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Genomic Integration of Bovine Leukemia Provirus

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Bovine leukemia virus (BLV) is an exogenous retrovirus responsible for enzootic bovine leukosis (EBL), a lymphoproliferative disease of cattle (Callahan et al. 1976; Kettmann et al. 1976, 1978). Molecular hybridization and immunological studies with purified BLV antigens have failed so far to detect any relatedness between BLV and other known retroviruses (for review, see Burny et al. 1978; Mussgay and Kaaden 1978). However, it has been shown recently that the amino-terminal sequence of the major internal protein (p24) of BLV is related to structural proteins (p30) of mammalian type-C viruses, suggesting that BLV shares a common ancestor with other type-C viruses (Oroszlan et al. 1979).

EBL is a complex disease. Soon after infection, a strong humoral antibody response develops; this seems to be dependent on virus dose and is currently used as a diagnostic indication of virus proliferation (Burny et al. 1978; Mussgay and Kaaden 1978). A peculiar manifestation of BLV infection, persistent lymphocytosis (PL), may then occur. This is characterized by a permanent increase in the number of peripheral lymphocytes (Götze et al. 1953; Bendixen 1963; Tolle 1964), and this form of the disease aggregates along some cattle herds that may be different from those along which lymph-node tumors aggregate (Abt et al. 1970, 1976; Ferrer et al. 1974, 1978). It follows that the two conditions are separate responses to BLV infection, although animals with PL have a much higher chance of developing lymph-node tumors (Bendixen 1963; Schmidt et al. 1970; Ferrer et al. 1979). The incidence of tumor development is rather low and seems to be under the control of multiple factors, such as age, genetic makeup, and environment.

The availability of good BLV-producing systems (Van Der Maaten and Miller 1976) and the development of the gel-filter transfer technique (Southern 1975) have recently allowed us to approach the host-virus interaction at the molecular level. Integration sites of the proviral DNA in the genome of the target cell, which is the B lymphocyte (Kenyon and Piper 1977; Paul et al. 1977), were analyzed by density gradient centrifugation of host-cell DNA in Cs_2SO_4/BAMD (3,6-bisacetatomercurimethyl dioxane) and by proviral DNA hybridization on restriction fragments of the fractions obtained by centrifugation

(Kettmann et al. 1979). This approach revealed that proviral integration occurs in a high-density fraction ($\rho = 1.708$ g/cm^3) of host-cell DNA. The existence of viral variants was also shown, as well as the existence of different integration sites for the provirus. Differences in the proviral integration sites were found not only in infected cells belonging to different animals, but also in those of the same animal. The presence of at least two different integration sites in the infected cells of one given animal (Kettmann et al. 1979) prompted investigations in which integration sites of circulating white blood cells of animals with PL were compared with those of infected cells of animals in the lymph-node-tumor stage of the disease. Our results indicate that viral integration in PL is found at many different sites in the genomes of different infected cells, whereas in the tumor stage only one or a very few integration sites of the provirus are found. This may suggest that a selection of one or a very few clones of infected cells takes place during the course of the disease, and that from an originally polyclonal population of infected cells, a mono- or oligoclonal situation develops.

MATERIALS AND METHODS

Bovine Tissues and Cells

Bovine tissues and cells were collected from 12 field cases of EBL (animals 351 628, 928, 641, 2586, 2587, 4, 7, 12, 15, 950, 92, and 82) and 4 field cases of sporadic bovine leukosis (SBL) (animals C7, C10, C11, C12). Animals 351, 628, 928, 641, 2586, 2587, 4, 7, and 12 had PL without tumors. Animals 15, 950, 92, and 82 were at the tumor phase of EBL. White blood cells (WBC) (W351, W628, W928, W641, W2586, W2587, W4, W7, W12) and tumors (T15, T950, T92, T-C7, T-C10, T-C11, T-C12) were used as sources of DNA. The thymus from a normal calf was used as a source of control DNA.

Cell Line

The BLV-producing fetal lamb kidney (FLK) cell line used as reference in the liquid-hybridization experiments was kindly provided by M. J. Van Der Maaten (Van Der Maaten and Miller 1976).

Preparation of Virus and Viral RNAs; cDNA Synthesis

BLV production and purification and viral RNA extraction and purification were done as described by Ghysdael et al. (1979). The two poly(A) 38S RNAs present in each viral particle were prepared from the 60S–70S viral RNA by heat denaturation and chromatography on oligo(dT) cellulose (Ghysdael et al. 1979). BLV [^{32}P]cDNA (4.2×10^8 cpm/μg) was synthesized as described by Kettmann et al. (1979), and BLV [^{3}H]cDNA (1.8×10^7 cpm/μg) was synthesized as described by Kettmann et al. (1978). The sizes of the cDNAs were determined by gel electrophoresis using native and denatured *Hae*III digests of ϕX174 DNA as molecular-weight markers and they were found to be 100–400 nucleotides long.

