Identification and phylogenetic characterization of a human T-cell leukaemia virus type I isolate from a native inhabitant (Rapa Nui) of Easter Island

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Human T-cell leukaemia virus type I (HTLV-I) is endemic in Melanesia, one of the three ethnogeographic regions of the Pacific; in the other two regions, Polynesia and Micronesia, the incidence of the virus is relatively low. In an effort to gain new insights into the prevalence of HTLV-I in the Pacific region, we did a seroepidemiological survey on Easter Island, which is located on the eastern edge of Polynesia. Of 138 subjects surveyed, including 108 Rapa Nui (the native inhabitants of this island), we identified one HTLV-I-seropositive Rapa Nui. The new HTLV-I isolate derived from this carrier (E-12) was phylogenetically analysed to ascertain the origin and past dissemination of HTLV-I in the island. The analysis demonstrated that isolate E-12 belongs to subgroup A of the Cosmopolitan group, and that it differs from HTLV-Is found in Melanesia, which are highly divergent variants. In subgroup A, E-12 grouped with South American HTLV-Is including those from Amerindians. This result suggests that this isolate originated in South America rather than in Melanesia.

Introduction

Human T-cell leukaemia virus type I (HTLV-I) is a human retrovirus and is considered to be the aetiological agent of adult T-cell leukaemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). This virus is endemic mainly in Africa, Japan, South America, the Caribbean basin and the Melanesian region (Hinuma et al., 1981; Yanagihara et al., 1990), although sporadic infections with the virus have been found in almost all parts of the world (Tajima & Hinuma, 1992). Phylogenetic studies on various HTLV-I isolates (‘HTLV-Is’) have indicated that HTLV-I can be classified into three major groups: the Cosmopolitan (HTLV-Ia) (Gessain et al., 1992), Central African (HTLV-Ib) (Gessain et al., 1992) and Melanesian (HTLV-Ic) groups (Gessain et al., 1993; Nerurkar et al., 1993). Distinct HTLV-I variants of the Central African group recently identified in Central Africa (Chen et al., 1995; Mahieux et al., 1997; Mboudjeka et al., 1997) have been proposed as a fourth group, HTLV-IId, by Mahieux et al. (1997). The Cosmopolitan group is further subdivided into four minor subgroups: the Transcontinental (A), Japanese (B), West African (C) and North African (D) subgroups (Miura et al., 1994; Vandamme et al., 1994; Yamashita et al., 1996). As their names suggest, these phylogenetic groups and subgroups are generally associated with the geographical origins and ethnic backgrounds of the virus carriers. For instance, HTLV-Is from the Melanesian region are members of the Melanesian group, while the vast majority of South American HTLV-Is belong to subgroup A of the Cosmopolitan group. This correlation is not unreasonable when we consider that the efficiency of transmission of HTLV-I is remarkably low and...
thus horizontal spread through a human population is rare. The low transmission rate is probably the result of a natural transmission mode that requires close and frequent contacts, such as breast feeding and habitual sexual intercourse.

The islands of the Pacific Ocean have been intensively surveyed for the presence of HTLV-I because of their geographical proximity to known endemic areas of infection. This region is not only adjacent to the two endemic foci of HTLV-I in Japan and South America, but it also includes another endemic focus, Melanesia, which is one of the three ethnogeographic regions (Melanesia, Micronesia and Polynesia) of the Pacific Ocean. In contrast, past seroepidemiological surveys have uncovered little evidence of this virus in the Micronesian and Polynesian regions (Tajima et al., 1991; Yanagihara, 1994), except for the cases of HTLV-I infection among some Japanese Americans living in Hawai (Blattner et al., 1986; Ureta Vidal et al., 1994a). During a recent survey of HTLV-I in Chile, we took advantage of an opportunity to conduct a seroepidemiological survey of the population of Easter Island, which is located on the eastern edge of Polynesia, thereby hoping to gain new insights into the prevalence of this virus in the Pacific region. During the survey, we identified one HTLV-I-seropositive case among the Rapa Nui, the native inhabitants of this island. A new HTLV-I isolate derived from this carrier (E-12) was phylogenetically analysed in an attempt to understand the origin and past dissemination of HTLV-I on the island.

