PRIMATOLOGY

Differences in STLV-1 Genome Structure in Baboons Dead from Malignant Lymphoma in Various Chronological Periods of a Many-Year Outbreak of the Disease

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The fragments of simian retrovirus genome STLV-1Ph detected in lymphomatous specimens from baboons dead from malignant lymphoma in different periods of a many-year (22 years) outbreak are studied. African and Asian subtypes of STLV-1Ph retrovirus circulate in the animals: the African type predominates during the first and Asian during the second half of the outbreak.

Key Words: *baboons; outbreak of malignant lymphoma; STLV-1Ph retrovirus; African and Asian subtypes*

Many-year (1967-1991) outbreak of malignant lymphoma among baboons (primarily *Papio hamadryas*) in the Sukhumi Breeding Center began with inoculation of human leukemic material to several animals and led to death of almost 400 adult baboons [1]. A group of baboons at a high risk of lymphoma was formed including a total of 4158 animals over the entire period of experiment. Control group consisted of 800 baboons brought to the center and living at an isolated territory, which had no contacts with lymphomatous animals. No cases of lymphoma were detected in the control group during at least 15 years. Immunophenotypically, malignant lymphomas of baboons were identified as non-Hodgkin's lymphomas (NHL) of T or B cell similar to human tumors. Testing for STLV-1 in 68 cases of T-cell NHL by PCR showed that retrovirus can be present in all cytological variants of identified T-cell baboon lymphomas [3,11].

Horizontal dissemination of lymphoma and the presence of type C retrovirus particles in lymphoid cells, lungs, kidneys, and salivary glands of sick animals confirmed viral origin of lymphoma. In further studies the virus was identified as simian T-lymphotropic retrovirus (STLV-1Ph or PTLV-1) [4,7] belonging to human HTLV-1 subfamily. Serum antibodies to STLV-1 were detected in 40-45% adult animals in the high-risk group and in almost 100% of baboons with T-cell malignant lymphomas [3]. Moreover, antibodies to STLV-1 were detected in the blood of 7% healthy adult animals in the control group. For detecting the possible biomolecular differences between the viruses isolated in the control and lymphomatous groups, we compared sequenced fragments of STLV-1 from lymphomatous baboons (STLV-1PhL) and healthy carriers (STLV-1PhN) in the control group. Different degree of homology with HTLV-1 and between STLV-1PhL and STLV-1PhN was detected in the sequenced env and tax sites [7,10]. The forest variant of STLV-1PhN had 96% homology with HTLV-1 and only 83% with STLV-1PhL. The data on the molecular structure of sequenced fragments of STLV-1/HTLV-1 were sufficient for phylogenetic analysis and grouping of viruses of this family into two

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major geographic subtypes: African and Asian, whose evolution was independent [8]. Analysis of the sequenced env fragments of STLV-1Ph [7] and the data of other authors [8] showed that previously examined STLV-1PhL was more homologous to the Asian subtype by its env site and was 100% homologous with STLV-1 isolated from *M. rhesus* by its tax fragment. Sequencing of individual fragments of STLV-1 provirus from lymphomatous baboons, who died during the late period of the outbreak showed an Asian subtype of *M. rhesus* STLV-1 retrovirus [9].

Different level of homology between different T-lymphotropic baboon retroviruses and to HTLV-1 suggested the emergence of recombinant variants of STLV-1Ph circulating in baboons. A retrospective analysis of malignant lymphomas in baboons was carried out, and animals were selected which died from lymphoma during different periods of many-year outbreak of the disease. Provirus DNA extracted from formalin-fixed material embedded in paraffin can be used for biomolecular studies [2].

We compared sequenced fragments of STLV-1 detected in baboons dead from malignant lymphoma in different periods of the outbreak.

