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Human T-Lymphotropic Virus Type II in Japan

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Serum specimens were assayed for human T-lymphotropic virus type II (HTLV-II) infection in 1,500 individuals known to be seropositive for HTLV-I and 30,000 blood donors in Japan. All HTLV-I-positive individuals were negative for HTLV-II. However, one of the blood donors was clearly seropositive for HTLV-II. Further, the donor was shown to be positive for HTLV-IIb. Here we report at least one case with HTLV-II in Japan and discuss the origin of the infection.

Key words: HTLV-II — Seropositivity for HTLV-II — Japanese blood donor

Human T-lymphotropic virus types I and II (HTLV-I/-II) are structurally closely related viruses that similarly infect and immortalize normal human T cells.[1–3] Although HTLV-I is endemic in Japan, studies to determine if HTLV-II infection also occurs have produced conflicting results. Initial studies using polymerase chain reaction (PCR) suggested that HTLV-II infection could be detected in HTLV-I-positive blood donors and patients with HTLV-I associated myelopathy (HAM) in the southern part of Japan.[4,5] However, in subsequent studies, patients with adult T-cell leukemia (ATL) or HAM have been shown to be seronegative for HTLV-II.[6,7] To further address this question, we have serologically analyzed blood specimens from 1,500 individuals known to be positive for ATL-associated antigens (ATLA), and serum samples from 30,000 blood donors in the western part of Japan.

Serum samples were screened by means of the gelatin particle-agglutination test (Serodia-ATLA, Fujirebio Inc., Tokyo) for HTLV in a primary screening assay, followed by type-specific HTLV immunoblotting (HTLV BLOT 2.3, Diagnostic Biotechnology Pte Ltd., Singapore).[8–10] One of 1,500 HTLV-I-positive sera was slightly positive for antibody to rgp46-II and strongly positive for antibodies to various HTLV antigens (Fig. 1A), suggesting cross-reactivity of an HTLV-I antibody to rgp46-II antigen or double infection of HTLV-I and HTLV-II. To determine whether the antibody binding to rgp46-II noted in the immunoblot reflected HTLV-II infection of the individual, we have amplified genomic DNA from the peripheral blood mononuclear cells (PBMC) of the individual by PCR specific for the HTLV-II *pol* gene.[11] The result was negative for HTLV-II infection, suggesting the presence of cross-reactive antibody to the HTLV-II antigen in the ATLA-positive serum (data not shown). In contrast, we have identified a case seropositive for HTLV-II but negative for HTLV-I by type-specific Western blotting for HTLV among blood donors (Fig. 1B).

A subtype-specific, peptide-based, enzyme-linked immunosorbent assay (ELISA) of HTLV-II was used to assay serum from the HTLV-II-seropositive donor as described previously.[9] For this ELISA, 4 serum specimens from HTLV-IIa-infected intravenous drug abusers (IVDAs) in South Vietnam and 9 serum specimens from HTLV-IIb-infected IVDAs were used as reference sera.[8–10] The HTLV-II-seropositive donor was shown to be clearly positive for HTLV-IIb using the reference sera (Fig. 2).

As PBMC of the HTLV-IIb-seropositive donor were not available for molecular virus analysis, we performed reverse transcription (RT) of ultracentrifuged DNase-nontreated plasma with 3′ outer primer (nt 807 to 831 of HTLV-II MoT cells = 5′-GGGAAAGCCCGGTTGGGATTTGGCCCCCAT-3′) to synthesize cDNA as a template for HTLV-II long terminal reports (LTR) nested PCR.[12] The positions of the oligonucleotides are numbered relative to the MoT isolate in the Entrez data base, National Center for Biotechnology Information, National Library of Medicine (National Institutes of Health, Bethesda, MD). HTLV-II genome was not detected in the plasma from the HTLV-II-seropositive donor, although a PCR signal was detected after PCR in 1 ml of supernatant of MoT cells cultured for 4 days (kindly supplied by Dr. T. M. Folks, Retrovirus Diseases

