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Immunology

HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy

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ABSTRACT **The risk of disease associated with persistent virus infections such as HIV-I, hepatitis B and C, and human T-lymphotropic virus-I (HTLV-I) is strongly determined by the virus load. However, it is not known whether a persistent class I HLA-restricted antiviral cytotoxic T lymphocyte (CTL) response reduces viral load and is therefore beneficial or causes tissue damage and contributes to disease pathogenesis. HTLV-I-associated myelopathy (HAM/TSP) patients have a high virus load compared with asymptomatic HTLV-I carriers. We hypothesized that HLA alleles control HTLV-I provirus load and thus influence susceptibility to HAM/TSP. Here we show that, after infection with HTLV-I, the class I allele *HLA-A*02* halves the odds of HAM/TSP ($P < 0.0001$), preventing 28% of potential cases of HAM/TSP. Furthermore, *HLA-A*02*$^+$ healthy HTLV-I carriers have a proviral load one-third that ($P = 0.014$) of *HLA-A*02*$^-$ HTLV-I carriers. An association of *HLA-DRB1*0101* with disease susceptibility also was identified, which doubled the odds of HAM/TSP in the absence of the protective effect of *HLA-A*02*. These data have implications for other persistent virus infections in which virus load is associated with prognosis and imply that an efficient antiviral CTL response can reduce virus load and so prevent disease in persistent virus infections.**

Host genetic factors are major determinants of susceptibility to infectious disease in humans. In 1974, Zinkernagel and Doherty (1) showed that the cytotoxic T lymphocyte (CTL) response to virus infections was restricted by class I alleles of the MHC. Surprisingly, however, it has been difficult to demonstrate a direct protective effect of class I MHC alleles against a viral infection in either human or animal populations. There have been reports of protective class I alleles in HIV-1 infected long-term nonprogressors (2–5), but the results have not been consistent. HLA class II alleles have been associated with both susceptibility to and protection from viral diseases, e.g., hepatitis B and human papilloma virus; the immunogenetics of infectious diseases has recently been reviewed by Hill (6).

In chronic virus infections such as HIV-1 and 2, hepatitis B virus, and hepatitis C virus, virus load is an important determinant of the outcome of infection and disease. Recent evidence suggests that provirus load is also an important factor in the outcome of human T-lymphotropic virus-I (HTLV-I) infection (7–10). HTLV-I is a persistent virus, infecting 10–20 million people worldwide. Most infected people remain healthy, but 1–2% develop a progressive paralytic myelopathy (HTLV-I-associated myelopathy; HAM/TSP) and a further 2–3% develop an aggressive T cell leukemia/lymphoma. The reasons for the different outcomes of infection are unknown. HAM/TSP is a chronic debilitating inflammatory disease of the central nervous system, characterized by axonal damage and demyelination, most pronounced in the midthoracic spinal cord (11). The HTLV-I proviral load is 10- to 100-fold greater in HAM/TSP patients than in asymptomatic healthy carriers (HCs) of the virus (9, 12), although the ranges overlap. The pathogenesis of this condition is not understood.

We have shown previously that no particular sequence of HTLV-I is associated with neurological disease (13), and we therefore concluded that the different outcomes of HTLV-I infection are caused mainly by differences in the host response to the virus rather than the virus itself (14, 15). HAM/TSP patients mount a very vigorous antibody (16) and CTL (17–19) response to HTLV-I. This has led to the suggestion (17, 20) that the anti-HTLV-I immune response, in particular the CTL, contributes to the tissue damage in the spinal cord that causes the syndrome of HAM/TSP. However, we have found a chronically activated CTL response almost entirely directed at the viral transactivator protein Tax (19) in the majority of both HCs of the virus and HAM/TSP patients. The CTL response exerts a significant selection pressure on the Tax protein, selecting variant sequences of Tax that escape CTL recognition (21). However, the variant sequences do not reach fixation in the viral population, because the putative escape mutations impair the function of the Tax protein (21). The selection on Tax is stronger in the HCs than in the HAM/TSP patients (22). We concluded that the CTL response in HTLV-I infection might be protective rather than pathogenic (23). According to this hypothesis, HCs are high CTL responders and HAM/TSP patients low CTL responders to HTLV-I. These conclusions were supported by mathematical models of the population dynamics of immune responses to persistent viruses (24), which made the nonintuitive prediction that the frequency of anti-HTLV-I CTL could be greater in the HAM/TSP patients than in the HCs, even though the CTL are responsible for the lower proviral load in HCs. Thus, polymorphic genes that control the efficiency of the anti-HTLV-I CTL response might account for the different outcomes of HTLV-I infection.

