indication of a profound deterioration of the immune system of patients who had no remission at all.

Thus, our investigations have shown that: a) regarding patients with head and neck cancer, antibodies to TP are determined exclusively in the uNPC group; b) within the uNPC group antibodies are detected in 30-40% only; c) preliminary data point to a relationship between the dynamics of TP antibodies in the course of treatment and the prognosis of the disease. Studies in this field are in progress.

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Detection of STLV-1 Integration in DNA Extracted from Formalin-Fixed and Paraffin-Embedded Specimens of *M. Arctoides* Lymphomas Using the Polymerase Chain Reaction

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The feasibility of using the polymerase chain reaction to analyze of DNA extracted by the conventional method from formalin-fixed and paraffin-embedded specimens of monkey lymphoid tissue was investigated. Using HTLV-1 primers that can amplify DNA fragments of various length (159 to 717 base pairs), we determined the size of formalin-destroyed DNA which appeared to be up to 500 base pairs in length. Analysis of four specimens of *M. arctoides* lymphomas that had been stored since 1969 revealed integrated STLV-1 provirus.

Key Words: *polymerase chain reaction; DNA; formalin fixation; primer; STLV-1*

The polymerase chain reaction (PCR) [11,13] has become one of the main methods of detecting various viral agents [3,4,8,15]. PCR-amplified products are easily visualized and can be used in a broad spectrum of analytical studies [6,9]. The high sensitivity of the PCR method in the detection of specific DNA sequences is attractive regarding retrospective investigations. However, formalin-induced fragmentation of conventionally extracted

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TABLE 1. Nucleotide Sequences of Primers to Different Fragments of HTLV−1 Viral Genome Used for PCR Amplification of *M. arctoides* STLV−1 Provirus

Fragment	Nucleotide sequences of primers (5'-3')	Positions*	Fragment length, bp
env	CTCCCTTCTAGTCGACGCTCCAGG GCCACCGTTACCGCTCGGCGGGAG	5684 −6151	467
tax	CGGATACCCAGTCTACGT GAGCCGATAACGCGTCCATCG	7358 −7517	159
tax	AACCTGTACACCCTCTGGG TATTTGGGGCTCATGGTCA	7743 −8324	580
LTR	CCCAAATATCCCCCGGGGGC TTTCGAAATTTCTCTCCTGAGAG	40 −757	717

Note. *: Primer positions are given on the basis of data of complete HTLV−1 genome sequencing [14].

DNA can pose a serious obstacle for the extensive use of the PCR method in the study of stored tissue specimens [12]. Therefore, the elaboration of new methods of DNA extraction from formalin-fixed and paraffin-embedded tissues as well as the modification of known methods are important prerequisites for conducting retrospective PCR analysis of archive material [6,7,15].

The present investigation explores the feasibility of using PCR for the analysis of DNA conventionally extracted from formalin-fixed and paraffin-embedded specimens of lymphoid tissue, and estimates the size of formalin-destroyed DNA fragments using HTLV-1 primers that can amplify DNA fragments of various length. The study was carried out on archive material of monkey lymphomas for the purpose of determining STLV-1 integration in DNA of the tumor cells.

MATERIALS AND METHODS

The object of the study was DNA extracted from formalin-fixed and paraffin-embedded lymph nodes affected with virus-induced malignant lymphomas from four brown macaques (*Macaca arctoides*). The lymphomas had been induced in experiments performed in 1969-1974 at the Sukhumi Primatology Center [2]. Electron microscopic study of ultrathin sections of hemopoietic organs, lymphocytes, and plasma sediments from macaques with lymphomas revealed the presence of retroviral C-type particles. The discovered virus differed from all mammalian C-type retroviruses known at that time according to viral-immunological and molecular biology characteristics [1] and was arbitrarily named "plasma virus." For histological study tissue was fixed in nonbuffered formalin for 5-10 days and embedded in paraffin. The specimens were analyzed in PCR after 18 to 23 years of storage.

