Detection of Integrated Type-C Viral DNA Fragments in Two Primates (Human and Gibbon) by the Restriction Enzyme Blotting Technique

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There have been reports of sporadic findings of type-C markers in human cells. Most commonly these markers were related to the two classes of primate type-C viruses: The simian sarcoma virus-simian sarcoma associated virus complex [SiSV (SiSAV)] and gibbon ape leukemia virus (GaLV) group and the baboon endogenous virus, BaEV (see Gallo elsewhere in this book). However, detection of proviral sequences related to either of these virus groups was rare, and a preliminary survey indicated that DNA from leukemic tissues, although hybridizing more viral probe (SiSAV and BaEV) than DNA from normal tissues, displayed a broad range of hybridization values which never approached the level of DNA from virus-infected tissue culture cells (Gallo, elsewhere in this book). One possible explanation for the low but possibly significant hybridization values found in fresh leukemic cells is that subgenomic fragments could have been integrated in the DNA of these tissues rather than complete provirus. However, unequivocal evidence for partial provirus integration of type-C RNA viruses is still lacking in the literature. In this report we show by restriction enzyme-blotting analysis, that (i) a few tissues from a gibbon ape exposed to GaLV contained an incomplete provirus; (ii) DNA from *all* human DNA contained sequences that hybridize specifically to SiSV-SiSAV genomes, suggesting a recombination event between these viruses and human DNA via infection; (iii) DNA from two leukemia DNA samples showed *extra*, presumably acquired, viral fragments related to BaEV. One of the samples was from the uncultured leukocytes of patient HL23 (Gallo, elsewhere in this book). In addition one other human DNA sample showed *extra* viral fragments related to SiSAV.

Partial Provirus in a Gibbon Exposed to GaLV

We obtained tissues from two gibbons from a colony in Hall's Island, Bermuda, G-1 was leukemic (acute T-cell leukemia) and viremic, and a distinct isolate of GaLV (designed $GaLV_H$) was isolated from its leukocytes after culture (Gallo et al., 1978; Reitz et al., 1979). G-4 although terminally ill with emaciation (cause unknown), was not frankly leukemic or viremic, but its serum contained high titer of antibody against GaLV antigens (Gallagher et al., 1978). When DNA from tissues of G-4 were examined for GaLV proviral DNA sequences by hybridization, all tissues were negative with the exception

of the spleen, liver and kidney. DNA from these three tissues hybridized 30 to 50% of $GaLV_H$ or SiSAV probes relative to DNA from G-1 tissues (Wong-Staal, Reitz, and Gallo, 1979) suggesting that these tissues may harbor an incomplete provirus, although other possibilities exist. We therefore decided to analyze the samples by restriction enzymes followed by the blotting technique described by Southern (1975) to determine whether proviral fragments may be present. A schema of the procedure used is presented in Fig. 1. DNA digested with the site specific endonuclease is fractionated on agarose gels and then the DNA is "blotted" onto a sheet of nitrocellulose filter paper. The DNA positioned on these filters is hybridized to excess ^{125}I viral RNA and autoradiographs are prepared. Discrete fragments containing DNA sequences that hybridize the labeled probes can be visualized. As shown in Fig. 2, after digestion of the cellular DNA's with Bam HI, two viral fragments were detected in tissues of G-1, corresponding to sizes of 2,0, and 1,5 $\times 10^6$ daltons. However, DNA from the kidney and liver of G-4 contained only the $1{,}5 \times 10^6$ dalton fragment (Fig. 2). Since this fragment is subgenomic in size, the result clearly indicates that an incomplete provirus is integrated in