DNA Extraction

DNAs for restriction endonuclease analysis were purified essentially as described by Kay et al. (1952). The molecular weights of all DNA preparations were found to be higher than 20×10^6 as estimated by 0.5% agarose gel

electrophoresis using undigested λ DNA and λ *Eco*RI digest as molecular-weight markers.

Cellular RNA Extraction

Extraction of cellular RNA was performed as described by Drohan et al. (1977), except that DNA was not spooled out before DNase-I treatment.

Restriction Endonuclease Digestion and Gel Electrophoresis

*Eco*RI (Boehringer Mannheim) digestions were carried out according to the method of Polisky et al. (1975); *Bam*HI and *Xba*I (New England BioLabs) digestions were carried out using the conditions given by the manufacturers. Horizontal agarose (Sigma) slab gels (0.4 × 20 × 25 cm) were used for gel electrophoresis. The *Eco*RI digest of λ DNA and the *Hae*III fragments of ϕX174 DNA were routinely used as molecular-weight markers.

Gel Transfer and Filter Hybridization

Gel transfer and filter hybridization were carried out as described by Meunier-Rotival et al. (1979) and Kettmann et al. (1979), except that BLV [^{32}P]cDNA was prehybridized in the hybridization mixture for 16 hours in the presence of 3 mg/ml of denatured, sonicated calf thymus DNA. Filters were exposed at −70°C to preflashed Kodak RP-Royal X-Omat film in the presence of Siemens "Special" intensifying screens for 2–5 days.

DNA Fractionation

DNA was fractionated by centrifugation in Cs_2SO_4/BAMD density gradients as described by Cortadas et al. (1977). Analytical CsCl density gradient centrifugations were done as described by Filipski et al. (1973).

Liquid Hybridizations

Liquid hybridizations were performed as described by Kettmann et al. (1978).

RESULTS

Persistent Lymphocytosis

As shown in Figure 1A, BLV [^{32}P]cDNA hybridized on *Eco*RI fragments from WBC DNA of animals with PL. Hybridization covered a range of molecular weights between 5×10^6 to 6×10^6 and 10×10^6 to 12×10^6. Some hybridization bands could be seen over the continuous background (this is not evident in the autoradiographs as reproduced); hybridization patterns were different for different animals. It should be noted that (1) the integrated bovine leukemia proviruses examined so far (Kettmann et al. 1979) have shown one or no *Eco*RI restriction sites, (2) unintegrated proviral DNA (m.w. 6.4×10^6) could not be detected in the DNAs examined (data not shown), and (3) the faint hybridization band (5.0×10^6) displayed by control DNA (and probably by WBC DNAs from animals with PL) corresponds to rDNA (Kettmann et al. 1979; Meunier-Rotival et al. 1979).

BLV [^{32}P]cDNA also hybridized on two *Bam*HI fragments, 2.0×10^6 and 1.3×10^6, from the above DNAs. Weak hybridization bands were also observed in some cases on both higher- and lower-molecular-weight fragments (Fig. 1B); the control DNA digest did not show any hybridization.

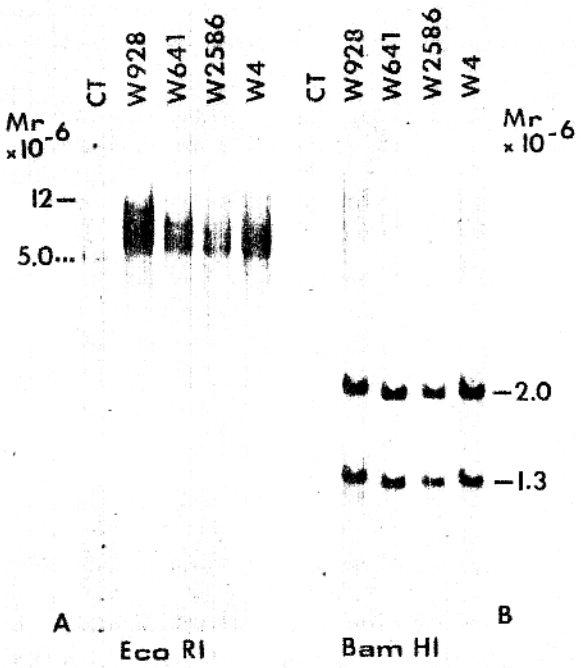

Figure 1
Hybridization patterns of BLV [^{32}P]cDNA on DNA restriction fragments from circulating WBC of animals with PL. DNAs (20 μg) of W641, W2586, W4, and calf thymus (CT) were exhaustively digested with *Eco*RI or *Bam*HI before electrophoresis on a 1% agarose gel, transfer, hybridization, and detection by autoradiography (see Materials and Methods). Autoradiographs are shown.

