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High Risk of Mother-to-Child Transmission of HTLV-I in p40^{tax} Antibody-positive Mothers

Takashi Sawada,[1,7] Junichi Tohmatsu,[1] Takashi Obara,[1] Atsushi Koide,[1] Shimeru Kamihira,[2] Michito Ichimaru,[2] Seizaburo Kashiwagi,[3] Wataru Kajiyama,[3] Nobuyuki Matsumura,[4] Kenichiro Kinoshita,[4] Michitami Yano,[4] Kazunari Yamaguchi,[5] Tetsuyuki Kiyokawa,[5] Kiyoshi Takatsuki,[5] Hirokuni Taguchi[6] and Isao Miyoshi[6]

[1]Tsukuba Research Laboratories, Eisai Co., Ltd., Tokodai 5-1-3, Tsukuba-shi, Ibaraki 300-26, [2]Nagasaki University School of Medicine, Sakamoto-cho, Nagasaki 852, [3]Kyushu University School of Medicine, Maidashi, Higashi-ku, Fukuoka 812, [4]Nagasaki Central National Hospital, Kuhara, Oomura-shi, Nagasaki 856, [5]Kumamoto University School of Medicine, 'Honjo, Kumamoto 860 and [6]Kochi Medical School, Oko-cho, Nankoku 781-51

A new enzyme-linked immunosorbent assay (ELISA) for detecting the antibody to a human T-lymphotropic virus type I (HTLV-I) *tax* gene product, p40^{tax}, has been developed. By this ELISA method, we have investigated the relationship between the presence of p40^{tax} antibody in HTLV-I-infected mothers and the virus transmission rate from mothers to their children. The rate of mother-to-child transmission of HTLV-I was higher in p40^{tax} antibody-positive mothers than in antibody-negative ones. Thus, the presence of p40^{tax} antibody may indicate an increased risk of vertical transmission of HTLV-I.

Key words: HTLV-I — p40^{tax} antibody — Mother-to-child transmission

Human T-lymphotropic virus type I (HTLV-I) is a unique retrovirus which possesses *tax* gene between *env* and 3′ long terminal repeat (LTR).[1)] A protein of 40 kD, named p40^{tax}, is a product of the *tax* gene[2-4)] and appears to activate the transactivation of the LTR[5-8)] and cellular genes of interleukin 2 (IL-2) and IL-2 receptor (IL-2R).[9-12)] Due to these mechanisms, p40^{tax} seems to play an important role in the virus replication and virus-induced lymphocyte transformation. Antibody to p40^{tax} was detected in some patients with adult T-cell leukemia (ATL) and HTLV-I carriers.[2,3)] However, the biological significance of this antibody response remains unknown. In order to clarify its significance, we have developed a new enzyme-linked immunosorbent assay (ELISA) and have tested p40^{tax} antibody among family members of HTLV-I carriers.

For the ELISA antigen, p40^{tax} expressed in *Escherichia coli* with a full-length HTLV-I *tax* gene was used.[13)] The purity of the p40^{tax} protein was about 90% as determined by SDS-polyacrylamide gel electrophoresis. The p40^{tax} was diluted to 0.25 μg/ml with 0.05 *M* tris-HCl buffer (pH 8.0) containing 0.01% sodium dodecyl sulfate (SDS) and 0.1% sodium azide. Each microtiter well was coated with the p40^{tax} solution (100 μl) for 15 h at 25°C, then washed 3 times with saline, and normal rabbit serum (100 μl) containing 10 m*M* EDTA buffered to pH 8.0 with 0.05 *M* tris-HCl was added. Test sera (20 μl) were then added to the wells. The first reaction was carried out for 60 min at 37°C. Then the wells were washed 3 times with saline containing 0.01% Tween-20. For the second reaction, 100 μl of alkaline phosphatase-labeled anti-human IgG (Fc) monoclonal antibody was added to the wells. After 60 min of incubation at 37°C, the wells were washed 3 times with saline containing Tween-20. The final enzyme reaction was carried out by adding 100 μl of *P*-nitrophenyl phosphate solution (4 mg/ml in 0.05 *M* carbonate buffer containing 4 m*M* $MgCl_2$, pH 9.5) and incubating the mixture at 37°C for 30 min. The reaction was stopped by adding 100 μl of 1 *N* NaOH and then the absorbance at 405 nm was measured using a spectrophotometer (light path length, 1 mm). The cut-off value was determined as the average ELISA absorbance value plus 3 times the standard deviation which was obtained by assessing 169 HTLV-I seronegative specimens. Antibody to HTLV-I was also examined by ELISA[14)] (Eitest-ATL; Eisai Co., Ltd., Tokyo) and ELISA-positive specimens were confirmed by western blotting.[15)]

The sensitivity of the ELISA method for p40^{tax} antibody was compared with that of the radioimmunoprecipitation (RIP) method[4)] by using diluted samples of a p40^{tax} antibody-positive specimen. For RIP antigens, [^{35}S]methionine- and [^{35}S]cysteine-labeled SLB-I cell[16)] lysate was used. Figure 1 shows the relationship between the ELISA absorbance and RIP analysis on a serially

[7] To whom communications should be addressed.

