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Verification of HTLV-I Infection in the Solomon Islands by Virus Isolation and Gene Amplification

Richard Yanagihara,[1,3] Andrew B. Ajdukiewicz,[2] Vivek R. Nerurkar,[1] Ralph M. Garruto[1] and D. Carleton Gajdusek[1]

[1]*Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, USA and* [2]*Ministry of Health and Medical Services, Central Hospital, Honiara, Solomon Islands*

We report the detection of human T-lymphotropic virus type I (HTLV-I) genomic sequences by polymerase chain reaction in lymphocyte cultures of three unrelated native Solomon Islanders, including a patient with HTLV-I myeloneuropathy, residing in widely separated regions. In addition, we have isolated HTLV-I from T-cell lines derived from two of these individuals. Virus-specific proteins of 15, 19, 24, 46 and 53 kilodaltons were detected by immunofluorescence and Western immunoblot, using serum from a Colombian patient with HTLV-I myeloneuropathy, sera from HTLV-I-infected rabbits, and monoclonal and polyclonal antibodies against HTLV-I *gag* and *env* gene products. Amplification of HTLV-I *gag*, *pol* and *env* sequences by polymerase chain reaction confirmed that the viral isolates were HTLV-I, not HTLV-II. Our data clearly demonstrate that HTLV-I does exist in Melanesia. Although the Solomon Islands viral isolates resemble prototype strains of HTLV-I, we believe they represent variants of HTLV-I, particularly in the light of our recent isolation of an HTLV-I variant from Papua New Guinea. Nucleotide sequence analysis of these viral strains, now in progress, should clarify the molecular epidemiology and phylogeny of HTLV-I.

Key words: HTLV-I — Retrovirus — Polymerase chain reaction — Melanesia

Whether or not infection with human T-lymphotropic virus type I (HTLV-I) is prevalent in Melanesia has been a controversial issue. On the one hand, high prevalences of antibodies against HTLV-I have been reported for several remote population groups in Papua New Guinea, West New Guinea, Solomon Islands and Vanuatu, using enzyme immunoassay and gelatin particle agglutination.[1-11] On the other hand, the claims of HTLV-I hyperendemicity in Melanesia have been contested because of the inability to verify screening results by confirmatory tests and the failure of Melanesian sera to neutralize a prototype strain of HTLV-I.[5,12] By applying stringent Western immunoblot criteria,[13] we have demonstrated HTLV-I seroprevalences of 2% to 10% among hospitalized patients from four provinces of the Solomon Islands.[14] In addition, we have recently identified the first case of HTLV-I myeloneuropathy in Melanesia in a life-long resident of Guadalcanal.[15] We now report the detection of HTLV-I genomic sequences by polymerase chain reaction (PCR) in lymphocytes obtained from this patient and the isolation of HTLV-I from T-cell lines derived from two other unrelated individuals residing in widely separated regions of the Solomon Islands.

[3] To whom requests for reprints should be addressed: National Institutes of Health, Bldg. 36, Rm. 5B-21, Bethesda, Maryland 20892.

In March 1990, blood specimens were collected, after obtaining prior informed consent, from three native Solomon Islanders, who were previously identified as being HTLV-I seropositive by immunofluorescence and Western analysis.[14] Patient 1 was a 38-year-old man with HTLV-I myeloneuropathy from Guadalcanal in Guadalcanal Province; patient 2 was a 40-year-old woman with a history of transfusion-acquired hepatitis from Marovo in Western Province; and patient 3 was a 60-year-old woman with diabetes mellitus and renal insufficiency from Bellona in Central Province. Peripheral blood mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation 36 h after collection in a laboratory in which HTLV-I and other human or animal retroviruses had not been handled. Cells were stimulated for 3 days with phytohemagglutinin (PHA) (Wellcome Diagnostics, Dartford, England) at 2 μg per 10^6 cells, after which they were cultured independently, as well as cocultivated with an equal number of PHA-stimulated umbilical cord blood mononuclear cells obtained from healthy, HTLV-I-seronegative Caucasian neonates (Advanced Biotechnologies, Inc., Columbia, MD). Cultures were maintained at 37°C under a 5% CO_2 atmosphere with biweekly media changes of RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum, 10% interleukin 2 (IL-2) (Advanced Biotechnologies), 2 m*M* *l*-glutamine and 50 μg of gentamicin per ml. At

