

## Isolation of STLV-I from Orangutan, a Great Ape Species in Southeast Asia, and Its Relation to Other HTLV-Is/STLV-Is

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To study the evolutionary origin of human T-lymphotropic virus type I/simian T-lymphotropic virus type I (HTLV-I/STLV-I), we isolated and characterized STLV-I from orangutans (*Pongo pygmaeus*). Plasma samples from 3 out of 41 animals examined were reactive by particle agglutination and immunofluorescence, and one of these three was confirmed to be anti-HTLV-I antibody-positive by western blotting (WB). Cultured peripheral blood mononuclear cells from the WB-positive orangutan were reactive to anti-STLV-I-positive rhesus monkey plasma. The proviral long terminal repeat region was amplified by polymerase chain reaction and sequenced. A phylogenetic analysis indicated that orangutan STLV-I is related to the Melanesian group of HTLV-Is and other Asian STLV-Is, but the degree of divergence is considerable.

Key words: STLV-I/HTLV-I — Orangutan — Phylogenetic analysis — LTR — Indonesia

The evolutionary origin of human T-lymphotropic virus type I (HTLV-I), the etiological agent of adult T-cell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis, is attracting much attention. Currently, the simian counterpart of this virus, simian T-lymphotropic virus type I (STLV-I), which constitutes a group of highly related retroviruses together with HTLV-I, is suspected to have been the origin of HTLV-I through transmission from non-human primates to humans.

Previous molecular epidemiological analyses have phylogenetically classified HTLV-Is into three major groups: the Melanesian group, the central African group and the cosmopolitan group.<sup>1-5)</sup> Many STLV-I isolates have been obtained from various non-human primates in Africa and Asia, and it has been reported that the chimpanzee STLV-I is genetically closely related to the central African group of HTLV-I, forming a tight cluster in the phylogenetic tree.<sup>6)</sup> In addition, a recent study has revealed that HTLV-I from a pygmy in central Africa belongs to the same subcluster as African green monkey STLV-Is.<sup>7)</sup> Thus, fairly recent interspecies transmissions between human and non-human populations are suggested to have occurred in the central African area.

Conversely, the Melanesian group of HTLV-I isolated from native people (aborigines) in Papua New Guinea and Australia is known to have diverged phylogenetically

earlier than any other group of HTLV-I<sup>8)</sup> and has been postulated to be an ancestor of all HTLV-Is in the world by Sherman *et al.*<sup>5)</sup> In Asian non-human primates, STLV-I sequences have been reported from several macaque species,<sup>9-11)</sup> and STLV-I from Celebes macaques (*Macaca tonkeana*) has been shown to be related to the Melanesian HTLV-I group.<sup>12)</sup> We thus became interested in the isolation and phylogenetic analysis of STLV-I strains from anthropoid apes in Asia. This paper reports the first isolation of STLV-I from orangutans (*Pongo pygmaeus*), a great ape species inhabiting Southeast Asia, and its phylogenetic characterization.

First, we conducted a serosurvey of forty-one orangutans kept in five zoos on the island of Java, Indonesia. Plasma samples from the orangutans were screened for HTLV-I antibodies using the particle agglutination (PA) test (Serodia HTLV-I, Fujirebio, Tokyo) and an indirect immunofluorescence assay (IFA) using MT-1 cells as antigens. Reactive specimens were then tested by a western blotting assay (WB; PROBLLOT HTLV-I, Fujirebio). Six of 41 plasma samples were reactive by PA, and three of these that were also reactive by IFA were named INA001, INA004, and INA111; only one (INA004) of the three was finally confirmed to be positive by WB and the remaining two (INA001 and INA111) were judged indeterminate according to the manufacturer's instructions because detection of viral component bands was partial (Fig. 1). These three animals showed no clinical or hematological abnormality. For detection of STLV-I

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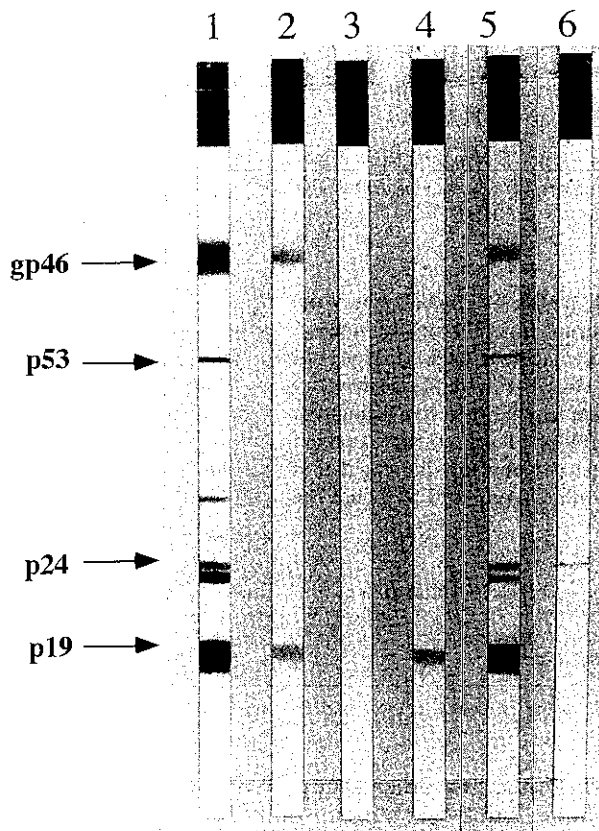


