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Antibodies against p40^{tax} Gene Product of Human T-Lymphotropic Virus Type-I (HTLV-I) under Various Conditions of HTLV-I Infection

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We investigated antibodies against pX gene product, p40^{tax}, by ELISA using recombinant p40^{tax} protein in HTLV-I seropositive carriers as well as patients with adult T cell leukemia (ATL) and HTLV-I-associated myelopathy (HAM). Seventy (49.0%) out of 143 HTLV-I healthy carriers were found to be positive for antibody against p40^{tax} antigen and the follow-up samples at two-year intervals revealed constant reactivity by ELISA in each carrier. The onset of antibody production was delayed 4 to 12 weeks as compared with anti-HTLV-I in primary infection cases. The anti-p40^{tax}-positive rate (90%) in HAM patients was significantly higher than that of healthy carriers, acute and chronic ATL patients and their family members. Furthermore, HAM patients and a few healthy carriers showed high reactivities by ELISA. Children from mothers with anti-p40^{tax} showed a higher anti-HTLV-I-positive rate than that of children from mothers without anti-p40^{tax} (54.5% versus 12.5%). Two men without anti-p40^{tax} and one female with low anti-p40^{tax} developed ATL during follow-up studies. These results suggest that HTLV-I carriers could be divided into 2 or 3 sub-populations according to antibody response to p40^{tax}. A smaller population with anti-p40^{tax}, especially a high antibody reactivity, could have a high risk of developing HAM and of transmission from mother to child. In addition, ATL may occur in a population with low or absent anti-p40^{tax}.

Key words: HTLV-I — Anti-p40^{tax} — Adult T cell leukemia — HTLV-I-associated myelopathy

Human T lymphotropic virus type-I (HTLV-I) has been described as a novel human retrovirus which may cause the development in humans of particular T-cell malignancies designated as adult T-cell leukemia (ATL).[1,2] HTLV-I also transforms normal T lymphocytes *in vitro*, lending credence to its etiologic role in these human malignancies. The virus genome is relatively simple, containing the genes *gag*, *pol*, *env*, and a 3′ region termed "X". This X region may be responsible for the transforming potential of the virus.[3–5]

Gessain *et al.*[6] and Osame *et al.*[7] have independently reported in the tropics and Japan that some HTLV-I-infected populations may develop chronic myelopathies, designated as tropical paraparesis or HTLV-I-associated myelopathy (HAM). In Japan, which is not tropical, HAM has been recognized to have a similar geographical distribution to ATL. We were very interested that the same pathogen produces the different disorders, ATL and HAM. HTLV-I infection of healthy people is persistent and the infections are mainly confirmed by the detection of antibodies to *gag* and *env* gene products.

As the antibody responsiveness between the virus and host has been discussed only in relation to antibodies against *gag* and *env* products until recently, the present study was undertaken to clarify the prevalence and physiological significance of the antibody to *tax* (pX-IV) gene product of the X gene, named p40^{tax}.

MATERIALS AND METHODS

Detection of antibodies to HTLV-I and p40^{tax} As a gelatin particle agglutination method (PA)[8] simultaneously defines virus-specific IgG and IgM antibodies, we screened first by PA and then the antibodies were reexamined by enzyme-linked immunosorbant assay (ELISA)[9] and western blotting (WB) using HTLV-I-infected MT2-cell lysates and a recombinant p40^{tax} protein as antigens.

PA for anti-HTLV-I antibody A PA was developed by Ikeda *et al.*[8] and was licensed by the Ministry of Health and Welfare. It has been used for the mass screening of anti-HTLV-I antibodies in sera from donors in all Red Cross Centers of Japan. The features and usefulness of the PA have been previously reported.[10] A final serum dilution of 1:16 or higher causing particle agglutination was interpreted as positive.

ELISA for anti-HTLV-I and p40^{tax} antibodies An ELISA kit to detect anti-HTLV-I antibodies developed

by Eisai Pharmaceutical Company (Tokyo) was licensed by the Ministry of Health and Welfare. The features of the HTLV-I ELISA using HTLV-I antigens from the MT2-cell line have been described elsewhere in detail.[9] Absorbance (A405) was measured with a Hitachi 557 photometer (Hitachi Electric, Tokyo). The levels of antibodies were estimated by using a cut-off index (CI); the sample A405 was divided by the sum of the control A405 and 0.33. When the CI was 1.1 or higher, the corresponding serum was considered as ELISA-positive.

