A Pathologic Study on Fetuses and Placentas from Cows Affected with Enzootic Bovine Leukosis with Reference to Transplacental Infection of Bovine Leukemia Virus

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Abstract. Histopathologic examination was performed on 47 pregnant cows affected with bovine leukemia and their 51 fetuses. All the dams examined were positive for serum antibodies against bovine leukemia virus (BLV) and BLV particles by electron microscopy. Antibodies to BLV were positive in 5 of 15 (33.3%) fetuses examined, and one of them showed higher antibody titer than that of the dam with infiltration of neoplastic cells in the fetal placenta. Generalized neoplastic lesions were recognized in other 2 fetuses, and one of them had lesions located at the fetal placenta, Wharton's jelly of umbilical cord and through the fetal subcutis. From the distribution of the lesions their occurrence in the fetus was thought to be originated from dam and metastatic in nature. In 11 among 36 (30.6%) fetuses of which sera were not examined for antibodies, hyperplastic lesions in the lymphatic tissues were demonstrated. The incidence of transplacental infections and the pathogenesis of fetal infections were discussed.

The possibility of vertical transmission has been reported by several investigators [1, 4, 5, 20, 31] on enzootic bovine leukosis (EBL). Since bovine leukemia virus (BLV) has been isolated in close association with the EBL [11, 13], serologic diagnostic procedures have been established using the viral antigens [12, 14]. Although there have been rather numerous patho-morphologic studies on EBL [18], none of them is concerned with fetuses from cows affected with tumors EBL.

This report is to describe the results of pathologic, virologic and serologic studies indicating the prenatal infection with BLV.

Materials and Methods

Forty seven pregnant cows autopsied from 1960 through 1979 and their 51 fetuses were subjected to histopathologic examination. The cows 2-14 years of age consisted of 25 Holstein-Friesian, 13 Japanese Black, and 11 Shorthorn. Sera from 15 fetuses examined antibodies to BLV at 4th (2 fetuses), 5th (2 fetuses), 6th (2 fetuses), 7th (3 fetuses), 8th (5 fetuses) or 9th (1 fetus) month of gestation. The remaining 36 fetuses, whose sera were not examined antibodies to BLV, examined pathologically at 3rd (1 fetus), 4th (4 fetuses), 5th (5 fetuses), 6th (10 fetuses), 7th (7 fetuses), 8th (6 fetuses) or 9th (3 fetuses) month of gestation.

All of the cows were killed and autopsied. Tissues from the fetuses as well as dams were fixed with 10% neutral formalin. Paraffin sections were made by a routine procedure, and they were stained with hematoxylin and eosin.

The stimulating culture of peripheral lymphocytes [13] was performed on 15 dams and it was examined for BLV particles by electron microscopy [13].

Sera from 30 dams were examined for antibodies to BLV by immunodiffusion test using BLV antigens containing both P- and gp-antigens [18].
body titration was made by 2-fold dilution system and the titers were expressed by the reciprocal of the highest dilution of the serum producing apparent precipitation with antigen [18].

Results

Macroscopic as well as histologic examinations revealed tumorous lesions of marked or moderate degree in various organs and tissues except for 4 cows which had only initial lesions in most of the lymph nodes [19]. Serum antibody to gp-antigen was detected in all of 30 cows examined and antibody to P-antigen was detected in 24 cows. C-type particles were demonstrated in the cultured lymphocytes from all of the 15 cows examined by electron microscopy (Fig. 1). Five among 15 (33.3%) fetuses, whose sera were examined, were positive for antibody to BLV, and they had apparent hyperplastic lesions in the lymph nodes. The antibody titers of the fetuses and dams were shown in Table 1. On the other hand, in fetuses with negative antibodies to BLV, any hyperplastic changes were hardly disclosed in the lymph nodes.

