CONCISE REPORT

Natural Antibodies in Sera From Japanese Individuals Infected With HTLV-I Do Not Recognize HTLV-III

By Toshio Hattori, Marjorie Robert-Guroff, Toru Chosa, Masao Matsuoka, Kazunari Yamaguchi, Toshinori Ishii, Robert C. Gallo, and Kiyoshi Takatsuki

Seventy-one sera from Japanese individuals infected with human T cell leukemia virus type I (HTLV-I) were examined for the presence of antibodies to HTLV-III by an enzyme-linked immunosorbent assay (ELISA) and by a strip radioimmunoassay based on the Western blot technique. The sera were from 23 healthy carriers and from 48 patients, including 18 with smoldering adult T cell leukemia (ATL), 13 with chronic ATL, and 17 with acute ATL. All people tested lived in the southwestern part of Japan, a known endemic area for HTLV-I infection. Antibodies against HTLV-I were detected in all sera both by indirect immunofluorescent methods and strip radioimmunoassay using cell lysates. Six sera were reactive in the ELISA assay for HTLV-III. But these sera did not react specifically to HTLV-III–related proteins (p15, p24, gp41) when analyzed by strip radioimmunoassay. Our data suggest that coincidental infection of HTLV-I and HTLV-III is quite rare in Japan.

THE ASSOCIATION of the human T cell leukemia (lymphotropic) virus (HTLV) with acquired immunodeficiency syndrome (AIDS) was first implicated by the findings of antibodies to cell membrane antigens associated with HTLV-I in patients with AIDS and the detection of proviral DNA of HTLV-I in peripheral blood lymphocytes of AIDS patients.[1,2] Recently, however, it was suggested that these observations might be due to the opportunistic and/or concomitant infection of HTLV-I in AIDS patients. Several converging lines of research have linked a new subtype of the HTLV family, HTLV-III, to the pathogenesis of AIDS.[3-5] HTLV-I was initially isolated from an American patient with an aggressive variant of mycosis fungoides, a form of T cell lymphoma.[6] Subsequently, the same virus was found in patients with adult T cell leukemia (ATL),[7] common in the southwestern part of Japan.[8,9]

ATL is characterized by malignant proliferations of the helper-inducer subset bearing the OKT4 surface marker.[10] This subset is depleted in patients with AIDS probably due to the cytopathic effect of HTLV-III.[4] Although profound immunodeficient states are not observed in patients with ATL and no definitive naturally occurring AIDS has been found in Japan, one case of ATL was reported that had an episode of *Pneumocystitis carinii* pneumonia and a low helper-suppressor ratio prior to the occurrence of overt leukemia, suggesting the coincidental infection of HTLV-I and HTLV-III.[11]

The exact origin and relationships of the HTLV subgroups have not been clarified yet. It has been reported that both types of virus are present in the Caribbean region and in Africa.[12-15] It is very important to determine whether antibodies against HTLV-III are present in the HTLV-I endemic area of Japan. We studied the sera from 71 Japanese people infected with HTLV-I and the sera from 24 patients with aplastic anemia.

MATERIALS AND METHODS

Materials. Sera from 48 patients with ATL and 23 healthy carriers infected with HTLV-I were collected. Acute, chronic, and smoldering ATL were classified as described previously.[16] In brief, patients with acute ATL suffered from increased numbers of ATL cells, skin lesions, lymphadenopathy, and hepatosplenomegaly with aggressive clinical features. Patients with chronic ATL showed increased white cell counts (more than 10^{10}/L); however, infiltration of leukemic cells to the skin, lymphadenopathy, and hepatosplenomegaly were not marked. Smoldering ATL is characterized by the long duration of a few ATL cells (0.5% to 3%) in the peripheral blood. These patients frequently have skin lesions as premonitory symptoms. Sera from 24 patients with aplastic anemia who received multiple blood transfusions (5 to 78 units) were also examined.