weekly intervals, cultured lymphocytes were spotted onto 10-well slides (Cell-line Associates, Newfield, NJ), then fixed with acetone for 10 min, and examined for the expression of HTLV-I antigens by the indirect immunofluorescent antibody technique, using monoclonal antibodies against HTLV-I p19 and p24 (Cambridge Biotech Corp., Rockville, MD), sera from rabbits experimentally infected with a Colombian strain of HTLV-I,[16] and sera from patients with HTLV-I myeloneuropathy.[15,17] Virus-specific antibodies were then detected using either rhodamine-labeled goat antibodies against mouse or rabbit IgG F(ab')2 (Accurate Chemical & Scientific Corp., Westbury, NY), or fluorescein isothiocyanate-labeled goat antibodies against human IgG (Cappel Laboratories, Inc., Cochranville, PA). Appropriate dilutions of HTLV-I antibody-negative sera, as well as HTLV-I-infected (MT-2)[18] and uninfected T-cells (MOLT-3) (American Type Culture Collection, Rockville, MD) served as controls.

Four IL-2-dependent T-cell lines, designated SI-1, SI-2, SI-3 and SI-4, were established from patients 2 and 3. All T-cell lines grew as clumpy suspensions, and one (SI-3 from patient 3) has been maintained in continuous culture for nearly 9 months. Of the four T-cell lines, only one (SI-1 from patient 2) expressed CD4 at high density (80%), while CD8 expression was predominant (80% to 95%) in the other three. By immunofluorescence, viral antigen was evident in more than 50% of cells, using autologous sera, sera from patients with HTLV-I myeloneuropathy, and sera from rabbits experimentally infected with HTLV-I, as well as monoclonal antibodies against HTLV-I *gag* proteins p24 and p19 (Fig. 1). No staining was observed with HTLV-I antibody-negative sera or with a monoclonal antibody against HTLV-II *gag*-encoded protein p24. Mature and immature viral particles resembling HTLV-I were readily observed by thin-section electron microscopy in all four T-cell lines (Fig. 2). Western analysis of viral pellets, prepared by centrifuging culture medium for 1 h at 35,000 rpm

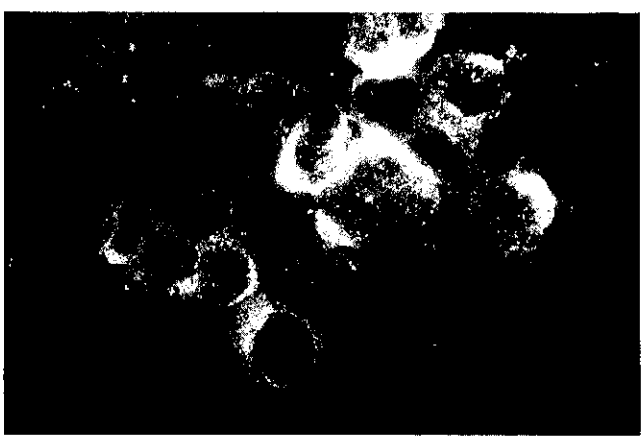

Fig. 1. Intracytoplasmic virus-specific fluorescence in SI-1, a T-cell line derived from a 40-year-old woman with a history of transfusion-acquired hepatitis, using a monoclonal antibody against HTLV-I *gag*-encoded protein p19. (Original magnification, ×500)

