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Markers of HTLV-I-Related Virus in Hamadryas Baboon Lymphoma

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A. Introduction

Our previous studies have shown that hamadryas baboons of the Sukhumi "high lymphoma" stock are infected with human T-lymphotrophic virus (HTLV)-I-related virus to a significantly higher degree than baboons of different lymphoma-free populations. Levels of anti-HTLV-I-related antibodies in prelymphomatous baboon sera were also significantly higher than those in matched controls [1, 2]. These studies posed the question of whether HTLV-I-like virus is etiologically related to baboon malignant lymphoma. The most informative indirect approach to the study of this possibility is the search for integrated provirus in baboon lymphoma DNA. Thus, we tested by Southern blotting *Pst*I, *Bam*HI, and *Eco*RI digests of high molecular weight baboon lymphoma DNA, using as a probe genome-length HTLV-I cloned in *Sst*I site of pSP-65 vector (this molecular clone was kindly provided by Dr. R. Gallo). Ten *Pst*I-digested lymphoma DNA samples (lymphomatous lymph nodes) were found positive (Table 1). The band pattern was similar to, but clearly different from, that characteristic for HTLV-I (cf. Figs. 1, 2, 4). At least three fragments (1.7 kb, 1.5 kb, and 1.1 kb) were observed in all samples (Table 1; Fig. 1). They were thought to be internal fragments [3]. In each positive sample "individual" bands were also found that suggest monoclonal (or oligoclonal) integration of HTLV-I provirus into different sites in baboon lymphoma DNA (Fig. 1). This suggestion was proved correct by Southern analysis of *Bam*HI and

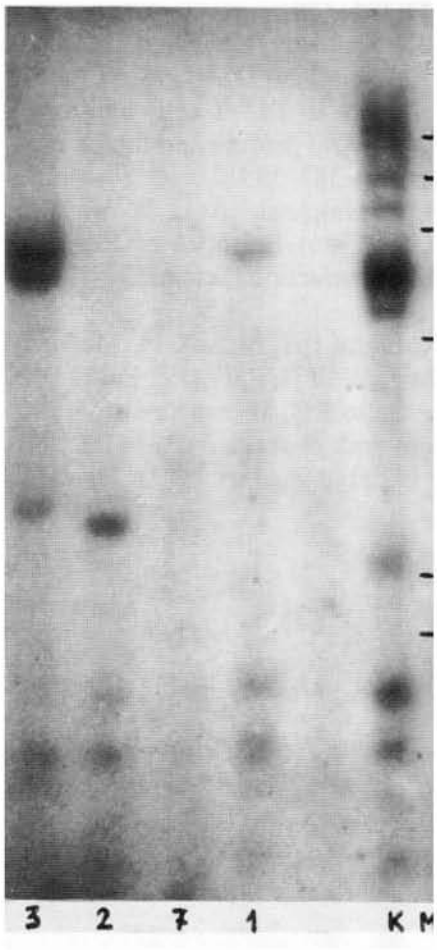

Fig. 1. Southern analysis of *Pst*I digests of baboon lymphoma DNA. 1–10, sample numbers the same as in Table 1; K, baboon cell line (594S-F9) producing HTLV-I-like virus; M, Molecular size markers: 23.4, 9.4, 6.5, 4.4, 2.3, and 2.0 kb

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Table 1. HTLV-I-related sequences in DNA of lymphomatous lymph nodes of hamadryas baboons

No.	Tumor no.	*Pst*I digests		*Bam*HI digests		*Eco*RI digests	
		+/−	internal fragments (in kb)	+/−	fragments (in kb)	+/−	fragments (in kb)
1.	PHL-1	++	1.7; 1.5; 1.1[a]	++	25; 15	++	20
2.	PHL-2	++	1.7; 1.5; 1.1[a]	++	24; 22	++	23.5
3.	PHL-3	++	1.7; 1.5; 1.1[a]	+++	14; 8; 4.1	+++	24; 5
4.	PHL-4	++	1.7; 1.5; 1.1[a]	+++	9	+++	23.4; 4.5
5.	PHL-5	++	1.7; 1.5; 1.1[a]	+	12; 7.5	NT	
6.	PHL-6	+	1.7; 1.5; 1.1[a]	+	13; 10; 7.6	+	23.5; 15
7.	PHL-7	+	1.7; 1.5; 1.1[a]	+	8.4; 5.8	−?	
8.	PHL-8	++	1.7; 1.5; 1.1[a]	++	8	+++	11.5; 9.4; 7
9.	PHL-9	+	1.7; 1.5; 1.1[a]	NT		++	24
10.	PHL-10	++	1.7; 1.5; 1.1[a]	NT		++	20; 12

+, weakly positive; ++, positive; +++, highly positive. NT, not tested.
[a] Junction fragments of different sizes were individual for each sample in Pst digests.

