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Expression of the Bovine Leukemia Virus and Its Internal Antigen in Blood Lymphocytes (39942)

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Introduction. Particles resembling mature forms of C-type viruses were detected by Miller *et al.* (1) in short-term cultures of peripheral blood lymphocytes (PBL) from leukemic cattle. Studies from this laboratory extended this finding and provided the first conclusive electron-microscopic (2, 3) and serological (4, 5) evidence of an indigenous C-type virus in the bovine species. On the basis of seroepidemiological studies (6-8) and transmission experiments (9, 10), this virus is now universally regarded as the etiological agent of the adult form of bovine leukemia and is commonly referred to as the bovine leukemia virus or BLV.

Studies on a small group of cattle showed that infected bovine lymphocytes begin to release BLV particles only after a few hours of *in vitro* cultivation. It was also found that in most, but not all cases, phytohemagglutinin (PHA) enhances the replication or expression of BLV in these cultures (2). Similar results were obtained when the immunofluorescent (IF) technique on acetone-fixed cells was applied to detect BLV antigens in lymphocytes from an infected cow (5). Subsequent studies demonstrated that the antigen detected by the IF technique on acetone-fixed cells is the major internal BLV polypeptide, which is now well characterized immunologically (4, 11, 12).

In the present work, we sought to determine whether *in vitro* cultivation is a general requirement for the expression of BLV in lymphocytes from cattle and sheep. The effect of PHA on this phenomenon and the presence of BLV in cells other than lymphocytes have also been investigated.

Materials and Methods. Animals. All cattle used were from multiple-case study herd BF (6, 13). The incidence of BLV infection in the adult population of this herd is 95% or more. In this study we also examined sheep that were inoculated at birth with BLV-releasing bovine lymphocytes or with cell-free preparations of BLV isolated from an experimentally infected monolayer cell culture (14).

Cultures. Peripheral blood was collected by jugular venopuncture and lymphocytes were separated after lysing the red cells by hypotonic shock. Cultures were set up at a density of 1-2 $\times$ 10^6 PBL/ml of Eagle's minimal essential medium supplemented with 10 or 20% heat-inactivated (56° for 30 min) fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). PHA (Burroughs-Wellcome, Greenville, N.C.) was used at the concentration of 0.02 ml/ml of medium. Further details on these procedures have been given in a previous publication (2).

Electron microscopy. Cells for electron-microscopic examinations were processed as described earlier (2). At least 100 cell sections, each representing a different cell, were examined in each PBL sample.

Immunofluorescent antibody test. The indirect immunofluorescent test for the detection of the major internal BLV antigen has been described in previous publications (5, 6).

Results. Samples of PBL from 32 BLV-infected cattle with or without leukemia (lymphosarcoma) or persistent lymphocytosis and from experimentally infected sheep were examined before and after short-term *in vitro* culture. As shown in Table I, virus particles were not observed in any of these samples before cultivation. However, after short-term cultivation, a variable percentage of the lymphocytes from each of the samples showed characteristic BLV particles. The differences in percentages of cells

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with virus particles in the cultures from the three groups of cattle examined were not statistically significant.

Table II presents the results obtained when the presence of the major internal BLV antigen was examined in uncultured or cultured PBL from infected cattle and sheep. The results clearly show that, as in the case of the BLV particles, expression of the viral antigen requires the *in vitro* cultivation of the cell. As shown by the results of a representative experiment (Table III), cells with the antigen are detected as early as 6 hr after incubation and their number is usually maximal after 48 hr. These findings are in agreement with the results obtained in experiments in which the time of appearance of cells with BLV particles was examined in similar cultures (2).

The effect of PHA on the expression of BLV particles was studied in 56 short-term (48- to 72-hr) PBL cultures derived from 20 infected cattle. In 41 (73%) of these cases the PHA-treated cultures had a larger percentage of cells with virus particles than the parallel control cultures without PHA.

Discussion. The present study shows that, as a rule, the expression of BLV and its major internal antigens in PBL from cattle and sheep requires *in vitro* cultivation. These findings, which extend observations previously made in a small number of cattle (2), indicate that in infected animals there is a "factor(s)" that inhibits both replication of the virus and expression of the viral genome.

Onuma *et al.* (15) have also reported that sera from some leukemic cattle and cattle inoculated with BLV inhibit the release of an ether-resistant antigen, presumably a

TABLE III. EXPRESSION OF THE MAJOR INTERNAL ANTIGEN[a] OF BLV IN BOVINE LYMPHOCYTES[b] AT VARIOUS TIMES AFTER *in Vitro* CULTIVATION.