In the twin fetuses of case No. 1 which had antibody to gp-antigen, the titers were almost equal to that of their dam, while antibody to P-antigen was undetected. In the Fetus No. 1-A which was located at the right uterine horn of the Cow No. 1, 20 or more subcutaneous tumor nodules having various sizes 3 to 30 mm in diameter were counted (Fig. 2). Tumor nodules were detected also in the perirenal adipose tissues, the cardiac lobe of the lungs and many other places. Some of the external as well as visceral lymph nodes were enlarged. Histopathology of this fetus revealed accumulation of neoplastic cells in the subcutaneous tissues with occasional infiltration into the corium or muscles (Fig. 3). Similar lesions were detected at Wharton's jelly along the umbilical cord (Fig. 4). Neoplastic lesions were observed also in the interstitium of the skeletal muscles, and in the peripheral as well as thoracic adipose tissues. In the lungs, neoplastic cells were accumulated at the interlobular tissues and some alveolar walls causing alveolar collapse. The infiltration and proliferation of neoplastic cell were evident at the leptomeninges of both cerebrum and cerebellum (Fig. 5). In the sinuses of most lymph nodes examined, reticular cells were proliferated and polymorphic lymphoblastoid cells were scattered. Furthermore, in the subiliac lymph nodes, a number of degenerated neoplastic cells infiltrated in the sinuses.

The Fetus No. 1-B obtained from the left uterine horn of the same Cow No. 1 showed no gross lesions. Histologic examination, however, disclosed slight but distinct neoplastic cell accumulations at the interlobular tissues of the lungs, interstitial tissues of the skeletal muscles and the mesen-
tery. The lymph nodes showed hyperplastic lesions similar to those observed in the Fetus No. 1-A.

Although numerous whitish neoplastic lesions were visible on the cotyledons through the transparent amnion, no gross lesion was detected on the amnion (Fig. 6) with clear amniotic fluid. Intense neoplastic cell proliferation was observed in the myometrium and endometrium of the dam. The proliferated cells were accumulated at the periglandular and perivascular portions of the functional layer of the maternal placenta. The infiltration occurred widely into the fetal placenta, cotyledons (Figs. 7 and 8) and subepithelial layers of the chorionic villi.

In case No. 2, antibody titer to gp-antigen in the fetus was higher than that of the dam, and no gross lesion was observed in the fetus. Histologic examination disclosed obvious follicular hyperplasia with a germin al center and the cortical area extended with many reticular cells as well as large polymorphic lymphoblastoid cells with basophilic cytoplasm in the medullary sinuses of most lymph nodes. In the mesenteric lymph nodes, immature cells with a large and pale nucleus were accumulated at the paracortical area through the medulla. Moderate number of plasma cells and a few of eosinophils and neutrophils were observed at the Peyer’s patches. In this case, intense infiltration and proliferation of neoplastic cells were observed at the lamina propria of the endometrium infiltrating into the functional layer. Neoplastic cells morphologically similar to those in the endometrium were demonstrated at the interstitial tissue of the fetal placenta, and the cells were accumulated at the nutritional sub-germinal cell layer as well as perivascular areas (Fig. 9).

In antibody-positive case No. 3, antibody titer to gp-antigen of the fetus was lower than that of the dam. In most of the lymph nodes, the cortex was expanded due to proliferation of lymphocytic and lymphoblastoid cells at the paracortical area with follicular hyperplasia (Fig. 10). Some eosinophils and neutrophils were present in the sinus.

The antibody titers of Cow No. 4 and the fetus were similar to those in case No. 3. In most of the lymph nodes, the cortical area was extended due to proliferation of lymphocytes and enlarged follicles. In the sinus large lymphoblastoid cells were present in moderate number (Fig. 11). Plasma cells were observed in the medullary cords (Fig. 12).