Abbreviations: HTLV-I, human T-lymphotropic virus-I; HAM/TSP, HTLV-I associated myelopathy; CTL, cytotoxic T lymphocyte; HCs, healthy asymptomatic HTLV-I carriers; F_p, preventive fraction; OR, odds ratio; CI, confidence interval; PBMC, peripheral blood mononuclear cell.

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MHC Class I and Class II as Candidate Genes in HAM/TSP. Both the class I MHC proteins (HLA-A, -B, and -C), which present viral peptides for recognition by virus-specific CTL (25), and class II MHC proteins (HLA-DR and -DQ), which present peptides to CD4 T cells, are likely to be important in the immune response to HTLV-I infection. The results of several previous studies (26–29) have suggested associations between various HLA class I or class II alleles and susceptibility to T cell leukemia/lymphoma or HAM/TSP. However, small sample size, mixed ethnicity, lack of adequate controls, or lack of staging in these studies precluded a definite conclusion. No previous study has indicated HLA-associated protection against HTLV-I-associated disease. We therefore set out to test two hypotheses, that (*i*) certain class I MHC alleles protect against the HTLV-I induced disease HAM/TSP and (*ii*) certain class I MHC alleles reduce HTLV-I provirus load.

The most consistent association between HLA and HAM/TSP has been found with *HLA-DRB1*0101* (26, 29–31), an apparent susceptibility allele for HAM/TSP. This allele is in linkage disequilibrium in the Japanese population with *HLA-B*07*, *Cw*07*, and *DQB1*0501* (32, 33). We therefore set out to test a third hypothesis, that (*iii*) *HLA-DRB1*0101* is associated with susceptibility to HAM/TSP.

We report here the results of a two-stage case-control association study of HAM/TSP in the population of Kagoshima Prefecture (1988 population: 1.7 million), southern Kyushu, Japan, where the seroprevalence of HTLV-I infection in adults is ≈10% (34, 35). The estimated prevalence of HAM/TSP in the HTLV-I positive population is <1% (36). We show that the *HLA-A*02* gene is associated with both protection from HAM/TSP and a significant reduction in provirus load in asymptomatic carriers of HTLV-I. The allele *DRB1*0101* is associated with susceptibility to HAM/TSP, but only in the absence of the protective effect of *HLA-A*02*. We conclude that a strong persistent class I-restricted CTL response to HTLV-I benefits the host by reducing the viral load. The results suggest that an effective vaccine against HTLV-I and other persistent viruses should elicit a strong antiviral CTL response.

METHODS

Study Population. Two hundred and thirty-two cases of HAM/TSP were compared with 201 randomly selected HTLV-I seropositive asymptomatic blood donors (HCs) from the Kagoshima Red Cross Blood Transfusion Service. All cases and controls were of Japanese ethnic origin and resided in Kagoshima Prefecture, Japan. The diagnosis of HAM/TSP was made according to World Health Organization diagnostic criteria (37).

HLA Class I Typing. A two-stage study was performed. In stage 1, 96 PCR–sequence-specific primer reactions were performed to detect all known HLA-A, -B, and -C specificities in an allele- or group-specific manner (92 possible alleles or groups of alleles) (38). In stage 2, a single pair of sequence-specific primers detecting all known subtypes of *A*02 (A*0201-0225*) was used to test an independent sample of cases and controls.

