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BLV RECEPTOR ACTIVITY IN PLASMA MEMBRANES FROM TUMOROUS LYMPH NODES OF BLV-INFECTED COWS

E. RISTAU, H. ROESSLER, H. BURKARDT, *D. SCHOLZ, S. ROSENTHAL

Academy of Sciences of the German Democratic Republic, Central Institute of Molecular Biology, 1115 Berlin-Buch, G.D.R. and *Humboldt-University, Institute of Virology, 1040 Berlin, G.D.R.

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Summary. — Plasma membranes of cells from lymph nodes of bovine leukaemia virus (BLV)-free cattle and of cells from tumorous lymph nodes of BLV-infected cattle have been investigated for their reactivity with iodine labelled BLV antigens gp51 and p24. It has been found that only the plasma membranes from cells of tumorous lymph nodes bound gp51 and p24. The binding could be abolished by addition of nonlabelled antigens. It has been calculated from Scatchard plot analysis that 10^5 molecules of gp51 or 10^4 molecules of p24 can be bound per tumour cell. The findings led to the conclusion that tumour cells of BLV-infected cattle are endowed with receptors for the BLV antigens gp51 and p24.

Key words: *cattle*; *tumour cells*; *receptors*; *bovine leukaemia virus*

Introduction

The bovine leukaemia virus (BLV) causes in cattle persistent lymphocytoses and/or tumorous leukoses. BLV-infected cattle has a high concentration of antiviral antibodies. The viral genome is integrated within the DNA of infected blood lymphocytes and tumour cells (reviewed by Burny *et al.*, 1980; Ghysdael *et al.*, 1984), but BLV production can be demonstrated only upon cultivation of these lymphocytes in vitro (Stock and Ferrer, 1972; Baliga and Ferrer, 1977; Kettmann *et al.*, 1982). Portetelle *et al.* (1978) have shown that noncultivated lymphocytes cannot be lysed by the cytotoxic antibodies present in the serum of BLV-infected cattle because antigenic sites of gp51 are not available on these cells. In an earlier paper we described that although in vitro cultivated tumour cells express gp51, they cannot be lysed by cytotoxic antibodies (Ristau and Weppe, 1984). We concluded that gp51 is present on tumour cells in a form hampering access for cytotoxic antibodies. In order to clarify further this problem, we have investigated the ability of plasma membrane preparations from tumorous lymph nodes of BLV-infected cattle to bind radioiodinated BLV gp51 and p24 antigens.

Materials and Methods

Tissues. The tumorous lymph nodes were obtained from cattle with tumorous leukosis. Normal lymph nodes were coming from cattle of a leukosis-free herd which did not show antibodies against BLV antigens at the time of slaughter.

Plasma membrane preparation and extraction. The preparation of plasma membranes from tumorous lymph nodes (PMT) and from normal lymph nodes (PMN) has been previously described (Ristau *et al.*, 1982). Briefly, the lymph node tissue was finely minced with scissors and suspended in the 10 fold volume of 0.15 mol/l NaCl in 10 mol/l Tris-HCl buffer, pH 7.4, using a Potter homogenizer. The homogenate was passed through a Nylon sieve and centrifuged at 5,000 g for 20 min. The plasma membrane fraction was obtained by centrifugation of the supernatant at 50,000 g for 1 hr. The plasma membranes were washed twice with 36 mmol/l phosphate buffer pH 7.5 and were finely resuspended in this buffer. Extraction of the plasma membranes with 2% Triton X-100 was carried out for 1 hr at room temperature and at a protein concentration of 6 mg/ml. Supernatant which was obtained after centrifugation for 1 hr at 100,000 g contained the solubilized membrane proteins.

Radioimmunoassays. Iodination of BLV gp51 and p24. The antigens gp51 and p24 were kindly provided by Dr. Portetelle (Department de Biologie Moleculaire, Universite Libre de Bruxelles). Iodination of the antigens was carried out according to the method of Greenwood *et al.* (1963). The iodinated p24 was separated from free NA_2 ^{125}I by gel permeation chromatography on Sephadex G 25 which was previously equilibrated with 0.2% human serum albumin in PBS (pH 7.2). The iodinated gp51 was further purified by affinity chromatography on lectin sepharose (*lens culinaris*) according to Devare and Stephenson (1977). The specific radioactivities amounted to 555 kBq/ng, each for gp51 and p24.