MATERIALS AND METHODS

Formalin-fixed tumor lymph nodes embedded in paraffin were examined. The material was collected from 14 *Papio hamadryas* dead from generalized malignant lymphoma in 1968-1990. The animals were sacrificed during the preagonal period by total exsanguination under general ketamine narcosis. In parallel, biopsy specimens of a peripheral lymph node from a healthy STLV-1 carrier from the control group were examined. Tumor material from some baboons dead after 1980 were immunophenotyped with monoclonal antibodies (MAb) in cell suspension [5]. Lymphomas from baboons dead during the initial periods of the outbreak were identified by immunophenotyping in histological paraffin sections with appropriate MAb to B and T cells (CD20, Serotec; κ, λ, Dako; βF1, Endogen), interacting with formalin-fixed antigens. Lymph node specimens were stained with hematoxylin-eosin and according to Giemsa. Biomolecular studies included DNA extraction and PCR amplification with appropriate primers. For isolation of genome DNA, material of 5 histological sections (10 μ) was used in each case with subsequent routine treatment. The sections were released from paraffin and treated with proteinase K (200 μg/ml, Sigma, in 200 μl PCR buffer: 50 μM KCl, 10 μM Tris-HCl, pH 8.4, 1.5 μM $MgCl_2$) at 56°C overnight and subsequent inactivation of proteinase for 10 min at 95°C. For PCR and sequencing of amplified STLV-1 fragments, oligonucleotide primers used before were taken: to env site gp46 (5706-5726, 5'-gga tat gac ccc atc tgg ctc-3', 5992-5973, 5'gct gga agc gct aac gat gg-3'); 5'-terminal orf-II pX (7358-7377, 5'-

TABLE 1. Material Analyzed

No.	Baboon No.	Sex	Age by death, years	Type of lymphoma	Immunological appurtenance	Material for typing
1	3 050	Female	8.5	Immunoblastic	n. d.	Histological sections
2	3 559	-"-	9	Immunocytoma	B cell	-"-
3	4 210	-"-	9	Prolymphocytic	T cell	-"-
4	3 105	-"-	10.5	Immunoblastic	B cell	-"-
5	1 705	-"-	16	Lymphocytic-prolymphocytic	T cell	-"-
6	3 482	Male	14	Immunocytoma	B cell	-"-
7	2 520	Female	16	Lymphocytic	T cell	-"-
8	4 216	-"-	14	-"-	-"-	-"-
9	4 214	-"-	10	Lymphocytic-prolymphocytic	B cell	-"-
10	11 111	Male	9	Large-cell anaplastic	T cell	-"-
11	13 977	Female	14	-"-	-"-	Cell suspension
12	17 912	-"-	9	Prolymphocytic	-"-	-"-
13	18 655	-"-	8	Immunoblastic	-"-	-"-
14	20 555	-"-	5	Lymphocytic	-"-	-"-
15	268	Male	6	Healthy carrier STLV-1	—	-"-

Note. PCR analysis showed STLV-1 in all animals. Here and in Table 2: n. d.: not determined.

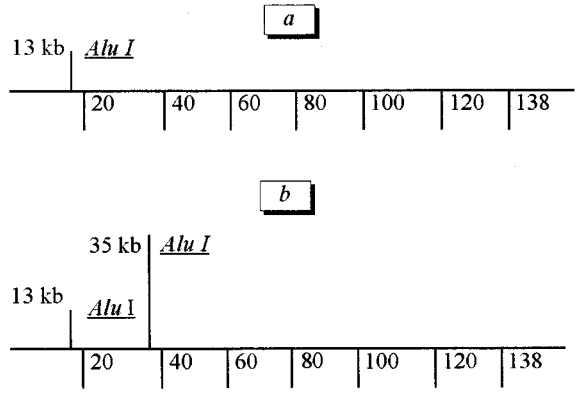

Fig. 1. Minirestriction map of *gag* (138 kb) fragments of African (*a*) and Asian (*b*) STLV-1 subtypes.

cgg ata ccc agt cta cgt gt-3', 7516-7496, 5'-gag ccg ata acg cgt ccf tcg-3'); sites of *gag* coding the amino-terminal protein p24 (bases 1423-1444, 5'-cca tca cca gca gct aga tag c-3', 1560-1537, 5'-agt tgc tgg tat tct cgc ctt aat-3'). Amplified STLV-1 fragments were electrophoresed in 2% agarose gel with ethidium bromide.

Based on the known sequences of STLV-1 *gag* sites, endonuclease *Alu* I specific for the Asian and African STLV-1 subtypes was selected by means of DNASIS software. For restriction mapping, 3-14 units of the enzyme and 1 μl of appropriate buffer (Boehringer Mannheim) were added to 8 μl of PCR product and the mixture was incubated for 2 h at 37°C. The product was analyzed in 12% acrylamide gel and visualized by ethidium bromide staining.