Anti-p40^{tax} antibodies were detected by the ELISA method, using as an antigen a recombinant p40^{tax} protein, which is coded by full-length HTLV-I *tax* gene and expressed in *E. coli*.[11] The procedure for coating the p40^{tax} protein as an alternative to using MT2-lysates as antigens is fundamentally the same as in the above ELISA kit for anti-HTLV-I antibodies. The cut-off value was determined as the average of the p40^{tax} ELISA absorbance value obtained from the HTLV-I-seronegative specimens, plus 3SD.

The specificity of the pX ELISA can be summarized as follows: (1) the data obtained by the ELISA were in good agreement with the results of radioimmunoprecipitation analysis using native p40^{tax} from SLB1 cells[11]; (2) anti-p40^{tax} antibody was detected only among HTLV-I carriers; (3) recombinant p40^{tax} protein used as the antigen has been purified[11]; (4) as shown in Fig. 4, it was reasonable that the development of anti-p40^{tax} was observed in seroconverted cases.

Western blotting (WB) Some sera, especially seroconverted samples and sera from HAM and ATL, were reexamined in detail by WB, performed according to the method developed by Laemmli.[12] HTLV-I antigens from MT2-cell lysates or recombinant p40^{tax} protein were fractionated by electrophoresis on 12.5% polyacrylamide slab gel in the presence of sodium dodecyl sulfate. The protein bands in the gel were electrophoretically transferred to a nitrocellulose sheet as described by Towbin *et al.*[13] Anti-HTLV-I or p40^{tax} antibodies (primary antibodies) which reacted to fractionated antigens on the sheet were stained by an avidin-biotin staining procedure as described previously.[14] The visualized bands were confirmed by mouse monoclonal antibodies, GIN-14[15] and H-15,[16] against the p19, p24, p28, and p53 antigens of HTLV-I *gag*-related proteins.

Serum samples Sera from donors who were healthy carriers without clinical manifestations were collected at the Red Cross Nagasaki Blood Center. Follow up samples from residents who lived in the Gotoh Islands of Nagasaki prefecture, an ATL-endemic area, were obtained at an interval of two years from 1986. Sera from seroconverted recipients, patients with ATL and HAM, and family members with or without ATL patients in their families were collected at Nagasaki University Hospital from April, 1986 to August, 1987. Acute ATL, chronic ATL and a preleukemic state of ATL (pre-ATL) were classified as described previously.[17, 18] The age of the subjects was 16 years or greater.

RESULTS

Rates of positive anti-p40^{tax} antibody among HTLV-I seropositive healthy populations One hundred and forty-three (19.1%) out of 400 blood donors and 350 residents in the Gotoh Islands were seropositive for HTLV-I by the PA and the HTLV-I ELISA methods. The anti-p40^{tax}-positive rate by the pX ELISA among 143 seropositive for HTLV-I was 49.0% (70/143; age range 16 to 80 years). Of the remaining 607 seronegative for HTLV-I, there were only two who reacted. Of the two who reacted, one was positive and one was negative by the pX WB analysis. As shown in Fig. 1, the percentage positive for anti-p40^{tax} among HTLV-I seropositives was almost constant with increasing age, ranging from 41% to 63.6% and the values (44.4% and 52.5%) of males and females were also not clearly different.

The positive rates and levels of anti-HTLV-I and anti-p40^{tax} antibodies Anti-HTLV-I testing and anti-p40^{tax} testing were performed on 82 patients with HTLV-I-associated disorders and 301 healthy people. The 301 healthy people were further classified into group-I and -II subpopulations according to whether or not ATL patients were included in their family.

As can be seen from Table I, all of the patients with HTLV-I-associated disorders such as ATL, pre-ATL, and HAM had anti-HTLV-I antibodies with or without anti-p40^{tax} antibody. However, anti-p40^{tax}-positive rates ranged from 33.3% to 90.0%. Only HAM patients showed a high positive rate (90% : $P<0.01$) as compared with the other patient group and the subpopulations of healthy people. Mean A405 levels of anti-HTLV-I and

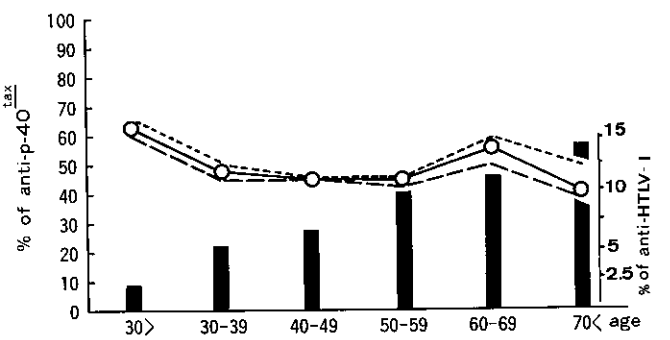

Fig. 1. Age-dependent positive rates of anti-p40^{tax} among anti-HTLV-I positives (linear graph) and anti-HTLV-I (histograph). ·····, females; – – –, males; ○——○, total.