Lymph-node-tumor Stage

As shown in Figure 2A, BLV [^{32}P]cDNA hybridized on two *Eco*RI fragments, 6.0×10^6 and 3.7×10^6, of W950 DNA; two *Eco*RI fragments, 6.0×10^6 and 1.0×10^6, of W82 DNA; and three *Eco*RI fragments, 17.0×10^6, 9.6×10^6, and 1.2×10^6, of W15 DNA. The latter DNA showed, in addition, a spurious hybridization band, 5.0×10^6, also shown by the control DNA and corresponding to rDNA (Kettmann et al. 1979; Meunier-Rotival et al. 1979). This band might also be present in the DNAs of W950 and W82. The results obtained with W15 are similar to those already reported (Kettmann et al. 1979), except that in previous work only the strong 17.0×10^6 and 1.2×10^6 bands were seen. It will be shown below that these bands correspond to the provirus-positive segments that predominate in these cells. The other provirus-positive segments originate in the 9.6×10^6 band and in another band, 0.6×10^6, that is too weak to be seen in Figure 2A. Previous work showed that spleen cells from animal 15 showed the same predominant provirus-positive bands as did WBC (Kettmann et al. 1979).

BLV [^{32}P]cDNA also hybridized on two common *Bam*HI fragments (2.0×10^6 and 1.3×10^6) of the DNAs from W15, W950, and W82 and on two different *Bam*HI fragments of the DNAs from W82 (17.0×10^6 and 13.0×10^6), W950 (9.6×10^6 and 5.9×10^6), and W15 (11.5×10^6 and possibly 2.0×10^6; the latter suggestion is derived from the strong relative intensity of the 2.0×10^6 band) (Fig. 2B).

BLV [^{32}P]cDNA also hybridized on the same *Xba*I fragments, 6.3×10^6, 2.6×10^6, and 2.3×10^6, from the DNAs of five lymph-node tumors of animal 950 (Fig. 3). A faint 1.0×10^6 band, present in all *Xba*I digests including that of the control DNA, corresponds to contaminating DNA (satellite or rDNA)

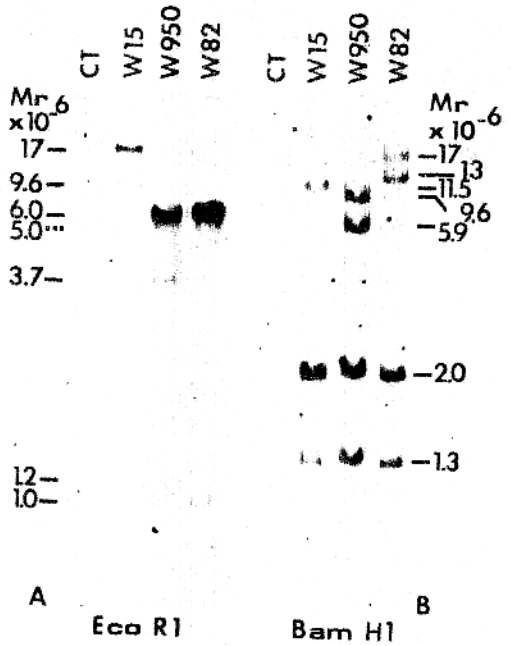

Figure 2
Hybridization patterns of BLV [^{32}P]cDNA restriction fragments from circulating WBC of animals in the tumor phase of EBL. DNAs (20 μg) of W15, W950, W82, and CT were treated as described for Fig. 1.

(Meunier-Rotival et al. 1979). The case of animal 15 was more complex than that of animal 950. In fact, hybridization of the viral probe occurred as follows (Fig. 3):

1. on four fragments, 7.1×10^6, 3.8×10^6, 1.7×10^6, and 0.7×10^6, from the DNAs of tumors 3, 4, and 5;
2. on four fragments, 3.1×10^6, 2.0×10^6, 1.7×10^6, and 0.7×10^6, from the DNA of tumor 2 (interestingly, the two smallest fragments were those already found in tumors 3, 4, and 5; these fragments, probably corresponding to internal proviral fragments, were not present in the DNAs from tumors of animal 950);
3. on six fragments from the DNA of tumor 1 (this pattern was equal to the sum of the other two patterns, the bands corresponding to the DNAs of tumors 3, 4, and 5 being predominant).