Fragments of STLV-1 tax gene were purified by the Jet Sorb Kit and sequences in two directions using *Taq* Dye Deoxy-Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) on an automated sequencer (Model 373A, Applied Biosystems Inc.).

RESULTS

An outbreak of NHL started at the end of 1967, when the first baboon (adult female) died from lymphoma. Specimens examined in this study (Table 1) were taken from lymphomatous baboons (n=14), adult animals, mainly females who died from malignant lymphoma at the age of 5-16 years. All animals included in the analysis belonged to the group at high risk of NHL and therefore directly or indirectly contacted with each other. Four baboons died during the first two years of the outbreak (1968-1969). Six baboons (including the above-mentioned) died during the first 5 years. Other baboons died between the seventh and twenty-third years of the outbreak. Fourteen examined baboons had B- and T-cell lymphomas of different cytological variants. Provirus STLV-1 sequences were

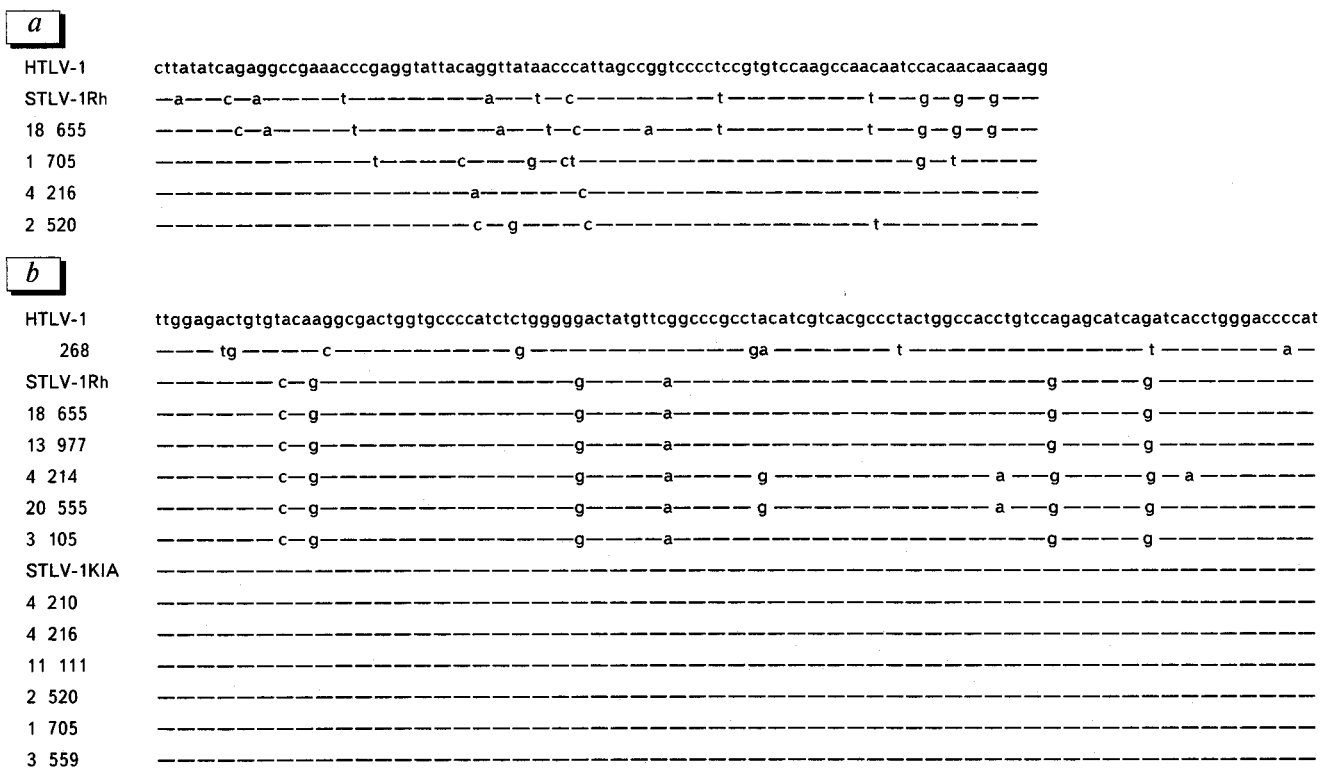

Fig. 2. Comparative analysis of nucleotide sequences. *a*) *gag* gene encoding p24 (1445-1535 kb); *b*) tax gene encoding pxII (7378-7495 kb) in sequenced STLV-1Ph (*Papio hamadryas*) in comparison with the prototype Asian (STLV-1Rh, *Macaca rhesus*) and African (STLV-1KIA, *Papio cynocephalus*) strains.

