Risk Factors for Adult T-Cell Leukemia Among Carriers of Human T-Lymphotropic Virus Type I

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The presence of circulating "flower cells" and a low prevalence of antibody to Tax regulatory protein of human T-lymphotropic virus type I (HTLV-I) are characteristics of adult T-cell leukemia (ATL). To examine the predictability of levels of HTLV-I antibodies and of flower cell-like abnormal lymphocytes (Ably) for the risk of ATL among asymptomatic HTLV-I carriers, we prospectively evaluated the levels of viral markers of five HTLV-I carriers who developed ATL and 38 age-, sex-, and screen-matched HTLV-I–positive controls in the Miyazaki Cohort Study. After accounting for matching factors, Ably level was slightly, but not significantly, higher among cases than among controls (P = .13). Anti–HTLV-I (odds ratio [OR] = 1.6 per twofold dilution; 95% confidence interval [CI] 0.94, 3.8) was associated with ATL diagnosis, but antibody to Tax regulatory protein (anti-Tax) was not (OR = 0.78; 95% CI 0.26, 1.7). Anti-Tax level was low for all ATL cases for up to 10 years preceding their diagnosis, independent of the level of anti–HTLV-I titer. HTLV-I carriers with a higher anti–HTLV-I titer and a lower anti-Tax reactivity may be at greatest risk of ATL.

ADULT T-CELL LEUKEMIA (ATL) is caused by human T-lymphotropic virus type I (HTLV-I) infection,[1,2] generally many decades after initial infection.[3-5] It is believed that carriers who have acquired infection early in life are at greatest risk of ATL.[6] One of the features of ATL is the unique morphology of leukemic cells with an indented or convoluted nucleus ("flower cells"). The level of flower cells is a marker to distinguish clinical subtypes of ATL and to monitor progression from smoldering to overt ATL.[7] Asymptomatic HTLV-I carriers often have a very low, but detectable, level of circulating abnormal lymphocytes (Ably) that closely resemble the flower cells.[8]

In the population-based Miyazaki Cohort Study, we have found that the presence and levels of Ably is highly correlated with HTLV-I proviral load.[9,10] The presence of Ably also is associated with a greater proportion of $CD4^+/CD25^+$ cells, a predominant phenotype of ATL cells.[11] Moreover, persistent proliferation of HTLV-I–infected clones has been shown primarily in $CD4^+$ cells in asymptomatic carriers with a high proviral load.[12] These data suggest that HTLV-I–infected T-cell clones are likely expanding among Ably-positive carriers and that the presence of Ably may correlate with the risk of ATL. High levels of Ably in the presence of the symptoms of immune suppression has been shown to predict the subsequent development of ATL.[13] However, it is uncertain if low levels of Ably among asymptomatic HTLV-I carriers predict the onset of ATL.

Although the precise mechanism of HTLV-I oncogenesis is unknown, the enhanced expression of Tax regulatory protein, a viral gene product that transactivates transcription of viral mRNA and other host genes that control cell proliferation, is believed to play a central role in HTLV-I oncogenesis.[14,15] However, the level of Tax mRNA has been shown to be low in ATL cells,[16] which has led to the hypothesis that a continuous expression of Tax gene product may not be required for the maintenance of the malignant phenotype.[17] This theory is supported by our observation of a lower prevalence of antibody to the Tax regulatory protein (anti-Tax) among ATL cases compared with asymptomatic HTLV-I carriers.[18] A lower prevalence of anti-Tax also has been found in the subset of HTLV-I carriers with a detectable level of Ably (>0.6%) compared with those without Ably,[10] suggesting that anti-Tax reactivity may be low among those at risk of ATL before their clinical diagnosis.

In this prospective study, we describe changes in prediagnostic viral markers of ATL cases and examine risk factors for ATL in a matched case-control study nested within the Miyazaki Cohort Study.

MATERIALS AND METHODS

Study subjects. The Miyazaki Cohort Study was established in 1984 in two HTLV-I endemic villages in Miyazaki Prefecture, Japan. Nearly 27% of 1,960 cohort members enrolled as of August 1996 are HTLV-I seropositive at baseline.[19] The cohort has been followed in the context of free health examinations offered annually by the government for those aged 40 or older. Those younger than 40 years who come to these examinations also are enrolled in the study. The annual screens consist of a physical examination, other routine health examinations, and blood tests. A detailed baseline questionnaire collects demographic data, as well as information on alcohol and smoking status. A shorter instrument completed at each follow-up screening updates information on interval health history and symptoms and current alcohol and smoking behavior. The study protocol was approved by the Institutional Review Boards of the Miyazaki Medical School and the Harvard School of Public Health. Informed consent was obtained from all study subjects.

