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Evidence that the Spontaneous Blastogenesis of Lymphocytes from Bovine Leukemia Virus-Infected Cattle Is Viral Antigen Specific

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Cattle lymphocytes cultured for 3 days were found to spontaneously incorporate thymidine (3STI). Under optimal conditions of culture, the median magnitude of 3STI activity in lymphocytes from bovine leukemia virus (BLV)-infected cattle was higher than that of BLV-free cattle, but the ranges of the values overlapped. However, the 3STI activity of most BLV-infected cattle was specifically inhibited by serum containing BLV antibodies, whereas the 3STI activity of BLV-free cattle was not. The 3STI inhibitor copurified with immunoglobulin, and its activity could be absorbed with BLV. Rabbit anti-BLV serum inhibited 3STI, but rabbit anti-BLV p25 did not. These results indicate that BLV infection induces or expands a BLV-specific lymphocyte population. Spontaneous blastogenesis may be indicative of an immune response which controls virus spread.

The adult or enzootic form of bovine lymphosarcoma is the most frequent malignancy of domestic cattle (6). It is caused by bovine leukemia virus (BLV) (6). BLV is a type C retrovirus that is found in many cattle, but it is probably not endogenous to the bovine species (6, 8, 17). BLV also causes persistent lymphocytosis, an apparently benign condition of cattle (6). Most BLV-infected cattle do not develop lymphosarcoma or persistent lymphocytosis; i.e., they are usually asymptomatic virus carriers. Whether BLV-infected cattle develop lymphosarcoma or persistent lymphocytosis seems to be genetically determined (10). BLV appears to infect only the lymphocytes of cattle (8), but the BLV-infected lymphocytes synthesize virus particles and viral antigens only after in vitro cultivation (1).

Spontaneous thymidine incorporation after 3 days in culture (3STI) has been reported to occur in cultures of blood lymphocytes taken from cattle with lymphosarcoma (26) or persistent lymphocytosis (20, 25). Recently, we reported that spontaneous blastogenesis occurs in cultures of blood lymphocytes taken from BLV-infected cattle without lymphosarcoma or persistent lymphocytosis (2, 15). Data obtained by Kenyon and Piper (16) suggest that the blastogenic lymphocytes are not infected with BLV. Kenyon (Ph.D. thesis, University of Pennsylvania, Philadelphia, 1976) has also found that the blastogenic activity of lymphocytes from cattle naturally infected with BLV can be inhibited by serum from other BLV-infected cattle. Recently, Takashima and Olson (23) have reported that inhibitable blastogenic activity can be induced in cattle by experimental infection with BLV. However, their study involved only three infected cattle, and the virus inoculum was derived from a cell line known to produce both BLV and bovine viral diarrhea virus. They also did not show that the inhibition was specific for BLV-infected cattle. Thus, it is not clear from the previous reports whether spontaneous blastogenesis was associated with BLV infection, another infection, or a lymphoproliferative disease.

To determine whether blastogenic activity was due to BLV infection, we optimized the conditions for inducing 3STI activity and analyzed the 3STI activity of cattle carefully screened for BLV infection and other common infestations or lymphoproliferative conditions.

MATERIALS AND METHODS

Animals. Cattle naturally infected with BLV were selected from the Jersey herd BF, Guernsey herds BI and BH (7), and a commercial Holstein herd M. Only cows older than 36 months were used.

Preparation of peripheral blood lymphocytes. Heparinized blood (Panheparin, Abbot Laboratories, North Chicago, Ill.; 1 to 4 U of Panheparin per ml of blood) was centrifuged at 750 × *g* for 25 min at 15°C. The plasma was removed, and the buffy coat was collected and suspended in minimal essential suspension medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 100 U of penicillin per ml and 100 μg of streptomycin per ml (minimal essential suspension medium). The buffy coat cells from 10 ml

of blood were suspended in 7 ml of minimal essential suspension medium. Each 7 ml of cell suspension was layered over a mixture of 2.0 ml of Lymphoprep (Nyegaard & Co., Oslo, Norway) and 2.0 ml of Lympholyte M (Accurate Scientific Co., New York, N.Y.) in a 12-ml siliconized conical tube. The tubes were centrifuged at 400 × g (interface force) for 25 min at 15°C. The interface bands were collected and washed (250 × g for 6 min) at least twice. To reduce platelet contamination, occasionally as many as five centrifugations were done. The resulting cell suspensions were about 95% lymphocytes (as identified by differential staining of cell smears) with a viability of 99%. The recovery of lymphocytes from blood was 70 ± 25% (mean ± standard deviation, $n = 40$).