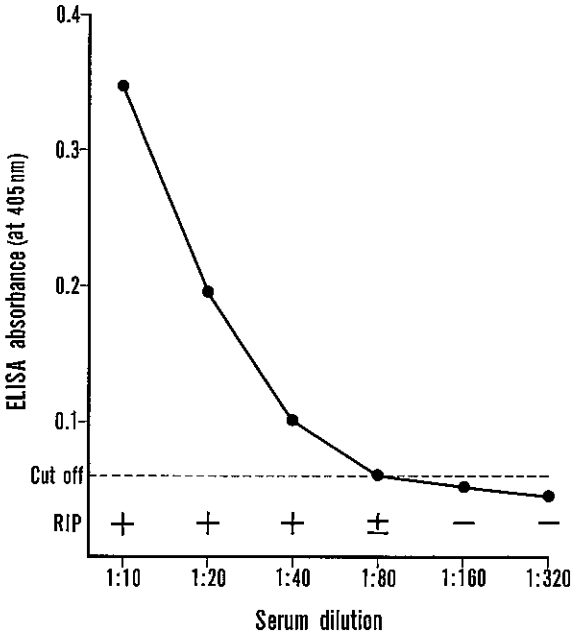

Fig. 1. Relationship between ELISA absorbance and RIP analysis of a serially diluted p40^{tax} antibody-positive human serum.

diluted p40^{tax} antibody-positive specimen. The ELISA was positive up to 1:40 dilution, and the RIP was also positive up to the same dilution. These results show that the sensitivity of the ELISA method is almost the same as that of the RIP method.

For the assessment of the relationship between the presence of p40^{tax} antibody in HTLV-I carrier mothers and vertical transmission rate from mothers to their children, a total of 121 healthy carrier mothers and 225 children, aged 2 to 55 years, were examined for p40^{tax} antibody and HTLV-I antibody by the ELISA methods. Among the children, in order to eliminate possible horizontal transmission of the virus, married women whose husbands were seropositive for HTLV-I and persons having a history of blood transfusion were excluded. The average ages of children from p40^{tax} positive and negative mothers were almost the same. As shown in Table I,

Table I. p40^{tax} Antibody in Sera of HTLV-I-seropositive Mothers and Vertical Transmission Rate of HTLV-I to Their Children

Mothers' sera (n=121)		Children's sera (n=225)		
p40^{tax} antibody	No.	HTLV-I seropositive	HTLV-I seronegative	Total No.
Positive	75	67 (50.8%)	65 (49.2%)	132
Negative	46	17 (18.3%)	76 (81.7%)	93

75 mothers were positive for p40^{tax} antibody and 67 (50.8%) of 132 children born to these mothers were seropositive for HTLV-I, whereas the other 46 mothers were negative for p40^{tax} antibody and only 17 (18.3%) of 93 children born to these mothers were seropositive for HTLV-I. These data suggest that the risk of mother-to-child transmission of HTLV-I is higher in p40^{tax} antibody-positive mothers than in antibody-negative ones (χ^2 test, $P<0.001$). Since p40^{tax} induces transcriptional transactivation of the HTLV-I LTR and activates the expression of HTLV-I, milk lymphocytes from mothers with p40^{tax} antibody may have been activated to express more HTLV-I antigens and this may increase the risk of HTLV-I infection by breast feeding in mother-to-child transmission of HTLV-I.

Breast feeding is thought to play an important role[17-19] and it has been reported that the transmission rate in children born to HTLV-I seropositive mothers is about 25%.[20] It should be noted that the majority of children (about 75%) are not infected with this virus, even when they had received breast milk. Factors determining the vertical transmission of HTLV-I are not clear. If the situation is analogous to that of hepatitis B virus e antigen-positive mothers,[21] the p40^{tax} antibody may serve as a parameter of infectivity in mother-to-child transmission of HTLV-I.

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