Hours in culture	No. of cells positive/total no. of cells examined	Percentage of cells positive
0	0/250	0
3	0/350	0
6	5/252	2
12	7/283	2.4
24	36/210	17.1
48	44/242	18.1

[a] As determined by the immunofluorescent antibody technique on acetone-fixed cells.

[b] Collected from peripheral blood of a cow with persistent lymphocytosis in the multiple-herd BF.

TABLE I. DETECTION OF BLV PARTICLES IN BOVINE AND OVINE PERIPHERAL BLOOD LYMPHOCYTES AFTER SHORT-TERM *in Vitro* CULTIVATION.

BLV-infected donors		Lymphocytes before cultivation, Cells with virus/total No. of cells (%)	Lymphocytes after cultivation[a]	
Category	No.		Cells with virus/total No. of cells (%)	Percentage range
Leukemic cattle[b]	14	0/1246 (0)	516/1387 (37)	3-60
Nonleukemic cattle with PL[b]	15	0/3162 (0)	733/2967 (25)	2-51
Nonleukemic cattle without PL[b]	4	0/943 (0)	236/873 (27)	2-54
Sheep[c]	7	0/625 (0)	190/1275 (15)	2-47

[a] Cells cultured for 48 or 72 hr.

[b] Cattle were naturally infected. PL, persistent lymphocytosis.

[c] Sheep were experimentally infected.

TABLE II. EXPRESSION OF THE MAJOR INTERNAL BLV ANTIGEN[a] IN BOVINE AND OVINE PERIPHERAL BLOOD LYMPHOCYTES UPON SHORT-TERM *in vitro* CULTIVATION.

BLV-infected donors		Lymphocytes before cultivation, Positive cells/total No. of cells (%)	Lymphocytes after cultivation[b]	
Category	No.		Positive cells/total No. of cells (%)	Percentage range
Leukemic cattle[c]	5	0/1000 (0)	310/1000 (21)	4-63
Nonleukemic cattle with PL[c]	6	0/1200 (0)	324/1200 (27)	2-45
Sheep[d]	21	0/4200 (0)	630/4200 (15)	5-35

[a] As detected by the immunofluorescence antibody technique on acetone-fixed cells.

[b] Cells cultured for 48 or 72 hr.

[c] Cattle were naturally infected. PL, persistent lymphocytosis.

[d] Sheep were experimentally infected.

BLV component, from a monolayer cell culture experimentally infected with the virus.

The nature of the factor responsible for the inhibition of BLV expression in the animal has not been studied, but it is likely that antiviral antibodies, known to be present in cattle and sheep infected with BLV (5, 8, 10), interfere with replication of the virus. It is less easy to postulate a mechanism by which antiviral antibodies can block expression of the internal BLV antigen in the cytoplasm of infected cells. Kenyon and Piper (16) have shown that in BLV-infected cattle the virus is present in only a fraction of the PBL population. It is possible that in the animal the subpopulation of infected cells is too small to be detected by the methods used in the present study, but that upon *in vitro* cultivation these cells divide more actively than the noninfected lymphocytes. However, the experiments of Kenyon and Piper (16) also indicate that most of the spontaneous incorporation of [^{3}H]thymidine observed in PBL from infected cattle after 3 days in culture is attributable to cells that do not replicate BLV.

Our results also confirm and extend the observation that in most cases PHA-treated cultures of PBL from infected cattle contain more virus than parallel untreated cultures of the same cells (2). This may indicate that the mitogen stimulates preferentially the BLV-infected subpopulation of PBL. Evidence for differences in susceptibility of human PBL subpopulations to the mitogenic effect PHA has been noted (17). The possibility that PHA may derepress a C-type genome by stimulating cellular DNA synthesis should also be considered.

The observations that BLV can be detected only in lymphocytes from infected cattle and that the *in vitro* cultivation and PHA treatment of these cells enhances the expression or replication of BLV are important from the diagnostic standpoint. It should be noted that the viruses responsible for the induction of spontaneously occurring leukemias in mice and cats, unlike BLV, replicate continuously in the infected lymphocytes, regardless of whether these cells are in the animal or in *in vitro* culture. Thus, the BLV system may be very useful for studying factors that control the expression of the genome of a leukemia virus in lymphoid cells.

The apparent ability of BLV to persist in a latent or masked state in the animal suggests that neither active nor passive immunization will be effective in arresting an established BLV infection. On the other hand, vaccination may very well be effective as a preventive measure.

Summary. The present study shows that the detection of the bovine leukemia virus (BLV) in naturally infected cattle or experimentally infected sheep requires the *in vitro* cultivation of the peripheral blood lymphocytes. This indicates that the infected animals possess a "factor" that inhibits replication of the BLV or expression if its genome. Addition of phytohemagglutinin to the lymphocyte cultures increases the expression of the virus in most, but not all, cases.

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