Viable lymphocytes were counted as either erythrosin B-excluding (24) or fluorescein diacetate-incorporating cells (21). Fluorescein diacetate was dissolved in 1 mg of acetone per ml and stored for up to 1 month at 4°C. Ten microliters was added to 100 μl of cell suspension. The cells were counted in a standard hemacytometer by a combination of incident fluorescent excitation (fluorescein isothiocyanate filters) and transmitted tungsten light.

Lymphocyte cultures. Lymphocytes were cultured in minimal essential suspension containing 10% serum, unless otherwise indicated. All sera were heated at 56°C for 45 min. Except where noted, 10^6 viable cells in a total volume of 0.6 ml were cultured in polystyrene tubes (12 by 75 mm) for 70 h in a CO_2 incubator at 37°C. Viable cell recovery was 26 ± 19% (mean ± standard deviation, $n = 98$). At 16 h before the termination of the culture, 0.1 ml of [^{3}H]thymidine (20 μCi/ml; [*methyl*-^{3}H]thymidine, 5 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) was added. Samples were harvested with a semiautomated cell harvester, dried, immersed in 3 ml of scintillation fluid, and counted in a Packard Tri-Carb scintillation counter. The coefficient of variation (standard deviation divided by the mean) of the counts per minute for 144 samples was 15%. The coefficient of variation for repeated tests of the same animal was 23%.

Serological tests and sera. Antibodies to the foamy-like bovine syncytial virus (BSV) were detected by immunofluorescence with acetone-fixed bovine syncytial virus-infected cells as described elsewhere (5). Virus-neutralization tests for antibodies against bovine viral diarrhea virus, parainfluenza 3, and infectious bovine rhinotracheitis virus were performed by the diagnostic laboratories at Pennsylvania State University and Cornell University.

BLV antibodies were detected by radioimmunoassay by use of BLV major internal (p25) or envelope glycoprotein (gp) virion antigens (4, 19).

BLV antibody-positive serum SR124 was drawn from BF138. Control serum SR3153 was drawn from BI594. The radioimmunoassay titers for BLV p25 and gp antibodies in SR124 serum were greater than 1: 10,000, whereas SR3153 serum was negative in both radioimmunoassay tests at a 1:5 final dilution. Cultured lymphocytes from BI594 were negative in a syncytium induction test (9) and in a competitive radioimmunoassay with radiolabeled p25 (19). Both SR124 and SR3153 sera were negative for bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, and parainfluenza antibodies.

A rabbit antiserum against whole BLV was prepared by the intradermal inoculation of density-gradient-purified BLV. The virus was isolated from cell line BLV-bat$_2$ clone$_1$, which is free of other known bovine viruses and adventitious agents (9). The procedure used to prepare the rabbit anti-BLV p25 serum has been described (12).

For absorption, serum was diluted 1:2 in phosphate-buffered saline, and 2 ml was mixed with 1.0 mg of purified BLV. About 1 ml of the mixture was sonicated (model W-22F; Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) for a total of 2 min at a setting of eight. Both the untreated and the sonicated mixtures were centrifuged at 10,000 × g for 60 min. The supernatant fluid was carefully removed and used.