Fig. 1. Western blotting of plasma samples from orangutans (*Pongo pygmaeus*). Three specimens which were positive by IFA were named INA001, INA004, and INA111, respectively. Lane 1, HTLV-I strongly positive control; lane 2, HTLV-I weakly positive control; lane 3, negative control; lane 4, INA001 showing weak reactivity to only p19; lane 5, INA004 showing reactivity to p19, p24, p53, and gp46; lane 6, INA111 showing weak reactivity to only p24.

antigens, peripheral blood mononuclear cells were separated from heparinized whole blood and co-cultivated with or without human cord blood lymphocytes. Only INA004 cultured cells were reactive by IFA using anti-STLV-I-positive rhesus plasma, resulting in the isolation of STLV-I from orangutan for the first time. For detection of proviral DNA, we extracted DNA from the cells and the viral long terminal repeat (LTR) region was amplified by polymerase chain reaction (PCR) using the primers ATLTR11 (5'-ACTAAGGCTCTGACGTCTCCCC) and ATLTR12 (5'-CGGTACTTGGCCGTGGCCCAAGCCG) as previously described.<sup>6,13</sup> A positive PCR signal was detected in INA004, but not in INA001 or INA111. To examine the genetic relationship of this isolate with other known strains, we performed a

phylogenetic analysis based on the sequences in the LTR region. This region is one of the most variable within the viral genome and is thought to be evolutionally neutral because it is a non-coding region. Therefore, it is suitable for clarifying phylogenetic relationships.<sup>6,13</sup> The PCR products of the LTR region of INA004 were subcloned into the *Sma* I site of pUC119. The plasmid DNA of three positive clones was extracted and sequenced by the Taq dye deoxy-terminator cycle sequencing method (Applied Biosystems, Inc., CA) on an automated sequencer (Model 373A, Applied Biosystems, Inc.). The sequenced fragments were 513 bp long and corresponded to positions 122-628 in ATK, a prototypic HTLV-I strain (GenBank accession number J02029).<sup>14</sup> From the LTR sequence data, the total number of nucleotide substitutions was estimated by the six-parameter method<sup>15</sup> and a phylogenetic tree was constructed by the neighbor-joining method.<sup>16</sup> Furthermore, the phylogenetic stability of the branching was statistically evaluated by bootstrap analysis (100 bootstrap replications).

In the phylogenetic tree (Fig. 2), we included two STLV-I strains from Asian monkeys, for which LTR sequences were previously reported.<sup>10,12</sup> One (TE4) was from Celebes macaques and the other (PTM3) was from pig-tailed macaques (*Macaca nemestrina*). The position of INA004 in the phylogenetic tree was closer to the Melanesian group (PNG1 and MEL5) of HTLV-I together with TE4 than to any other HTLV-I/STLV-I. But the bootstrap probability of INA004 was not high (43% to TE4 and 39% to the Melanesian group) and it diverged very early, resulting in a unique position distinct from the Melanesian group of HTLV-I.

A sequence analysis of INA004 revealed that, although the known functional regions such as the TATA box and poly (A) signal were conserved, there were many sequence differences even within the 513-base fragment. Within this fragment, there were 73, 69, 62 and 50 base substitutions, 14, 10, 8 and 17 insertions, and 0, 1, 0 and 0 deletions when compared with TE4 (16.8% divergence), PNG1 (15.8% divergence), MEL5 (13.7% divergence) and PTM3 (16.8% divergence), respectively. These results indicated that the strain INA004 has significantly diverged from other Asian HTLV-I/STLV-I strains. Our findings appear to show that the orangutan STLV-I has undergone an intrinsic evolution over a very long period within this species. This is in marked contrast to the close relationship between the chimpanzee STLV-I (ChM114-1) and central African HTLV-I strains. Interestingly, all the STLV-I strains of Asian origin commonly possessed very long horizontal lines in the tree. Further study of STLV-I strains in other monkey and ape species in Southeast Asia is necessary to shed new light on the origin of the primate retrovirus group.