In any of the 36 fetuses whose serum antibody was not examined, no tumors were recognized at autopsy. However, lymphocytes increased in number at the cortical area of the lymph nodes examined from 2, 4, 1, and 1 fetuses of 6th, 7th, 8th, and 9th month of gestation, respectively. Follicle formation with the germinal center was recognized in 3 and 1 fetuses of 7th and 8th month of gestation, respectively. Increase in number and size of sinus reticular cells was observed in a single fetus of 6th month of gestation. Large blastoid cells with wide basophilic cytoplasm were scattered in the lymph sinuses of most lymph nodes examined in 1, 3, 2, and 1 fetuses of 6th, 7th, 8th, and 9th month of gestation, respectively. Among these, in 2 fetuses, one each of 7th and 8th month of gestation, respectively, showed every 3 kinds of these hyperplastic changes described above. Any of these lesions showing activated functions was detected in the lymph nodes of 11 fetuses (30.6%) which were 6th month of gestation or older. Besides, the moderate infiltration of eosinophils and neutrophils was noted in many
of the lymph nodes of 9 fetuses.

**Discussion**

Hyperplastic changes in the lymphatic tissues were recognized in 11 fetuses among 36 (33.3%) of which no serologic examination was performed. Even if some of the reactive changes observed in the lymph nodes of the fetuses might be due to unidentified antigenic effects other than BLV, the fetuses were born from EBL cows, and they showed significant proliferation of immature cells and/or follicular hyperplasia in the lymph nodes. Their ages were 6th to 9th month of gestation.

In the 15 fetuses whose sera were examined for antibodies to BLV, 5 (33.3%) of 5th to 9th month of gestation were positive. One of them with neoplastic cell infiltration into the fetal placenta showed a higher antibody titer than that of the dam. Two having antibody titers similar to those of their dam showed multiple tumors or neoplastic lesions microscopically. Other 2 fetuses having lower antibody titers than those of their dams also showed hyperplastic changes in the lymph nodes.

It is known that the transfer of immunoglobulins through the placenta is incapable in the ungulate [21], and that bovine fetus is endowed to produce antibodies by itself around 100 days of gestation [21, 26-28]. Therefore, hyperplastic changes recognized in the fetal lymphatic tissues suggest that the fetal antibody would have been produced by the fetuses themselves.

Tumorous lesions were frequently observed in the uterine wall approximately in 30-40% cases of the tumorous EBL [18]. In most of these cases, neoplastic cells usually involved in the deep layer of the endometrium and the myometrium, and rather uncommon in the upper layer of the endometrium. This finding is enough to suggest the presence of a barrier in the maternal placenta protecting their fetuses against the invasion of neoplastic cells. Sano [25] examined the uteri of 57 EBL cows and demonstrated no lesions in the upper functional layer of the endometrium. In the present studies, infiltration of neoplastic cells was recognized at the upper layer of the endometrium in 8 cows, 4 each from both groups with or without examination for antibodies to BLV, with slight or moderate hyperplasia in the fetal lymph nodes.

It is accepted that free BLV particles are rarely detected directly from the infected cattle except using a procedure of stimulating lymphocyte culture, and that lymphocytes with BLV genes will provide a source of infection [3]. The same will be applied in case of transplacental infection of BLV. The present examinations demonstrated that the neoplastic cells were infiltrating into the maternal as well as fetal placentas in 5 fetuses. The subcutaneous lesions of Fetus No. 1-A seemed to develop sequentially from the fetal placenta through the Wharton’s jelly of the umbilical cord.

The occurrences of congenital tumors were reported in fetuses and neonates by several authors [2, 7-10, 22, 23, 29, 30], and it is of interest that the cutaneous lesions similar to those of the present case were demonstrated in 4 cases [2, 7-9]. Accordingly, transplacental metastasis from the dam cannot be neglected on the pathogenesis of the neoplastic lesions in fetuses from pregnant cows at tumorous stage of EBL. On the other hand, infection with BLV was evidenced in Fetus No. 3, though the dam had only initial lesions in the lymph nodes [19]. The possibility of fetal infection due to lymphocytic transfer from the dam is still not to be denied, when the dam is at pretumorous stages of the disease.
FETUSES AND PLACENTAS FROM EBL COWS

Although only 9 fetal or new born cases with tumorous lesions were previously reported [2, 7-10, 22, 23, 29, 30], the twin fetuses of case No. 1 in the present paper would be the first case in which transplacental BLV infection was proved. Transplacental infection was proved in 5 of 15 (33.3%) fetuses whose sera were tested for antibodies to BLV, and similar tendency in the rate of infection was suggested histologically in the fetuses whose sera were not tested in the present studies. These figures were somewhat different from those of obtained by previous authors [6, 24]. This is probably due to the fact that the present data were obtained from the fetuses whose dams were at the tumorous stage and not from those apparently healthy or subclinical.