Serum fractionation. Two methods were used to fractionate sera, ammonium sulfate precipitation and immunoadsorbents. For method 1, 1 volume of BLV reference serum was mixed with 2 volumes of saturated ammonium sulfate, pH 6.5, and stirred for 4 h at 20°C. The precipitate was collected by centrifugation, suspended, and dialyzed against 0.01 M phosphate buffer, pH 7.8. This material was passed through a diethylaminoethyl cellulose column equilibrated with the same buffer. Fractions were collected, pooled, and concentrated by vacuum dialysis. For method 2, Affigel-10 (Bio-Rad Laboratories, Richmond, Calif.) was conjugated with bovine immunoglobulin (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M $NaHCO_3$ as described by the Bio-Rad instructions. The conjugated gel was washed with 0.58% (vol/vol) glacial acetic acid in 0.15 M NaCl and finally with 0.15 M NaCl.

Rabbit anti-bovine immunoglobulin G (Miles Laboratories, Inc., Elkhart, Ind.) was passed through the column, and the bound material was eluted with 0.58% (vol/vol) glacial acetic acid in 0.15 M NaCl. The eluted material was precipitated with 33% saturated ammonium sulfate, suspended, and dialyzed against 0.1 M $NaHCO_3$. This material was then coupled to Affigel-10, as described above, and washed with the glacial acetic acid solution and finally with 0.15 M NaCl.

BLV antibody-positive serum SR124 was diluted with an equal volume of 0.15 M NaCl passed through the Affigel-10–rabbit anti-bovine immunoglobulin G immunoadsorbent. The column was washed with 0.15 M NaCl, and the bound material was eluted with glacial acetic acid solution. The eluted material was dialyzed against 1,500 volumes of minimal essential suspension medium.

Detection of immunoglobulin was done either by immunodiffusion in agar with commercially prepared immunoglobulin (Sigma Chemical Co.) or quantitatively with a commercial radial immunodiffusion kit (Miles Laboratories, Inc.). Protein concentrations were estimated by the Bio-Rad protein assay reagent and by absorbance at 260 and 280 nm.

RESULTS

Optimal conditions for assessment of spontaneous blastogenesis. Cattle peripheral blood cells were cultured in the presence of fetal or adult bovine sera. The 3STI activity was highest with certain sera from BLV-free cattle

and certain fetal bovine sera (FBS). Titrations of one lot of FBS and two adult bovine sera are shown in Fig. 1. The 3STI activity was highest in the presence of 3% FBS or 3 to 10% BLV antibody-negative serum SR3153. There was no significant response in the presence of BLV antibody-positive serum SR124. For many, but not all, cattle, the 3STI activity was consistently higher with 3% FBS than with any concentration of serum from BLV-free cattle. Cell recovery was better at serum concentrations greater than 2%. Autoradiography showed that more then 50% of the thymidine-incorporating cells were blastoid. The 3STI activity was completely inhibited by incubation of the lymphocytes before culture with 30 μg of mitomycin C per ml for 30 min at 37°C.

The dependence of 3STI activity on cell concentration is shown in Fig. 2. The maximal 3STI activity was seen in the range 1×10^6 to 3×10^6 cells per tube, and it was much reduced at relatively small deviations from this range. The optimal range for 3STI activity in microtiter plates was 0.3×10^6 to 1×10^6. However, the incorporation per cell cultured was three to five times higher in tube cultures than in microtiter plate cultures.

The kinetics of thymidine incorporation as measured in 12-h intervals are shown in Fig. 3. The incorporation shown in the first 12-h interval was seen in cultures from three of five cows. Another peak of incorporation occurred in the interval of 60 to 72 h, the usual period for assessment of 3STI activity. As shown in Fig. 3, thymidine incorporation in the 60- to 72-h period did not occur in the presence of BLV antibody-positive serum SR124.