Isolation of DNA. Pieces of formalin-fixed and paraffin-embedded tissue weighing approximately 2 mg were cut from the block with a disposable scalpel, minced, and placed in 10-ml plastic centrifuge tubes. Specimens were deparaffinized by incubation in 1 ml of xylene for 10 min at 55°C, and sedimented by centrifugation at 8000 rpm (300 g) for 5 min. This procedure was repeated two times, xylene was decanted, and its residues were removed by the addition of 1 ml of 95% ethanol. The material sedimented from ethanol was mixed with 2 ml of buffered solution containing 150 mM NaCl, 10 mM Tris-HCl (pH 7.5), 10 mM EDTA (pH 8.0), and 2% SDS, and shaken. Then 40 μl of proteinase K (500 μg/ml) were added and the mixture was incubated for 20 min at 65°C. After subsequent incubation for 12 hours at 37°C, 2 ml of buffered solution containing 650 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 10 mM EDTA were added to the material. DNA was isolated by means of routine phenol-chloroform extraction and precipitated with a solution of 0.1 M Na acetate (pH 5.2) in 10 ml ethanol. Precipitated DNA was sedimented by centrifugation for 30 min at 4000 g, washed with 70% ethanol, dried, and resuspended in a buffered solution of 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA (pH 8.0). The DNA concentration was recorded on the spectrophotometer at wavelength 260 nm. Specimens were stored at 4°C before use in PCR.

PCR amplification. Amplification was performed according to the Gene Amply Kit protocol (Perkin-Elmer-Cetus) with the following modifications. To 1 μg of genomic DNA 40 μl of the mixture containing 100 ng of each primer, 0.25 mM of each deoxynucleotide (Pharmacia), 25 mM Tris-HCl (pH 9.0), 2 mM MgCl2, 50 mM NaCl, and 2 units of Tag (*Thermus aquaticus*) DNA-polymerase (Perkin-Elmer-Cetus) were added. In order to prevent evaporation, the mixtures were covered with 50 μl mineral oil (Sigma). Forty cycles of amplification of the fragments of genomic DNA were conducted in an automatic thermal cycler

TABLE 2. Results of PCR with Specimens of DNA Extracted from a Collection of Tumor-Involved Lymph Nodes of *M. arctoides* Using HTLV-1 Primers

DNA specimen	env, 467 bp	tax, 580 bp	tax, 159 bp	LTR, 717 bp
1	+	–	+	–
2	+	–	+	–
3	+	–	+	–
4	+	–	+	–
MAL-1*	+	+	+	+

Note. *: DNA of STLV-1 obtained from an STLV-1-positive MAL-1 cell culture.

(Perkin-Elmer-Cetus) according to the following regime: 94°C - 2 min, 60°C - 1 min, 72°C - 1 min. The samples of amplification product (10 μl) were visualized by electrophoresis in 2% minigel with 0.5 μg/ml ethidium bromide. DNA extracted from STLV-1/HVMA positive MAL-1 culture served as a positive control, and PCR product in the absence of DNA as a negative control.

Oligonucleotide primers. On the basis of data of complete HTLV-1 genome sequencing [15], four pairs of primers were chosen for amplification of conserved fragments of STLV-1 provirus LTR, env, and tax (Table 1). Synthesis of primers was carried out on an Applied Biosystems 381A synthesizer with subsequent routine purification.

RESULTS

PCR with HTLV-1 env primers (467 base pairs - bp) and tax primers (159 bp) and samples of DNA extracted from stored specimens of *M. arctoides* tumor-involved lymph nodes disclosed integration of HTLV-1 related T-lymphotropic STLV-1 virus. At the same time, PCR with the same DNA specimens and primers that amplify longer fragments of DNA (tax - 580 bp, LTR - 717 bp) yielded negative results (Table 2).

It is known that formalin causes the formation of methyl bonds between DNA purine bases and histones [16]. This reaction affects the efficiency of extraction and induces DNA fragmentation, thus lowering the sensitivity of PCR [10]. PCR sensitivity also drops as a function of the fixation time [12] and the duration of specimen storage [5]. Therefore, in work with materials stored as paraffin blocks for more than 5 years it is recommended to use primers that amplify DNA fragments of no more than 200 bp length [6,15]. In the present study we have shown that DNA isolated from routinely fixed tissue by means of conventional phenol-chloroform extraction is quite suitable for amplification of fragments of 467 bp length. Long-term storage of paraffin blocks is not an obstacle for their use in PCR, as is illustrated by our discovery of STLV-1 integration in tumor DNA of *M. arctoides* that had died of malignant lymphoma in 1969-1974.

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