Tumorous lesions in fetuses indicate that the occurrence of EBL can be very early in life. Virologic and histopathologic examinations are required for differential diagnosis between EBL and the calf type leukosis, especially in calves [17].

Although the vertical transmission might play an important role in epizootiology of BLV infection [20], approximately 20% of new born calves were shown to be positive for serum antibodies already before ingesting colostrum [6, 24]. In our experience only a single case was found to be infected with BLV among 16 calves born from BLV positive dams [15]. These data would indicate that horizontal infection is rather common and important in natural transmission of BLV [16].

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References


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要約

地方病性ウシ白血病牛の胎児および胎盤に関する病理学的研究とくにウシ白血病ウイルスの従胎盤感染について：大島寛一・高橋喜和夫・岡田幸助・沼宮内・茂・香川裕一・南野久晃（岩手大学農学部家畜病理学講座）——ウシ白血病腫瘍期にある妊娠牛47例およびその胎児51例について，病理組織学的検索を行なった。母ウシについては，血清BLV抗体および風疹によるBLV粒子の検索を伴せ行ない，検査した全例が陽性であった。血清BLVの検索を行なった15例の胎児では，5例（30.3%）が陽性で，そのうち1例は母ウシより高い抗体価を示し，かつ胎児胎盤に腫瘍細胞の浸潤を認めた。別の2例では全身に腫瘍組織が認められ，そのうち1例では胎児胎盤から臓帯を経て，胎児死下に腫瘍組織が見られ，母ウシからの浸潤とともに転移を思わせるものがあった。また，血清を検査しなかった36例の胎児のうち，11例（30.6％）のリンパ組織に活性化が認められた。BLVの従胎盤感染を思わせた。これらの所見にもとづいて，従胎盤感染の頻度，病理発生などについて考察を加えた。

Explanation of Figures

Fig. 1. BLV from peripheral lymphocyte culture of Cow No. 3. ×49,000.
Fig. 2. Subcutaneous tumors of Fetus No. 1-A. ×0.33.
Fig. 3. Subcutaneous proliferation of neoplastic cells in Fetus No. 1-A. H-E stain. ×50.
Fig. 4. Neoplastic cell proliferation in Wharton’s jelly of Fetus No. 1-A. U: Urachus. H-E stain. ×50.
Fig. 5. Infiltration of neoplastic cells in the cerebellar meninges of Fetus No. 1-A. M: Molecular and granular layer. H-E stain. ×240.
Fig. 6. Multiple whitish neoplastic lesions (arrows) in the cotyledons visible through transparent amnion of Cow No. 1. ×0.57.
Fig. 7. A cut surface of the cotyledon shown in Fig. 6. ×1.2.
Fig. 8. Neoplastic cell infiltration in the lamina propria of chorionic villi of the cotyledon of Cow No. 1. H-E stain. ×120.
Fig. 9. Neoplastic cell infiltration in the fetal (upper) and maternal (left bottom) placentas of Cow No. 2. H-E stain. ×50.
Fig. 10. Follicular hyperplasia of the intestinal lymph node of Fetus No. 3. H-E stain. ×120.
Fig. 11. Large lymphoblastoid cells in the sinus of the bronchial lymph node of Fetus No. 4. H-E stain. ×480.
Fig. 12. Plasma cell proliferation with large basophilic cells in the medullary cord and sinus in the iliac lymph node of Fetus No. 4. H-E stain. ×480.