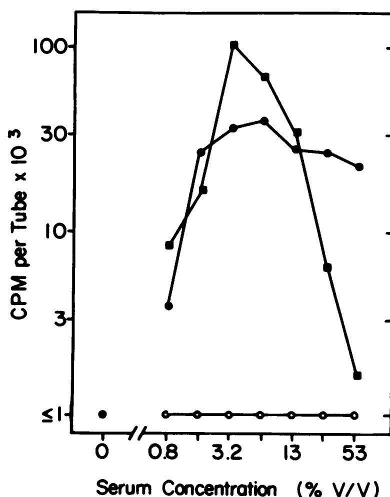

FIG. 1. *Dependence of 3STI activity on serum concentration. Lymphocytes were cultured for 72 h in medium supplemented with the indicated concentrations of serum. Symbols:* ■, *FBS;* ○, *BLV antibody-positive serum SR124;* ●, *BLV antibody-free serum SR3153.*

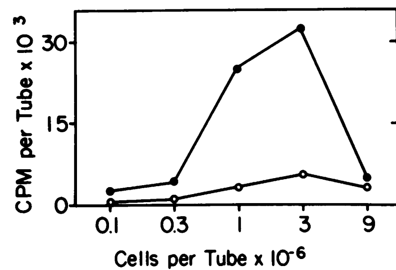

FIG. 2. *Dependence of 3STI activity on lymphocyte concentration. The indicated number of lymphocytes from one BLV-infected cow were cultured for 70 h with BLV antibody-positive serum SR124* (○) *or BLV antibody-negative serum SR3153* (●).

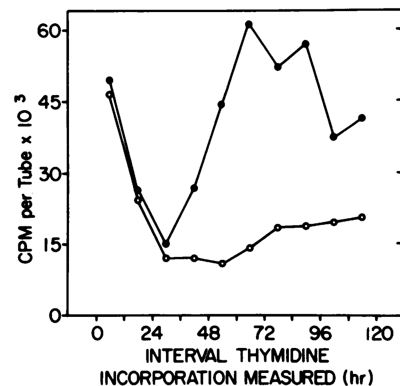

FIG. 3. *Kinetics of spontaneous thymidine incorporation. Multiple lymphocyte cultures were labeled and harvested in each of the indicated 12-h intervals. Cultures were incubated with BLV antibody-positive serum SR124* (○) *or BLV antibody-negative serum SR3153* (●).

Several other variables were tested for their influence on 3STI activity: (i) 3STI activity was slightly higher with minimal essential suspension medium rather than RPMI 1640; (ii) the amount of thymidine incorporation was independent of the thymidine concentration above 20 nmol (5 Ci/mmol) per 2×10^6 cells in 0.6 ml of medium; (iii) higher-specific-activity (25 Ci/mmol) [^{3}H]thymidine made the test slightly, but not proportionally, more sensitive; and (iv) 2-mercaptoethanol had no effect on 3STI activity at concentrations ranging from 10^{-2} to 10^{-9} M.

Figure 4 shows the distribution of thymidine incorporation by lymphocytes from BLV-infected and BLV-free cattle cultured 3 days under optimal conditions with phosphate-buffered saline. 3STI activity occurred in all cattle. There seems to be a higher level of spontaneous blast-

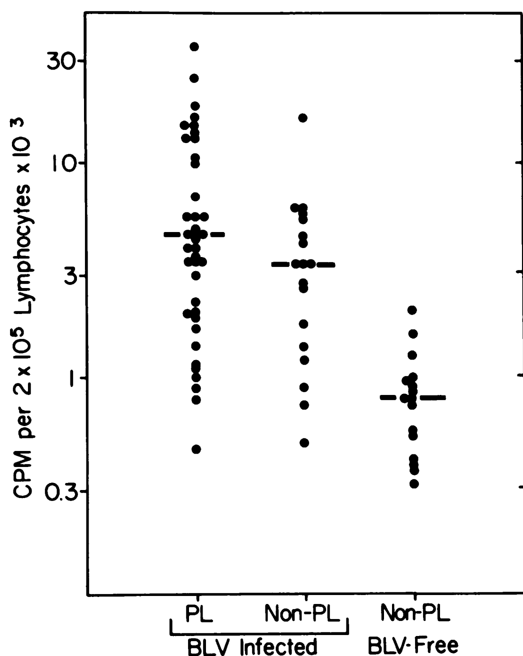

FIG. 4. *Distribution of 3STI activity in BLV-infected and BLV-free cattle. Lymphocytes from the indicated groups of cattle were cultured for 72 h in 3% FBS. The bars indicate the median values. PL, Persistent lymphocytosis.*

ogenesis in lymphocytes from BLV-infected than from BLV-free cattle, but the values overlap.

