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# ANTIBODY PRODUCTION AGAINST BLV-p24 IN CALVES FOLLOWING APPLICATION OF CELL EXTRACTS FROM TUMOROUS LYMPH NODES OF CATTLE WITH ENZOOTIC BOVINE LEUKOSIS

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*Summary.* — In the cell extract from tumorous lymph nodes of bovine leukosis virus (BLV)-infected cattle (tumour cell extract) and from lymph nodes of BLV-free cattle (control cell extract) neither the gp51 nor the p24 antigens were detectable. The tumour cell extract contained receptors for the BLV antigens gp51 and p24. The immunogenicity of the cell extracts was tested in calves. All calves treated with the tumour cell extract developed antibodies against p24 but not against gp51. Administration of the control cell extract, in contrast, induced no antibodies against p24.

*Key words: cattle; tumour; bovine leukosis virus; p24; lymph nodes*

## *Introduction*

The enzootic bovine leukosis (EBL) is aetiologically linked to infection with the bovine leukaemia virus (BLV). A characteristic feature of the BLV-infected cattle is the persisting antibody level against the virus coat glycoprotein gp51 and against the internal virus structural protein p24. Furthermore, there may occur in the infected cattle persistent lymphocytosis (PL) and tumorous leukosis (for survey see Burny *et al.* 1980). Virus particles or virus antigens can mostly be detected only after short-term cultivation of the blood lymphocytes or tumour cells in vitro (Stock and Ferrer, 1972; Baliga and Ferrer, 1977). On the other hand, the permanent production of BLV-specific antibodies can be explained only by continuous or discontinuous presence of viral antigens in vivo. In an earlier paper (Ristau *et al.*, 1986) we showed that cells from lymph node tumours of BLV-infected cattle revealed receptors for BLV-antigens gp51 and p24. We assumed that low antigen production may remain undiscovered in noncultured tumour cells if antigens gp51 and p24 are bound to plasma membrane receptors, so that they escape immunological detection. To test this hypothesis, extracts from the cells of tumorous lymph nodes of BLV-infected cattle were inoculated to calves. The sera of the calves were investigated for the presence of antibodies against gp51 and p24.

## *Materials and Methods*

*Tissues and cell extracts.* The tumorous lymph nodes were obtained from cattle with tumorous leukosis. Normal lymph nodes were obtained from cattle of a leukosis free herd; the sera of these cattle did not contain antibodies to BLV antigens at the time of slaughter. Cell extracts were obtained as described by Onuma and Olson (1977). The protein content of the extracts was 25 mg/ml.

*Immunization schedule.* The investigations were carried out on 5 month old calves. At the start of the experiment the calves showed no clinical, haematological or serological signs of leukosis. The calves were injected seven times with each 20 ml tumour or normal cell extract at biweekly intervals. Ten ml of the extract were administered i.m. twice with Freund's complete adjuvant (VEB Kombinat Veterinärimpfstoffe Dessau), and then 10 ml was given by s.c. route.

*Radioimmunoassays (RIA).* The antigens gp51 and p24 were kindly provided by Dr. H. Roessler (Central Institute of Molecular Biology, Berlin-Buch). The iodination of the antigens was performed as described (Ristau *et al.*, 1986).

*Antibody detection in bovine sera by RIA.* Four µl of serum and 1 ng of $^{125}$I-labelled p24 or 0.5 ng of $^{125}$I-labelled gp51 were mixed with 400 µl of 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 30 hr incubation at 4 °C, 200 µl of rabbit anti-bovine Ig was added in excess, and the sample was further incubated for 1 hr at 37 °C and for 4 hr at 23 °C. Upon addition of 4 ml of 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 3000xg, the supernatants were poured off and the sediments washed once again with the same buffer. The radioactivity of the sediments was determined in a gamma-counter model NZ-310A (Gamma, Budapest, Hungary).

*Competition RIA for detection of BLV antigens in cell extracts.* The reaction mixture and experimental procedure were identical with the assay described above, except that the cell extract was incubated with bovine serum in a dilution which led to about 30 to 50 % binding of the labelled antigen.