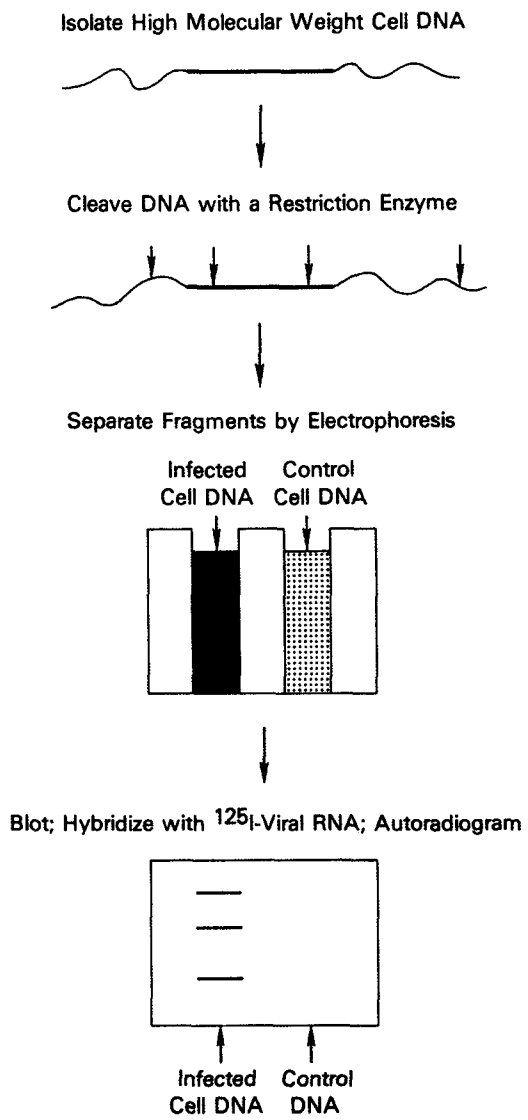

Fig. 1. Scheme for detection of integrated viral sequences by restriction enzyme-blotting. High molecular weight DNA is purified from cells or tissues and cleaved with a given restriction enzyme. Cleavage sites for the enzyme are distributed all over the DNA, including the provirus (indicated here by the dark straight line). The resultant heterogeneous collection of DNA fragments is then separated by electrophoresis on agarose gels for fractionation on the basis of size. After electrophoresis, staining with ethidium bromide reveals a broad distribution of DNA. However, after transfer of the DNA to nitrocellulose filter paper, only the fragments containing part or all of the provirus will hybridize to ^{125}I-viral RNA. In the hypothetical case presented in the schema, there are three fragments. These are visualized by autoratiography

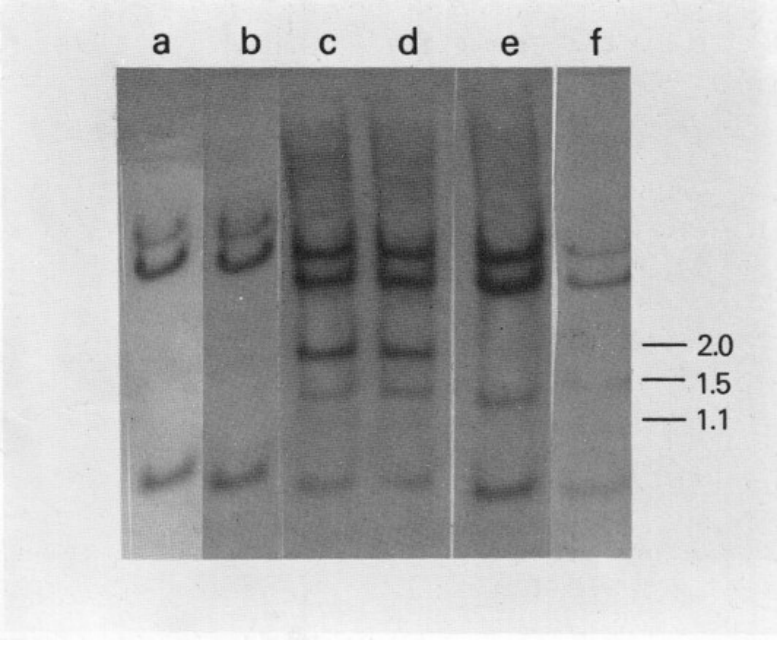

Fig. 2. Cleavage pattern of integrated viral sequences in DNA from tissues of G-1 and G-4. 25 μg of each cellular DNA was digested with Bam HI and electrophoresed in 0,8% agarose. Transfer of the DNA to nitrocellulose filters was carried out according to Southern (1975). Hybridization was carried out with ^{125}I-ribosomal RNA as control or ^{125}I-SiSAV RNA in 50% formamide, 3XSSC, 0,5% sodium dodecyl sulfate, 5 mg/ml tRNA, 0,02% each of ficoll, polyvinylpyrrolidone and BSA at 37°. Molecular weights are given in daltons $\times 10^{-6}$ *a)* Normal gibbon DNA hybridized to ^{125}I-rRNA as control. *b)* Normal gibbon spleen DNA × ^{125}I-SiSAV RNA. *c)* G-1 heart DNA × ^{125}I-SiSAV RNA. *d)* G-1 lymph node DNA × ^{125}I-SiSAV RNA. *e)* G-4 liver DNA × ^{125}I-SiSAV RNA. *f)* G-4 kidney DNA × ^{125}I-SiSAV RNA

the DNA from these tissues. DNA from a normal gibbon spleen did not contain any viral specific fragments. (The three bands shown in normal gibbon spleen DNA are ribosomal DNA bands due to small amounts of RNA in the viral RNA preparation. See ^{125}I-rRNA control.) Thus the $1{,}5 \times 10^6$ dalton detected in DNA from kidney and liver of G-4 represents an acquired partial provirus by the animal.