Inhibition of spontaneous thymidine incorporation. The 3STI activity of cells from one BLV-infected cow ranged from 100 to 700 cpm in the presence of sera from 14 BLV-infected cattle, including the serum from the lymphocyte donor, but the activity of the same cells in the presence of nine sera from BLV-free cattle was 17,500 to 26,800 cpm. The mean cell recoveries in the lymphocyte cultures with BLV antibody-positive serum SR124 (21%) and with BLV antibody-negative serum SR3153 (22%) were not significantly different.

Correlation of 3STI activity with BLV infection. From the coefficient of variation calculated from all assays done with SR124, SR3153, and autologous sera, inhibition of 3STI activity by BLV antibody-positive serum in any one test had to exceed 50% to be significant ($P < 0.025$). Whether a given animal had consistent evidence of inhibitable 3STI activity was determined by the Wilcoxon rank sign test ($P < 0.025$).

A total of 58 BLV-infected cattle were tested at least three times for 3STI activity. Fifty cattle (86%) had 3STI activity inhibitable by serum from BLV-infected cattle (Table 1). Cattle with or without persistent lymphocytosis had inhibitable 3STI activity. Inhibitable 3STI activity was not detectable in cattle that had a BLV p25 titer less than 1:50, but the absolute amount of inhibitable 3STI activity did not correlate with p25 or gp antibody titers. None of the 16 BLV-free cattle (including 2 infected with BSV) tested had inhibitable 3STI activity. None of the cattle had antibodies diagnostic of bovine viral diarrhea virus, parainfluenza 3, or infectious bovine rhinotracheitis virus infection or had clinical signs of any disease.

Characterization of the inhibitor of 3STI activity. Titration experiments were carried out to determine the relative concentration of the inhibitor of 3STI activity. All sera were mixed with 3% FBS. A typical result is shown in Fig. 5. BLV antibody-positive serum SR124 showed inhibition even at a concentration of 0.03%. Inhibition (relative to FBS alone) was also observed with many, but not all (not shown), sera from BLV-free cattle, but this inhibition was always much weaker than that of BLV antibody-positive sera. Furthermore, the inhibition did not exceed 50% even at concentrations of 50%. The inhibition by serum from many BLV-free cattle was observed even when the serum and lymphocytes were from the same animal. However, the 3STI activity of BLV-free animals was not inhibited by BLV antibody-containing serum any more than by BLV antibody-free serum.

The specificity of the serum inhibitor was further examined by absorption experiments. Incubation with purified BLV almost completely removed the inhibitory activity of BLV antibody-positive serum (Table 2). Sonication of BLV did not significantly change its absorption capacity.

Antiserum prepared against whole BLV, but not antiserum prepared against p25, had inhibitory activity for the 3STI activity of BLV-infected cattle (Table 3). The titers of both rabbit antisera in the radioimmunoassay with p25 an-

TABLE 1. *Correlation between BLV infection and inhibitable 3STI activity*

Cattle	Hematological status	Inhibitable 3STI activity (positive/total [%])
BLV infected	PL[a]	39/40 (98)
	Non-PL	11/18 (61)
BLV-free[b]	Non-PL	0/16 (0)

[a] PL, Persistent lymphocytosis (6, 18).

[b] These cattle had no BLV p25 or gp antibodies as determined by radioimmunoassay at a 1:5 serum dilution, and they were from leukemia-free herds.

tigen were greater than 1:20,000. This titer is higher than the titers of many of the bovine sera which had inhibitory activity.

The fractionation of BLV antibody-positive serum with ammonium sulfate or by an affinity adsorbent showed that the inhibitory activity was in the immunoglobulin-enriched fraction (Table 4).