Table I. Comparison of the Rate and the Absorbance Value at 405 nm (A405) of Anti-HTLV-I and Anti-p40^{tax} Antibody-positive Sera in HTLV-I Associated Disorders and Healthy Carriers

Subjects	No. of cases	Anti-HTLV-I		Anti-p40^{tax}	
		Positive (%)	A405[a]	Positive (%)[b]	A405[c]
ATL	50	100	1.55±0.55	67.6	0.74±0.68
Acute	36	100	1.62±0.58	63.8	0.44±0.38
Chronic	14	100	1.53±0.50	71.4	1.04±0.97
Pre-ATL[d]	12	100	1.40±0.63	33.3	0.64±0.51
HAM	20	100	1.98±0.33	90.0	2.03±0.97
Healthy population					
group-I[e]	101	36.6	1.24±0.77	40.5	1.05±0.86
group-II[f]	200	9.5	1.60±0.54	52.6	0.84±0.88

a) Mean A405 values of sera from anti-HTLV-I-positive persons.
b) Anti-p40^{tax} positive rates among anti-HTLV-I-positive persons.
c) Mean A405 values of sera from anti-p40^{tax}-positive persons.
d) Pre-leukemic state of ATL.
e) Group having relative(s) and family member(s) with ATL.
f) Group having no relative or family member with ATL.

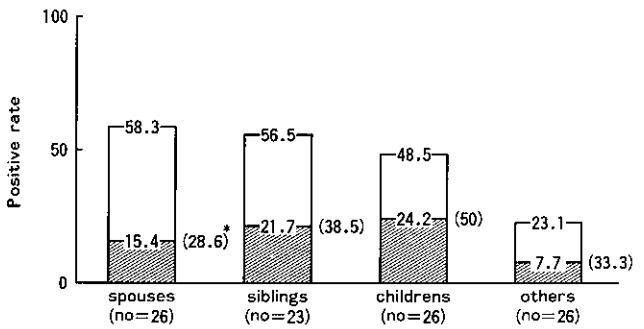

Fig. 2. Positive rates of anti-HTLV-I and anti-p40^{tax} in 101 members and relatives of families having ATL family members. □, anti-HTLV-I; ■, anti-p40^{tax}; ( )*, percent positive for anti-HTLV-I antibodies.

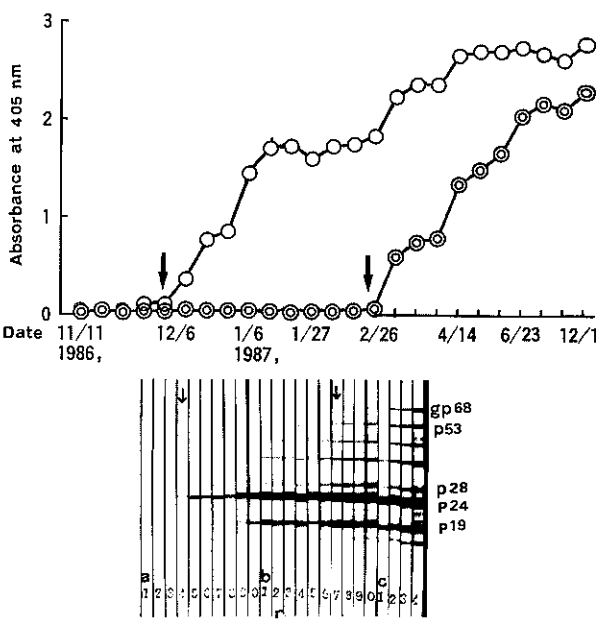

Fig. 3. Follow-up studies of the development of anti-HTLV-I and anti-p40^{tax} by ELISA with HTLV-I (○), with p40^{tax} (◎) and WB analysis using MT2-lysates as the viral antigens. Lane a-4 (same sample as sera on Dec. 6, 1986): *gag* antibody was initially demonstrated (see ↓). Lane b-7 (same sample as sera on Feb. 26, 1987): *env* antibody was initially demonstrated (see ↓).

anti-p40^{tax} also were statistically higher in HAM than in the other group ($P<0.01$).

Of healthy people, the group-I population showed a higher HTLV-I seropositive rate than the group-II population (36.6 versus 9.5%: $P<0.01$). On the other hand, anti-p40^{tax}-positive rates among HTLV-I seropositives out of the group-I and -II populations were not different.