**Methods**

- **Subjects.** In 1996, we performed a seroepidemiological study on HTLV-I infection among 138 inhabitants of Easter Island. Most of the inhabitants studied were Rapa Nui (35 males (mean age 49.7 years) and 73 females (mean age 45.2 years)), while the others included 26 people of mixed blood (6 males (mean age 39.8 years), 20 females (mean age 34.1 years)), 3 Chilean males (mean age 45.7 years) and 1 Caucasian female (age 39 years). To detect and titrate HTLV-I antibodies in plasma, a particle agglutination (PA) test (Fujirebio, Tokyo, Japan) was used. For confirmation, we used an enzyme-immunoassay (EIA) and a Western blot assay (HTLV-I/II BLOT2.4; Diagnostic Biotechnology, Singapore). In the Western blot assay, a serum sample was considered HTLV-I-positive when it reacted to Gag (p19 or p24) and two Envs. One of the Envs was the HTLV-I envelope recombinant gp46 peptide (MTA-1) and the other was a common yet specific HTLV-I and HTLV-II epitope, recombinant gp21 protein (GD21). Human leukocyte antigen (HLA) haplotypes of seropositive subjects were determined as described previously (Blank et al., 1995).

- **PCR.** DNA was extracted by a conventional method, using proteinase K, from peripheral blood mononuclear cells which were obtained from whole blood samples by the Ficoll gradient method. Thereafter, DNA was subjected to nested PCR to amplify a part of the long terminal repeat (LTR) region approximately 590 bp long and corresponding to positions 99 to 685 in ATK, the prototype Japanese HTLV-I strain (Seiki et al., 1983). The nested PCR conditions and the oligonucleotide primers for amplification were described previously (Yamashita et al., 1995). In addition, we amplified a part of the env gene (522 bp long) which includes the carboxyl terminus of gp46 and almost the entire transmembrane protein gp21, as described previously (Mboudjeka et al., 1997). Special care was taken in the PCR procedure to avoid contaminating the amplified products. All the genomic DNAs were manipulated in a room free from the amplified products, and a negative control was used in each PCR experiment.

- **Subcloning and sequencing.** The amplified LTR region fragments were subcloned into plasmid vector pUC119 using the TA cloning method. Approximately 500-bp-long sequences of the LTR region (positions 122 to 628 in ATK) were determined from the cloned PCR products. We sequenced one clone, since nucleotide sequences from different clones of a sample were virtually identical in our previous investigation. The fragment was sequenced in both directions, yielding a 507-bp-long nucleotide sequence. A fragment of the partial env gene was directly sequenced after purification of the PCR products by phenol-chloroform extraction. The nucleotide sequences were determined by using an automated DNA sequencer (Applied Biosystems). The new nucleotide sequences in the present study have been deposited in GenBank (accessions nos AF013221 and AF132300).

- **Phylogenetic analysis.** For construction of phylogenetic trees, each set of nucleotide sequences, both newly obtained and previously reported, was aligned by using the computer software CLUSTAL W (Thompson et al., 1994) and minor manual modifications. All phylogenetic
trees in the present study were constructed by using three methods: the neighbour-joining (NJ) method (Saitou & Nei, 1987), the maximum parsimony (MP) method and the maximum likelihood (ML) method. We used CLUSTAL W for construction of the NJ trees and PHYLIP version 3.52 (Felsenstein, 1993), a phylogeny inference package, for construction of the MP trees. In order to ascertain the robustness of the constructed NJ trees, bootstrapping (Felsenstein, 1985) was done to generate 100 resamplings of the original sequence alignments and pairwise genetic distances were estimated for each resampling by Kimura’s two-parameter method (Kimura, 1980). For the MP trees, 100 resamplings of the original alignment were generated using the SEQBOOT program and then the most parsimonious trees were generated from bootstrapped sequence data using the DNAPARS program. The majority-rule consensus MP tree was generated using the CONSENSE program. The ML trees were generated using the DNAML program (global rearrangements, randomized input order, and outgroup rooting options ‘on’). The empirical transition/transversion bias was 4.5 for the partial LTR (507 bp) analysis and 3.2 for the gp21 env (522 bp) analysis. Since the statistical evaluation of the branch length and branching nodes is a built-in feature of the DNAML program, bootstrapping was not done in the ML method. The trees were visualized with TREEVIEW (Page, 1996). In this report, only the phylogenetic trees constructed by the NJ method are shown because the trees constructed by the MP and ML methods were virtually identical to those constructed by the NJ method.