DISCUSSION

The results that we obtained can be summarized as follows: (i) optimal conditions were determined for inducing 3STI activity; (ii) under optimal conditions, 3STI activity was found in lymphocyte cultures of all cattle tested, and there was no statistical difference between BLV-infected and BLV-free cattle; (iii) in contrast, 3STI activity that was inhibitable by BLV antibodies was found only in lymphocyte cultures of BLV-infected cattle, not in BLV-free cattle; and (iv) the inhibitor of 3STI activity was identified as BLV-specific immunoglobulin.

It is unlikely that 3STI activity is induced by FBS (14, 27), because it occurs in cultures containing only BLV antibody-free adult serum. Heterologous serum stimulation is also unlikely, because lymphocytes from a single donor cultured with 9 different BLV antibody-free sera supported 3STI activity, whereas 14 different BLV antibody-containing serum did not. Furthermore, 3STI activity was inhibited by mixing adult cattle serum without BLV antibodies with BLV antibody-positive serum (e.g., Table 2).

BLV-associated spontaneous blastogenesis seems to be primarily specific for gp (Table 3). This apparently contradicts our other finding that only cattle with p25 antibody titers of 1:50 or greater have 3STI activity (all BLV-infected

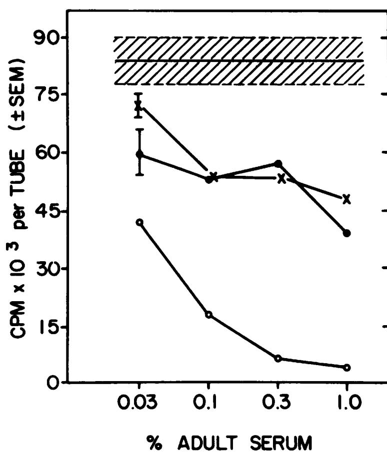

FIG. 5. *Titration of the inhibitor of 3STI activity. Lymphocytes from one BLV-infected cow (BF327) were cultured for 70 h in the presence of the indicated serum concentrations. In addition, each culture had a final concentration of 3% FBS. The cross-hatched area represents the mean ± standard deviation of cultures with no adult serum (3% FBS only). Symbols: ●, BLV antibody-negative serum SR3153; ×, BLV antibody-negative serum from BI615; ○, BLV antibody-positive serum SR124. SEM, Standard error of the mean.*

TABLE 2. *Absorption of inhibitor of 3STI activity with BLV antigens*[a]

Bovine serum	Absorption	3STI inhibitory activity
BLV antibody-positive SR124	None	3,000[b]
	Whole BLV	75
	Sonicated BLV	200
BLV antibody-negative SR3153	None	100[c]
	Whole BLV	100[c]

[a] Lymphocytes from a BLV-infected cow (BF327) were cultured for 70 h in the presence of various concentrations of the indicated serum. The final serum concentration was made up to 10% with SR3153 serum.

[b] Reciprocal titer necessary to inhibit 3STI activity by 50%.

[c] Higher concentrations did not inhibit 3STI activity by any more than 1:100.

TABLE 3. *BLV p25 antibody does not inhibit 3STI activity*

Rabbit immunization	BLV antibody[a]		3STI inhibitory activity[b]
	gp	p25	
Whole BLV	+	+	100
Purified p25	−	+	20
None	−	−	10

[a] Sera were tested in BLV p25 and gp radioimmunoassays. Sera precipitating less than 20% of the appropriate antigen at a 1:5 final dilution were considered negative.

[b] Lymphocytes from a cow (BF314) were cultured for 72 h in the presence of various concentrations of the indicated serum. All of the cultures contained 3% FBS. Shown is the reciprocal titer of the serum necessary to inhibit the control (27,000 cpm) by 50%.

TABLE 4. *Copurification of immunoglobulin and the inhibitor of 3STI activity*[a]

Inhibitor[b]	Immunoglobulin (%)[c]	Sp act[d]
Whole serum	30 ± 5	30 ± 15
$(NH_4)_2SO_4$ supernatant	3 ± 0.5	4 ± 2
$(NH_4)_2SO_4$ precipitate	80 ± 5	100 ± 30
DEAE[e]-adsorbed $(NH_4)_2SO_4$ precipitate	95 ± 5	110 ± 20
Affigel-10–anti-bovine immunoglobulin eluate	98 ± 2	110 ± 20

[a] Each of the indicated inhibitors was assayed for total protein concentration and total immunoglobulin concentration and titrated for inhibition of 3STI activity. FBS (3%) was added to all cultures.

