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DEMONSTRATION OF A THYMUS CELL TUMOUR IN BLV-INFECTED CATTLE

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Summary. — Occurrence of thymus cell tumours was followed in cattle with enzootic leukosis using a thymus-specific antiserum. Among 32 tumorous lymph nodes investigated, one could be identified as a thymus cell tumour. In the DNA extract from this tumorous lymph node BLV-specific sequences have been demonstrated. This finding disproved the hitherto assumption that BLV-induced lymph node tumours of cattle were derived exclusively from B-lymphocytes.

Key words: *cattle*; *bovine leukosis*; *thymus*; *lymph node tumour*

Introduction

Enzootic bovine leukosis (EBL) is a tumorous disease of the lymphatic system induced by bovine leukaemia virus (BLV). According to existing results only the B-cell system is affected by BLV infection and BLV-induced transformation (Muscoplat *et al.*, 1974; Weiland and Straub, 1975; Kumar *et al.*, 1978; Kenyon and Piper, 1977; Paul *et al.*, 1977; Takashima *et al.*, 1977; Onuma *et al.*, 1978; Esteban *et al.*, 1985). However, the occurrence of T-cell tumours in BLV-infected cattle could not be excluded with certainty so far, because appropriate immunological methods for the indentification of T-cell tumours had not been applied in major extent (Koyama *et al.*, 1983). In this paper the previously described test system for detection of a thymus-specific antigen in plasma membranes (Ristau, 1984*a*) has been applied to investigate the occurrence of thymus cell tumours in cows with enzootic bovine leukosis. The results led to the conclusion that thymus cell tumours can also arise in BLV-infected cattle.

Materials and Methods

Tissues. Normal lymph nodes and thymus showing no pathological alterations were obtained from leukosis-free calves. The tumorous lymph nodes were removed from adult animals with tumorous leukosis. The tissues were stored for a maximum of 6 month at −20 °C until use.

Plasma membranes. Plasma membrane preparations were obtained from normal and tumorous lymph nodes and from thymus tissue as described earlier (Ristau *et al.*, 1982; Ristau, 1984*b*).

Antisera. Thymus-specific antiserum was prepared in rabbits with plasma membranes from calf thymus. The serum was absorbed to plasma membranes from normal lymph nodes as described

earlier (Ristau, 1984*a*). Rabbit antiserum against bovine IgG was provided by Dr. H. Rössler (Department of cell differentiation of the Central Institute of Molecular Biology).

Enzyme-linked immunosorbent assay (ELISA). Thymus-specific antigen and IgG in plasma membranes were identified by ELISA using PVC-adsorbed plasma membranes and a sheep anti-rabbit IgG labelled with horse-radish peroxidase (the enzyme-antibody conjugate was obtained from Dr. B. Porstmann, Humboldt-Universität, Berlin). Details of the method were described in earlier papers (Ristau, 1984*a*, *b*).

Detection of BLV provirus sequences by the DNA-hybridization technique. DNA from tumour tissue and from BLV-infected foetal lamb kidney (FLK) cells was isolated according to the method of Jeffreys and Flavell (1977). 10 or 20 µg DNA in a total volume of 40 µl water were boiled for 10 min. After quick chilling of the sample on ice, the DNA was denatured by addition of 5 µl 0.2 mol/l Na_2EDTA and 5 µl 2 mol/l NaOH for 20 min at room temperature. Subsequently, the reaction mixture was neutralized by addition of 20 µl 1 mol/l Tris-HCl-buffer pH 8.0, 10 µl mol/l HCl and 80 µl of a solution consisting of 0.15 mol/l NaCl and 0.015 mol/l sodium citrate. Immediately after that, the samples were sucked off through nitrocellulose filters with a pore size of 0.45 µm (Sartorius, FRG). The filters were dried in vacuum for 2 hr at 80 °C. Filter-bound DNA was hybridized with a proviral BLV-clone from a Belgian tumour case (Deschamps *et al.*, 1981), recloned in pBR 322. This DNA was kindly provided by J. Deschamps (Universite de Bruxelles). After labelling this probe with ^{32}P-dATP (Amersham, specific radioactivity 1×10^{14} Bq/mmol) by nick translation procedure the specific activity amounted to 8×10^7 dpm/µg DNA. The filter-bound DNAs were prehybridized at 65 °C for 12 hr in 10 ml of 3 × SSC (SSC = 0.15 mol/l NaCl plus 0.015 mol/l sodium citrate), Denhardt's solution (Denhardt, 1966), 0.1% SDS (SERVA) and 100 µg sonicated salmon sperm DNA (SERVA). The ^{32}P-dATP-labelled probe (200 ng) was added to this solution and the hybridization continued for 24 hr. Thereafter, the filter-bound DNAs were washed at 65 °C for 3 hr with 3 changes of the washing solution (0.2 mol/l SSC, 0.1% SDS) and subsequently autoradiographed with 2 Dupont intensifying screens (ORWO) and an ORWO X-ray film HS-11 at −70 °C for 6 days.