Radioimmune binding precipitation test. From 5 to 200 µl plasma membrane suspension and 1 ng of ^{125}I-labelled p24 or 0.5 ng of ^{125}I-labelled gp51 (amounting to about 30,000 cpm) were filled up to a vol of 400 µl with 0.04 mol/l phosphate buffer pH 7.2, containing 0.2 ml Triton X-100, 0.5 g NaN_3, and 5 g human serum albumin per liter. After incubation period of 30 hr at 4 °C, 200 µl of a titrated excess of rabbit anti-cattle-Ig serum or of a rabbit antiserum against PMN was added, and the samples were further incubated for 1 hr at 37 °C and for 4 hr at 23 °C. Upon addition of 4 ml ice-cold 8 mmol/l phosphate buffer, pH 7.2, containing 10 mmol/l NaCl and 0.01 ml Triton X-100 per liter, the samples were centrifuged for 30 min at 3,000 g, the supernatants were decanted and the sediments washed once again with 4 ml of the same buffer. The radioactivity of the pellets was determined in a gamma-counter model NZ-310A (Gamma, Budapest, Hungary).

Enzyme-linked immunosorbent assay (ELISA). Reactivity of plasma membranes with BLV antigens immobilized on polyvinylchloride solid phase was examined by enzyme-linked immunosorbent assay under conditions as described by Scholz *et al.* (1986). The binding of the plasma membranes to the BLV antigens was evaluated by using a horse-radish peroxidase anti-bovine Ig conjugate (VEB Kombinat für Veterinärimpfstoffe, Dessau).

Results

Radioimmune binding precipitation assay of ^{125}I-p24

PMT and PMN were investigated for their ability to bind ^{125}I labelled p24. All PMT (n = 9) tested bound p24, while with the 4 PMN no p24 binding could be demonstrated (Table 1). A significant binding of ^{125}I-p24 occurred already at protein concentrations below 125 µg/ml. With protein concentrations above 500 µg/ml, most of the PMT bound the whole immunologically active p24, and thus no increase in binding was observed with increasing protein concentration. With 2 PMT, a significant p24 binding could be observed only at protein concentrations around 1000 or 2000 µg/ml, respectively, but it was here also clearly above the binding capacity of PMN (Table 1). The difference in the binding capacity of PMT and PMN for p24 could be demonstrated also if a rabbit antiserum against PMN was used

Table 1. BLV-p24 binding capacity of plasma membrane preparations from tumorous lymph nodes (PMT 1—9) and from normal lymph nodes (PMN). The number listed are the cpm in the precipitates obtained on precipitation of the membrane-p24 complexes with anti-cattle immunoglobulin

Plasma membrane preparation	Membrane protein concentration (mg/ml)					
	0.063	0.125	0.250	0.500	1.000	2.000
PMT-1	467	657	1 107	2 027	3 380	6 098
PMT-2	788	1 126	2 176	3 634	6 094	9 779
PMT-3	2 450	3 200	5 000	7 400	11 350	n.d.
PMT-4	3 100	5 971	9 859	10 435	11 679	11 639
PMT-5	3 240	5 799	9 578	11 647	11 117	11 989
PMT-6	3 726	7 283	10 706	10 984	11 673	11 839
PMT-7	4 838	7 194	9 124	8 462	8 731	11 020
PMT-8	5 969	9 787	11 390	11 073	11 411	11 993
PMT-9	10 417	11 417	12 517	12 199	13 148	12 590
PMN MW ± ± SD (n = 4)	433 ± 164	506 ± 168	463 ± 109	557 ± 117	625 ± 99	761 ± 65

n.d.: not determined

instead of the anti-cattle-Ig serum for the precipitation of the ^{125}I-p24 plasma membrane complex (Fig. 1).

When replacing the rabbit anti-cattle-Ig serum by normal rabbit serum, no binding of ^{125}I-p24 to PMT was demonstrated (Fig. 1-c). If Triton X-100 extracts from PMT and PMN were investigated instead of plasma membrane preparations, ^{125}I-p24 binding occurred again only to membrane proteins from tumour tissue (Fig. 2). Binding of 30% of the radioactivity used (10,000 cpm) was observed at lower protein concentration (230 µg/ml) comparing to non-solubilized plasma membrane preparation (838 µg/ml, PMT-3, Table 1). The binding of ^{125}I-p24 to PMT could be completely suppressed by addition of unlabelled p24 whereas bovine serum albumin or plasma membranes

