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IMMUNOLOGICAL CHARACTERIZATION OF BLV PROTEINS SYNTHESIZED IN *ESCHERICHIA COLI*

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Summary. — Hybrid proteins composed of β-galactosidase and polypeptides of the bovine leukaemia virus (BLV) including those of the main core protein p24, the envelope protein gp51 and the transmembrane protein gp30 were produced in *Escherichia coli* and immunologically characterized. The hybrid proteins were immunologically reactive with sera from cattle naturally infected with BLV, demonstrating a possible use for diagnosis of BLV infection. Detection of antibodies was most sensitive with the p24 derivative.

Key words: *bovine leukaemia virus*; *bacterial expression*; *fusion proteins*; *diagnosis*

Introduction

Bovine leukaemia virus (BLV) is a B-cell lymphotropic retrovirus which causes the enzootic bovine leukosis (EBL). EBL is the most frequent tumorous disease of cattle and causes severe economical losses (Burny *et al.*, 1985). BLV is transmitted horizontally in cattle herds and induces shortly after infection antibodies against the major viral proteins gp51, p24, and gp30 (Bex *et al.*, 1979; Mammerickx *et al.*, 1980; Bossmann *et al.*, 1989).

Serological diagnosis and separation of infected animals is an efficient way to control the disease (Mammerickx *et al.*, 1978). Alternatively, protection by vaccination with BLV envelope proteins gp51 and gp30 which are able to induce virus-neutralizing (Závada *et al.*, 1978; Bossmann *et al.*, 1989) and cytolytic antibodies (Portetelle *et al.*, 1978) could be tried. For both strategies large amounts of viral proteins have to be produced.

Recently we have demonstrated the expression of hybrid proteins consisting of N-terminal β-galactosidase and C-terminal *gag* and *env* polypeptides in *Escherichia coli* (Ulrich *et al.*, in press) (Fig. 1). In the present study we investigated the possibility to use these fusion proteins for the detection of antibodies in BLV-infected cattle.

Materials and Methods

Plasmids. The pEX expression vectors were used to construct plasmids which encode amino acids (aa) 46—200 of p24 (pGAG1), aa 55—103 (pENV1), aa 103—265 (pENV2), aa 55—265 (pENV3) of gp51, aa 135—265 of gp51, and 1—45 of gp30 (pENV4), and aa 45—195 of gp30

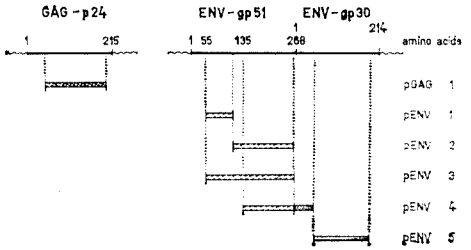

Fig. 1
Fragments of BLV polypeptides contained in fusion proteins synthesized by *Escherichia coli*

(pENV5) (Ulrich *et al.*, in press). The aa positions were deduced from nucleotide (Rice *et al.*, 1984; Rice *et al.*, 1935) and protein sequence data (Oroszlan *et al.*, 1979; Schultz *et al.*, 1984).

Electrophoresis and Western blot analysis. Bacterial cells transformed with the plasmids pENV1—5 and pEX were grown at 30 °C and induced at 42 °C for 2 hr. The proteins were subjected to SDS polyacrylamide electrophoresis (Laemmli, 1970) and electrophoretically transferred onto nitrocellulose filters (Burnette, 1981). The blots were preincubated for 1 hr at room temperature with phosphate-buffered saline containing 0.1% of Tween 80 (PBST) and then further incubated at 4 °C overnight with the indicated monoclonal antibodies (MoAb) or bovine sera at final dilutions of 1 : 1 000 or 1 : 50 in PBST, respectively. The blots were washed five times in PBST for 20 min each and incubated at 4 °C overnight with anti-mouse or anti-cattle immunoglobulins coupled to horseradish peroxidase. Washing of the blots was performed with PBST and PBS each three times. The binding of antibodies was detected by adding the substrate solution (12 mg 2-brome-1-naphthol, 40 µl H_2O_2 (30%) per 100 ml PBS).

Immunoprecipitation. The native and the denatured viral gp51 were isolated according to Portetelle *et al.* (1980) and Uckert *et al.* (1986), respectively. Iodination and immunoprecipitation were performed according to Portetelle *et al.* (1980). Appr. 15 000 c.p.m. of the protein were incubated overnight at 4 °C with MoAb specific for gp51-epitopes A—H at a final dilution of 1 : 10 000. After incubation anti-mouse antiserum was added and incubation continued for 15 hr at 4 °C. The precipitates were collected by centrifugation and radioactivity was counted in a Gamma-Counter.