As it is well known that patients with ATL are frequently clustered in families and areas, we studied the frequency of anti-p40^{tax} antibody according to the relationship of 101 members and relatives of 26 families with ATL family members (group-I, see Table I and Fig. 2). As shown in Fig. 2, the HTLV-I seropositive rates ranged from 23.1% to 58.3% among the group having family members with ATL, which was significantly higher than that (9.5%) in the group where no family members had ATL. However, the p40^{tax} seropositive rates in HTLV-I

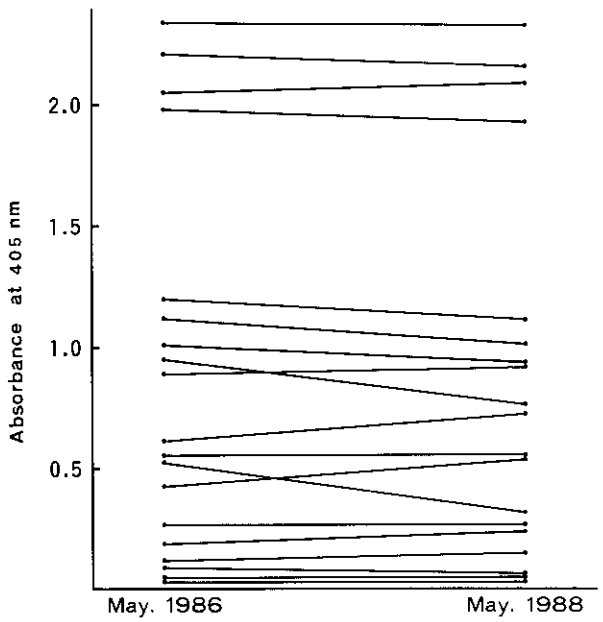

Fig. 4. Follow-up studies of the A405 levels on pX ELISA at a two-year interval.

seropositive members of each group were almost equal, ranging from 28.6% to 50.0%.

Follow-up studies of anti-p40^{tax} In 4 cases which had received blood products before HTLV-I screening and had seroconverted, we followed the development of anti-HTLV-I and anti-p40^{tax} antibodies by pX and HTLV-I ELISA and WB analysis. Fig. 3 shows the time course of the development of the antibodies in a recipient. The appearance of anti-p40^{tax} in the 4 cases was delayed 4 to 12 weeks as compared with anti-HTLV-I. Furthermore, to study the anti-HTLV-I antibody profile, we carried out a further analysis by WB. As shown in Fig. 3, antibodies to *gag* proteins p19, p24, p28, and/or p53 were initially demonstrated and then antibodies to *env* proteins gp68 appeared about 12 weeks after the antibodies against *gag* proteins. At the same time, with the development of antibodies against *env* proteins, anti-p40^{tax} antibodies could also be detected. On the other hand, we also tried to study the time course of levels of anti-p40^{tax} in healthy carriers over time. The p40^{tax} reactivity in the ELISA at an interval of two years is illustrated in Fig. 4. These follow-up results revealed that the A405 values of individuals at the two-year interval were almost constant, i.e. the high, low, or negative A405 of each carrier remained high, low or negative. Interestingly, three carriers developed ATL during the follow-up studies of HTLV-I seropositive residents in the Gotoh Islands. Two were

Table II. Comparison of the Prevalance of Anti-HTLV-I-positive Populations in Children Born from HTLV-I Seropositive Mothers with or without Anti-p40^{tax}

	Anti-HTLV-I (positive/tested)	Rate (%)
Children of seropositive mothers		
with anti-p40^{tax}	6/11	54.5*
without anti-p40^{tax}	3/24	12.5*
Total	9/35	25.7

* $P<0.05$ by chi-square.

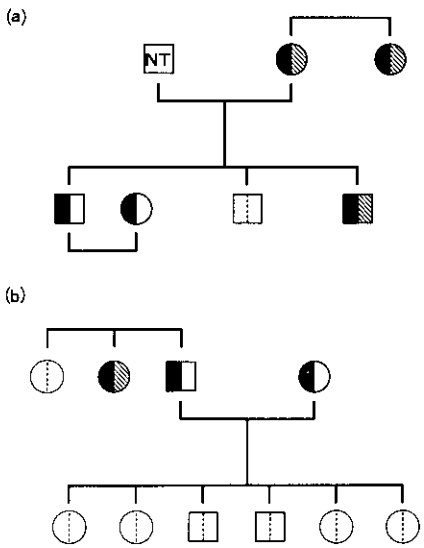

Fig. 5. Pedigree patterns of family members and relatives in the transmission from mothers with positive (a) and negative (b) anti-p40^{tax} to their children. □, ○, anti-HTLV-I- and anti-p40^{tax}-negative males and females, ◧, ◐, anti-HTLV-I-positive and anti-p40^{tax}-negative males and females; ◙, ●, anti HTLV-I- and anti-p40^{tax}-positive males and females.

negative and one was slightly reactive for p40^{tax} in sera obtained before the development of ATL.