The hybridization pattern exhibited by the *Eco* RI digest of the latter DNA (data not shown) was similar to that shown by the WBC of the same animal (Fig. 2A); however, the 1.7×10^6 and 1.2×10^6 bands were weaker than the 9.6×10^6 and 0.6×10^6 bands, another indication of the existence of two sets of provirus-positive segments in animal 15.

Annealing kinetics in liquid solutions were performed with BLV [^{3}H]cDNA and leukocyte DNA from PL animals (Fig. 4A). The results were compared with those obtained with DNAs from circulating WBC and tumor cells of animals at the terminal stage of EBL (Fig. 4B). In both cases, DNA from FLK-BLV cells and a 1:5 dilution of that DNA were taken as reference annealing curves. Rates of annealing of the probe to FLK-BLV DNA and T15-2 DNA are identical. All other curves obtained are quite comparable and express a much slower rate of hybridization, slightly higher than the rate measured with the 1:5 dilution of FLK-BLV DNA.

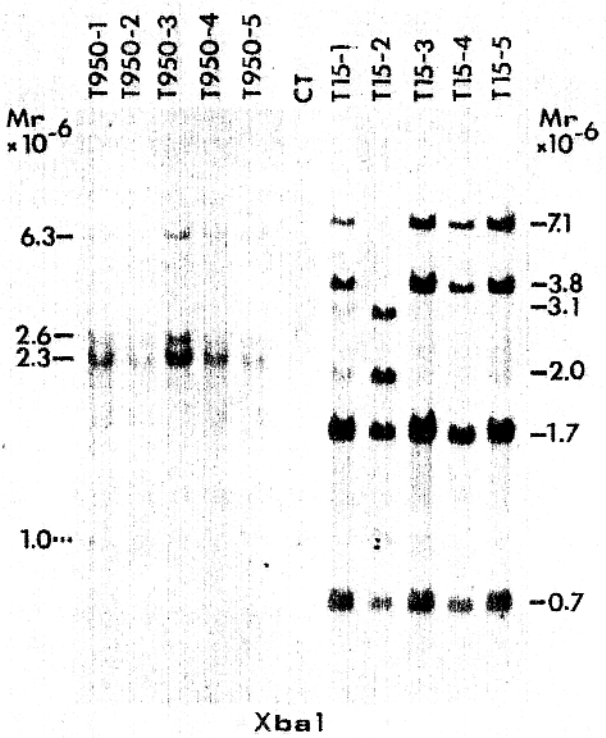

Figure 3
Hybridization patterns of BLV [^{32}P]cDNA on DNA restriction fragments from EBL tumors. DNAs (20 μg) of T950-1 to T950-5, CT, and T15-1 to T15-5 were digested with *Xba*I and treated as described for Fig. 1.

BLV Variants

To investigate the occurrence of viral mutants in the BLV population further, DNAs from circulating WBC of EBL animals belonging to two different BLV foci of infection were digested with *Xba*I. Animal 15, in the tumor stage of EBL, and animals 2586 and 2587, in the PL stage, belonged to focus 1; animal 82, also in the tumor stage of EBL, and animals 4, 7, and 12, in the PL stage, belonged to focus 2. As shown in Figure 5, the same two internal viral fragments (1.7×10^6 and 0.7×10^6) were observed after *Xba*I digestion of the DNAs of animals from focus 1. These fragments were not found in the DNA patterns of animals from focus 2; the DNAs of these animals shared the same unique internal viral fragment of 2.3×10^6.

Location of the Bovine Leukemia Provirus in the Host Genome as Determined by Cs_2SO_4/BAMD Density Gradient Centrifugation

As in the murine (Steffen and Weinberg 1978; Cohen et al. 1979) and avian (Collins and Parsons 1977; Hughes et al. 1978) retrovirus systems, many sites seemed possible for BLV integration in the host-cell genome. Cs_2SO_4/BAMD density gradient centrifugation (Cortadas et al. 1977) was used to determine whether BLV integration occurs at random in the bovine genome. The profile of such a fractionation of T15-3 DNA based on the GC content of cellular DNA is

