Detection of Bovine Leukemia Viruses (BLV) in Mammary Tissues of BLV Antibody-Positive Cows Affected by Subclinical Mastitis

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ABSTRACT. The mammary tissues of 6 cows with bovine leukemia virus (BLV) antibody and subclinical mastitis were investigated histopathologically, and their organ cultures were ultrastructurally observed. Numerous BLV particles, 110 to 120 nm in diameter, were seen around lymphocytes, which had infiltrated into mammary alveoli and showed blastogenesis under culture. Particles budding from the cell membrane were also found. — **KEY WORDS:** BLV, organ culture, subclinical mastitis.

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Adult type of bovine leukosis is an infectious neoplastic disease caused by bovine leukemia virus (BLV), and carrier cattle are the most important latent source of infection. Transmission of BLV occurs by contact [1, 2, 8, 12], by biting insects [2, 11], iatrogenically [10] or via milk [3, 5, 13].

The presence of BLV particles in the mammary tissue was demonstrated morphologically in only few reports [3, 5, 13]. The purpose of this study is to show BLV particles in organ cultures of the mammary glands of BLV antibody-positive cows with subclinical mastitis.

Cows, which showed positive agar gel immunodiffusion reactions for BLV antigen [7] and appeared clinically normal, were examined histologically, and 6 cows with subclinical mastitis were selected for the present study. The mammary tissues were fixed in 10% buffered formalin and embedded in paraffin wax. Sections were cut 6 μ m thick and stained with hematoxylin and eosin (HE). Small pieces of the mammary tissues from the 6 cows were sliced into 3 mm³ cubes after washing with Hank's solution. They were placed in minimum essential medium (MEM) containing 20% of fetal calf serum, and incubated for 24 hr in 5% CO₂. The samples were cut into 1 mm³ cubes, fixed in a mixture of 1.5% phosphate buffered paraformaldehyde and 0.5% glutaraldehyde, postfixed with 0.05 M cacodylate buffer solution containing 1% osmic acid, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7000 transmission electron microscope.

No macroscopical lesions were detected in the mammary glands of 6 cows, but histological lesions were found in some lobules of the mammary gland of all cows. These lesions were composed of an infiltration of lymphocytes, plasma cells and neutrophils into alveoli and interlobular connective tissue, and the alveoli also contained numerous macrophages and desquamated alveolar lining cells (Fig. 1). After incubation of small pieces of mammary tissues for 24 hr, alveolar epithelial cells from five of six animals frequently extended cytoplasmic processes toward the basement membrane, and the nuclei of the cells were enlarged with markedly swollen nucleoli. Many lymphocytes showed distinct blastogenesis in the alveoli

(Fig. 2). Ultrastructurally, numerous C-type viral particles, 110–120 nm in diameter, were present around the blastogenic lymphocytes in three of six animals, occasionally with budding particles on the cell membranes (Figs. 3 & 4).

Since BLV is integrated mainly with the genomes of B-lymphocytes as provirus, BLV-infected animals, whether or not having lymphosarcoma, are a very important source of infection [6]. Experimental transmission of BLV using milk from BLV-infected cows was successful in sheep [10]. BLV infection occurred, when 10⁶ BLV-infected lymphocytes in colostrum free of BLV-specific antibody were given to calves born to BLV-negative dams. In contrast, the infection was inhibited, when 10⁷ to 10⁹ infected lymphocytes in colostrum containing BLV-specific antibody were given to similar calves [13]. These findings suggest that specific immunoglobulin in colostrum neutralizes the virus, and that antibody titers in colostrum are sufficiently high enough to protect calves from BLV infection [4]. It is probable, however, that BLV infection occurs if ordinary milk from BLV-infected cows were fed to calves without BLV-specific antibody, or if many BLV-infected lymphocytes are given after calves lose maternal antibody protection. In the present study, the cows positive for BLV antibody had subclinical mastitis [14], and numerous C-type viral particles considered as BLV [9] were detected in the lymphocytes of the mammary glands. We should be cautious with milk from BLV-infected cows with subclinical mastitis, because such milk contains many lymphocytes capable of producing BLV particles.

REFERENCES

1. Abt, D. A., Marshak, R. R., Ferrer, J. F., Piper, C. E., and Batt, D. M. 1976. *Vet. Microbiol.* 1: 287–300.
2. Bech-Nielsen, S., Piper, C. E., and Ferrer, J. F. 1978. *Am. J. Vet. Res.* 39: 1089–1092.
3. Dutcher, R. M., Larkin, E. P., and Marshak, R. R. 1964. *J. Natl. Cancer Inst.* 33: 1055–1063.
4. Ferrer, J. F. and Piper, C. E. 1978. *Ann. Rech. Vét.* 9: 803–807.
5. Ferrer, J. F., Kenyon, S. J., and Gupta, P. 1981. *Science* 213: 1014–1016.

Fig. 1. An alveolus containing many neutrophils and lymphocytes. HE stain. $\times 230$.

Fig. 2. Many lymphocytes showing blastogenesis after incubation for 24 hr. Toluidine blue stain. $\times 800$.

Fig. 3. Numerous extracellular BLV particles. Bar=100 nm.

Fig. 4. A BLV particle (arrow) budding from plasma membrane of a lymphocyte. Bar=100 nm.

6. Kettman, R., Burny, A., Cleuter, Y., Ghysdael, J., and Mammerichx, M. 1978. *Ann. Rech. Vét.* 9: 837-844.
7. Koyama, H., Nakanishi, H., Kajikawa, O., Yoshikawa, H., Tsubaki, S., Yoshikawa, T., and Saito, H. 1983. *Jpn. J. Vet. Sci.* 45: 471-475.
8. Lucas, H. H., Dawson, M., Chasey, D., Wiberley, G., and Robert, D. H. 1980. *Vet. Rec.* 106: 128.
9. Miller, J. M., Miller, L. D., Olson, C., and Gillette, K. G. 1969. *J. Natl. Cancer Inst.* 43: 1297-1305.
10. Miller, J. M. and Van Der Maaten, M. J. 1979. *J. Natl. Cancer Inst.* 62: 425-428.
11. Ohshima, K., Okada, K., Numakunai, Y., Sato, S., and Takahashi, K. 1981. *Jpn. J. Vet. Sci.* 43: 79-81.
12. Schultz, R. D. 1973. *Cornell Vet.* 63: 507-535.
13. Van Der Maaten, M. J., Miller, J. M., and Schmerr, M. J. F. 1981. *Am. J. Vet. Res.* 42: 1498-1500.
14. Yamagiwa, S., Ono, T., Uemura, T., and Ida, T. 1957. *Jpn. J. Vet. Res.* 5: 146-165.