Results and Discussion

In order to use BLV-polypeptides synthesized in *E. coli* for immunological tests, one has to make sure that at least some of the epitopes are sequentially rather than conformationally determined. We have tested different MoAb (designated A—H) which were raised against gp51 by Bruck *et al.*

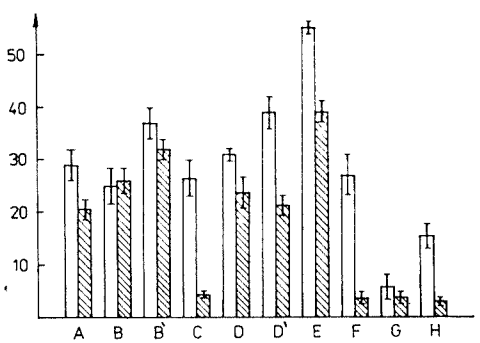

Fig. 2
Immunoprecipitation of iodinated native and denatured gp51 by monoclonal antibodies
Open and hatched columns represent the average of three precipitations with native and denatured gp51, respectively. The bargs give standard deviations.

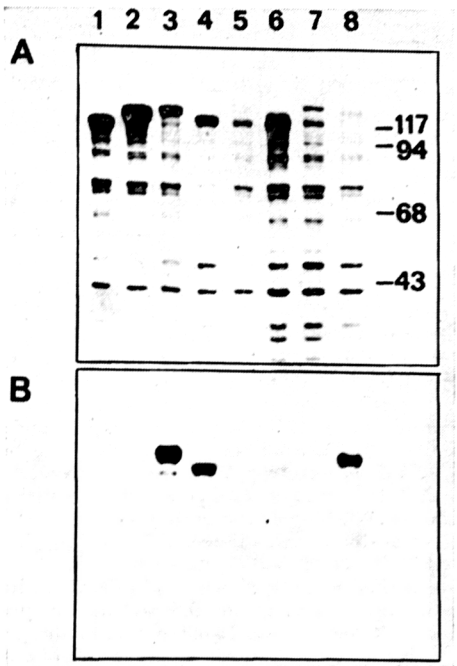

Fig. 3
Reactivity of bacterially synthesized proteins with monoclonal antibodies
(A) Coomassie blue-stained proteins; (B) Western blots using MoAb specific for epitopes B (a), D (b), and E (c). Total lysates corresponding to appr. 2×10^7 NF1 cells transformed with pEX (lane 1), pENV1 (lane 2), pENV2 (lane 3), pENV3 (lane 4), and pENV4 (lane 5) were loaded on each track. In each case only the high molecular weight region of the gel is shown (68–200 kD).

(1982*a*; 1982*b*) for their ability to recognize the native and denatured protein. The native gp51 was precipitated by MoAb specific for epitopes A, B/B′, C, D/D′, E, F, and H, but not by those directed against epitope G. Heat denaturation of viral gp51 completely abolished its reactivity with MoAb against the epitopes C, F, and H, whereas the reactivity of antibodies to the epitopes A, B/B′, D/D′, and E was not affected (Fig. 2). This indicates that four out of the eight epitopes represent sequential epitopes (A, B, D, E), the others being dependent on the native conformation of the gp51 (sites C, F, G, H). In experiments comparing the immunological reactivity of native and denatured p24 we could not find such differences suggesting that p24 exposes mainly sequential determinants (Platzer *et al.*, in preparation).

To localize the sequence-determined antigenic sites in the gp51 molecule we analysed the bacterially synthesized fusion proteins by Western blotting (Fig. 3). The gp51 derivatives produced in pENV2, pENV3, and pENV5, transformed bacterial cells were recognized by MoAb specific for epitopes B, D, and E (Fig. 3B, lanes 3, 4, 5). They reacted weakly with the MoAb specific for epitope A and, as expected, not at all with the MoAb directed against the conformationally dependent epitopes C, F, G, and H (not shown). The smallest gp51 derivative coded by pENV1 which covers amino acids 55 to 103 did not react with any of these MoAb (Fig. 3B, lane 2). Controls with a nonrecombinant plasmid (lysates of pEX transformed cells) were