Detection of anti–HTLV-I antibody. Detection of anti–HTLV-I antibody in the sera was done by indirect immunofluorescent (IF) assay as described by Hinuma et al using MT-1 cells.[7] Positive sera usually stained 2% to 3% of cells, and these positive cells were readily observed in the midst of large number of IF-negative cells. Sera were serially diluted in phosphate-buffered saline (pH 7.4) in order to determine the antibody titer, defined as the maximum dilution that gave positive results. Normal human serum was always used as a negative control. The presence of anti–HTLV-I antibody was also confirmed by a recently established strip radioimmunoassay using cell lysates from MT-2 cells (T. Chosa, T. Hattori, K. Takatsuki: MS in preparation) that produce large amounts of HTLV-I.[17]

Detection of anti–HTLV-III antibody. The ELISA assay was performed essentially as described by Saxinger and Gallo[18] using Immulon 1 (Biotech Laboratories, Bethesda, MD) plates coated with proteins of disrupted HTLV-III. Assays were done in duplicate, and sera with an absorbance reading greater than three times the average of four normal negative control readings were further tested by strip radioimmunoassay.

The virus strip radioimmunoassay using purified HTLV-III was done by the methods described previously,[5] using nonfat dry milk instead of bovine serum albumin to prevent nonspecific binding of proteins to the nitrocellulose.

RESULTS AND DISCUSSION

All 71 sera were positive for anti–HTLV-I antibody by IF assay. The titer of antibody was defined using serially diluted sera (Fig 1). Antibodies to HTLV-I–related proteins (p19, p24, p28, gp46) were found in all the sera by strip radioimmunoassay using cell lysates of the HTLV-I–producing cell line, MT-2, as described previously.[19] The profiles of recognized proteins were similar for most of the patients (Fig 2A), although some bands could not be seen, especially in sera of patients with acute ATL (unpublished observations, Febru-

From the Second Department of Internal Medicine, Kumamoto University of Medical School, Japan; and the Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Md.

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Address reprint requests to Dr Toshio Hattori, Second Department of Internal Medicine, Kumamoto University Medical School, 1-1-1 Honjyo, Kumamoto 860, Japan.

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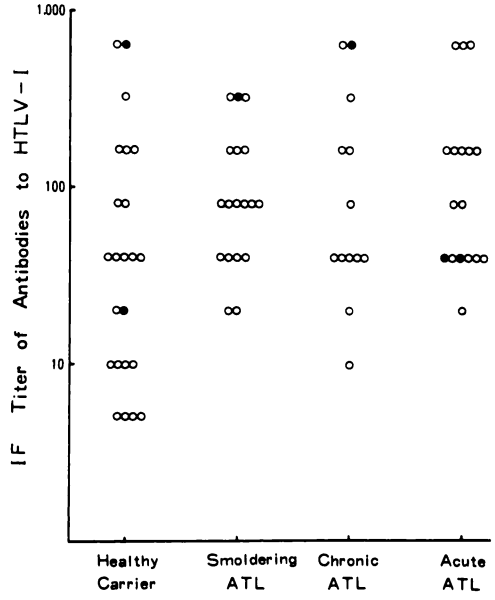

Fig 1. Titer of anti-HTLV-I antibody by IF assay and results of ELISA assay for anti-HTLV-III antibody. The titer of anti-HTLV-I antibody was determined using serially diluted sera. (O) indicates nonreactive and (●) indicates reactive for anti-HTLV-III in the ELISA assay.

ary, 1985). Samples from six were reactive in the ELISA for HTLV-III (Fig 1). However, the absorbance readings of all these sera were less than five times the average of the negative control readings, in contrast to the very high readings that have been frequently observed using sera from patients with hemophilia (data not shown). Recently Weiss et al called cases with similar absorbance readings "borderline" in their extensive studies on ELISA for HTLV-III.[20] Thus we call these sera reactive rather than positive. Furthermore, these sera failed to react to HTLV-III-related proteins (p15, p24, gp41) when analyzed by the virus strip radioimmunoassay, which is more specific than the ELISA assay (Fig 2B). An additional 20 anti-HTLV-I-positive sera also failed to react specifically with HTLV-III antigens in the virus strip radioimmunoassay. Finally, the individuals whose sera reacted in the ELISA had no symptoms of severe immunodeficiency such as *Pnemocystitis carinii* pneumonia or Kaposi's sarcoma. Two of 24 sera from patients with aplastic anemia were also reactive in the ELISA assay; however, all proved to be negative for antibody to HTLV-III antigens by the virus strip radioimmunoassay.