The subjects of this analysis are the five HTLV-I carriers who developed ATL during the follow-up through December, 1995, and 38 HTLV-I–positive controls. ATL cases were identified through annual census or reports from next-of-kin. The diagnosis was confirmed by medical records for three of the five cases; two others were confirmed by reports from the local government nurses. Using a nested case-control sampling,[20] the controls were selected from within the cohort among HTLV-I carriers who were alive and free of ATL diagnosis when

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the case died and were matched to the cases by age (± 5 years), sex, and study screens attended. The number of controls selected per case varied based on availability of eligible controls (Table 1).

Laboratory methods. For each serum sample, positivity of anti–HTLV-I was tested by passive particle agglutination assay (Serodia-HTLV, Fujirebio, Tokyo, Japan) at a dilution of 1:16. Positive sera were titered by serial twofold dilution. Anti-Tax reactivity was measured by a Western blot assay using a recombinant Tax gene product as the antigen.[21-23] Reactivity to recombinant-Tax protein was quantified by comparison with anti-Tax positive control sera at dilutions of 1:100, 1:500, 1:2500, or higher (+++, ++, +, or ±), where a lower dilution corresponds to a higher anti-Tax reactivity. Multiple measurements of viral markers were available for subjects who attended more than one screening during the follow-up. Because the standard method of viral marker measurement has changed over time, all sera from the screens selected for case-control comparison in the present study were retested for anti–HTLV-I titer and anti-Tax reactivity in 1996 to attain internal consistency.

Peripheral blood smears were obtained from either the ear lobe or a finger prick, by smearing one drop onto a glass slide. The slides were fixed by methanol and stained with Giemsa. All blood smears were read blinded to ATL diagnosis. The identification of Ably followed the criteria by Kondo et al.[8] The number of Ably among 500 leukocytes was recorded in percent.

Statistical analysis. The mean age, leukocyte count, Ably level, and geometric mean of anti–HTLV-I titer were compared between cases and controls using Wilcoxon signed-rank test. The prevalence of smoking and the presence of anti-Tax reactivity were compared using McNemar's test. The odds ratio (OR) and 95% confidence interval (CI) for the association of ATL diagnosis with viral markers and Ably level were estimated from exact conditional logistic regression (LOGXACT, 1996, Cytel, Cambridge, MA). For cases with multiple observations, the first measurement of HTLV-I viral markers, Ably, and smoking status were used for calculation of the ORs. In the logistic regression models, anti–HTLV-I titer by serial twofold dilution and anti-Tax reactivity were treated as ordinal categorical variables with 10 and five levels, respectively. Leukocyte count, Ably level, and age were treated as continuous variables. Smoking status was dichotomized, with current- and ex-smokers combined as ever-smokers. Statistical significance was based on two-sided tests.

RESULTS

Five ATL cases (3 men, 2 women) developed during the study period. One additional patient was diagnosed with ATL before his enrollment in the study. The characteristics of these ATL cases are summarized in Table 1. The mean age at death of these six cases was 73 years. Samples had been taken from these cases up to 10 years preceding their death. Four of the five incident cases had multiple measurements of prediagnostic viral markers, which showed virtually no change in level over time. Anti-Tax reactivity was relatively low or undetectable for all cases. Only one case (case 3) had detectable level of Ably (>0.6%) before diagnosis. All male cases were long-term smokers, whereas both female cases were never-smokers.

Because ongoing therapy may have modified the levels of Ably and of viral markers, the prevalent ATL case (case 1) was excluded from further analysis. The characteristics of the five ATL cases as compared with the 38 controls are summarized in Table 2. After accounting for the matching factors, neither the geometric mean of anti–HTLV-I titer (1,782 *v* 467, $P = .31$) nor prevalence of anti-Tax reactivity (20% *v* 32%, $P = .57$) was different between cases and controls. The prevalence of ever-smokers and the mean leukocyte count were also similar for cases and controls. The mean Ably level was slightly, but not significantly, higher among cases compared with controls (0.36% *v* 0.24%, $P = .13$). Table 3 shows the distribution of anti-Tax reactivity among cases and controls by their anti–HTLV-I titer. Among controls, the proportion of anti-Tax positivity increased with anti–HTLV-I titer level. The positive correlation between the levels of anti–HTLV-I titer and anti-Tax reactivity were comparable to those in the entire study cohort of HTLV-I carriers (Table 3). In contrast, there was no apparent correlation between these two viral markers among ATL cases.