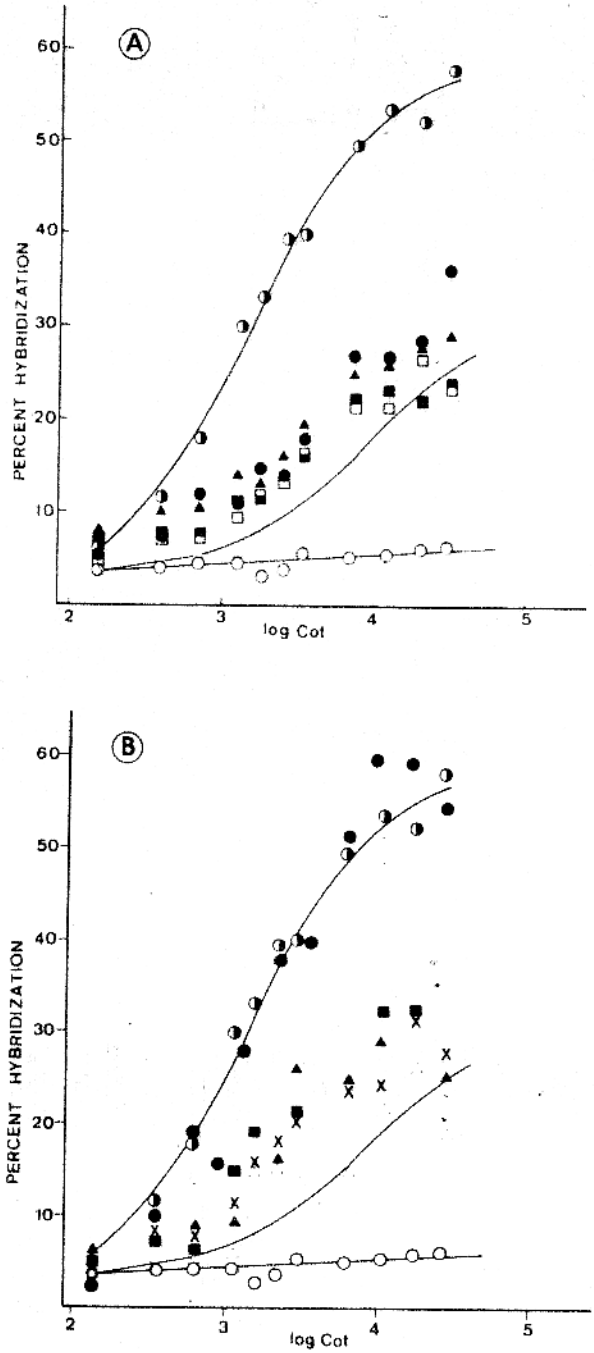

Figure 4

Kinetics of annealing of BLV [^{3}H]cDNA and cellular DNAs. BLV [^{3}H]cDNA (2500 cpm) and cellular DNA (500 μg) were hybridized in 0.4 M sodium phosphate (pH 6.8) and 0.05% SDS. At various C_0t values, samples were assayed for nuclease-S1 resistance. (*A*) DNAs of FLK cell line (◑), FLK diluted fivefold with normal bovine DNA (△), W928 (□), W641 (▲), W2586 (■), W4 (●), and CT (○); (*B*) DNAs of FLK cell line (◑), FLK diluted fivefold (△), T15 (●), T950 (▲), W15 (x), W82 (■), and CT (○).

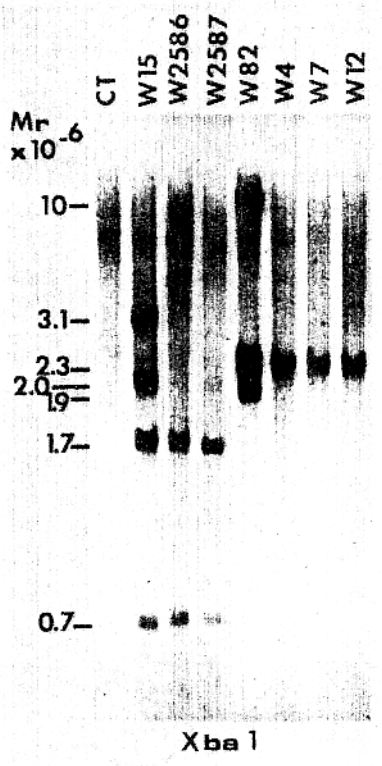

Figure 5
Hybridization patterns of BLV [^{32}P]cDNA on DNA restriction fragments from circulating WBC of BLV-infected animals from two foci of infection. WBC DNA (20 μg) from animals 15, 2586, and 2587 (focus 1) and from animals 82, 4, 7, and 12 (focus 2) were digested with *Xba*I and treated as described for Fig. 1.

shown in Figure 6A. Pools were made and their densities were determined by CsCl centrifugation (Fig. 6B). Viral sequences were detected by the Southern (1975) filter-blotting technique (data not shown). Bovine leukemia proviral sequences were detected mainly in large GC-rich fragments having buoyant densities in CsCl close to 1.708 g/cm^3.

