MOLECULAR CLONING OF PROVIRUS DNA FROM BOVINE LEUKAEMIA LYMPHOCYTES AND ITS APPLICATION AS A PROBE FOR DIAGNOSTIC PURPOSE

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The provirus DNA isolated from lymphocytes of a cow infected with the bovine leukaemia virus (BLV; positive immunodiffusion test), was subjected to molecular cloning and identified by comparing with the \(^{32}\)P-labelled provirus cDNA isolated in Belgium. Hybridization revealed a clone containing 8.5 kb DNA fragment of the BLV provirus.

The probe based on the “Polish fragment” of leukaemia virus was tested on 10 cows with a positive serological response. The presence of provirus DNA in the cellular genome of lymphocytes was confirmed.

Diagnosis of enzootic bovine leukaemia virus is based mainly on detection of specific antibodies, and only rarely on estimation of the lymphocyte count or visualization of virus particles. Negative results of serological and virological tests do not exclude infection since the only reliable criterion is the presence of provirus DNA in cell genome. Therefore, application of a sensitive molecular probe is needed to detect the integrated provirus DNA.

MATERIALS AND METHODS

Bovine lymphocytes were obtained from the animals showing a positive serological response (BLV+) in the immunodiffusion test on agarose gel. As control, DNA from serologically negative (BLV-) animals was used. The lymphocytes were washed, suspended in 10 mM Tris/HCl buffer, pH 8.0, containing 10 mM EDTA, digested with pronase, extracted twice with phenol-chloroform mixture and, finally, the digest was dialysed against the same buffer. Genomic DNA was digested with endonuclease SacI (Amersham), and 10 \(\mu\)g of the digested DNA was electrophoresed in 0.7% agarose gel. Endonuclease SacI is able to excise almost the whole molecule of the provirus, leaving only the terminal fragments of the regulatory sequences of about 170-260 bp [1].

DNA was transferred onto nitrocellulose filter according to Southern [2] and hybridized [3]. The molecular probe was prepared on the basis of the
DNA fragment of the bovine leukaemia provirus derived from (BLV +) Belgian cattle. On autoradiography it was found that hybridization occurred in the DNA corresponding to about 8.5 kb. DNA from this area was extracted from the gel and used for cloning. Bacteriophage λgt WesαB was used as a vector. Dephosphorylation of the phage was performed as described by Maniatis et al. [3] with CIP (Boehringer, Mannheim) at the concentration of 0.001 unit per μg DNA. About $6 \times 10^5$ recombinant phages were tested and a single clone containing the desired DNA was obtained. With the use of the constructed probe lymphocytes from 10 BLV + and 2 BLV − cows were tested.

RESULTS AND DISCUSSION

As a result of cloning of DNA from lymphocytes of a BLV + cow, a single single clone of recombinant λgt WesαB phage was obtained; it contained the SacI-SacI fragment of genome of the Polish strain of bovine leukaemia provirus. The presence of this fragment in the recombinant phage genome was confirmed by electrophoresis of the phage DNA digested by endonuclease SacI. In this way, in addition to two fragments corresponding to phage λ arms, a single DNA fragment of about 8.5 kb hybridizing with the Belgian probe, was obtained (Fig. 1).

Two variants, American and Belgian, of bovine leukaemia provirus have been described [4-6]. They differ by the presence or absence of a single nucleotide sequence recognized by endonuclease SacI within the presumed gene “env”. The provirus cloned in our laboratory does not contain the site recognizable by SacI. Therefore it seems that the “Polish variant” resembles the “Belgian” virus. This suggestion should be more conclusively evidenced by restriction analysis.

Examination of the DNA samples taken from 10 serologically positive cows confirmed the presence of provirus DNA which on electrophoresis was located in the area corresponding to about 8.5 kb (Fig. 1B). This DNA fragment was lacking in the serologically negative animals (Fig. 1). Although in all experiments equal amounts of the digested genomic DNA were applied onto electrophoretic gels, the intensity of autoradiographic signals showed large variation (Fig. 1B). It is known that percentage of the infected lymphocytes is approximately constant and corresponds to 20–25% of the whole lymphocyte population [7] (Fig. 1B a, b, c). It seems, therefore, that the differences in the

Fig. 1 A. Hybridization of the 8.3 kb provirus DNA from the Belgian clone, $^{32}$P labelled by the “random priming” method (sp. act. about $9 \times 10^8$ d.p.m./μg DNA), to SacI-digested DNA samples of bovine lymphocytes. Lane b, positive response; lanes a and c, negative response. B. Hybridization of provirus DNA from the Polish clone, $^{32}$P labelled as described above (sp. act. about $9.4 \times 10^8$ d.p.m./μg DNA), to SacI-digested DNA samples of bovine lymphocytes. Positive response: lane a, of high intensity; lane b, of low intensity; lane c, of intermediate intensity of the signal.
radioactive response might be due to a different number of DNA provirus copies integrated in the host genome [8].

It seems that the method proposed might be very useful for diagnosis of leukaemia virus carriers in the serologically negative animals.

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REFERENCES