Detection of an Endogenous Human DNA Fragment That Hybridized Specifically to SiSAV-GaLV

When DNA from human cells (including fresh tissues of spleen, liver, kidney, placenta, leukocytes or tissue culture cells) was digested with Bam HI, and hybridized to ^{125}I-RNA from different type-C viruses, RNA from SiSV-SiSAV detected a fragment at $8{,}0 \times 10^6$ daltons in size in the DNA of all tissues tested. These results cannot be explained by human cellular nucleic acids contaminating the viral ^{125}I-RNA probe because the hybridizations were performed with three different strains of SiSV-SiSAV grown in different hosts, marmoset, human and rat and probes from each of these viruses gave the same result.

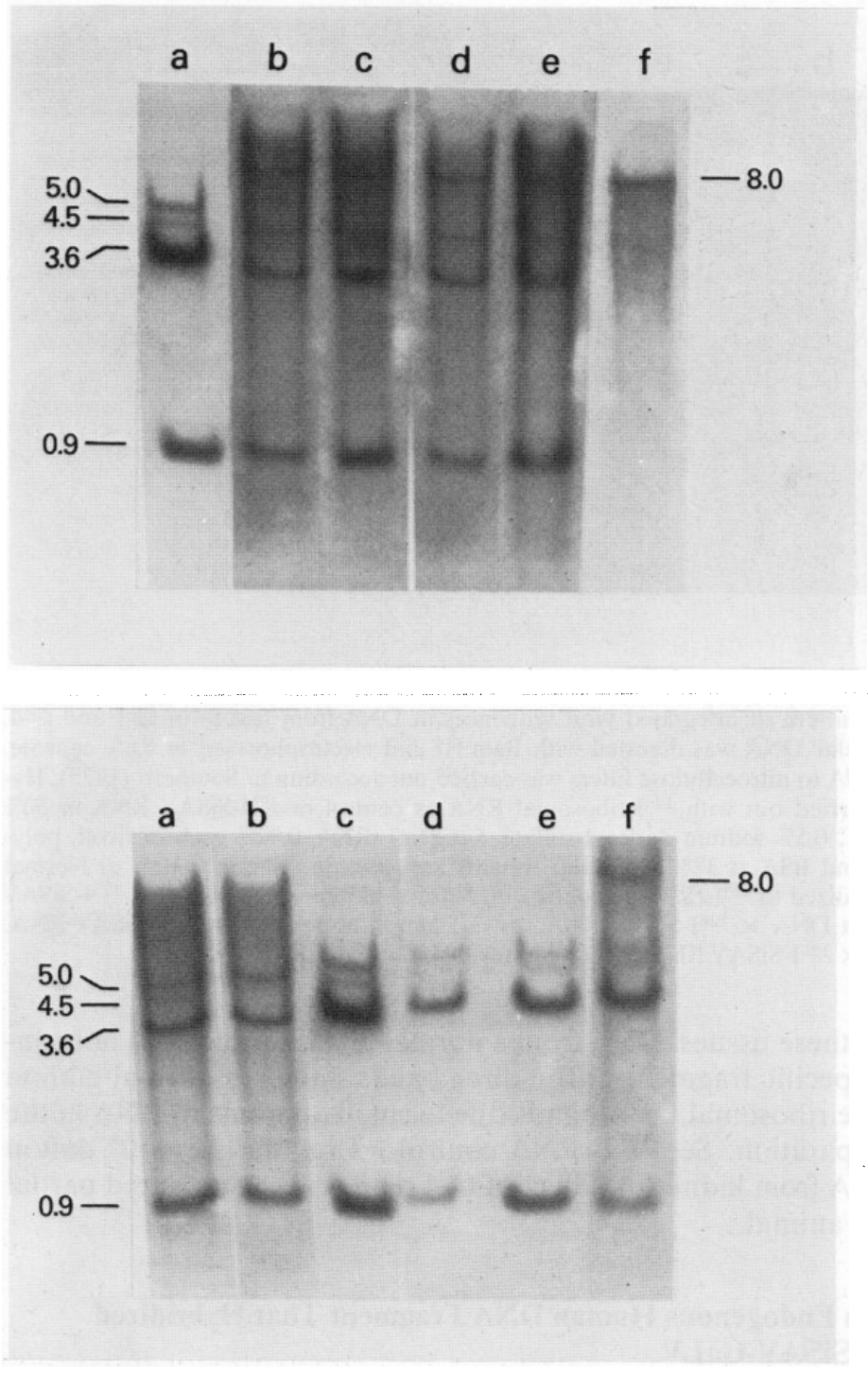

Fig. 3. Detection of an endogenous human DNA fragment that hybridized to genomic RNA of SiSAV-GaLV. Experiments were carried out as described in legend to Fig. 2. Forty different human DNA samples, including fresh tissues of spleen, liver, kidney placenta as well as various tissue culture cell