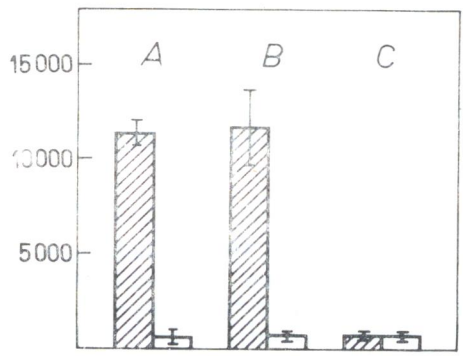

Fig. 1.
Binding of ^{125}I-p24 to plasma membranes from tumorous lymph nodes (shaded fields) and normal lymph nodes (open fields)
Precipitation of the plasma membrane ^{125}I-p24 complex by rabbit anti-cattle-Ig serum (A), by rabbit anti-PMN serum (B) or by normal rabbit serum (C). Mean values ± standard deviation from 5 determinations.
Ordinate: ^{125}I-p24 bound (cpm).

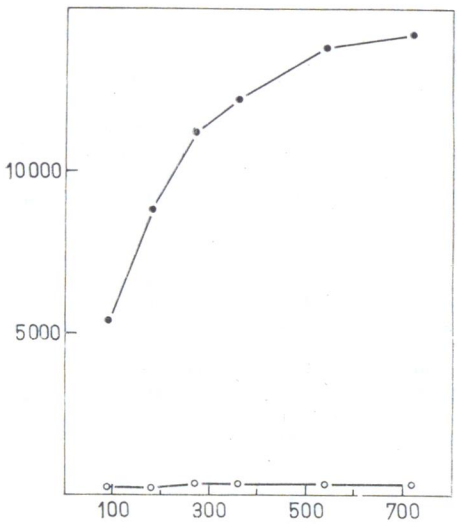

Fig. 2.
Binding of ^{125}I-p24 to Triton X-100 extract from plasma membranes of a tumorous (— ● —) and a normal lymph node (— ○ —)
Abscissa: protein concentration (µg/ml); ordinate: ^{125}I-p24 bound (cpm).

from Ehrlich ascites carcinoma cells did not diminish the p24-binding capacity (Fig. 3-I). The data represented in Fig. 3-I were transferred into a Scatchard plot (Fig. 3-II). From the linear part of the curve it was calculated that 1×10^4 molecules of p24 can be bound per tumour cell. This calculation is based on the finding that 1 g tumour tissue contains about 5×10^8 cells and yields about 3 mg plasma membrane protein.

Radioimmune binding precipitation assay of ^{125}I-gp51

Fifteen PMT and 15 PMN were investigated for their capacity to bind ^{125}I-gp51. Only the PMT revealed a significant binding capacity for gp51 (Fig. 4). The binding of iodinated gp51 to PMT could be inhibited by the adding of unlabelled gp51 (Fig. 5-I). Transfering the data from Fig. 5 into a Scatchard plot (Fig. 5-II) and evaluating the linear part of this plot we found that 12×10^4 gp51 molecules can be bound per tumour cell.

Binding of plasma membranes to BLV antigens in ELISA

The different binding capacity of PMT and PMN for BLV antigens was detectable also in the ELISA (Fig. 6). The binding of PMT to BLV antigens was inhibited by sheep serum containing antibodies to gp51 and p24, whereas serum from a BLV free sheep did not inhibit binding of PMT to BLV antigens (Fig. 7).

Discussion

It has been shown by the radioimmune binding precipitation assay that PMT specifically bind iodine-labelled BLV-gp51 and -p24. By adding of nonlabelled gp51 and p24, respectively, the binding of ^{125}I-gp51 and -p24 was prevented. In contrast, PMN did not bind BLV antigens. From these data