Subsequently, further class I typing was undertaken with a reduced number of PCR–sequence-specific primer reactions (42 possible alleles or groups of alleles), designed to detect all of the HLA-A, -B, and -C specificities occurring at a gene frequency of ≥5% or an odds ratio (OR) of ≤0.5 or ≥2.0 from the initial stage 1 study. After typing 100 cases and controls in total, the alleles *HLA-B*0702*, *Cw*0702*, and *Cw*0710* were selected for further analysis in an independent sample. The PCR primers used were unable to distinguish between *Cw*0702* and *Cw*0710*, a rare suballele.

HLA Class II Typing. Class II typing was performed in an unstaged manner by using the methods of Olerup (39, 40) and Bunce (38).

HLA-A*02 and HLA-B*07 Subtyping. The design of sequence-specific primers for *A*0201–A*0225* alleles and *B*0702–0708* was based on published gene sequences (41) updated from HLA informatics pages available on the internet (http://www.anthonynolan.com/HIG/index.html). PCR methods were as described (38).

Proviral Load Measurement. The HTLV-I provirus load in peripheral blood mononuclear cells (PBMC) was measured in all patients and HCs as described (9). A quantitative PCR reaction was performed by using an ABI 7700 sequence detector (Perkin–Elmer Applied Biosystems). All DNA standards and samples were amplified in triplicate. A standard curve was generated by using the β-actin gene from HTLV-I-negative PBMC and the Tax gene from TARL-2, a cell line containing a single copy of HTLV-I proviral DNA. The amount of HTLV-I proviral DNA was calculated as follows: copy number of HTLV-I (tax) per 10^4 PBMC = [copy number of tax/(copy number of β-actin/2)] × 10^4. The lower limit of detection was 1 copy per 10^4 PBMC.

Statistical Analysis. The χ^2 test, the Mann–Whitney *U* test, and the odds ratio (GraphPad, San Diego) were used for statistical analysis. The Bonferroni method (42) was used to correct for multiple comparisons. The population attributable risk was calculated according to Schlesselman (43). To identify the significant independent variables associated with disease risk, we carried out a standard logistic regression analysis (44).

To calculate the prevented fraction (F_p) of disease, consider the 2 × 2 contingency table

	G^+	G^-
D	a	b
H	c	d

where D = disease, H = health, G^+ = positive for protective genotype, G^- = negative for protective genotype. By Bayes' theorem of conditional probabilities, the fraction (F_p) of potential cases of disease D in the population that is prevented by the genotype G^+ is given by $F_p = (1 - R) \times [1 - (dr_1/br_2)]$, where R = prevalence rate of disease D in the population, $r_1 = a + b$ and $r_2 = c + d$. In the case of HAM/TSP, R is estimated as ≤1% of the HTLV-I-infected population. F_p is approximately normally distributed: the standard deviation is

Table 1. *HLA-A*02* reduces the odds of HAM/TSP

Stage	HAM/TSP, No.		HCs, No.		$\chi^{2\dagger}$	*P*	Odds ratio‡	CI, 95%
	A*02$^+$	A*02$^-$	A*02$^+$	A*02$^-$				
1	12	38	32	24	10.6	0.001	0.24	0.10–0.55
2	57	125	68	77	7.6	0.006	0.52	0.33–0.81
All subjects	69	163	100	101	17.3	<0.0001	0.43	0.29–0.63

A*02$^+$ and A*02$^-$ denote the presence or absence of the *A*02* gene in the subjects studied. In total, 232 HAM/TSP patients and 201 HCs were studied. Stages denote independent, consecutive case-control studies, and do not refer to clinical stage.

†With Yates correction.

‡Using the approximation of Woolf.

Table 2. *HLA-A*02* and subtype *A*0206* proviral loads in HCs

	HAM/TSP			HCs		
HLA subtype	Median proviral load	No. of subjects	*P*	Median proviral load	No. of subjects	*P*
A*02$^+$	524.8	67	0.18	16.8	100	0.014
A*02$^-$	616.6	155		50.1	101	
A*0206$^+$	631.0	28	0.42	10.5	52	0.004
A*0206$^-$	562.3	194		43.7	149	
A*02$^+$ A*0206$^-$	426.6	39	0.026	38.9	48	0.40
A*02$^-$	616.6	155		50.1	101	

*HLA-A*02* and subtype *A**0206 were associated with a reduced provirus load in HCs. Other *A*02* subtypes were associated with a reduced provirus load in HAM/TSP patients.
Median proviral load given as proviral copy number per 10^4 PBMC. P reported as two-tailed, uncorrected Mann–Whitney *U* test.

given by SD (F_p) = $(1 - R - F_p) \times \sqrt{[(c/dr_2) + (a/br_1)]}$. A full derivation of these formulae is available on request.