lines gave identical results, therefore only a typical pattern is shown. Human DNA digested with Bam HI was hybridized to ^{125}I-RNA of various viruses. *A.* Viruses of the SiSV-SiSAV group: *a)* rRNA control; *b)* SiSAV (A204), grown in human rhabdomyosarcoma cells; *c)* 705 RNA of SiSV (SiSAV) (71AP1), produced by a marmoset tumor cell line; *d)* 355 RNA of SiSV (SiSAV) (71API); *e)* SiSAV (M55), grown in normal rat kidney cells; *f)* same as e), but selected on oligo-dT cellulose column for poly (A). *B.* Other viruses: *a)* GaLVH; *b)* the endogenous cat virus RD114; *c)* Feline leukemia virus, strain Rickard, $FeLV_R$; *d)* Murine leukemia virus, strain Rauscher, $MuLV_R$; *e)* Baboon endogenous virus, BaEV (M7); *f)* Filter from e) rehybridized to SiSAV (M55)

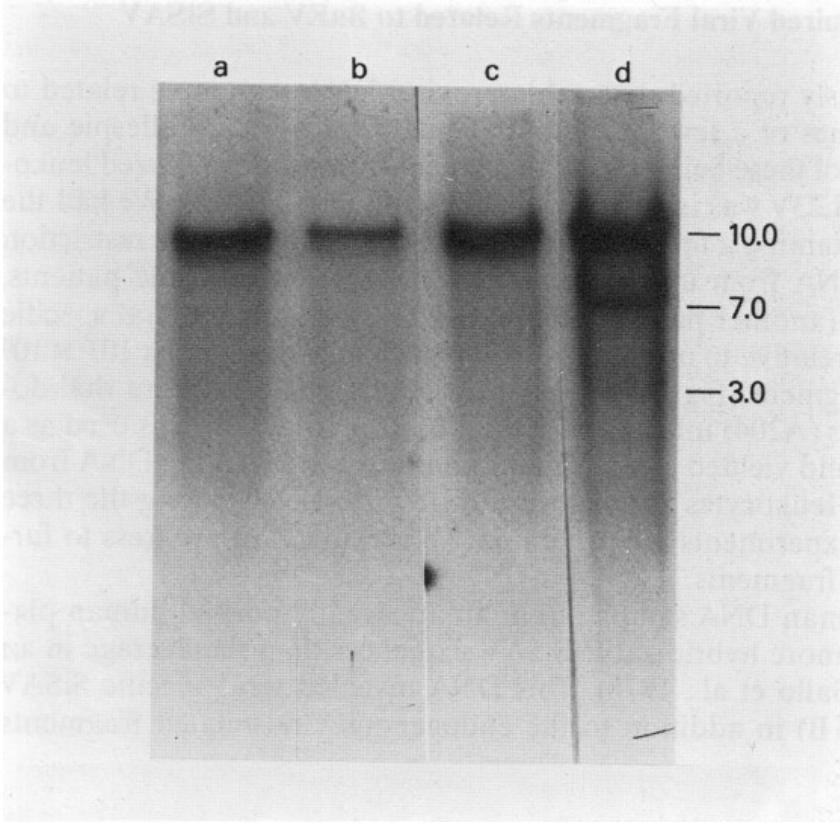

Fig. 4. Digestion of the endogenous SiSAV-related human DNA with Hind III human DNA digested with Hind III was hybridized to: *a)* ^{125}I ribosomal RNA: *b)* ^{125}I BaEV RNA: *c)* ^{125}I FeLV RNA: *d)* ^{125}I SiSAV (M55) RNA