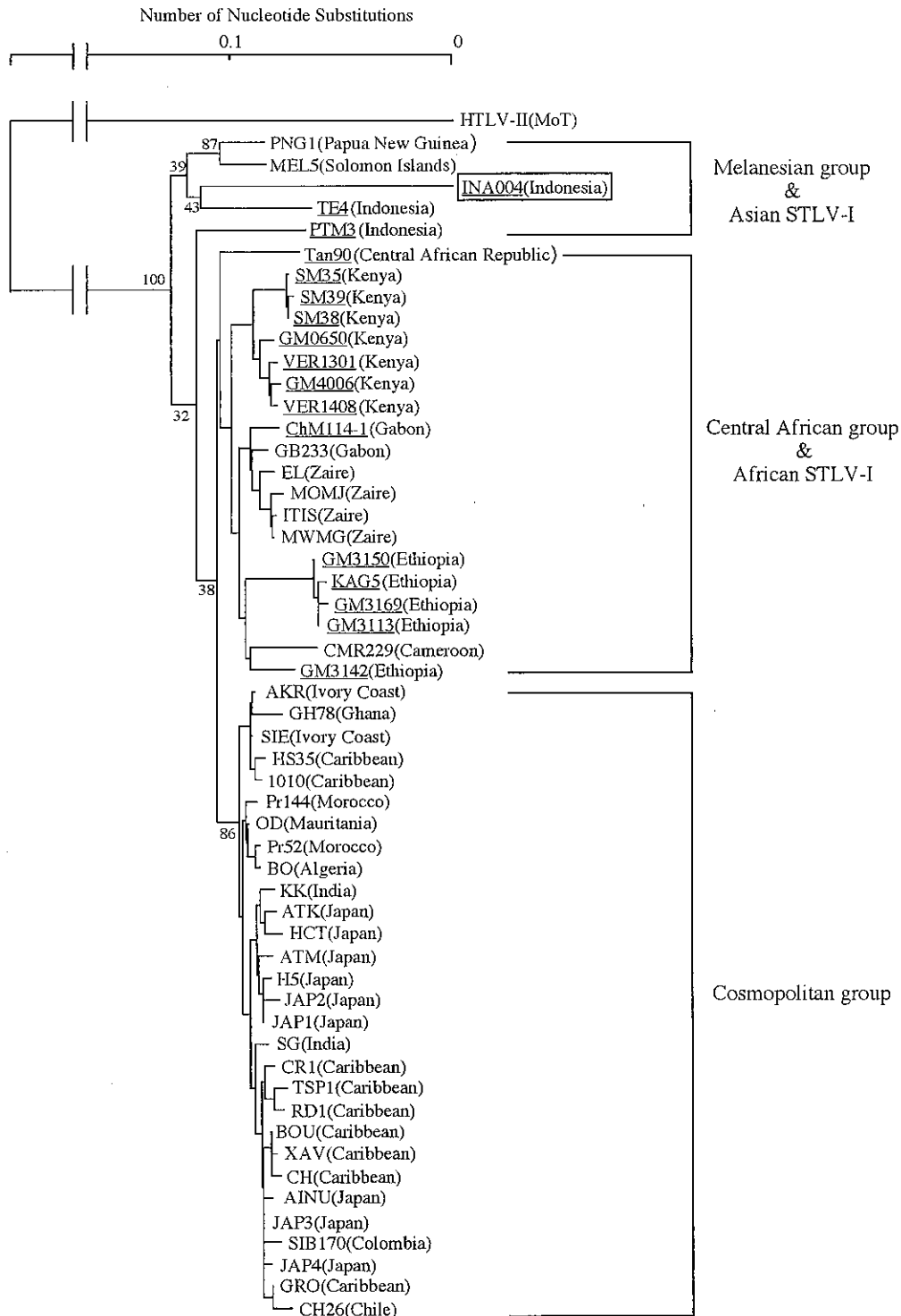


Fig. 2. Phylogenetic tree of HTLV-Is/STLV-Is based on sequences in the LTR region. The tree was constructed by the neighbor-joining method with a fragment of 507 bp of the LTR as described.<sup>6,13)</sup> The number of nucleotide substitutions per site was calculated by the six-parameter model.<sup>16)</sup> The bootstrap statistical analysis was performed by 100 bootstrap replications and the values (in %) are indicated beside the branches. The STLV-I isolates are underlined in this tree. INA004 (boxed) is an STLV-I isolate from an orangutan (*Pongo pygmaeus*). The GenBank accession number for INA004 is U53562.

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