Phenotypic Analysis of $Tax_{11–19}$-Specific CTL. In the chronically activated CTL response to HTLV-I, several peptides derived from the immunodominant Tax protein of the virus are restricted by *HLA-A*02* (45, 46). $Tax_{11–19}$ is a dominant *A*02*-restricted epitope (47). To compare the frequency of HTLV-I-specific CTL in HAM/TSP patients and HCs, we used the recently developed technique of soluble peptide-MHC tetramers (48). Analysis of PBMC for the presence of $Tax_{11–19}$-specific CD8$^+$ CTL was performed by using fluorescent-labeled tetramers of *HLA-A*0201* + β_2 microglobulin + $Tax_{11–19}$ in 19 *HLA-A*02*$^+$ HAM/TSP patients and 19 *HLA-A*02*$^+$ HTLV-I HCs from the Kagoshima study population. The cells were incubated with $Tax_{11–19}$ tetramer at 37°C for 20 minutes and anti-CD8 antibody (Caltag, South San Francisco, CA) on ice for 30 minutes then washed three times in ice-cold phosphate-buffered saline before fixation in 1% freshly made paraformaldehyde for 30 minutes at 4°C. The antigen-specific T cells were quantified by flow cytometry on a Coulter Epics XL (Beckman Coulter).

RESULTS

The median age of HAM/TSP patients (59 years) was greater than that of the HCs (41 years). The sex ratio of males/females in the HAM/TSP group was 1:2, with a 1:1 ratio in the HCs. However, there was no correlation between the HTLV-I proviral load and age at blood sampling (HAM/TSP patients: $r = -0.096$, $P = 0.22$; HCs: $r = 0.081$, $P = 0.25$, Spearman's rank correlation) or duration of disease in the Kagoshima population (see ref. 9 for details). Because the prevalence of HAM/TSP in Kagoshima is low (≤1%) among HTLV-I seropositives, very few HCs would be expected to develop HAM/TSP. χ^2 and logistic regression analyses confirmed that the frequency of *HLA-A*02*, *B*0702*, and *DRB1*0101* and the proviral load were unaffected by age or sex (44) (data not shown).

In the first 50 cases and 56 controls, the genotype frequency of *HLA-A*02* was significantly lower (24%) among the cases of HAM/TSP compared with the controls (57%, $P = 0.001$, uncorrected) (Table 1). Because of the large number of alleles tested for, it was necessary to confirm this association in an independent sample. We therefore retested the association with *HLA-A*02* in an independent sample by using a single pair of sequence-specific primers to detect all known subtypes of *A*02*. The frequency of *HLA-A*02* was again lower in the patients (33%) than the controls (47%, $P = 0.006$) (Table 1), confirming the association. The two data sets, when combined, indicate that the possession of *HLA-A*02* is associated with a reduction in the odds of disease by >2-fold ($P < 0.0001$) and prevents (F_p) ≈28% (±5.8% SD) of potential cases of HAM/TSP in the study population.

It is likely that *HLA-A*02* is associated with protection against HAM/TSP because *HLA-A*02*-restricted, anti-HTLV-I CTL kill HTLV-I-infected cells and so reduce the provirus load of HTLV-I. We therefore tested the hypothesis that HTLV-I proviral load is lower in *A*02*$^+$ subjects than *A*02*$^-$ subjects. In the whole sample, there was a 16-fold greater median provirus load in the HAM/TSP patients ($n = 222$) than in the HCs ($n = 201$) (575 copies per 10^4 PBMC, compared with 35 copies per 10^4 PBMC, $P < 0.0001$, Mann–Whitney *U* statistic, two-tailed), in agreement with other studies (7). There was a 3-fold lower median provirus load in the *A*02*$^+$ HCs compared with the *A*02*$^-$ HCs (Table 2, $P = 0.014$).