The reasons for the different results of the two assays for HTLV-III antibodies are not clear. It is possible that patients' sera react with some unknown cellular proteins because some of the patients received multiple blood transfusions. Alternatively, the results may reflect weak cross-reactivities of antigens of HTLV-I and HTLV-III because the genome of HTLV-III has significant homology with that of HTLV-I[21] and the weak immunologic cross-reactivities of HTLV-I and HTLV-II p24 with HTLV-III p24 have been recently observed in the Western blot system using hyperimmunized rabbit antisera.[22] We think that the latter possibility is less likely because there is no clear association of the titer of anti-HTLV-I and the results in the ELISA for anti-HTLV-III (Fig 1). Nevertheless, the extent of the immunologic relationship between each protein of HTLV-I and HTLV-III has not yet been fully explored using natural antibodies.

The low frequency of antibodies to HTLV-I in patients with AIDS in the United States has been already reported.[23] Our results suggest that concomitant infection with HTLV-III is rare in individuals infected with HTLV-I in Japan as well. Although the number of sera examined is small, the absence of anti-HTLV-III antibodies in the sera from patients with aplastic anemia who received multiple blood transfusions suggest that HTLV-III is not naturally prevalent in Japan.

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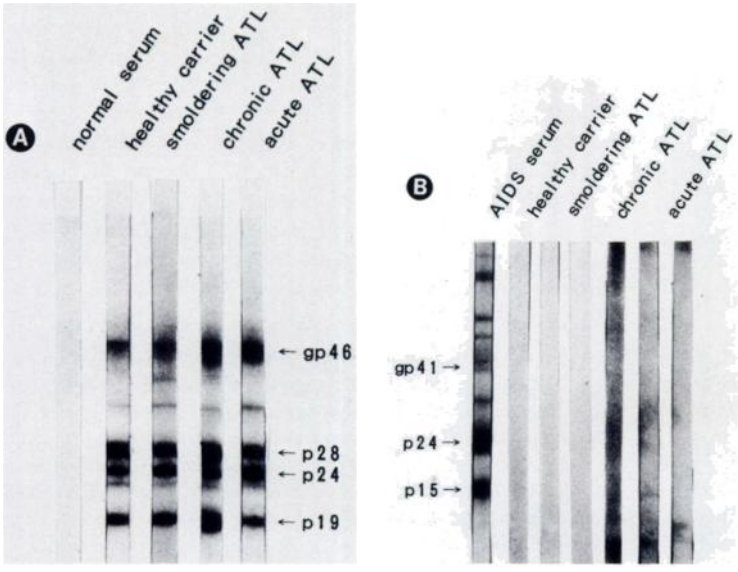

Fig 2. Western blotting strip assay for HTLV-I (A) and HTLV-III (B). (A) Cell lysates were obtained by incubating 10 million cells in 1 mL of lysing buffer [10 mmol/L Tris-HCl, pH 7.2, containing 0.9% NaCl, 0.5% NP-40 and 2 mmol/L phenylmethylsulfonylfluoride phenylmethylsulfonylfluoride (PMSF)] for 20 minutes on ice. After removing cell debris at 15,000 rpm for ten minutes, recovered proteins were diluted 1:1 with Laemmli sample buffer and electrophoresed on 12% polyacrylamide in the presence of sodium dodecyl sulfate (SDS). The Western blotting strip assay was performed exactly the same way as for HTLV-III. The sera that gave typical results are shown. (B) The sera from two healthy carriers and from one smoldering ATL, one chronic ATL, and two acute ATL patients who were reactive in the ELISA assay for HTLV-III were analyzed using disrupted virus preparations (20 μg per lane) as described in the text.

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