Bovine Leukemia Virus–Related Antigens in Lymphocyte Cultures Infected with AIDS-Associated Viruses

Abstract. *An earlier finding that lymphocytes from African patients with the acquired immune deficiency syndrome (AIDS) react with rabbit antiserum to purified antigens of bovine leukemia virus (BLV) prompted a study of the possible cross-reactions between a BLV-infected ovine cell line and human lymphocytes inoculated with a strain of lymphadenopathy syndrome–associated virus (LAV). A solid-phase radioimmunoassay was used to detect antigenic markers of the retroviruses. Crude extracts from short-term cultures of lymphocytes infected with LAV bound rabbit antisera to the LAV glycoprotein gp13 (molecular weight 13,000) and the BLV proteins p24 and gp51, but did not bind antibodies to the p24 of human T-cell leukemia virus type I (HTLV-I). Antiserum to LAV gp13 reacted with an ovine cell line producing BLV but also weakly with virus-free ovine cells. Lymphocyte cultures from four African patients with AIDS expressed BLV-related antigens within 6 to 10 days of culture, at the moment when particle-bound reverse transcriptase was produced. BLV-related antigens were induced in lymphocyte cultures from healthy individuals by addition of filtered supernatant or irradiated cells of the original culture. The antisera to BLV used in this study may prove useful for the detection of AIDS-associated viruses in short-term cultures of lymphocytes from AIDS patients or their contacts.*

Lymphadenopathy syndrome–associated retroviruses (LAV) have been isolated from patients with the acquired immune deficiency syndrome (AIDS) in France (*1*) and human T-cell leukemia viruses type III (HTLV-III) have been cultivated from AIDS patients in the United States (*2*). There appear to be no differences between the two groups of isolates and their major core proteins (p25) are identical by competitive radioimmunoassay (*3*). That AIDS in Central Africa is probably caused by similar viruses is indicated by studies in which an LAV-related virus was isolated from a Zairian married couple, one with AIDS and one with pre-AIDS (*4*), and antibodies to disrupted virions of LAV were found in most Zairian patients with AIDS (*5*).

We were thus surprised to find that lymphocytes cultivated from tissues of African patients with AIDS treated in Belgium (*6*) reacted with rabbit antiserum to purified antigens of bovine leukemia virus (BLV) when tested by a solid-phase radioimmunoassay. This prompted the study of possible cross-reactions between a BLV-infected ovine cell line and normal lymphocytes inoculated in this laboratory with an LAV strain (*7*). Cells infected with HTLV-III were not available at the time of this study. Lymphocytes from ten healthy adults were purified in Ficoll-Hypaque and grown for 3 days with purified phytohemagglutinin (PHA; 1 μg/ml). Washed lymphocyte suspensions containing 2×10^6 cells per milliliter and Polybrene (2 μg/ml) were inoculated with LAV in amounts equivalent to a reverse transcriptase activity of 10,000 cpm per milliliter of cell suspension. After a 3- to 4-hour adsorption period, the suspensions were diluted 1:2 and transferred into RPMI 1640 medium with 10 percent fetal calf serum, 10 percent human T-cell growth factor (TCGF or interleukin-2), Polybrene (2 μg/ml), and sheep antibody to α-interferon diluted 1:1000. Unadsorbed virus was removed after 1 day of culture and cells were grown in the same medium. Cell extracts were prepared after 5, 7, and 10 days of LAV infection. Results obtained on day 7 are shown in Table 1. The LAV-infected lymphocytes bound immunoglobulins of a rabbit antiserum that contains antibodies to LAV gp90 and to a gp13 component of this virus (*8*); rabbit antisera to purified BLV p24 and gp51 reacted with the LAV-infected cells at least to the same extent as the antiserum to LAV. The monospecificity of these polyclonal antisera to purified BLV p24 and gp51 has been demonstrated previously (*9*). Of ten virus-free lymphocyte cultures run in parallel, none adsorbed the antisera to BLV, but lymphocytes of patient 7 strongly reacted with the antiserum to LAV gp13. It is possible that the antigen inoculated into the rabbit also contained a histocompatibility polypeptide from the lymphocytes in which LAV was grown.