Table2. African (Afr) and Asian (As) STLV-1 subtypes of Baboon NHL in Different Chronological Periods of Disease Outbreak (Minirestriction Mapping and Sequencing)

No.	Baboon No.	Year of death	Time of death from beginning of outbreak, years	Minirestriction	Sequencing of STLV-1
1	3 050	1968	1	Afr	N. d.
2	3 559	1969	2	Afr	Afr
3	4 210	1969	2	Afr	Afr
4	3 105	1969	2	As	As
5	1 705	1971	4	Afr	Afr
6	3 482	1972	5	Afr	N. d.
7	2 520	1974	7	Afr	Afr
8	4 216	1974	7	Afr	Afr
9	4 214	1975	8	As	As
10	11 111	1979	12	Afr	Afr
11	13 977	1988	20	As	As
12	17 912	1989	21	As	As
13	18 655	1990	22	As	As
14	20 555	1990	22	As	As
15	268	Biopsy 1989	Healthy control	Afr	Afr

Note. PXII (159 kb), *pol* (186 kb), and *gag* (138 kb) were detected in all animals.

detected by PCR amplification in tumor cell DNA of all animals.

Restriction mapping revealed two STLV-1 subtypes in tumor tissue of lymphomatous baboons (Fig. 1). In some baboons STLV-1 DNA was cleaved by *Alu* I in the same site between the 13 and 20 kb, in others two restriction sites were observed: between 13 and 20, and between 35 and 40 kb. This indicates the presence of the African and Asian STLV-1 subtypes in these two groups of animals. The presence of two subtypes in the examined baboons was confirmed by sequencing of provirus DNA tax gene (Fig. 2).

Analysis of the results (Table 2) showed predominance of the African subtype in baboons dead during the first half of the outbreak (12 years), though in some baboons the Asian STLV-1 subtype was detected in NHL cells. During the two last years of the outbreak, only the Asian subtype of STLV-1 was detected in the lymphoma DNA of dead baboons. African STLV-1 subtype was detected in the lymph node DNA from a healthy carrier in the control group; however, sequence analysis showed that it differed from the analogous subtype in lymphomatous baboons (Fig. 2).

Therefore, restriction mapping and sequence analysis of lymphomatous material from STLV-1 carriers showed 2 subtypes of the retrovirus: one of them (A) was genetically related to the African (prototype strain STLV-1KIA, *P. cynocephalus*), while the other (B) to the Asian (prototype strain STLV-1Rh *Macaca rhesus*) subtypes of primate retroviruses. Listing of baboons in the order of their deaths from NHL in different periods of many-year outbreak demonstrates the time course of predominant circulation of the first (A) or second (B) subtypes of STLV-1Ph in the lymphomatous baboon group. The mechanisms of this phenomenon are not clear. Subtype A seems more appropriate for baboons originating from Africa. This subtype was detected in healthy control baboons. The majority of the known HTLV-1 strains detected in humans belong to the African subtype of retroviruses [8]. Detection of genetic sequences similar to STLV-1 sequences of *M. rhesus* in STLV-1 from lymphomatous baboons of the Sukhumi group suggested that the outbreak of lymphoma among baboons was caused by *M. rhesus* retrovirus [9]. Our results are at variance with this hypothesis. During the first 3 years of the outbreak, when attempts were made at transplanting lymphoma from one baboon to another, the possibility of contamination inside the breeding center could not be ruled out, despite all precautions for keeping baboons away from macaques. However, our results indicate that during this period only the African STLV-1 subtype was isolated from baboons. Variability of the retrovirus is therefore a more probable cause, and another cause is genetic recombination of isolated strains. High genetic variability of T-lymphotropic viruses of primates (PTLV) is well known [4]. The ecology in a group of baboons creates prerequisites for reinfection

and multiple infection [6]. This hypothesis can be confirmed after investigation on extensive data from different chronological periods of outbreak of baboon lymphoma and examination of longer fragments of STLV-1Ph provirus genome in the lymphoma DNA.

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