Results

Of the 138 inhabitants of Easter Island, one 58-year-old Rapa Nui female (E-12) was found to be PA- and EIA-positive. The titre of HTLV-I antibodies in this subject was 1/256 in the PA test. Seropositivity of the subject was confirmed by a Western blot, showing specific HTLV-I antibodies with p24, p19, rgp46-I and GD21 seroreactivity (Fig. 1). The other subjects tested were seronegative by the PA test. The seropositive subject had no history of blood transfusion and denied intravenous drug use. We had no information about
any history of homosexual or nonmonogamous heterosexual activity in this subject. The sister of E-12 (34 years old) was seronegative by the PA test, while the subject’s husband and parents were not studied. HLA analysis of E-12 indicated that the inferred HLA haplotypes are A2 B55/B56 DR12/A2 B39 DR4, suggesting that this patient is of mixed blood with Amerindian and Maori ancestry (Imanishi et al., 1992). Maoris are the indigenous inhabitants of New Zealand. These observations indicate that HTLV-I does exist but is rare on Easter Island.

In order to ascertain the evolutionary relationships of this new HTLV-I isolate with other HTLV-Is of various geographical origins, we phylogenetically characterized the new isolate. A phylogenetic tree constructed with the NJ method and based on the partial LTR region (507 bp) is shown in Fig. 2. The new HTLV-I isolate (E-12) is clearly a member of the Cosmopolitan group and furthermore, it belongs to the Transcontinental (A) subgroup. Phylogenetic trees constructed with the MP and ML methods gave essentially the same results. Despite the subject’s geographical location (Easter Island) and ethnic origin, the new isolate did not show strong similarity to HTLV-Is from the Melanesian region. In fact, the LTR sequence of this isolate differed from MEL5 (an isolate of the Melanesian group) by 8.3%, whereas only 2.6% difference was observed between E-12 and ATK (the prototypic strain of HTLV-I, which belongs to the Cosmopolitan group). This was also confirmed by the restriction fragment length polymorphism (RFLP) profile according to the classification system proposed by Ureta Vidal et al. (1994b). The RFLP pattern of E-12 indicated that the LTR of E-12 had the MaeII, Ndel and SacI sites, but not the AvaI and MaeIII sites, which was consistent with the RFLP pattern of subgroup A and differed from that of the Melanesian group. These findings were further confirmed by comparison of the gp21 env gene nucleotide sequences of E-
Fig. 4. Phylogenetic tree of HTLV-Is based on the partial env gene (522 bp). The tree was constructed by using the NJ method. Mel5 was used as the outgroup. For other details, see the legend to Fig. 2.

12 and Melanesian HTLV-I (data not shown). The partial env of E-12 belonged to the Cosmopolitan group based on an encoded amino acid substitution that is specific to this group: the E-12 env encodes a V at position 230 in ATK, while the other groups have M or I. These results indicate that E-12 belongs to subgroup A of the Cosmopolitan group but differs markedly from the Melanesian group of HTLV-I (HTLV-Ic).