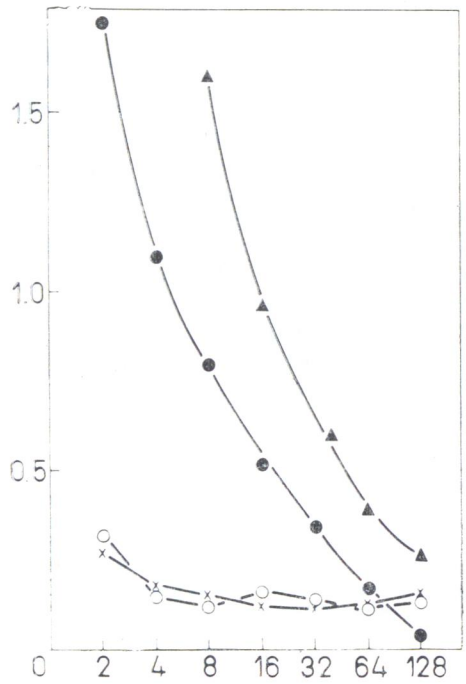

Fig. 1.
Reactivity of thymus-specific antiserum Plasma membranes from tumour T-11 (−▲−), from one of the other 31 investigated tumours (− × −), from calf thymus (− ● −) and from normal lymph nodes (− ○ −).
Abscissa: Serum dilution reciprocal; ordinate: absorbance at 492 nm.

Results

Plasma membranes from tumorous lymph nodes of 32 animals were investigated by the ELISA for the presence of thymus-specific antigen. From these tumours there was only one (T-11) which presented the thymus--specific antigen (Fig. 1). Besides the detection of thymus-specific antigen, the appearance of bovine immunoglobulin was also monitored by ELISA. It could be shown that plasma membranes from tumour T-11, as plasma membranes from calf thymus, did not react with antiserum directed to bovine immunoglobulin (Fig. 2). The absence of immunoglobulin further emphasizes the thymus cell nature of the tumour T-11. In retrospective it has been found that this thymus cell tumour originated from a 6-year-old cow from a heavily BLV-infected herd in which more than 60% of the animals had persistent lymphocytosis. Serological investigation for antibodies against BLV-antigens was not performed. Therefore, the BLV-infection of the tumour-bearing animal was ascertained by DNA-hybridization. It could be demonstrated (Fig. 3) that DNA isolated from tumour T-11 hybridizes with a BLV-specific probe. These findings confirm the presence of the BLV-genome in the thymus-cell tumour.