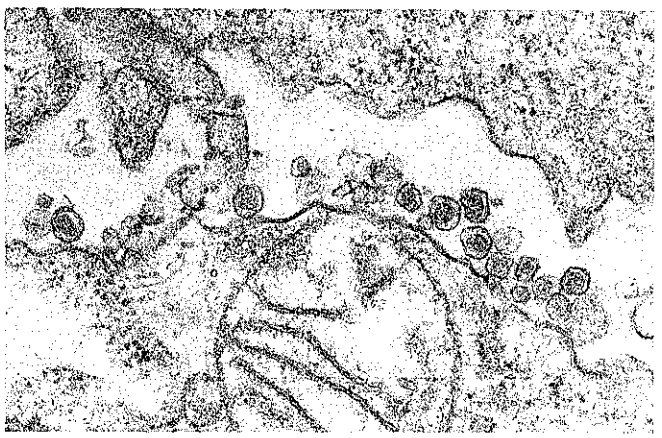

Fig. 2. Extracellular virus particles resembling HTLV-I in SI-1, a T-cell line derived from a native Solomon Islander (patient 2). (Original magnification, ×36,000)

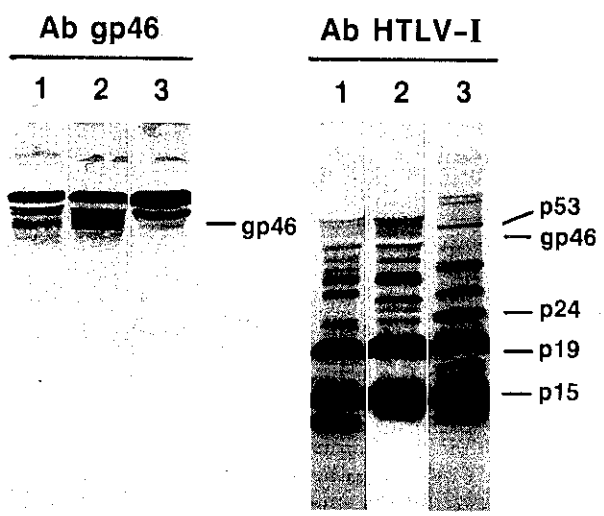

Fig. 3. Western analysis of (lanes 1 and 2) HTLV-I isolates from two Solomon Islanders (patients 2 and 3) and of (lane 3) a prototype strain of HTLV-I (MT-2), using a serum from a rabbit immunized with an 18-residue synthetic peptide spanning the extreme C-terminus of the major envelope glycoprotein gp46[19] (Ab gp46) and serum from a rabbit experimentally infected with HTLV-I[16] (Ab HTLV-I).

(100,000*g*) in a Beckman 50.2 Ti rotor, revealed virus-specific proteins with molecular weights of 15, 19, 24, 46 and 53 kilodaltons (Fig. 3), using monoclonal and polyclonal antibodies against HTLV-I structural proteins, including a rabbit antiserum prepared against the C-terminus of the major envelope glycoprotein gp46.[19] An HTLV-I-infected cell line was not established from the Solomon Islander with HTLV-I myeloneuropathy (patient 1). Instead, after 14 weeks in culture, an IL-2-independent B-cell line infected with Epstein-Barr virus (as determined by PCR and electron microscopy) resulted.

To verify further that the viral isolates were HTLV-I rather than HTLV-II, high-molecular-weight DNA was extracted from approximately 10^7 cells from each culture at 4 weeks and from each T-cell line, using a non-organic method (Oncor, Gaithersburg, MD), and the DNA was subjected to PCR analysis. Oligonucleotide primer pairs, specific for *gag* (bases 863–886 and 1375–1397 for p19; bases 1423–1444 and 1537–1560 for p24),[20] *pol* (SK54, bases 3365–3384; SK55, bases 3465–3483)[21] and *env* (bases 5684–5707 and 6128–6151)[20] sequences of a prototype strain of HTLV-I,[22] were synthesized on a PCR-Mate DNA synthesizer (Applied Biosystems, Foster City, CA). The reaction mixture consisted of 1 μM each oligonucleotide primer pair, 1 μg of DNA, 2.5 units of *Thermus aquaticus* DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 0.05% Nonidet P-40, and 0.2 mM each of dATP, dCTP, dGTP and dTTP. Following denaturation at 93°C for 4 min, the reaction mixtures were cycled 35 times at 93°C for 1 min, 55°C for 2 min and 72°C for 3 min. Amplified DNA was size-fractionated by agarose-gel electrophoresis and transferred to nylon membranes (Nytran, Schleicher & Schuell, Dassel, Germany) for hybridization, under high stringency, with oligoprobes end-labeled with [α-^{32}P]dCTP or with a full-length HTLV-I probe labeled with ^{32}P by nick translation (Oncor). At 4 weeks, HTLV-I *gag* and *env* sequences were detected in cultures from patients 2 and 3, and HTLV-I *gag* but not *env* sequences were detected in patient 1. As verification that the isolates were HTLV-I, DNA from the T-cell lines derived from patients 2 and 3 contained HTLV-I-specific *pol* gene sequences (Fig. 4).