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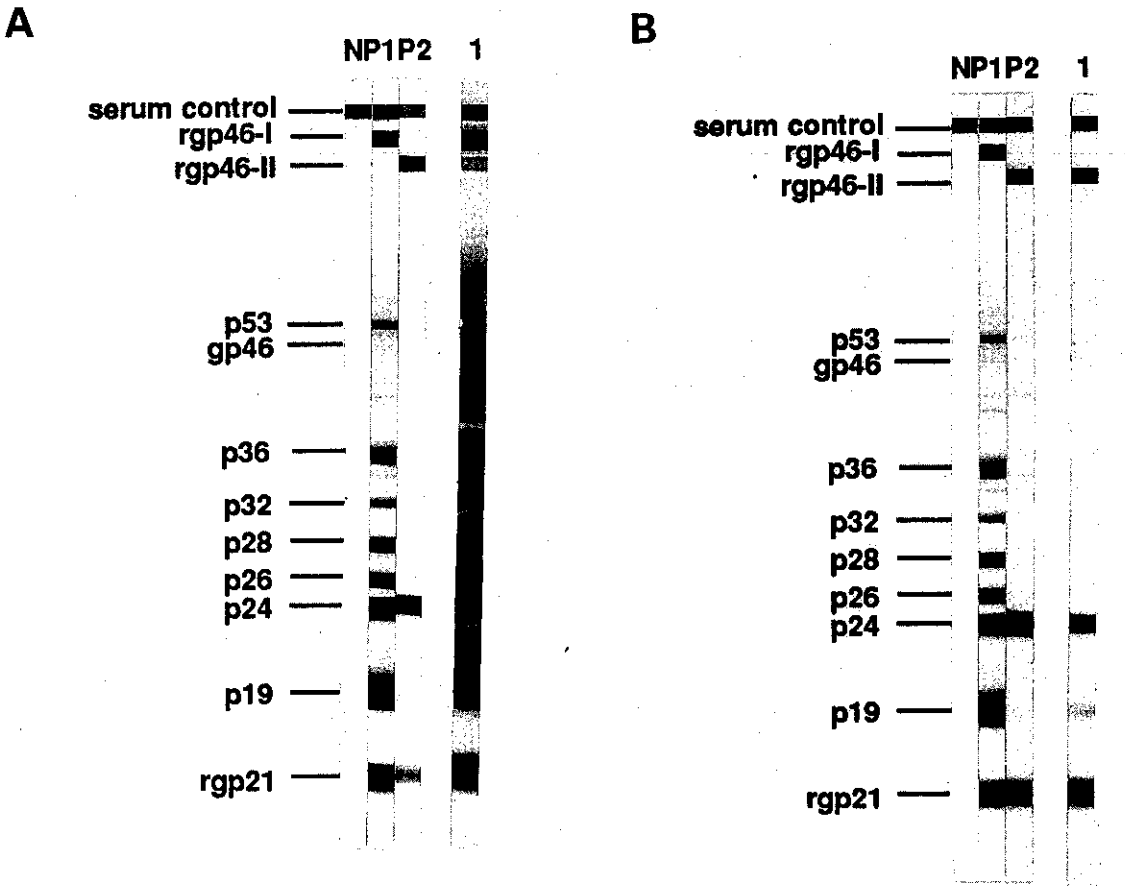

Fig. 1. Study of HTLV-II infection by type-specific Western immunoblotting of HTLV-II and HTLV-I antibodies in an HTLV-I-positive individual (A) and a healthy blood donor (B) in Japan. Lane 1 (N), negative control of the assay; lane 2 (P1), positive control for HTLV-I; lane 3 (P2), positive control for HTLV-II. Lane 1 of panel A shows an HTLV-I-infected donor whose serum was cross-reactive with HTLV-II, whereas lane 1 of panel B shows serum from a blood donor seropositive for HTLV-II, since the recombinant glycoprotein 46-II, more than one of the gag proteins of HTLV, and more than one of the env proteins of HTLV were reactive in the blotting. No bands were detected in 30 sera of normal healthy controls (data not shown).

Branch, Centers for Disease Control and Prevention, Atlanta, GA), which was used as a reference specimen of HTLV-II (data not shown). The PCR failed to detect the HTLV-II genome in the HTLV-II-positive serum, as well as in genomic DNA of CEM cells or genome of normal human serum (Fig. 3). These results indicate the absence of leaked genomic DNA of HTLV-II from HTLV-II-infected cells or of viral RNA in the plasma, or at least the presence of an amount less than that of one MoT cell. HTLV-II genome has sometimes been detected in sera positive for HTLV-II from Brazil by the identical PCR for HTLV-II (Hall *et al.*, personal communication), suggesting that storage conditions of blood specimens might influence the detection of the virus. Further, human immunodeficiency virus (HIV)-seropositivity was not detected in the HTLV-IIb-positive donor by immunoblotting for HIV-1 (Immunoblot HIV, Bio-Rad, Richmond, CA) (data not shown).

The HTLV-IIb-seropositive blood donor was a 29-year-old healthy Japanese man living in the western part of Japan. He denied any history of IVDA or blood transfusion, though he had once lived in Spain for several months.