Infection rate of children born from HTLV-I seropositive mothers with or without anti-p40^{tax} We examined the relationship between transmission and a p40^{tax} seropositive mother in 35 children born from 15 HTLV-I seropositive mothers. As shown in Table II, 6 (54.5%) out of 11 children from p40^{tax} seropositive mothers were positive for HTLV-I and only 3 (12.5%) out of 24 children from p40^{tax} seronegative mothers were positive; the former was significantly high ($P<0.05$ by chi-square). Typical transmission patterns from p40^{tax}

seropositive (Fig. 5a) and seronegative (Fig. 5b) mothers to their children are illustrated in Figs. 5a and b. In the former family with a $p40^{tax}$ seropositive mother, 2 out of 3 children were anti-HTLV-I positive, and in the latter family with a HTLV-I seropositive mother without anti-$p40^{tax}$, none of the children was seropositive.

DISCUSSION

It remains an enigma why HTLV-I is associated with two distinct diseases, ATL and HAM, in small populations among HTLV-I carriers. The fact that the same virus produces the two different diseases suggests that the host also contributes some causative factor(s) to develop either ATL or HAM.

From this standpoint, we studied the prevalence and physiological significance of the antibody against *tax* (pX-IV) gene product, named $p40^{tax}$, in a population of an ATL- and HAM-endemic area. The pX gene product appears to exhibit-transcriptional *trans*-activation of the HTLV-I long terminal repeat and may have important roles in the virus replication and in the early stage of the transformation of virus-infected cells.[3-5] Antibodies against $p40^{tax}$ have already been demonstrated in sera from anti-HTLV-I-positive persons, using native or recombinant $p40^{tax}$.[11, 19] However, the pathophysiological significance of the antibody is not yet fully known. Therefore, we would like to discuss the question of whether immune responsiveness against $p40^{tax}$ and susceptibility to ATL or HAM are causally related.

As mentioned above, about 50% of individuals among an anti-HTLV-I-positive population had anti-$p40^{tax}$ antibodies. The positive reaction and reactive intensity in the ELISA were stable for a long time among individual carriers.

In contrast to anti-HTLV-I, the positive rates of anti-$p40^{tax}$ in anti-HTLV-I seropositives did not increase with increasing age (see Fig. 1) and, in primary infections, the appearance of this antibody was delayed 4 to 12 weeks in contrast to the antibody response against *gag* and *env* gene products. The presence of the antibody and the level of reactivity were stable not only in healthy carriers but also in some patients with HTLV-I-associated disorders.

Consequently, there could be individual variations in antibody responsiveness among HTLV-I carriers, and the anti-HTLV-I-positive populations could be mainly classified into 2 groups in terms of the anti-$p40^{tax}$ antibody profile; responders and non-responders. Responders can also be divided into high and low responders and the high or low level of $p40^{tax}$-specific immune responsiveness is stable in each responder group.

Various HTLV-I-infected patients, i.e., those with HAM, a small number of healthy carriers and patients who have seroconverted from blood transfusions frequently had a high immune response against pX protein as compared with patients with ATL and most healthy carriers, including family members of ATL patients.

Three of the followed-up carriers in the Gotoh Islands, an ATL endemic area, developed ATL. Two were non-responders and one was a low-responder. On the other hand, the transmission rate from mothers of the responder group to their children was higher than that of mothers in the non-responder group. This evidence suggests that high-responders have a high risk of developing HAM and of virus transmission from mother to child.

Usuku *et al.*[20] pointed out, that there are two ethnic groups, HAM ethnic and ATL ethnic, according to the differences in HLA haplotypes. They have found different susceptibilities to HAM and ATL in terms of HLA haplotyping, and we have likewise observed an association with HLA haplotype by means of analyzing serological manifestations of anti-$p40^{tax}$, in agreement with their proposal.

These findings are highly suggestive of individual variations in the specific immunological relationship between the virus antigens and immunogenetic background of the host, and follow-up observation over an extended time will be necessary to obtain meaningful answers.

We are now investigating anti-$p40^{tax}$ in residents in ATL- and HAM-endemic areas from the point of view mentioned above. We would like to stress the importance in measuring anti-$p40^{tax}$ antibodies to reveal the potential of virus transmission and the susceptibility to ATL or HAM in individual carriers.

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