We also analyzed cross-reactions between cells infected with BLV, LAV, and HTLV-I (Table 2). With radioimmunoassays using ^{125}I-labeled goat antiserum to rabbit immunoglobulins, we demonstrated the binding of rabbit antibodies to BLV p24 with fetal lamb kidney (FLK) cells (a BLV-producing line) and, to a lesser extent, with LAV-infected primary lymphocyte cultures. There was also a low but significant reaction with MT_2 cells producing HTLV-I. Antibodies to BLV gp51 bound to LAV-infected lymphocytes and to FLK cells, but not to MT_2 cells, while antiserum to LAV gp13 bound even more to FLK cells than to those infected with LAV.

There are two possible explanations

Table 1. Reaction of LAV-infected lymphocytes with antiserum to BLV p24, BLV gp51, and LAV gp13. Lymphocytes grown for 7 days with LAV were adjusted to a concentration of 10^6 cells per milliliter with protease inhibitors (2 m*M* tosyl lysine chloromethylketone and phenylmethylsulfonyl fluoride); they were frozen at −80°C for at least 2 hours and then thawed and sonicated. Cell extracts were distributed in the wells of Microtest III flexible assay plates (Falcon 3911, 50 μl per well) and left to dry overnight. We then added 50 μl of a 5 percent bovine serum albumin solution. After 1 hour of incubation at 37°C, we added 50 μl of rabbit antiserum diluted 1:1000 in 40 percent fetal calf serum. After 2 hours at 37°C, wells were washed three times with phosphate buffered saline (PBS) and then filled with 50 μl of PBS containing 25,000 cpm of goat antiserum to rabbit immunoglobulin (059-03, ATAB). After 1 hour at room temperature, wells were washed three times, dried for 20 minutes at 70°C, cut, and distributed into tubes for counting in a gamma counter. Antisera and labeled immunoglobulins were diluted and incubated in 40 percent fetal calf serum before use. Results show mean of quadruplicate tests. Standard deviations were ±10 percent, as well as the index of ^{125}I bound calculated as the ratio of counts per minute in test wells to the counts in control wells with no antisera added.

Serum	Binding of ^{125}I-labeled goat antiserum to rabbit immunoglobulin			
	With LAV		Without virus	
	Radioactivity (cpm)*	Index	Radioactivity (cpm)*	Index
LAV grown in lymphocytes from patient 3				
Antiserum to BLV p24	724	4.2	145	1.2
Antiserum to BLV gp51	481	2.8	138	1.1
Antiserum to LAV gp13	671	4.0	98	0.8
Nonimmune serum	170		123	
LAV grown in lymphocytes from patient 7				
Antiserum to BLV p24	510	6.0	89	0.9
Antiserum to BLV gp51	595	7.0	104	1.0
Antiserum to LAV gp13	935	11	784	8.0
Nonimmune serum	85		98	

*Standard deviations are ±10 percent.

for these results. First, reaction of the antiserum to LAV with FLK cells was not completely specific, since control, virus-free ovine cells (OVK) also reacted with this serum, although significantly less than did the FLK cells. Second, FLK cells produced more virus particles than LAV-infected lymphocytes, as judged by the amount of released particle-bound reverse transcriptase activity (not shown). When Protein A was used as a labeled reagent, the reaction of the antiserum to LAV was also partly nonspecific, and the nonspecificity was much higher if the antiserum was not diluted in 40 percent fetal calf serum, indicating that this rabbit had been partly immunized against calf serum proteins. By contrast, rabbit antisera to BLV p24 and gp51 did not react with virus-free cells, and the Protein A assay with antiserum to BLV p24 confirmed a small degree of cross-reactivity between FLK and MT_2 cells. Conversely, goat antiserum to HTLV-I also bound to some antigens of FLK cells. Of two mouse monoclonal antibodies to HTLV-I p24, one, antibody 493, revealed an epitope common to BLV- and HTLV-I–infected cells.