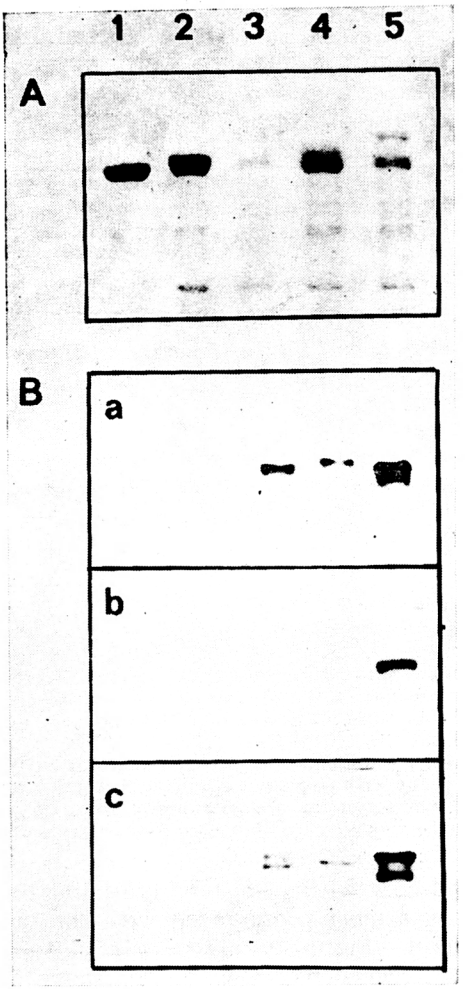

Fig. 4
Recognition of bacterially synthesized BLV proteins by antibodies in a serum of a BLV-infected cow
(A) Coomassie blue-stained proteins, (B) Western blot analysis was performed by using a serum of a naturally BLV-infected cow with persistent lymphocytosis. Prior to filter incubation preadsorption of the serum was carried out by addition of pEX-transformed *E. coli* extracts and incubation at 4 °C overnight. Lanes 1 to 8 present lysates of *E. coli* transformed with 1: pEX (negative control); 2: pRD114 encoding two thirds of the transmembrane protein p20 of the endogenous cat retrovirus RD114 (negative control); 3: pGAG 1; 4: pENV1; 5: pENV2; 6: pENV3; 7: pENV4; 8: pENV5. Molecular weight markers are β-galactosidase (117 kD), phosphorylase b (94 kD), bovine serum albumin (68 kD) and ovalbumin (43 kD).

negative (Fig. 3B, lane 1). From these results it can be concluded that the sequence specific epitopes B, D, and E must be located in the C-terminal half of gp51. Since Bruck *et al.* (1982*b*) have found that epitopes A—D and E—H can be separated by limited proteolysis it is likely that the conformation-dependent epitopes, F, G and H are localized in the N-terminal part of gp51.

In order to test whether microbially produced BLV polypeptides are recognized by naturally occurring antibodies in the sera from BLV-infected cattle, the fusion proteins containing derivatives of p24, gp51, and gp30 were analysed by Western blotting using a serum of a naturally BLV-in-

Table 1. Recognition of bacterially synthesized BLV proteins by antibodies in cattle sera at different stages of BLV-infection

Derivate	—	RD114-p20 (1.-126.aa)	p24 (45.-200.aa)	gp51 (55.-103.aa)	gp51 (135.-268.aa)	gp30 (45.-195.aa)
clone	pEX	pRD	pGAG 1	pENV 1	pENV 4	pENV 5
Bovine sera						
N 81	—	—	—	—	—	—
N 41	—	—	—	—	—	—
N 96	—	—	—	—	—	—
S 3	—	—	+ + +	+ +	—	+ +
S 7	—	—	+ +	+	—	—
S 10	—	—	+ +	+	—	—
S 11	—	—	+ +	—	—	—
PL 80	—	—	+ + +	+ +	—	—
PL 91	—	—	+ + +	+ +	+ +	—
PL 08	—	—	+ +	—	—	—
PL 69	—	—	+ + +	+	—	—
PL 5	—	—	+ + +	—	+ +	+ +
T 2	—	—	+ + +	+ +	+ +	+ + +
T 3	—	—	+ +	+	—	—
T 4	—	—	+ +	+	+	+

Sera from uninfected cattle (N), from seropositive cattle without haematological disorders (S), from cattle with persistent lymphocytosis (PL), and from cattle with tumours (T) were analyse l by Western blotting. The number of crosses gives a semi-quantitative estimate of the extent of reaction in Western blots. For further explanations see also Legend to Fig. 4.

fected cow with persistent lymphocytosis (Fig. 4). The immunological reaction was most pronounced with the fusion proteins containing p24, a short segment of gp51 (amino acids 55 to 103) and gp30 (Fig. 4B, lanes 3, 4, and 8, respectively). The gp51 derivative covering amino acids 135 to 265 was also recognized by the cattle serum, but with low efficiency (Fig. 4B, lane 7). A gp51 fusion protein overlapping the sequences contained in the constructions mentioned before, showed a weak immunological band at a molecular weight smaller than expected for the fullsized product (Fig. 4B, lane 6). Most likely, the antibodies recognized a proteolytic breakdown product. A lysate containing a gp51 derivative covering the amino acids 103—265 did not show any reactivity probably owing to the low expression level (Fig. 4B, lane 5). Controls with β-galactosidase and with an unrelated fusion protein were negative (Fig. 4B, lanes 1 and 2).

In order to determine the applicability of the immunological test, sera from cattle at different stages of EBL were analysed (Table 1). All sera of BLV-infected cattle recognized the p24 containing fusion protein, whereas

only some of them reacted with *env* derivatives. In some cases gp51 containing fusion proteins covering amino acids 55—103 or 135—265 gave a positive reaction which, however, could not be related to the stage of the disease. These antibodies are probably directed against sequence determinants. They must comprise only a small fraction of the total antibody population directed against gp51 in infected animals since the denatured viral protein showed an about 100-fold lower reactivity as compared with the native one (not shown). These data are in agreement with those of Bruck *et al.* (1984) who also found that most antibodies are directed against the conformational epitopes F, G and H.

In summary, we investigated the possibility to use BLV proteins which are synthesized in *E. coli* for the detection of antibodies in BLV-infected cattle. Fusion proteins consisting of β-galactosidase and BLV-p24 seem to be applicable in immunological tests after their purification (Siakkou *et al.*, in press).

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