The identification of HTLV-II subtypes can be useful to estimate the origin of the virus. In this respect, it is interesting to note that HTLV-IIb is the most prevalent HTLV-II subtype in Spain[13], which might suggest that the Spanish visit was the origin of the infection of this Japanese blood donor. Recently, HTLV-IIa has been shown to be serologically and/or genetically positive in

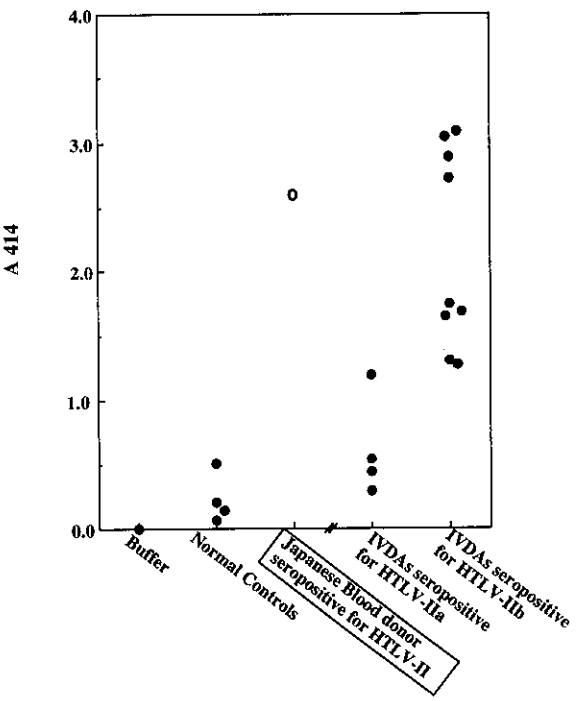

Fig. 2. Subtype analysis of HTLV-II in the HTLV-II-seropositive blood donor by HTLV-IIb-specific peptide-based ELISA. A Japanese blood donor seropositive for HTLV-II (circled) was assayed for subtype assessment by synthetic peptide-based ELISA. Four sera from IVDAs seropositive for HTLV-IIa and 9 sera from IVDAs seropositive for HTLV-IIb in Vietnam were used as reference sera.

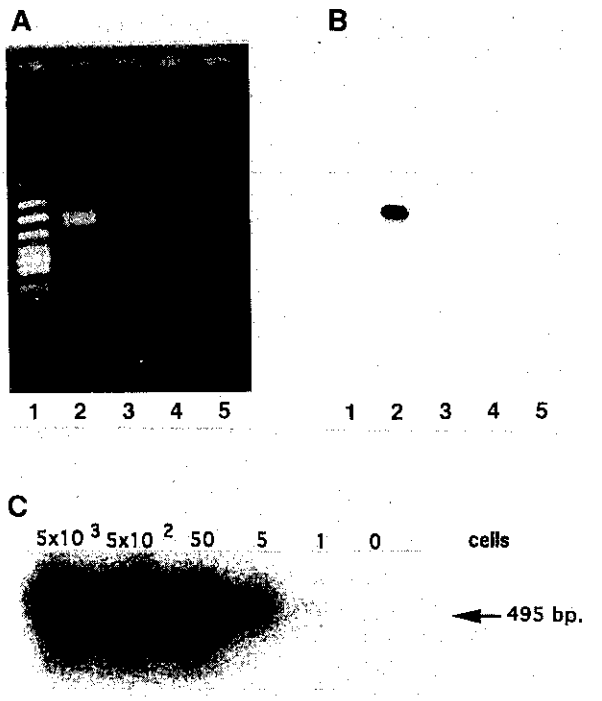

Fig. 3. Analysis of a serum from the HTLV-IIb-seropositive subject by RT-PCR. Gel electrophoresis (A) and Southern blot (B) after DNase nontreatment and RT-nested PCR with HTLV-II LTR primer pairs on genome from culture supernatant from HTLV-II-infected MoT cells (lane 2) or that from HTLV-II-negative CEM cells (lane 3), genome from HTLV-IIb-positive serum (lane 4), or normal human serum (lane 5). Lane 1 was ϕX174/*Hae*III digest. (C) PCR amplification of HTLV-II genome of MoT cells. The MoT cells, which expressed HTLV-II genome, were counted and diluted to afford 5×10^3 (lane 1), 5×10^2 (lane 2), 50 (lane 3), 1 (lane 4) and 0 cells (buffer; lane 5) for PCR amplification in the presence of a constant background of 1.0 μg of genomic DNA of CEM cells.

Mongolia[14] and Indonesia.[15] Further, we showed that approximately 60% of IVDAs were seropositive for HTLV-II, and both HTLV-IIa and HTLV-IIb subtypes were identified in this population.[9] The HTLV-IIs in South Vietnam IVDAs appeared to have been introduced from IVDAs from the United States during the Vietnam conflict.[8–10] Thus, it seems possible that HTLV-IIa may have been initially introduced into these geographical regions, and a mixture of both subtypes a and b, or subtype b alone, may have been newly introduced from distant regions where the virus is prevalent. Our findings demonstrate that there is at least one HTLV-II-seropositive person in Japan, and this HTLV-II infection might have originated from Spain.

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