Serum from an animal with a BLV tumor and containing BLV antibodies reacted specifically with FLK cells, although weakly because of the low affinity of Protein A for bovine immunoglobulins. Protein A could not be used in radioimmunoassays with human lymphocyte primary cultures since this reagent binds directly to Fc fragments of lymphocyte subpopulations. It was possible to study reactions of LAV-infected lymphocytes with mouse and ovine antibodies by using labeled antisera to mouse and goat immunoglobulins. Epitopes for HTLV-1 p24, recognized by the monoclonal antibodies 493 and 6G9, were not detected in LAV-infected lymphocytes, nor were the HTLV-I p19 epitopes recognized by monoclonal antibody 12/1-2. Actually, LAV-infected lymphocytes did not reveal any antigenic component of HTLV-I p24 when assayed with goat antiserum to HTLV-I p24. Neither did they react with BLV antibodies in the serum from the animal with a BLV tumor.

These results are consistent with data showing that amino acid sequences of HTLV-I p24 are homologous to BLV p24 (*10*). However, immunological cross-reactions between the two viruses have been demonstrated previously, although LAV has been shown to be related to equine infectious anemia virus (EIAV); in particular, LAV p24 was immunoprecipitated by horse sera containing antibodies to EIAV, but not by antibodies to core proteins of various other retroviruses, including BLV (*11*).

Our data do not apply to purified virions. They leave open the possibility that common antigenicity between BLV and HTLV group may be more readily detected in virus-infected cells, possibly because exposition of the shared components depends on the steric configuration of precursor polyproteins.

We found that rabbit antisera to BLV were useful for the early detection of retrovirus markers in lymphocyte cultures from AIDS patients. The four patients studied here belonged to a group of black patients from Central Africa treated in Brussels and documented according to the criteria described (*6*). A specimen from lymph node biopsy and a blood sample were obtained on the same day from patient A, and the lymphocytes were cultivated for 3 days with PHA (1 μg/ml), washed, and grown in RPMI 1640 medium with TCGF and Polybrene. Supernatants were collected periodically for assay of particle-bound reverse transcriptase activity. Cell extracts were prepared and assayed for reactivity with antiserum to BLV p24 (Table 1). Transient expression of an antigen related to BLV p24 occurred in both lymph node and blood cultures, with peak expression corresponding to peak reverse transcriptase production (Fig. 1). Disappearance of both retrovirus markers coincided with degeneration of the lymphocyte cultures. Early expression of BLV p24–related antigens within 5 days of culture was also observed with lymph node cells of patient B, as well as with two blood samples from this patient obtained 7 and 17 days later. A third blood sample, taken 1 month after the lymph node biopsy, yielded negative results. For the next two patients, C and D, cell extracts from the lymphocyte cultures were assayed in parallel with antibodies to BLV gp51 and to BLV p24 (Fig. 1, C and D). Lymph node cell culture of patient C simultaneously produced reverse transcriptase and expressed BLV-like gp51

Table 2. Binding of antibodies to various virus-infected and virus-free cells. The LAV-infected lymphocytes were as described in Table 1. The FLK and OVK cell lines are, respectively, BLV producers and virus-free cells. MT_2 is a lymphoid T-cell line producing a Japanese isolate of HTLV-I (ATLV), while the T cells of the HSB_2 line do not produce virus. Of the antibodies, goat antiserum to HTLV-I p24 as well as mouse monoclonal antibody 493 and 12/1-2 were obtained from R. C. Gallo and F. de Noronha; antibody 5G9 was from B. F. Haynes. Bovine and sheep sera of animals with BLV tumors contained antibodies to BLV. Binding of the various antibodies was revealed as in Table 1 or with labeled Protein A (Pharmacia, Uppsala), affinity-isolated goat antiserum to mouse immunoglobulins (TAGO Inc., Burlingame) or rabbit antiserum to goat immunoglobulins (Janssens Pharmaceutica, Belgium). Iodination of these products was performed each month in this laboratory. Results show indices of ^{125}I bound, as defined in Table 1. Some tests were not feasible (NF) because of nonspecific adsorption of Protein A to lymphocytes.

