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Genomic Integration of Bovine Leukemia Provirus and Lack of Viral RNA Expression in the Target Cells of Cattle with Different Responses to BLV Infection

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A. Introduction

Enzootic bovine leukosis (EBL) is a contagious lymphoproliferative disease whose etiological agent is a retrovirus, the bovine leukemia virus (BLV). EBL is a complex disease. Soon after infection a strong humoral antibody response develops and persists for the animal's entire life. Such BLV-infected cattle can remain asymptomatic virus carriers for many years. They can also at a given time develop persistent lymphocytosis (PL) characterized by a permanent large number of peripheral lymphocytes. A variable but always significant percentage of PL animals develop lymphoid tumors, the terminal tumor phase of EBL. The remnant tumor cases develop suddenly in BLV carriers without any previous hematologic disorder. In general, the fate of BLV-infected animals is variable and depends upon several factors, including age, genetic make-up, environmental factors, and immunologic surveillance (see Burny et al. 1980 for a review).

In the present investigation we studied BLV integration sites in DNA preparations from target tissues of BLV-infected animals and viral RNA expression in the same cells. DNA was digested by bacterial restriction endonucleases and submitted to electrophoresis in agarose gels. After transfer to nitrocellulose paper, the DNA fragments were annealed to a specific BLV (^{32}P) cDNA probe. Genomic DNA fragments containing viral information appear after autoradiographic development as individual bands and sometimes as smears.

The results of our study allow comparison of BLV provirus integration and viral RNA expression in *leucocytes* of animals showing different responses to BLV infection, namely, in asymptomatic BLV carriers (Ab^+ änimals), animals in PL (PL^+ animals), and tumor cases (T^+ animals). Our major findings are: (1) No BLV proviral sequences are detected in Ab^+ cases by the technique used; (2) Circulating leucocytes of PL animals accomodate BLV provirus at many possible sites; (3) Lymphocytes infected by BLV and found in EBL tumors constitute monoclonal populations of cells carrying one copy of the proviral genome which is integrated at one genomic site; and (4) In the vast majority of cases studied, no viral RNA expression was detectable in the lymphoid tumor cells or circulating leucocytes of affected animals.

B. Results and Discussion

I. Viral DNA Content of BLV-Infected Cells

As shown in Fig. 1, no EcoRI fragment containing viral information was detected in the DNA samples from Ab^+ animals (animals no. 19, 33, and 34). The weak hybridization band of 5.0×10^6 which was common to the control DNA and all the other bovine DNAs tested corresponded to ribosomal DNA (Kettmann et al. 1979). Since no hybridization occurred with leucocyte DNA from Ab^+ animals, and taking account of the sensitivity of the method, we can conclude that less than 5% of the total leucocyte population can harbor the provirus. It should be noted here that EcoRI cleaves once at one kilobase distance from the 3′ end of the BLV unintegrated provirus with a mol.wt. of 6.0×10^6 (Kettmann, unpublished results). Previously, we have reported that 25% to 40% of total leucocytes of animals in PL harbor BLV proviral sequences (Kettmann et al. 1980).