Anti–HTLV-I titer was associated with ATL diagnosis (OR = 1.6 per twofold dilution; 95% CI 0.95, 3.8), albeit not to a statistically significant degree ($P = .09$) (Table 4). Anti-Tax was not predictive (OR = 0.78; 95% CI 0.26, 1.7). With mutual adjustment for anti–HTLV-I titer and anti-Tax reactivity, the OR for anti–HTLV-I titer and anti-Tax reactivity was 1.6 (95% CI 0.95, 3.5) and 0.74 (95% CI 0.21, 1.8), respectively. Smoking and leukocyte counts were not significant predictors of ATL. We were unable to obtain a stable estimate for the association of Ably level and the risk of ATL in the logistic regression analysis due to sparse data.

DISCUSSION

The rare occurrence and long latency period of ATL has posed substantial difficulties in community-based, prospective

Table 1. Prediagnostic Changes in Viral Markers, Ably, and Leukocyte Count Among the Six ATL Cases in the Miyazaki Cohort Study

Case No.	No. of Controls	Sex	Date of Diagnosis	Date of Death	Age at Death	Smoking	Anti–HTLV-I Titer	Anti-Tax Reactivity	Ably Level in %	Leukocyte Count	Screen Year
1*	—	M	05/1982	08/1987	66	Yes	8,192	−	0.0	7,400	1986
2	10	F	NA	02/1987	69	No	512	±	0.2	4,300	1985
3	11	M	NA	08/1990	64	Yes	8,192	−	1.1	6,400	1985
							8,192	−	NA	9,600	1987
4	3	M	08/1991	04/1992	78	Yes	8,192	−	0.6	4,300	1984
							8,192	−	0.6	5,100	1985
5	5	M	10/1994	12/1994	83	Yes	1,024	+	0.2	4,800	1985
							2,048	+	0.6	4,800	1989
6	9	F	07/1994	05/1995	77	No	512	±	0.4	5,800	1985
							256	±	0.6	4,300	1989
							512	±	NA	3,800	1992

Abbreviation: NA, not available.

*Measurements of all markers for this prevalent case were posttreatment. This case is not included in the case-control analysis.

Table 2. Comparison of Demographic Characteristics and HTLV-I Viral Markers Between Five ATL Cases and 38 Age-, Sex-, and Screen-Matched Controls in the Miyazaki Cohort Study, Adjusted for the Matching Factors

	Case (n = 5)	Controls (n = 38)	*P* Value
Age (mean, years)*	79	76	.13
Smoker†	3 (60%)	18 (47%)	.73
Ably level (mean, %)‡	0.36	0.24	.13
Leukocyte count (mean, /mL)‡	4,200	5,300	.99
Geometric mean anti-HTLV–I titer§	1,782	467	.31
Positivity for anti-Tax reactivity†§	1 (20%)	12 (32%)	.57

*Age of subjects was determined at the death of the index case.

†Viral markers and smoking status were determined at the first screen at which data were available for the index case.

‡The mean value was based on data from the screens attended by the index case.

§Anti-Tax reactivity (+++, ++, +, and ±) was defined as positive; (−) as negative.

studies of this malignancy. The present study examined the characteristics of ATL cases in a well-defined adult population endemic for HTLV-I infection. The mean age of ATL mortality, 73 years, was much higher than the national average of 57 years among the general Japanese population,[24] reflecting the high average age (60 years) of the HTLV-I–positive cohort members in the Miyazaki Cohort.

Our analysis found a 1.6-fold increase in the risk of ATL per twofold increase in anti–HTLV-I titer, indicating that those carriers with the highest titer (≥1:8,192) will have an approximately 70-fold risk of developing ATL relative to those with the lowest titer level (1:16). Given a strong positive correlation between anti–HTLV-I and proviral load among asymptomatic carriers in our cohort,[25] the observed association of anti–HTLV-I with ATL diagnosis may imply a similar association of proviral load with this malignancy.

As would be predicted from our previous finding of a lower prevalence of anti-Tax reactivity among ATL cases,[18] the analysis of prediagnostic sera of the ATL cases in the present study showed that anti-Tax reactivity was low or undetectable for all cases for up to 10 years preceding their diagnosis. Given the observed higher anti–HTLV-I titer among the ATL cases compared with asymptomatic carriers, as well as our previous report of a strong correlation between anti–HTLV-I titer and anti-Tax positivity among asymptomatic carriers,[21] it seems possible that a loss of anti-Tax occurs in the process of ATL development. Those carriers at risk of ATL also may be inherently more likely to have low anti-Tax reactivity.