[b] SR124 serum was the source for all inhibitor fractions.

[c] Percentage of total protein (mean ± estimated range).

[d] Inhibition units × 100 per milligram of total protein. One inhibition unit equals the micrograms of protein necessary for 50% inhibition (mean ± estimated range).

[e] DEAE, Diethylaminoethyl.

cattle tested had gp antibodies). One explanation can be derived from the following hypothesis: the spontaneous blastogenesis of BLV-infected cattle results from stimulation of BLV-specific memory lymphocytes by BLV produced de novo in culture, but blastogenesis is detectable only in cattle given a sufficient amount of sensitization. By coincidence, a p25 titer of 1:50 corresponds with the minimum sensitization necessary to produce detectable 3STI activity. Experiments are in progress to test this and other hypotheses.

Two independent methods of fractionating serum showed that immunoglobulin and inhibitory activity copurified. It is likely, therefore, that the inhibitor of 3STI activity is a BLV antibody.

Spontaneously occurring thymidine incorporation has been noted in peripheral blood lymphocyte cultures of people infected with Epstein-Barr (22), measles (11), or influenza (3) virus and in spleen cells of mice in which murine leukemia virus had been expressed for long periods (13). It is possible that viral infections, especially persistent ones, induce a memory lymphocyte population which may have a role in the control of virus infection.