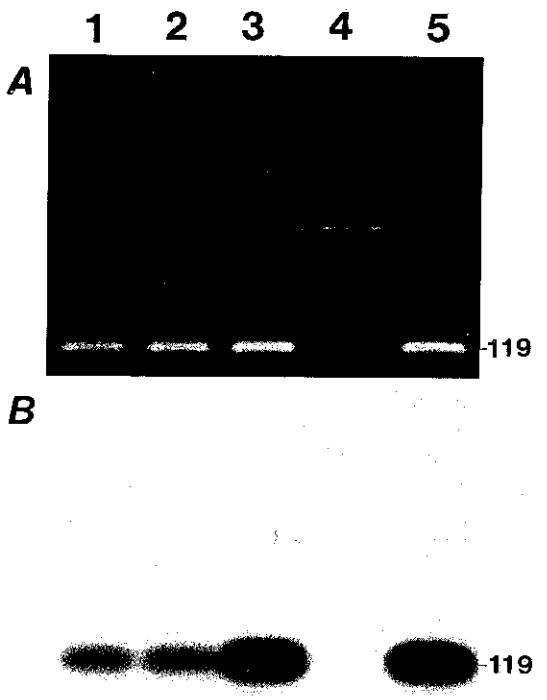

Fig. 4. (A) Ethidium bromide-stained agarose gel showing the 119-bp HTLV-I *pol* sequence amplified by PCR and (B) Southern analysis of the amplified product hybridized, under high-stringency conditions, with a ^{32}P-labeled HTLV-I *pol*-specific 35-mer oligoprobe (SK56, bases 3426–3460).[21] PCR was performed on DNA isolated from (lanes 1–3) SI-1, SI-2 and SI-3, three T-cell lines derived from two Solomon Islanders (patients 2 and 3), (lane 4) MOLT-3, an uninfected T-cell line derived from a patient with acute lymphoblastic leukemia and (lane 5) MT-2, an HTLV-I-infected T-cell line derived from a Japanese patient with adult T-cell leukemia.[18]

Our earlier studies of more than 3500 sera, collected between 1956 and 1988 from 34 Melanesian populations, for antibodies against HTLV-I indicated high prevalences of infection, as verified by strict Western immunoblot criteria, in several remote population groups having had no contact with Japanese or Africans and minimal or no contact with Europeans prior to our bleedings.[1, 3, 9, 10, 14] By contrast, some Micronesian populations having had intense contact with Japanese for more than five decades have no evidence of infection.[4, 9] These data argue against the dissemination of HTLV-I in the Pacific basin by the Japanese. The detection of HTLV-I genomic sequences by PCR and the successful isolation of HTLV-I from unrelated individuals from widely separated provinces in the Solomon Islands, reported here, provide further evidence in support of our serological data.

We have long maintained that the high prevalences of HTLV-I infection in Melanesia is due to a variant or variants of HTLV-I rather than to prototypical strains of HTLV-I. Although the viral isolates from native Solomon Islanders resemble prototype HTLV-I by immunofluorescence, Western immunoblot and PCR, our recent isolation and characterization of an HTLV-I variant from Papua New Guinea,[23, 24] suggest that the Solo-

mon Islands viral strains may also diverge significantly from prototype HTLV-I. Nucleotide sequence analysis of these viral isolates and comparisons with prototype strains, currently under way, are expected to provide important insights into the phylogeny of HTLV-I.

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