All other viruses, including $GaLV_H$ and BaEV, did not detect any viral specific DNA fragments in DNA from normal human tissues. Representative results using these viral RNA probes are depicted in Fig. 3. To further verify the results with Bam HI, another enzymes, Hind III, was used to analyze the human DNA sequences that interact with SiSV-SSAV, and the results are shown in Fig. 4. Hind III yielded two fragments of 7,0 and $3{,}5 \times 10^6$ daltons. These sequences were not derived from a conserved set of mammalian DNA sequences, since DNA from two primates, gibbon and baboon, as well as two lower species, cat and dog, did not reveal any specific fragments hybridizing SiSV-SSAV (see earlier results for normal gibbons, other data not shown). The negative data with DNA from these animals also indicates that the positive results with human DNA are not from cellular nucleic acids contaminating the viral probe.

Since two of the three probes used SiSV (71API), SiSV (NRK) were never passaged through human cells in the laboratory, a plausible explanation is that viruses of the SiSV-SiSAV group have infected humans in the past, and have recombined with human cellular sequences. We plan to study the nature of these sequences and their mode of expression in human cells after they are purified and amplified by cloning them in bacteria.

Detection of Acquired Viral Fragments Related to BaEV and SiSAV

We have previously reported detectable proviral DNA sequences related to the BaEV in tissues of a few leukemic patients (Wong-Staal, Gillespie and Gallo, 1976) one of these being HL23, the patient from whose cultured leukocytes the virus HL23V was isolated (Gallagher and Gallo, 1975). We had the opportunity to examine a few of these samples by "blotting" after restriction with Hind III. DNA from uncultured leukocytes of two leukemic patients, HL23 and HL49 (another patient with AML), revealed several viral specific DNA fragments relative to normal controls (which only displayed a $10{,}0 \times 10^6$ dalton rDNA fragment (Fig. 5A). DNA from human tissue culture rhabdomyosarcoma cells (A204) infected with the M7 strain of BaEV, was used as a positive control and yielded a very similar banding pattern as the DNA from HL23 and HL49 leukocytes although there are differences among the three samples. These experiments are preliminary and work is in progress to further define these fragments.

One other human DNA sample, from an apparently normal human placenta contained more hybridizable SiSAV sequences than the average in an earlier survey (Gallo et al., 1978). This DNA revealed viral specific SiSAV fragments (Fig. 5B) in addition to the endogenous viral-related fragments described earlier.

