Fig. 14. Lymphosarcoma of skin, Sheep V40. Note variable size of lesions in area from which wool has been clipped. These were red-gray and raised. These were red-gray and raised.

Fig. 15. Diffuse lymphoid tumor infiltration of corium from epidermis to s.c. fat in a skin lesion of Sheep V40. × 26.

Fig. 16. Primitive-type reticular lymphoid tumor cells in an imprint preparation from the enlarged posterior mediastinal lymph node of Sheep V31. May-Grünwald-Giesma, enlarged from a Kodachrome.

Fig. 17. Lymphosarcoma in Sheep V44. Top, mucosal aspect of esophagus with a 6- x 5- x 3-cm tumor; adjacent abomasum at right with a smaller tumor nodule; lower left, laryngeal region with a 5- x 4- x 4-cm tumor and enlarged suprathyroid lymph nodes; lower center, small intestine with tumor at mesenteric attachment; and lower right, a tumor (8 x 6 x 5 cm) in abomasum near pyloric valve.

Fig. 18. Large tumor of body wall that was 20 x 19 x 16 cm and weighed 3.2 kg (Sheep V44).

Fig. 19. Tumor (16 x 10 x 10 cm) that was quite firm and dense, thought to be thymic tissue and adjacent enlarged suprathyroid lymph node (Sheep V42).

Fig. 20. Sheep V44 with protrusion of right eye from a 4- x 3- x 2-cm tumor behind the eye.
Pathology of Lymphosarcoma in Sheep Induced with Bovine Leukemia Virus

Carl Olson and Lois E. Baumgartener


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