*Detection of BLV receptors in the cell extracts.* The radioimmune binding precipitation test for detection of receptors for the BLV antigens gp51 and p24 was made as described (Ristau *et al.* 1986).

*Reverse transcriptase activity assay.* Isolation of the leukocytes from bovine blood, the short time cultivation of the leukocytes and the investigation of the cell culture supernatants by the exogenous reverse transcriptase test were conducted according to Roessler *et al.* (1980).

*Immunodiffusion test (IDT).* The IDT for detection of BLV-gp51 and p24 was done as described (Wittmann *et al.*, 1983). The preparation of BLV antigen for detection of antibodies against gp51 was performed according to Wittmann *et al.* (1983). The preparation of BLV antigen for detection of antibodies against p24 was performed according to Miller and Van der Maaten (1986).

## *Results*

Prior to the application of the cell extracts, the sera of calves revealed no antibodies against gp51 and p24. Likewise, the application of control cell extract failed to induce antibodies in calves against the two BLV-antigens in question. However, after application of the tumour cell extract antibodies against p24, but no antibodies against gp51 could be demonstrated in the calf sera (Table 1). Antibodies against p24 were detected by RIA only after the 4th immunization dose of the tumour cell extract (Fig. 1). When this serum was concentrated five times by lyophilization, the antibodies against p24 could also be detected in immunodiffusion test. In the cultivated blood lymphocytes of calves treated with the cell extract no BLV-specific reverse transcriptase activity was found (Table 1).

When examined in competitive RIA neither the normal nor the tumour cell extract showed the presence of BLV-antigens gp51 and p24. Similarly,

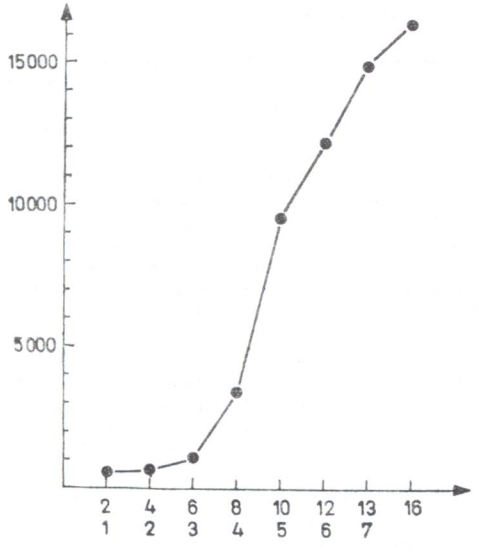


**Fig. 1**
Liquid phase radioimmunoassay for detection of antibodies against BLV-p24 in the serum of a calf at various intervals after application of the tumour cell extract

The serum was used in a dilution of 1 : 400. The liquid phase radioimmunoassay was performed as described in Materials and Methods.
Abscissa: upper line: time interval in weeks after starting the experiment; lower line: number of vaccinations. Ordinate: $^{125}$Ip24 bound (cpm)

the normal cell extract contained no receptors for these two BLV-antigens. In contrast, the tumour cell extract contained receptors for both gp51 and p24 (Figs. 2-I,II).

## *Discussion*

After application of the tumour cell extract the antibodies reacting with the BLV antigen p24 occurred in calves. Assuming that the production of these antibodies was brought about by a product coded by the BLV genome present in the tumour cell extract, then this gene product could be either the p24 itself, or some precursor protein encoded by the *gag*-gen and showing

**Table 1. Detection of antibodies against BLV in the sera of calves or of reverse transcriptase activity in the blood lymphocytes following application of extract from cells of tumours or normal lymph nodes**

| Material | Time of testing | Antibodies against gp51* | Antibodies against p24* | Reverse transcriptase activity |
|---|---|---|---|---|
| none | | 0/5** | 0/5** | 0/5*** |
| tumour cell extract | before immunization | 0/14 | 0/14 | 0/12 |
| control cell extract | | 0/7 | 0/7 | 0/7 |
| none | | 0/5 | 0/5 | 0/5 |
| tumour cell extract | 14 days following the 7th application of the tumour cell extract | 0/12**** | 12/12**** | 0/12 |
| control cell extract | | 0/7**** | 0/7**** | 0/7 |