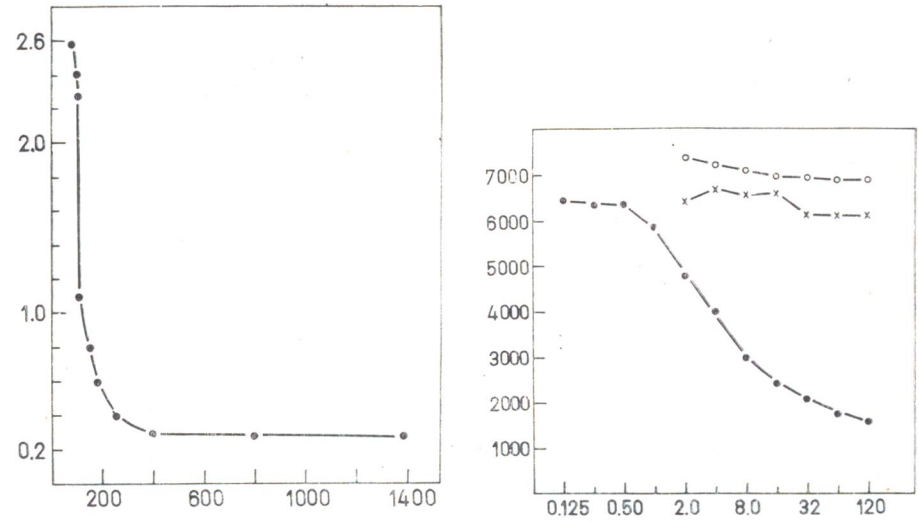

Fig. 3.
Competition of plasma membranes from a tumorous lymph node with nonlabelled p24 for the binding of ^{125}I-p24
I. (in the right) — The samples contained 1 ng ^{125}I-p24 with 30,000 cpm and 35 µg plasma membrane protein (— ● —). The plasma membrane control corresponding to 100% binding showed a value of 7,100 cpm. In reference experiment the same amount of ^{125}I-p24 and plasma membranes protein was incubated with bovine serum albumin (— ○ —; 3 — 500 µg/ml) or plasma membranes from Ehrlich ascites cells (— × —; 18 — 2400 µg/ml).
II. (in the left) — Scatchard analysis of the p24 binding to plasma membranes from tumorous lymph nodes using the data from Fig. 3-I.
Abscissae: protein p24 in ng or in fmol/ml, respectively; ordinates: ^{125}I-p24 bound in cpm (right) or the ratio of bound to free fmol/ml p24. (in the left).

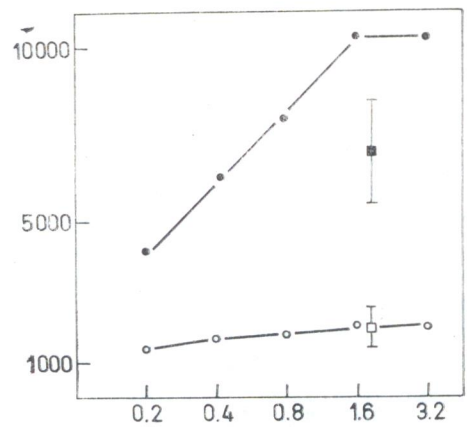

Fig. 4.
Binding of ^{125}I-gp51 to plasma membranes from a tumorous (— ■ —) and a normal lymph node (— ○ —)
Mean values and standard deviation of bound of ^{125}I-gp51 to plasma membrane preparations from each of 15 tumorous (— ■ —) and 15 normal lymph nodes (— □ —) at a constant protein concentration of 2 mg/ml.
Abscissa: protein concentration (mg/ml); ordinate: ^{125}I-gp51 bound (cpm).

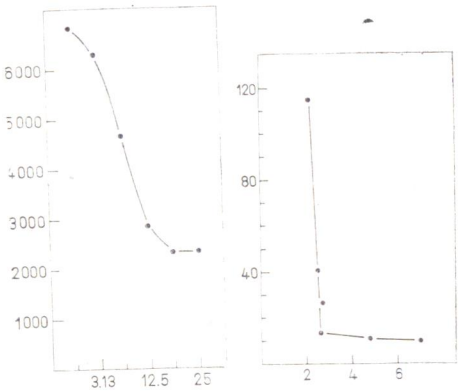

Fig. 5.
Competition of plasma membranes from a tumorous lymph node with nonlabelled gp51 for the binding of ^{125}I-gp51
I (in the left) — The samples contained 1 ng ^{125}I-gp51 with 18,000 cpm and 100 µg plasma membrane protein. The plasma membrane control corresponding to 100% binding showed a value of 4,500 cpm.
II (in the right) — Scatchard analysis of the gp51 binding to plasma membranes from tumorous lymph nodes using the data from Fig. 5-I.
Abscissa: I — gp51 added (ng) and II — bound pmol × 10^2/ml, respectively; ordinate: I — ^{125}I-gp51 bound (cpm) and II — ratio of bound to free pmol/ml gp51 × 10^2.

we conclude that cells of tumorous lymph nodes of BLV-infected cattle possess receptors for gp51 and p24, respectively. Plasma membrane receptors are required for the binding of the virus to the cells and for its penetration into the cells. In vitro BLV infection of the target cells can be inhibited by antibodies against gp51 (Závada *et al.*, 1978; Bruck *et al.*, 1982). Therefore, gp51 could be important for the early steps of infection. BLV-infected tumour cells also must be equipped with receptors for gp51. Receptors for binding of BLV and gp51, respectively, so far were demonstrated on mouse erythrocytes only (Sentsui *et al.*, 1982). While p24 is localized in the virus