An *A*02* subtyping method was developed to identify the *A*02* subtypes present in the population and to examine whether a particular *A*02* subtype was associated with disease protection or a reduction in proviral load. *A*02* subtypes *A*0201*, *0203, 0206, 0207*, and *0210* were detected. The genotype frequencies (as % of *A*02*$^+$ subjects) were as follows—*A*0201*: HAM/TSP 36%, HCs 36%; *A*0203*: HAM/TSP 3%, HCs 0%; *A*0206*: HAM/TSP 43%, HCs 53%; *A*0207*: HAM/TSP 20%, HCs 22%; *A*0210*: HAM/TSP 3%, HCs 2%. Four HAM/TSP patients and 11 HCs were heterozygous for *A*02* subtypes. The possession of *A*0206* was significantly associated with disease protection (*A*0206*$^+$; 30 of 232 HAM/TSP patients, 52 of 201 HCs, $P = 0.001$ (two-tailed), OR = 0.43, 95% CI (confidence interval) = 0.26–0.70). This association remained significant after correction for multiple comparisons ($P_{corrected} = 0.005$). The possession of *A*0206* was associated with a 4-fold reduction in median provirus load in the HCs ($P = 0.004$, Table 2), (F_p=15% ± 4.1% SD). The possession of *HLA-A*02* subtypes other than *A*0206* (i.e., *A*0201, 0203, 0207*, and *0210*) also was signif-

Table 3. *HLA-DRB1*0101* increases the odds of HAM/TSP in *A*02* negative (*A*02*$^-$) but not in *A*02* positive (*A*02*$^+$) subjects

	HAM/TSP, No.		HCs, No.					
Subjects	DRB1$^+$	DRB1$^-$	DRB1$^+$	DRB1$^-$	χ^2	*P*	Odds ratio	95% CI
All	34	161	20	163	2.8	0.049	1.72	0.95–3.12
A*02$^-$	27	107	10	83	2.9	0.044	2.09	0.96–4.57
A*02$^+$	7	54	10	80	0.005	0.47	1.03	0.37–2.89

χ^2 reported as one-tailed with Yates correction. Odds ratio used the approximation of Woolf.

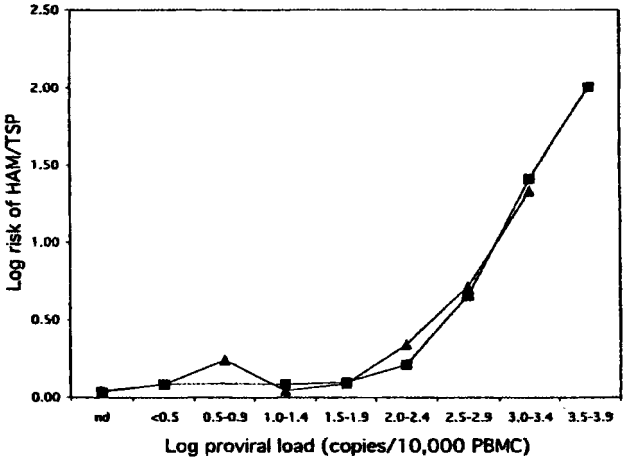

FIG. 1. Risk of HAM/TSP according to possession of *A*02*. ▲, log p(HAM | L) if *A*02*$^+$, ■, log p(HAM | L) if *A*02*$^-$, nd, not detectable by this assay, —, no *A*02*$^-$ HAM/TSP patient had a logarithmic proviral load of 0.5–0.9. At a given provirus load, the risk of HAM/TSP is not affected by the presence or absence of *A*02*. However, as the possession of *A*02* reduces the proviral load, the effect of *A*02* is to reduce the risk of disease. For the calculation of HAM/TSP risk at a given proviral load, we used Bayes' theorem. By using the standard notation for conditional probability, where p(HAM | L) denotes the probability of HAM/TSP in an HTLV-I-infected person with a given provirus load (L), we write: p(HAM | L) = [p(HAM) × p(L | HAM)]/[p(HAM) × p(L | HAM) + p(HC) × p(L | HC)]. We estimated p(L | HAM] and p(L | HC) from the distribution of proviral load in the HAM/TSP and HC cohorts in the present study. p(HAM), the prevalence of HAM/TSP in the HTLV-I-positive population, is taken as 0.01.