*determined with the immunodiffusion test, **number of sera with positive reactions/total number of sera investigated, ***number of lymphocyte samples with positive reaction/total number of samples investigated ****determined with the radioimmunoassay

p24-antigenicity (Mamoun *et al.* 1983), or not yet identified fusion proteins containing portions of the *gag*-gen coded products from the BLV genome. Ferrer *et al.* (1980) and Kettmann *et al.* (1980) failed to detect BLV-antigens and BLV-specific RNA in noncultivated blood lymphocytes and tumour cells. Noetzel *et al.* (1980), however, using labelled BLV-cDNA probe were able to show by in situ hybridization that 0.96 to 1.5 % of the noncultivated lymphocytes of tumour animals expressed BLV-specific RNA.

We could not detect p24 in the tumour cell extract but the extract contained receptors for the BLV-antigens gp51 and p24. This result is in agreement with the assumption that tumour cells would express BLV-antigens in vivo and that these antigens were masked by simultaneously present receptors thus escaping direct immunological detection.

The possibility should be also considered that the antibodies against p24 were induced not by a product of the BLV genome but by a product(s) of cellular genes or by gene products of other viruses. BLV is not an endogenous bovine retrovirus (Deschamps *et al.* 1981). It cannot be ruled out, however, that the nucleotide sequence of the BLV genome exhibits certain regions homologous with cellular DNA sequences. BLV p24 shows no antigenic cross reaction with the bovine syncytial virus, the bovine maedi-like virus (Schmerr *et al.* 1980) and the core proteins of the feline, murine and avian retroviruses (Gilden *et al.* 1975; McDonald and Ferrer, 1976). It could be shown that antibodies against p24 react with cells infected with HTLV-I

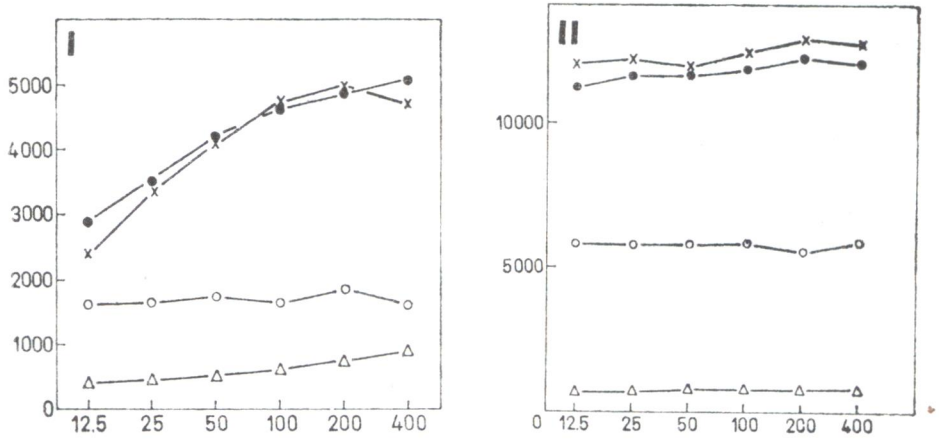


**Fig. 2.**

Binding of tumour cell extract and control cell extract with $^{125}$Igp51 (I) or $^{125}$Ip24 (II)
I: The tumour cell extract or control cell extract were incubated with $^{125}$Igp51 in the presence (tumour cell extract: —●—; normal cell extract: —○—) or absence (tumour cell extract: —×—; normal cell extract —△—) of bovine anti-gp51 serum and immunoprecipitated with rabbit anti-bovine Ig serum as described in Materials and Methods.
II: Incubation of the cell extracts with $^{125}$Ip24 and bovine anti-p24 serum. Symbols as above. Abscissae: cell extract concentration (µg protein/ml); ordinates: $^{125}$Igp51 (I) and $^{125}$Ip24 (II) bound (cpm)

or HTLV-III (Thiry *et al.* 1985). At the moment it is impossible to define clearly the genetic basis of the component present in the tumour cell extract, which induces in cattle the production of antibodies reacting with the p24 of BLV. The finding that this component is not present in normal lymph nodes suggests that it could be involved in tumourigenesis.

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