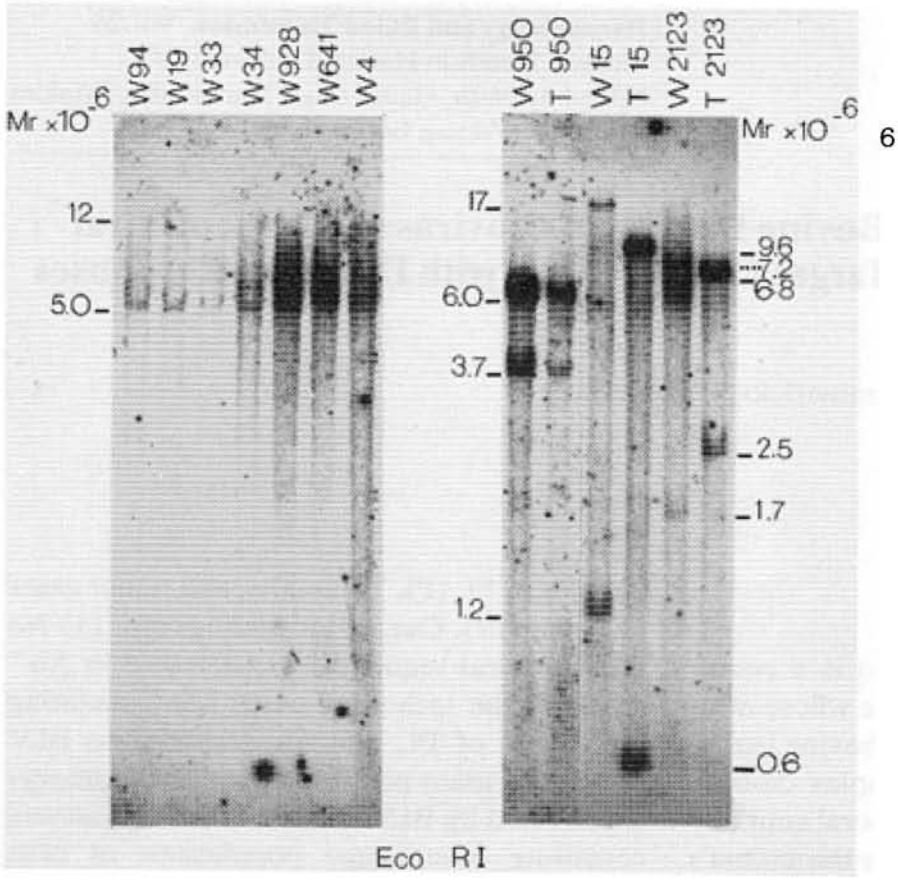

Fig. 1. Hybridization patterns of BLV (^{32}P) cDNA on DNA restriction fragments from circulating leucocytes *(W)* and tumors *(T)* of BLV-infected animals. DNAs (20 μg) of W19, W33, and W34 (Ab^+ animals), of W928, W641, and W4 (PL^+ animals), of W950, T950, W15, T15, W2123, and T2123 (T^+ animals), and of W94 (an Ab^- animal) were exhaustively digested by EcoRI before electrophoresis on a 0.8% agarose gel, and then transferred, hybridized, and detected by autoradiography (see Kettmann et al. 1980). Autoradiographs are shown

Here we show that less than 5% of total leucocytes of Ab^+ animals can carry the provirus. Taken together these results demonstrate that *PL is not an amplification of a pre-existing situation found in AB^+ animals.*

The pattern of proviral integration in the circulating leucocytes of animals carrying lymph node tumors (animals no. 950 and 15) was completely different from that described above in that a number of well-defined provirus-positive bands were present in the EcoRI digests. These fragments had mol.wts. of 6.0×10^6 and 3.7×10^6 for animal 950 DNA and 17×10^6 and 1.2×10^6 for animal 15 DNA. These results imply that in contrast to the multiplicity of integration sites found in the DNA from circulating leucocytes from animals in PL, only a very limited number of sites accomodate BLV provirus in the DNA of circulating leucocytes from tumor bearing animals. The EcoRI pattern obtained for the circulating leucocytes of animals 2123 was more complex and reminiscent of that obtained with leucocyte DNAs from animals in PL.

In animal 950 the same restriction pattern was found for the circulating leucocyte DNA and a tumorous lymph node. These results showed that the same BLV-infected clone was present in both affected tissues. In contrast, for animals No. 15 and 2123 two different patterns were found in the circulating leucocyte DNA and in a tumor DNA of the same animal, thus showing that in these animals two clones of BLV-infected leucocytes were detected.

II. Viral RNA Content of BLV-Infected Cells

Using liquid hybridization techniques and BLV (^3H) cDNA as a probe, we looked for viral RNA sequences in various total RNAs samples from BLV-infected cells. Genomic 35S RNA and total RNA from the virus-producing cells were used as positive controls. The results of Table 1 clearly showed that in all cases tested but one (leucocytes of animal 15) transcription of the integrated viral genome did not occur or occurred at a very low level. Our data are, however, still compatible with either one of the following possibilities: (1) lymph node tumor cells and circulating lymphocytes of PL^+ or T^+ animals express at a low rate a very small region of the BLV genome; or (2) a small percentage of BLV-carrying cells express the entirely or in part the viral information at a low rate. In situ hybridization experiments are being performed to solve this problem.

Animal number[a]	Tissue	Percentage of hybridization	Crt $\times 10^{-3}$	Number of viral copies[b]
94	Leucocytes	7.4%	61	0[c]
33	Leucocytes	8.6%	25	<1
34	Leucocytes	5.8%	38	<1
928	Leucocytes	7.2%	36	<1
4	Leucocytes	6.3%	34	<1
950	Leucocytes	7.0%	40	<1
	Tumor cells	6.9%	39	<1
2123	Leucocytes	7.9%	45	<1
	Tumor cells	7.1%	33	<1
15	Leucocytes	16.0%	32	1 to 2
	Spleen cells	7.8%	37	<1
	Tumor cells	7.0%	18	<1

Table 1. Viral RNA content of circulating leucocytes and tumor cells

[a] Animal 94 is normal; animals 33 and 34 are Ab^+; animals 928 and 4 are PL^+; animals 950, 2123 and 15 are $tumor^+$

[b] Estimations were made from hybridization experiments using increasing dilutions of BLV 35S genomic RNA and assuming a cellular RNA content of 10 pg. FLK cells were shown to contain 30 copies of the BLV RNA genome

[c] Hybridization reactions run in the same conditions with E. coli total RNA showed a background level of 7.1% at a Crt value of 60×10^3

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