Ten μg of high molecular weight DNA was digested with the enzymes *Pst*I, *Bam*HI, and *Eco*RI under optimal conditions (5u/μg, 37° C overnight), electrophoresed through 1.2% (*Pst*I) or 0.8% (*Bam*HI, *Eco*RI) agarose, blotted to nitrocellulose filter in 20 × SSC according to Southern blotting. Hybridization was carried out with ^{32}P-labeled DNA of pMT-2 plasmid (pSP 65 vector containing genome-length insert of HTLV-I which represented approximately 70% of pMT-2) at 42° C for 40 h in the following solution −4 × SSC, 2 × Denhardt's solution, 0.5% Sodium dodecyl sulfate, 0.02M EDTA, 100 mg/ml sonicated denatured salmon sperm DNA, 50 mg/ml yeast RNA, 50% formamide 5×10^7 cpm probe DNA. Before this, filters were prehybridized in the same solution, but without probe DNA, for 16 h. Washing was carried out twice for 10 min at room temperature in 2 × SSC, five times for 15 min at 55° C in 2 × SSC + 0.1% SDS, and finally five times for 15 min at 55° C in 0.1 × SSC + 0.1% SDS. Filters were exposed to X-ray film with intensifying screens at −70° C for 5–10 days.

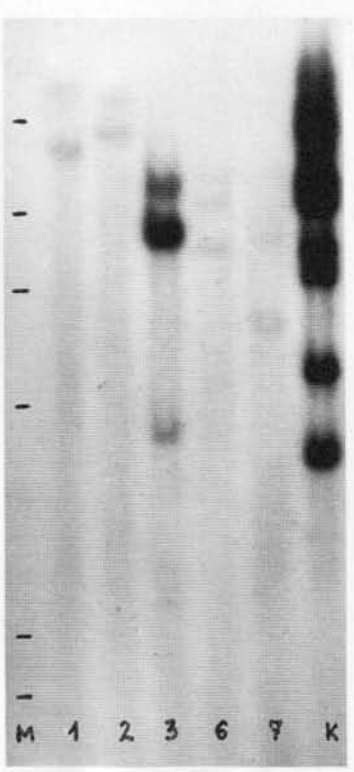

Fig. 2

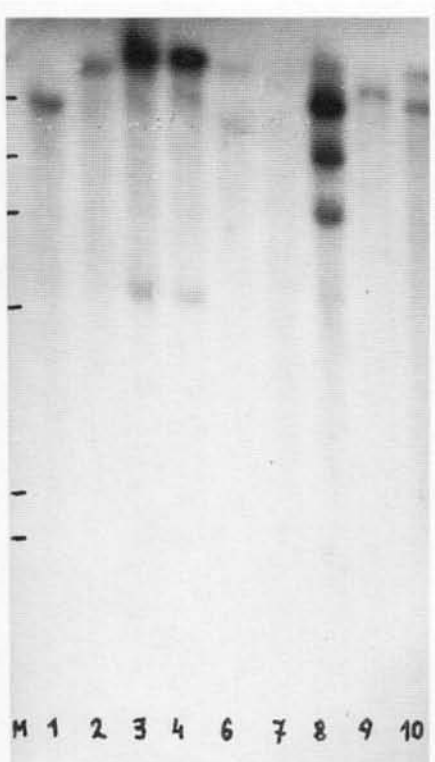

Fig. 3

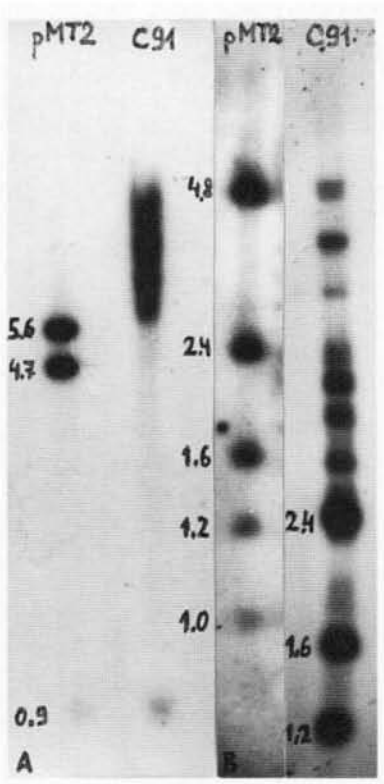

Fig. 4

Fig. 2. Southern analysis of *Bam*H1 digests of baboon lymphoma DNA
Fig. 3. Southern analysis of *Eco*R1 digests of baboon lymphoma DNA
Fig. 4. Southern analysis of *Pst*I (B) and *Bam*HI (a) digests of positive-control cellular and plasmid DNA. C91, human cell line (C91-PL) producing HTLV-I

*Eco*RI digests of the same lymphoma DNA (Table 1; Fig. 2, Fig. 3). One to three large fragments were observed in each sample, all with individual size. In some cases, fragments were found which were smaller than the expected HTLV-I-like provirus size. These data suggested multiple integration of HTLV-like provirus(es) in some baboon lymphomas or the oligoclonal origin of these tumors, as well as integration of defective provirus(es) in several cases. HTLV-I-related sequences were not found in one sample of baboon normal lymph node DNA and three samples of muscle DNA isolated from lymphomatous animals.

B. Conclusions

1. HTLV-I-like provirus is integrated into DNA of baboon malignant lymphomas.
2. Baboon HTLV-I-like provirus is closely related to, but distinct from, HTLV-I.
3. In most cases the integration of HTLV-I-like provirus is multiple monoclonal, or the origin of these tumors is oligoclonal.
4. Defective HTLV-I-like proviruses are integrated into DNA of some baboon malignant lymphomas.

References

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