icantly associated with protection against HAM/TSP (*A*02*$^+$ *A*0206*$^-$*;* 39 of 202 HAM/TSP patients, 48 of 149 HCs, P = 0.008 (two-tailed), OR = 0.50, 95% CI = 0.31–0.82). *HLA-A*02* subtypes other than *A*0206* also were associated with a reduction in median provirus load in the HAM/TSP patients (P = 0.026, Table 2) but not in HCs (P = 0.40, Table 2); the 1.3-fold reduction in median proviral load in HCs did not reach statistical significance.

The risk (prevalence) of HAM/TSP at a given proviral load can be calculated from the data on proviral load in the HAM/TSP and HC cohorts (Fig. 1). Fig. 1 shows that the risk of HAM/TSP rises exponentially as the provirus load increases above an apparent threshold of 1% PBMC ($\log_{10}$ proviral copy number per 10^4 PBMC = 2). However, the risk of HAM/TSP at any given provirus load was not affected by the presence or absence of *A*02*. This result suggests that the mechanism by which *A*02* reduces the risk of HAM/TSP is by reducing the provirus load of HTLV-I.

These data on the protective effect of *A*02* in HAM/TSP are supported by data from an independent population of HAM/TSP patients and HTLV-I-infected asymptomatic carriers from London. In this population, 4 of 15 HAM/TSP patients were *HLA-A*02*$^+$, compared with 10 of 14 HCs [P (one-tailed) = 0.02, Fisher's exact test]. Twenty-seven of the 29 subjects were of Caribbean origin and 2 were of Caucasian origin.

By using fluorescent-labeled tetramers of *HLA-A*0201* + β_2 microglobulin + Tax_{11-19}, tetramer-positive CTL were found in both HAM/TSP patients and HCs: Fig. 2 indicates that CD8$^+$ cells from *A*0201*$^+$, *A*0206*$^+$ and *A*0207*$^+$ individuals bound the *A*0201* Tax_{11-19} tetramer. The mean frequency of tetramer-positive cells in the CD8$^+$ population in HAM/TSP patients (1.9% ± 0.5% SE, n = 19) was not significantly different from the mean frequency in HC (0.9% ± 0.2% SE, n = 19; P = 0.58, Mann–Whitney). Mean tetramer binding in

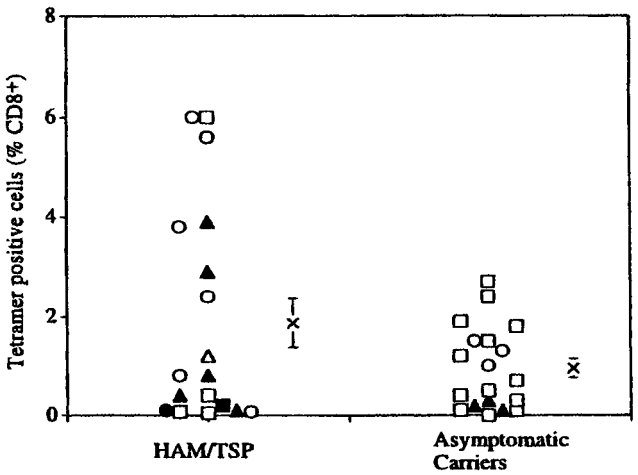

FIG. 2. Tetramer-positive CTL were found in both HAM/TSP patients and HCs. The mean frequency of HTLV-I-specific CTL does not differ between HAM/TSP patients and HCs (P = 0.58; two-tailed Mann–Whitney U-statistic). CD8$^+$ cells from both *A*0201*$^+$, *A*0206*$^+$, and *A*0207*$^+$ individuals bound the *A*0201*-Tax_{11-19} tetramer. ■, *A*0206*; ▲, *A*0207*; ○, *A*0201*; ●, *A*0210*, Δ *A*0201/A*0206*; ■, *A*0201/A*0207*; ×, mean % tetramer ± SE.