Viral RNA Content of BLV-infected Cells

Using liquid-hybridization techniques and BLV [^{3}H]cDNA as a probe, we looked for viral RNA sequences in various total RNAs of BLV-infected cells. Genomic 35S RNA and total RNA from the virus-producing cells were used as positive controls. Figure 7 shows annealing kinetics obtained with BLV 35S RNA, total FLK RNA, and total RNAs from tumors, circulating WBC, and normal tissues. From the comparison of the $C_0t_{1/2}$ values obtained with 35S RNA and total FLK RNA, one could deduce that the FLK cells contained 0.002% of viral RNA sequences, i.e., approximately 30 copies of genomic viral RNA molecules per cell. No hybridization could be detected with RNA samples from different tumors and circulating WBC under conditions where a few molecules (three to four) of viral 35S RNA per cell could have been detected.

Search for Bovine Leukemia Proviral Sequences in Tumors of Animals with SBL

Besides EBL induced by BLV, SBL, a noninfectious form of bovine leukosis, is occasionally found in very young animals. No bovine leukemia proviral

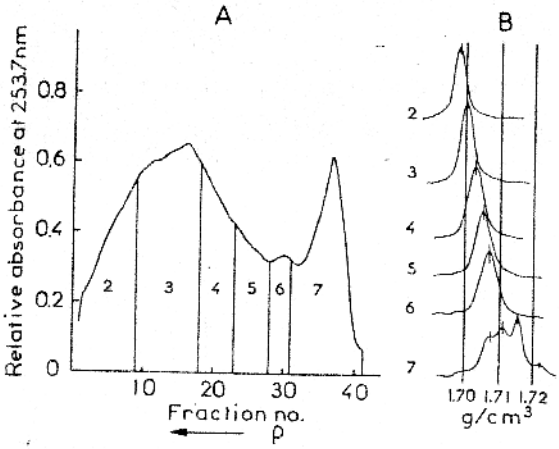

Figure 6

Analysis of T15 DNA by density gradient centrifugation. (*A*) Fractionation of T15 DNA in Cs_2SO_4 density gradient in the presence of BAMD at a BAMD/nucleotide molar ratio of $M_I = 0.1$. Fractions were collected and the relative absorbance was monitored at 253.7 nm. Arrow indicates the direction of density increase in the gradient; vertical lines determine the limits of pooled fractions 2–7 (fraction 1 is the pellet). (*B*) Analytical CsCl density gradient centrifugation profiles of pools 2–7.

sequences were detectable in tumors from those animals by liquid-hybridization experiments (Kettmann et al. 1978). Such results are in agreement with the generally accepted idea that BLV is not implicated in SBL. However, in order to search for an eventual presence of partial bovine leukemia provirus or for DNA sequences complementary to a minor component of the ^{32}P probe, DNAs from thymic (T-C7) and multicentric (T-C10, T-C11, T-C12) forms of SBL were analyzed by the Southern technique. As shown in Figure 8, no hybridization above background (obtained with calf thymus DNA) occurred with any of the DNAs from SBL.

DISCUSSION

Restriction Patterns

The results obtained in the present work bear essentially on the sites of integration of bovine leukemia provirus in the two different forms of EBL; the data obtained upon hybridization of the BLV probe with DNAs from WBC of animals with PL (Fig. 1) are consistent with the presence in these genomes of a large number of integration sites for the provirus. Hybridization of the probe with DNA degraded by *Eco*RI shows a smear covering a wide molecular-weight range, between 5×10^6 to 6×10^6 and 10×10^6 to 12×10^6, with some bands emerging above the continuous background. The latter finding suggests that a number of identical integration sites are used by the genomes of the host cells. Most of the *Eco*RI fragments were large enough to contain approximately one full-length copy of the provirus, and they might well do so. In the two previously explored cases (Kettmann et al. 1979), one proviral genome (animal 92) contained no *Eco*RI site and the other one (animal 15) contained one *Eco*RI site