Antibody		Index of binding to different cells					
		Human lymphocytes					
Source	Specificity	Infected with LAV	Control	FLK/BLV	OVK	MT_2	HSB_2
Goat antiserum to rabbit immunoglobulin							
Rabbit	BLV p24	3.9	1	11	1	2.2	1
	BLV gp51	4.7	1	3	1	1	
	LAV gp13	2.9	1	7.5	2	1	1
Protein A							
Rabbit	BLV p24	NF	NF	30	1	1.8	1
	BLV gp51	NF	NF	3	1	1	1
	LAV gp13	NF	NF	6.5	2.4	2	2
Goat	HTLV-I p24	NF	NF	3	1	28	1
Mouse monoclonal	493 HTLV-1 p24	NF	NF	2.5	1	3.5	1
	6G9 HTLV-I p24	NF	NF	1	1	5.9	1
Bovine	With BLV tumor	NF	NF	1.8	1	1	1
	Virus free	NF	NF	1	1	1	1
Goat antiserum to mouse immunoglobulin							
Mouse monoclonal	493 HTLV-I p24	1	1	1.8	1	2.2	1
	6G9 HTLV-I p24	1	1	1	1	3	1
	12/1-2 HTLV-I p19	1	1	1.8	1	3	1
Rabbit antiserum to goat immunoglobulin							
Goat	HTLV-I p24	1	1	2.2	1	12	1
Sheep	With BLV tumor	1	1	2	1	1	1
	Virus free	1	1	1	1	1	1

and p24. Fresh lymphocytes from a healthy adult were added after 8 days of culture, at a moment when retrovirus markers were already decreasing. Their synthesis resumed after addition of the fresh target cells. Induction of BLV-related gp51 and p24 could be transferred to other lymphocyte cultures, when filtered supernatant of the 15-day-old original culture was added with Polybrene (2 μg/ml) to a lymphocyte culture from an adult donor. Similar results were obtained with lymphocyte cultures of a blood specimen from patient D. In addition, cells of the 14-day-old original culture were irradiated and cocultivated with normal adult lymphocytes. The production of BLV-related antigens could be sustained provided that fresh target lymphocytes were added to the culture.