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LITERATURE CITED

1. **Baliga, V., and J. F. Ferrer.** 1977. Expression of the bovine leukemia virus and its internal antigen in blood lymphocytes. Proc. Soc. Exp. Biol. Med. **156**:388-391.
2. **Bloom, J. C., S. J. Kenyon, and T. G. Gabuzda.** 1979. Glucocorticoid effects on peripheral blood lymphocytes in cows infected with bovine leukemia virus. Blood **53**: 899-912.
3. **Buckley, C. E., II, M. J. Zitt, and T. R. Cate.** 1973. Two categories of lymphocyte unresponsiveness to phytohemagglutinin. Cell. Immunol. **6**:140-148.
4. **Devare, S. G., and J. R. Stephenson.** 1977. Biochemical and immunological characterization of the major envelope glycoprotein of bovine leukemia virus. J. Virol. **23**: 443-447.
5. **Diglio, C. A., W. C. D. Hare, D. C. Dodd, R. R. Marshak, and J. F. Ferrer.** 1975. Cytogenetic, cytological and virological characteristics of a bovine lymphosarcoma. Cancer Res. **35**:3628-3635.
6. **Ferrer, J. F.** 1980. Bovine lymphosarcoma. Adv. Vet. Sci. Comp. Med. **24**:1-68.
7. **Ferrer, J. F., D. A. Abt, D. M. Bhatt, and R. R. Marshak.** 1974. Studies on the relationship between infection with bovine C-type virus, leukemia and persistent lymphocytosis in cattle. Cancer Res. **34**:893-900.
8. **Ferrer, J. F., C. Cabradilla, and P. Gupta.** 1980. Bovine leukemia: a model for viral carcinogenesis. Cold Spring Harbor Conf. Cell Proliferation **7**:887-899.
9. **Ferrer, J. F., C. Cabradilla, and P. Gupta.** 1981. Use of a feline cell line in the syncytia-induction assay for detection of bovine leukemia virus infection in cattle. Am. J. Vet. Res. **42**:9-14.
10. **Ferrer, J. F., R. R. Marshak, D. A. Abt., and S. J. Kenyon.** 1979. Relationship between lymphosarcoma and persistent lymphocytosis in cattle: a review. J. Am. Vet. Med. Assoc. **175**:705-708.
11. **Finkel, A., and P. B. Dent.** 1973. Abnormalities in lymphocyte proliferation in classical and atypical measles infection. Cell. Immunol. **6**:41-48.
12. **Gupta, P., and J. F. Ferrer.** 1980. Detection of a precursor-like protein of bovine leukemia virus structural polypeptide in purified virions. J. Gen. Virol. **47**:311-322.
13. **Ihle, J. N., J. C. Lee, L. Enjuanes, L. Cicurel, I. Horak, and L. Pepersack.** 1980. Chronic immunostimulation as a possible mechanism in type-C viral leukemogenesis. Cold Spring Harbor Conf. Cell Proliferation **7**:1049-1064.
14. **Johnson, G. J., and P. S. Russell.** 1965. Reaction of human lymphocytes in culture to components of the medium. Nature (London) **208**:343-345.
15. **Kenyon, S. J., and C. E. Piper.** 1977. Cellular basis of persistent lymphocytosis in cattle infected with bovine leukemia virus. Infect. Immun. **16**:891-897.
16. **Kenyon, S. J., and C. E. Piper.** 1977. Properties of density gradient-fractionated peripheral blood leukocytes from cattle infected with bovine leukemia virus. Infect. Immun. **16**:898-903.
17. **Kettman, R., D. Portetelle, M. Mammerickx, Y. Cleuter, D. Dekegel, M. Galoux, J. Ghysdael, A. Burny, and H. Chantrenne.** 1976. Bovine leukemia virus: an exogenous RNA oncogenic virus. Proc. Natl. Acad. Sci. U.S.A. **73**:1014-1018.
18. **Marshak, R. R., W. C. D. Hare, D. A. Abt, J. E. Croshaw, J. W. Switzer, I. Ipsen, R. M. Dutcher, and J. E. Martin.** 1963. Occurrence of lymphocytosis in dairy cattle herds with high incidence of lymphosarcoma. Ann. N.Y. Acad. Sci. **108**:1284-1301.
19. **McDonald, H. C., and J. F. Ferrer.** 1975. Detection, quantitation and characterization of the major internal antigen of the bovine leukemia virus by radioimmunoassay. J. Natl. Cancer Inst. **57**:875-882.
20. **Muscoplat, C. C., I. Alhai, D. W. Johnson, K. A. Pomeroy, J. M. Olson, V. L. Larson, J. B. Stevens, and D. K. Sorenson.** 1974. Characteristics of lymphocyte response to phytomitogens: comparison of responses to lymphocytes from normal and lymphocytic cows. Am. J. Vet. Res. **35**:1053-1055.
21. **Rotman, B., and B. W. Papermaster.** 1966. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. Proc. Natl. Acad. Sci. U.S.A. **55**:134-141.
22. **Steel, C. M., J. Philipson, E. Arthur, S. E. Gardiner, M. S. Newton, and R. U. McIntosh.** 1977. Possibility of EB virus preferentially transforming a subpopulation of human B lymphocytes. Nature (London) **270**:729-731.
23. **Takashima, I., and C. Olson.** 1980. Effect of mitogens and anti-bovine leukosis virus serums on DNA synthesis of lymphocytes from cattle. Eur. J. Cancer **16**:639-645.
24. **Thorn, R. M., J. C. Palmer, and L. A. Manson.** 1974. A simplified ^{51}Cr-release assay for killer cells. J. Immunol. Methods **4**:301-316.
25. **Weiland, F., and O. C. Straub.** 1976. Differences in the *in vitro* response of lymphocytes from leukotic and normal cattle to concanavalin A. Res. Vet. Sci. **20**:340-341.
26. **Yang, T.-J., W. C. D. Hare, and D. A. Abt.** 1967. DNA and RNA synthesis in cultures of peripheral blood mononuclear leucocytes from normal and lymphosarcomatous cows. Br. J. Haematol. **13**:855-861.
27. **Zielske, J. V., and S. H. Golub.** 1976. Fetal calf serum-induced blastogenic and cytotoxic responses of human lymphocytes. Cancer Res. **36**:3842-3846.