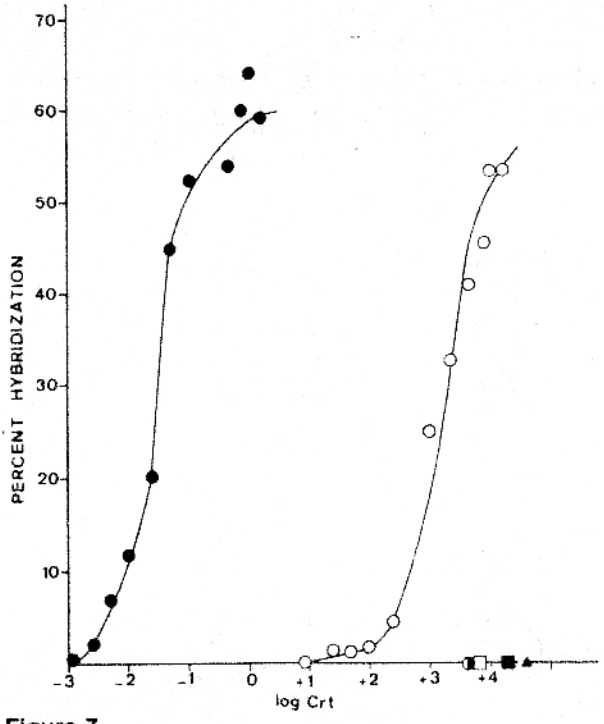

Figure 7

Hybridization between BLV [^{3}H]cDNA and various RNA samples. Hybridization conditions were as described in Materials and Methods. The hybridization mixtures contained genomic BLV 35S RNA (●); total RNA from FLK cells producing BLV (○); total WBC RNA from animals 4 (△) and 351, 628 (◑), all in the PL stage; polyadenylated RNA from a tumor of animal 92 (□); total RNA from a tumor (T15-1) (■) and from the enlarged spleen (▲) of animal 15; total WBC (▲) and tumor (▲) RNAs of animal 950; and total kidney RNA (▲) from a normal animal.

near one end of the provirus. Some large fragments, however, may contain only a segment of provirus, and some other fragments are just too short to contain more than that. In these cases, the other cellular DNA fragment containing a proviral segment may not be detected because either (1) it is too short (our experiments did not detect fragments smaller than 0.5×10^6), or (2) the proviral segment is too short, or (3) hybridization does not detect proviral fragments that are not sufficiently represented in cellular DNA (see below), these different reasons not being mutually exclusive.

The multiplicity of integration sites in the cells of animals with PL is confirmed by the experiments carried out on *Bam*HI fragments. In this case, the viral probe hybridized mainly on two restriction fragments having identical molecular weights (2.0×10^6 and 1.3×10^6) in the DNAs from the WBC of four different animals. This indicates that the fragments may be internal proviral fragments and suggests that the same viral variant may be involved in the PL of the four animals. Since the sum of the molecular weights of the two fragments is lower than that of the provirus (6.3×10^6), other chromosomal fragments possibly contain proviral segments. A few faint bands of different molecular weights are, in fact, seen in the restriction enzyme digests of different DNAs.

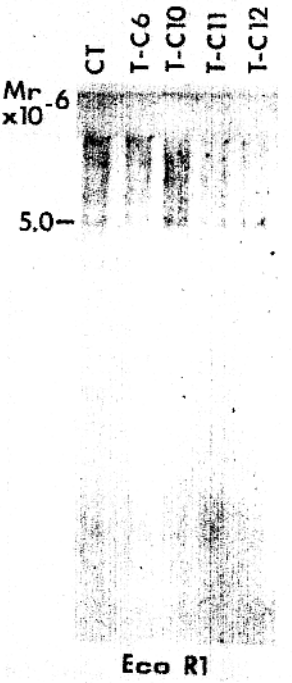

Figure 8
Hybridization patterns of BLV [^{32}P]cDNA on DNA restriction fragments of SBL tumors. DNAs (20 μg) of CT, T-C7, T-C10, T-C11, and T-C12 were digested with *Eco*RI and treated as described for Fig. 1.

Clearly, however, these correspond to some predominant flanking sequences and indicate the use of some identical integration sites, like the strong *Eco*RI bands emerging above the continuous hybridization background. The majority of individual provirus-positive flanking sequences are probably present in one copy per many cell genomes and are therefore not detected.

The pattern of proviral integration in the WBC of animals carrying lymph-node tumors is completely different from that described above, in that a well-defined number of provirus-positive bands are present in both *Eco*RI and *Bam*HI digests (Fig. 2). The latter digests show two common hybridizing bands (2.0×10^6 and 1.3×10^6) in all DNAs. These bands, probably corresponding to internal proviral fragments, were also found in the four animals with PL (Fig. 1), a preliminary indication that the same viral variant was present in all cases. In addition, *Bam*HI digests revealed two additional provirus-positive cellular DNA fragments, which differed from animal to animal and corresponded to flanking sequences that were different in different animals. The results obtained with *Eco*RI digests agree with the interpretation that W950 and W82 appear to hybridize the probe on one fragment ($\sim 6 \times 10^6$) containing most of the provirus and on one flanking fragment of different molecular weight. The case of W15 is interesting in that two different sets of provirus-positive fragments appear to be present, 17×10^6 and 1.2×10^6 on the one hand and 9.6×10^6 and 0.6×10^6 on the other. The first set is largely predominant over the other one and was the only set to be detected in earlier experiments (Kettmann et al. 1979); this set of fragments corresponds to the only one seen by hybridization on *Bam*HI fragments.