In a tree, constructed with the NJ method, that includes almost all the HTLV-I isolates of subgroup A (Fig. 3), the new isolate (E-12) clustered with HTLV-Is of South American origin. Although the bootstrap value supporting this cluster was not very high, we observed two common nucleotide variations among all (old and new) HTLV-Is in the cluster: C at position 479 and G at position 528 (the positions correspond to those of ATK), whereas all the other HTLV-Is of subgroup A have T at position 479 and A at position 528. It is interesting that most HTLV-Is from Colombian Amerindians have been shown to belong to the same cluster that the new isolate is in (Miura et al., 1994). Phylogenetic trees based on the MP and ML methods reproduced this cluster, supporting the close relatedness between E-12 and South American HTLV-Is.

Cosmopolitan HTLV-Is have been identified in Bellona Island, a Polynesian outlier within the Solomon archipelago (Yanagihara et al., 1991). These HTLV-Is were genetically analysed based on the partial env gene (Gessain et al., 1991; Nerurkar et al., 1993). To compare the new HTLV-I (E-12) with these isolates, we sequenced a part of its env gene (522 bp) (Fig. 4). The NJ tree based on the partial env gene showed that E-12 did not group with HTLV-Is from Bellona (BEL1 and BEL2), which showed more similarity to Far-East Asian isolates (MT2 and CMC). The trees based on the MP and ML methods also failed to disclose a relatedness between the HTLV-Is of Bellona and Easter islands. Instead, the HTLV-Is from Bellona Island have two nucleotide variations in common with two Far-East
Asian HTLV-Is (MT2 and CMC) (G at position 6168 and T at position 6219, whereas all the other HTLV-Is of the Cosmopolitan group, including E-12, have A at position 6168 and C at position 6219). Thus, these results do not reveal any strong association among Polynesian HTLV-Is.

Discussion

What is the source of the new HTLV-I isolate found in the Rapa Nui woman in this study? It is commonly believed that the Rapa Nui descended from Polynesians who came to Easter Island in about 400 AD. However, there has been extensive contact between the Rapa Nui and South Americans. For example, Peruvian slavers carried away almost all the island's males in the middle of the 19th century. The subsequent repatriation of some males introduced smallpox to Easter Island, which devastated the remaining native inhabitants. In addition, annexation of Easter Island by Chile in 1888 brought massive human migrations from South America. Thus, it is uncertain whether the present native inhabitants are really pure-blooded natives or not. Given the extensive contacts between Easter Islanders and South Americans during the past few centuries, and the generally accepted idea that HTLV-I was present in South America at least by the 19th century (Gessain et al., 1996; Yamashita et al., 1996), it is very likely that the new HTLV-I originated in South America. This is also supported by the infected subject's HLA haplotypes, which are frequently found in Amerindians. This may mean that the virus was from an Amerindian, some of whom are known to be infected with HTLV-Is that are closely related to the new isolate.

In contrast, the possibility for direct dissemination of HTLV-I from other Pacific islands to Easter Island must be quite low. The marked difference between the new HTLV-I from Easter Island and the HTLV-Is of the Melanesian region argues against the introduction of the new isolate from the Melanesian region. In addition, although Polynesians are considered to be the first settlers of Easter Island, a Polynesian origin of the new isolate also seems unlikely, as the two HTLV-Is of the Cosmopolitan group from Bellona Island were not closely related to the new isolate, based on the gp21 env gene sequence. Our recent study, in which HTLV-I isolate BEL-I was analysed based on the LTR region, supported the env-based phylogenetic analysis, showing that E-12 clusters with BEL-I (data not shown). Nonetheless, the present results do not completely exclude the possibility that the new isolate is a relic of a past endemic of the virus in Polynesia, since there are still few data on the prevalence of HTLV-I in Polynesia. On the other hand, sporadic infections recently identified in residents of the Marshall Islands and Nauru (Miller et al., 1998; Nicholson et al., 1992), both of which are situated in the Micronesian region, leave open the possibility of some transmission of HTLV-I from Micronesia to Easter Island. In summary, our findings strongly suggest that the new isolate of HTLV-I in Easter Island originated in South America. However, it is evident that more data are needed to understand the past dissemination of this virus in the Pacific region.

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References


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