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

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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 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 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. 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. 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 10^9/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 premorbid 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. 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.

Detection of anti-HTLV-III antibody. The ELISA assay was performed essentially as described by Saxinger and Gallo using Immubon I (Becton Dickinson, Mountain View, CA) 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, 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. 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
Healthy Smoldering
Chronic
Carrier ATL
Acute
ATL

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. (○) indicates nonreactive and (●) indicates reactive for anti-HTLV-III in the ELISA assay.

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 (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.

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 and the weak immunologic cross-reactivities of HTLV-I and HTLV-III p24 with HTLV-III p24 have been recently observed in the Western blot system using hyperimmunized rabbit antisera. 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. 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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