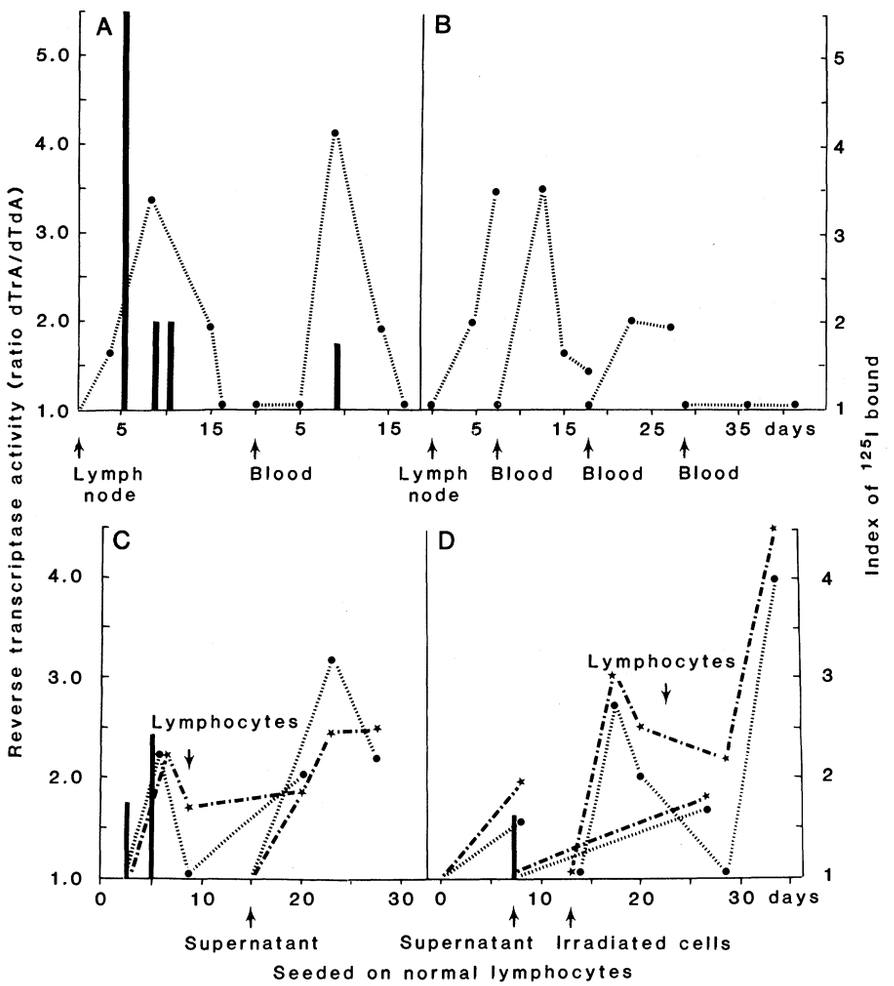

Fig. 1. (A and B) Binding of rabbit antiserum to BLV p24 (●) to lymphocyte cultures from AIDS patients A and B and detection of particle-bound reverse transcriptase in the supernatant (black bars). Antibody binding was assayed as in Table 1 and indices of ^{125}I bound were calculated from the radioactivity (counts per minute) of labeled goat antiserum to rabbit immunoglobulin bound to cell extracts treated with antiserum to BLV p24 divided by the radioactivity bound to cell extracts treated with normal rabbit serum or without rabbit serum. Reverse transcriptase activity was determined on concentrates (100 times) of tissue culture fluids suspended in assay buffer containing Triton X-100 (2 percent) and Tween 80 (0.1 percent). Assays were performed in 0.05*M* tris-HCl buffer, *p*H 7.9, containing 10 μ*M* ^{3}H-labeled dTTP (50 Ci/mmol), 130 μ*M* deoxyadenosine triphosphate, 50 μg each of $dT_{12-18}rA$ and $dT_{12-18}dA$ per milliliter, 5 m*M* $MgCl_2$. 0.1*M* NaCl, and bovine serum albumin (5 mg/ml). The assays were carried out at 30°C for 30 minutes. The results were expressed as the ratio of the enzymatic activity obtained on both synthetic template-primers ($dT_{12-18}rA$)/($dT_{12-18}dA$). Standard deviations are ±10 percent. (C and D) Binding of rabbit antisera to BLV p24 (●) and BLV gp51 (⋆) to lymphocyte cultures of ganglia biopsy from AIDS patient C and blood sample from patient D. Supernatants of the cultures were filtered at the times indicated by arrows and inoculated with Polybrene (2 μg/μl) into normal lymphocyte cultures that had been grown for 3 days with PHA (1 μg/μl). The cells were washed and resuspended in RPMI 1640 medium with 10 percent fetal calf serum, TCGF, and Polybrene before being inoculated with the filtered supernatants. Lymphocytes of the 13-day-old culture of patient D were irradiated with 2000 Rad from a cobalt source, and equivalents of 5 × 10^6 cells were cocultivated with 5 × 10^6 normal target lymphocytes, previously exposed to PHA. Standard deviations are ±10 percent.

Lymphocyte cultures from 25 healthy Belgian donors grown with PHA and TCGF did not bind rabbit antisera to BLV p24 or gp51 but four of these cultures bound the antiserum to LAV gp13. The use of this antiserum to detect viral antigens in crude whole cell extracts is valid only if virus-free control cultures are studied simultaneously.

It is striking that BLV markers were detected in the 20 Zairian patients tested thus far. The markers appeared within 5 to 8 days in the lymph node cultures and in five of six blood samples tested and reported here. Whether the change from positive to negative results in the blood tests for patient B has some prognostic meaning will be interesting to follow. These tests may prove useful in indicating the presence of LAV or HTLV-III in AIDS patients and their healthy contacts.

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