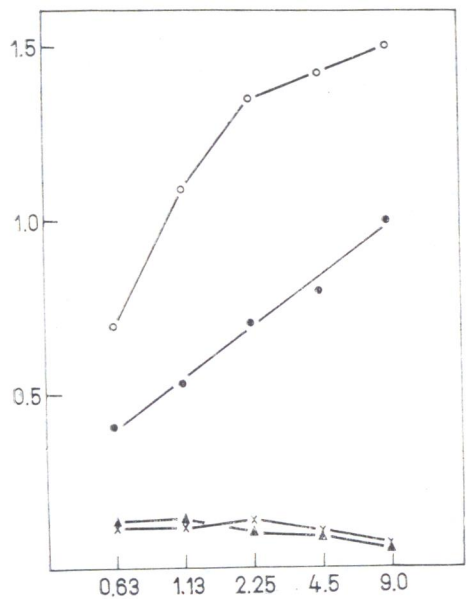

Fig. 6.
Binding of plasma membranes of lymph nodes from tumorous (—○—; —●—) and normal cattle (—▲—; —×—) in the ELISA.
The binding was detected with peroxidase -labelled anti-cattle Ig.
Abscissa: protein concentration (mg/ml); ordinate: absorbance at 492 nm.

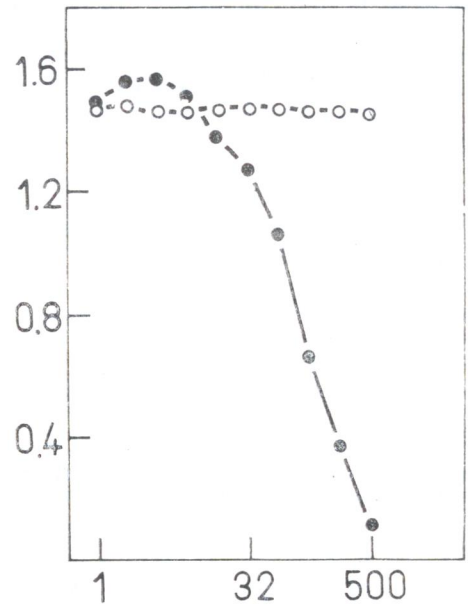

Fig. 7.
Competition binding to BLV-antigens between PMT (2.5 mg/ml) and sheep serum containing antibodies against gp51 and p24 (— ● —) or serum from a BLV-free sheep (— ○ —).
Abscissa: serum concentration (µl/ml); ordinate: absorbance at 492 nm.

core, the existing receptors for p24 on the PMT should not be of significance for the early steps of virus infection. On the other hand, our results are also compatible with the possibility that the receptors for gp51 and p24 play a role for the sustained proliferation or even for the malignant transformation of lymphocytes.

Receptors for the BLV antigens were not demonstrable in PMN. Possibly the antigen binding cells in vivo are in minority, e. g. on precursor cells or on a lymphocyte subpopulation only. Baird *et al.* (1977) have shown that in the case of mouse leukaemia virus about 2% of the normal lymphocytes, but 90% of the tumour cells had binding sites for the virus. The BLV antigen receptors could account for differentiation or maturation markers. Then, aside from the BLV infection, other factors, influencing the proliferation or differentiation stage, could lead to amplification of receptor bearing cells. For encephalomyocarditis virus it was shown that receptor activity was induced in previously receptor-negative murine splenic lymphocytes by cultivation of the lymphocytes in the presence of phytohaemagglutinin, Concanavalin A or *E. coli* lipopolysaccharide (McClintock *et al.*, 1983). We could not induce BLV receptor activity by cultivation of blood lymphocytes of cattle in the presence of *E. coli* lipopolysaccharide (data not shown), but this problem needs further investigation. BLV-infected cattle show a persisting antibody level against gp51 and p24. Attempts to demonstrate BLV antigen production in noncultivated blood lymphocytes or tumour cells have so far been unsuccessful. We suppose that a low BLV expression in tumour cells could be undiscovered because gp51- and p24-antigens are

bound to plasma membrane receptors and therefore escape direct immunological detection. After inoculation of calves with cell extracts from tumour tissue, antibodies reacting with p24 occurred (Ristau *et al.*, 1986), although no p24 was detectable in the tumour cell extract with RIA. This result supports our assumption that BLV antigens are present in noncultivated tumour cells.

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