The results obtained with lymph-node-tumor DNA are similar to those just described in that they indicate one well-defined hybridization pattern for five tumors in animal 950. For animal 15, three sorts of patterns are found in different tumors. Two patterns correspond to two different integration sites, and the third pattern corresponds to a mixed cell population in which both different

integration sites are represented. Hybridization patterns of an *Eco*RI digest of the latter DNA (data not shown) indicate that the two proviral patterns present in this case are the same as those found in the WBC of the same animal but that their relative proportions are reversed. Finally, it should be noted that both proviral restriction patterns present in the tumors of animal 15 shared two *Xba*I bands (1.7×10^6 and 0.7×10^6) already found in the WBC and spleen of the same animal (Kettmann et al. 1979), an indication of a common proviral variant in all the infected cells of this animal.

In conclusion, the main result of this investigation is that PL and the tumor stage of EBL are characterized by a polyclonality of infected cells in the first case and by a mono- or oligoclonality in the second case with regard to BLV integration. Under these circumstances, it is likely that a selection of BLV-positive cells takes place during the course of EBL. Since available indications do not suggest that such a selection depends on genetic differences in the provirus, we may wonder whether a selective advantage is not conferred upon some BLV-positive cells by the particular integration site used by the provirus.

Liquid Hybridization

Cytotoxicity experiments (Portetelle et al. 1978) show that all cells of our FLK-BLV cultures are BLV-infected. On the other hand, restriction patterns of T15-2 DNA strongly suggest that one copy of BLV proviral DNA is integrated per haploid cell genome. Taken together with the observed annealing rates (Fig. 4), these results strongly suggest that all cells of T15-2 do indeed carry the provirus. In contrast, all the other DNAs tested, whether from leukocytes of animals with PL or from tumor cells, annealed the probe to a much smaller extent. This indicates that in all these tissues only a fraction (~30%) of the cell population harbored the provirus. Similar conclusions were reached from electron microscopic quantification of BLV-producing cells among PL leukocytes kept in vitro (Paul et al. 1977). Interestingly, in our study, the fraction of cells carrying the BLV provirus seemed to be independent of the number of lymphocytes.

BLV Variants

From previous studies in other systems it was known that some restriction enzymes were better tools than others for detecting variants or mutant strains of the virus. In avian sarcoma virus systems, *Bam*HI digestion and blotting analysis uncovered viral mutants (Shank et al. 1978). In the bovine system, the *Xba*I digests analyzed so far allowed classification of seven WBC samples into two groups, which corresponded exactly to two different geographical foci of BLV infection (Fig. 5).

Location of Bovine Leukemia Provirus in the Host Genome

Preliminary studies were carried out to initiate characterization of the integration site(s) of the proviral DNA in PL and tumor cells and to analyze viral RNA molecules possibly present in the same types of cells. Experiments aiming

at a better identification of integration sites led to the observation that bovine leukemia provirus was detectable only in very few components of the bovine genome, having a density of 1.708 g/cm^3 in CsCl. This conclusion applied to tumor DNA as well as to PL DNA and indicated that the many possible integration sites were indeed restricted to a given portion of the host DNA.

Viral RNA in BLV-infected Cells

Attempts to identify viral RNA in total RNA extracted from PL or tumor cells have been unsuccessful. From the level of sensitivity of our hybridization conditions, it could be inferred that only a small fraction of cells, located in tumors or circulating, were synthesizing viral RNA at any given time. Most cells seemed to be at rest in the G_0 phase. Similar observations were reported by Haas et al. (1977) in the visna system. They confirmed that exogenous viruses can be fully controlled by the invaded cell.

BLV and SBL

Finally, blotting techniques were applied to DNAs of sporadic cases of bovine leukosis. Such techniques applied with simian sarcoma virus–simian sarcoma-associated virus probes allowed detection of viral information in normal DNA (Gallo and Wong-Staal 1980). We hypothesized that if a similar situation would prevail in the bovine, it also could be approached by the same methods. However, results were negative and again reemphasized that BLV probably plays no role in SBL induction.

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