Discussion

Four forms of tumorous leukosis are distinguished in cattle (survey see Burny *et al.*, 1980), of which only the adult enzootic bovine leukosis is BLV--induced. The other three forms, the leukosis of calf-, thymus and skin type

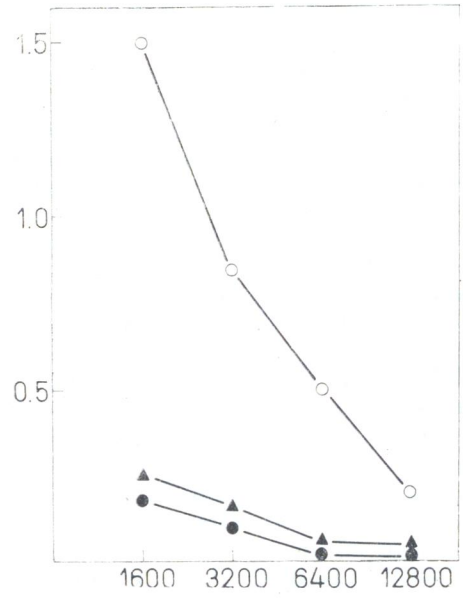

Fig. 2.
Reactivity of rabbit anti-cattle immunoglobulin
Plasma membranes from tumour T-11 (—▲—), from normal lymph nodes (— ○ —), and from calf thymus (— ● —). Abscissa: serum dilution reciprocals; ordinate: absorbance at 492.

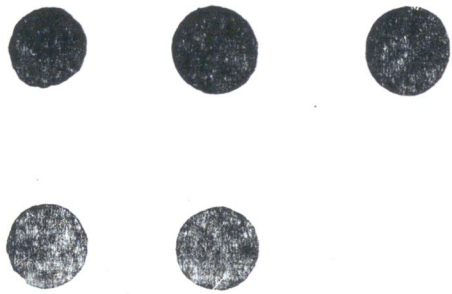

Fig. 3.
Spot hybridization of filter-bound DNAs with BLV-DNA as a probe
DNA was isolated from the thymus cell tumour T-11, from another bovine tumorous lymph node (T-1), from a Rous sarcoma virus-induced tumour of chicken (RAV-2) and from the BLV producing cell line FLK. These DNAs were hybridized with ^{32}P-dATP labelled DNA of a proviral BLV-genome from a Belgian tumour case, recloned in pBR322.
1, 2: T-11-DNA, 10 and 20 µg each; 3, 4: T-1-DNA, 10 and 20 µg each; 5: FLK--DNA, 20 µg; 6: RAV-DNA, 20 µg (neg.).

are aetiologically unclear. They occur sporadically and are differentiated by the age of the animals infected and by the localization of the tumour. It is typical for thymus-type leukosis that it occurs between 6 and 30 months of age and that only single lymph nodes are involved (Dungworth *et al.*, 1964). In the cases of thymus-type leukosis hitherto described no serum antibodies against BLV antigens were found, thus, the animals were obviously not infected with BLV (Mammerickx *et al.*, 1981; Parodi *et al.*, 1982; Markson *et al.*, 1982). Now we demonstrated the thymic origin of a lymph node tumour in BLV-infected cow by immunological method. Plasma membranes from the cells of this tumour carried thymus-specific antigen and contained no immunoglobulin. The cells of this tumour contained sequences of the BLV genome.

The role of the thymus in the pathogenesis of enzootic bovine leukosis is still unclear. Urbaneck *et al.* (1968) found tumorous alterations in the thymic area in 32% of cattle slaughtered due to leukosis. Van Der Maaten and Miller (1978) were unable to isolate BLV from the thymus of BLV--infected calves. On the other hand, Onuma and Olson (1977) detected BLV-antigens in the culture fluid from a thymus cell culture of a cow with tumorous leukosis. Takashima *et al.* (1977) separated blood lymphocytes into an EAC-positive and an EAC-negative fraction and demonstrated the production of BLV antigens in both groups of cells following long-term cultivation. They concluded that both B- and T-cells produced BLV.

It is known from the example of virus-induced cat leukosis that infection of different animals with one and the same virus may give rise to either B- or T-cell tumours (Essex and Grant, 1979). Thymus lymphomas may develop if young cats are infected with feline leukaemia virus. Spontaneous BLV--infection occurs only in few cows under 2 years of age (Burny *et al.*, 1979) and tumorous alterations develop after several years only. Possibly the critical BLV dose required is reached in most animals when the physiological involution of the thymus is already accomplished. This could explain the rare occurrence of thymus cell tumours in BLV-infected cattle.

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