Table 3. Distribution of Anti-Tax Reactivity by the Level of Anti–HTLV-I Titer Among Cases, Controls, and All HTLV-I Carriers in the Study Cohort

	No. of Anti-Tax Positive Subjects (%)		
Anti–HTLV-I Titer	Case (N = 5)	Control (N = 38)	Study Cohort* (N = 306)
4,096 ≤ titer	0/2 (0%)	5/6 (83%)	49/59 (83%)
1,024 ≤ titer ≤ 2,048	1/1 (100%)	7/8 (88%)	52/77 (68%)
128 ≤ titer ≤ 512	2/2 (100%)	8/17 (47%)	68/134 (51%)
16 ≤ titer ≤ 64	0/0 (UD)	2/7 (29%)	12/36 (33%)

For cases and controls, cross-classification was by the observation of viral markers at the first screen at which data were available for the index case.

Abbreviation: UD, undefined.

*Includes all HTLV-I–positive cohort subjects whose anti-Tax reactivity was available.

Table 4. Univariate Association of ATL With Viral Markers, Smoking, and Leukocyte Count Using Exact Conditional Logistic Regression

Variables*	OR	95% CI
Anti–HTLV-I titer	1.6	0.94-3.8
Anti-Tax reactivity	0.78	0.26-1.7
Leukocyte count	0.73	0.40-1.2
Ever-smoker	1.7	0.04-198.4

Abbreviations: OR, odds ratio; CI, confidence interval.

*The ORs for anti–HTLV-I titer and anti-Tax reactivity are for 1 unit increase in level; smoking status is dichotomous. Referent categories are: anti–HTLV-I titer 1:16, anti-Tax reactivity (−), and never-smokers, respectively. Leukocyte count is a continuous variable.

ATL cells are less likely to express detectable level of Tax mRNA.[26] Because Tax protein is a known target of cytotoxic T-lymphocyte (CTL) immunity,[16,27] it seems plausible that ATL cells that do not express Tax are likely to escape cell lysis mediated by the CTL.[2] Thus, one explanation for the observed low anti-Tax reactivity among ATL cases may be their low CTL response against HTLV-I. Determinants of CTL response, such as the human leukocyte antigens, may play a role in determining the host's susceptibility to ATL.[28-30] In addition, a large proportion of ATL cells has been found to carry deleted HTLV-I genomes, raising the possibility that genetic events that render an altered immunogenecity to the Tax protein may be crucial in the development of ATL.[31] Mutations in the Tax gene that lead to an altered CTL response against Tax protein[32] or changes in viral transcription and translation[33] also could result in the loss of anti-Tax reactivity. We were unable to evaluate these possibilities due to the lack of preserved lymphocyte specimens.

Ably level has been shown to spontaneously fluctuate in ATL patients.[13] Because of the large amount of within-individual variability, there have been arguments for and against the use of Ably level as a marker for risk of ATL.[9,34] In the present study, the mean level of prediagnostic Ably of the ATL cases was only slightly higher than that of their matched controls. The possibility exists that the predictability of Ably depends on clinical ATL diagnosis. However, due to small number of cases, no further analysis could be conducted. Thus, our findings must be interpreted with caution.

Although the use of population-based controls allowed the comparison of characteristics between cases and controls with minimal selection bias, there are several important limitations to the present study. A few eligible controls could not be included in the analysis because one or more serum samples were no longer available. However, exclusion of these subjects is unlikely to have affected the ORs substantially, as unavailability of specimens was random. Any misclassification of explanatory variables such as Ably measurement and smoking history would be unrelated to ATL diagnosis and thus have biased the estimates only towards the null. We were unable to evaluate our data with additional adjustment for proviral load and Tax mRNA level in multivariate analysis because preserved lymphocyte specimens were unavailable for the majority of ATL cases and controls. Statistical power to detect significant differences

was clearly limited by the relatively small number of observations.

In sum, the present analysis suggests that HTLV-I carriers with a higher anti–HTLV-I titer are at greatest risk of ATL and that the level of anti–HTLV-I and anti-Tax reactivity is discorrelated before diagnosis. Additional analysis of Tax mRNA expression, proviral load, HTLV-I clonality, as well as direct measurement of CTL response is needed to provide further insights into the oncogenic process of this virus infection. Investigation of correlates of the host immune response in this population in relation to HTLV-I viral markers may be useful to shed light on the association between host factors and the risk of ATL.

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