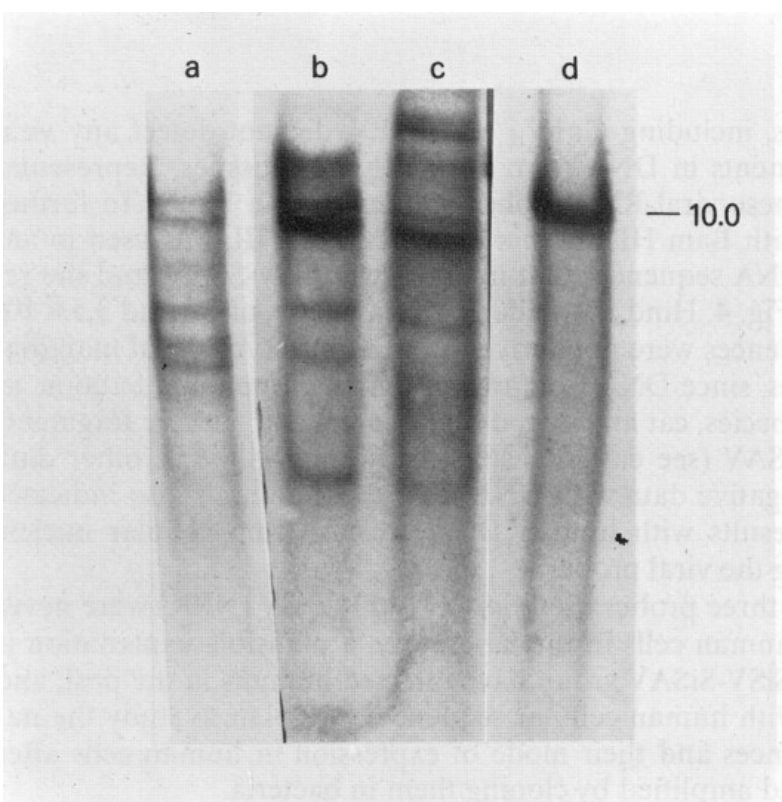

Fig. 5A

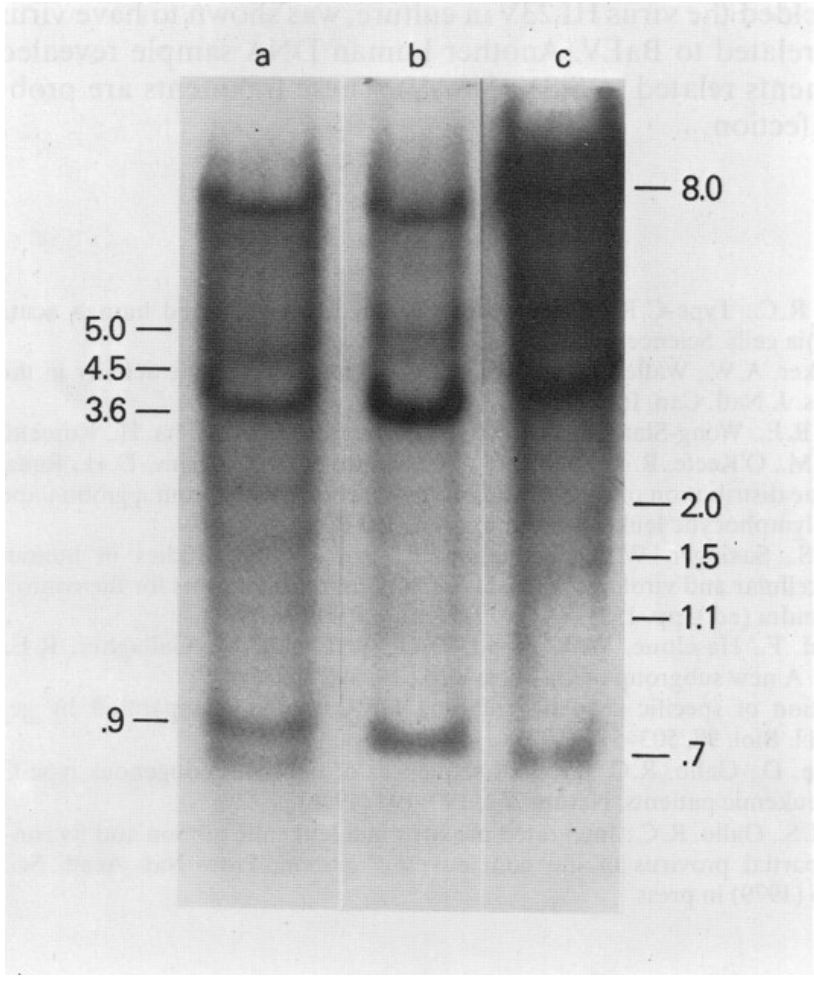

Fig. 5B

Fig. 5. Detection of "acquired" DNA fragments related to BaEV and SiSAV in several human DNA samples. *A.* DNA from uncultured leukocytes of two patients. HL23 and HL49. was treated with the restriction enzyme Hind III and processed as described in Fig. 2. A204 (M7) a human rhabdomyosarcoma cell line infected with BaEV (P. cynocephalus). and normal human spleen were used as positive and negative controls respectively. Hybridization was carried out with ^{125}I-BaEV RNA; *a)* A204 (M7); *b)* HL49; *c)* HL23; *d)* normal human spleen. *B.* DNA from 2 normal placentas and from NC37 (SiSAV). a human lymphoid cell line infected with SiSAV. were digested with Bam HI and hybridized to ^{125}I SiSAV RNA; *1.* NC37 (SiSV); *2.* normal placenta # 6; *3.* normal placenta called NP3. (NP3 was previously shown to hybridize significantly higher levels of SiSV probes than found with other DNA from normal human tissues)

Summary

We have shown that 1. partial provirus integration can be a possible result of a natural infection. and may serve as a model in animal systems where a viral etiology is implicated but detection of a major fraction of the virus genome is rare; 2. All human DNA contains some sequences that hybridize specifically with genomes of SiSV-SiSAV. suggesting that viruses of this group have infected humans in the past and recombined with human cellular DNA. 3. Finally. DNA from uncultured leukocytes of two leukemic patients. one be-

ing HL23, which yielded the virus HL23V in culture, was shown to have virus specific fragments related to BaEV. Another human DNA sample revealed virus specific fragments related to SiSV (SiSAV). These fragments are probably acquired by infection.

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