*HLA-A*02*-negative controls of mixed ethnic origin was 0.1% ± 0.1% SE (4 HAM/TSP patients, 1 HC).

*HLA-DRB1*0101* was associated with susceptibility to HAM/TSP [Table 3, P = 0.049 (one-tailed, because of the previously observed association)]. Possession of this allele also was associated with a significantly lower virus load in the HAM/TSP patients [Table 4; P = 0.024 (two-tailed)], but not in HCs. The *DRB1*0101*-associated susceptibility to disease and reduced provirus load was not seen in *HLA-A*02*$^+$ HAM/TSP patients, but was significant in *HLA-A*02*$^-$ patients (Tables 3 and 4). The population attributable risk conferred by *DRB1*0101* (i.e., the excess fraction of cases of HAM/TSP in the sample that would not have occurred had *DRB1*0101* been absent) was 7%. This increased to 11% in *A*02*$^-$*–DRB1*0101*$^+$ subjects, and fell to 0.4% in *A*02*$^+$*–DRB1*0101*$^+$ subjects.

The association of *B*0702* with HAM/TSP was examined in a staged study. In stage 1, 21 of 100 patients and 8 of 100 controls were found to have *B*0702* [P = 0.016 (two-tailed), OR = 3.1, 95% CI = 1.28–7.28; $P_{corrected}$ was not significant]. This association was not replicated, however, in an independent sample [20 of 130 patients, 15 of 101 controls, P = 0.46 (one-tailed)], and was only significant in the whole population by using a 1-tailed test of significance (P = 0.046, OR = 1.66, 95% CI = 0.96–2.88). Among *B*0702*$^+$ subjects, 49 of 57 (86%) also were positive for *DRB1*0101*, and 49 of 54 (91%) *DRB1*0101*$^+$ subjects also were positive for *B*0702*. All *B*0702*$^+$ subjects possessed *Cw*07*, and all *DRB1*0101*$^+$ subjects possessed *DQB1*0501. Cw*07* and *DQB1*0501* were not more significantly associated with HAM/TSP than *DRB1*0101* (data not shown).

In the 100 patients and 100 controls examined for all class I specificities, only *HLA-A*02, -B*0702*, and *-Cw*0702/10* differed significantly in frequency between patients and controls at the $P < 0.05$ level (uncorrected). We are therefore unable to confirm the suggestion made by Nishimura *et al.* (29) that *HLA-A*31* is associated with HAM/TSP.

DISCUSSION

This study demonstrates that the risk of HAM/TSP is strongly associated with the equilibrium provirus load of HTLV-I, and that *HLA-A*02* reduces the risk of disease by reducing provirus load. The data reported here and the previous findings in

Table 4. HTLV-I provirus load associated with *HLA-DRB1*0101* in the presence or absence of *HLA-A*02*

	HAM/TSP			HCs		
	Median proviral load	No. of subjects	*P*	Median proviral load	No. of subjects	*P*
DRB1*0101$^+$	331.1	34	0.024	49.0	20	0.33
DRB1*0101$^-$	602.6	161		34.7	163	
A*02$^-$ DRB1$^+$	338.8	27	0.03	109.6	10	0.22
A*02$^-$ DRB1$^-$	631.0	107		41.7	83	
A*02$^+$ DRB1$^+$	331.1	7	0.41	21.9	10	0.81
A*02$^+$ DRB1$^-$	524.8	54		21.9	80	

A significant reduction in provirus load is observed in the HAM/TSP patients, but not in the *A*02*$^+$ patients or in the HCs. Proviral copy reported as number per 10^4 PBMC. *P* level reported using two-tailed Mann–Whitney *U* test.

healthy HTLV-I carriers of strong anti-HTLV-I CTL responses, low proviral load, and viral escape mutants, can be interpreted as follows. HCs mount a strong CTL response to HTLV-I and so limit the proviral load to a low level. That is, their virus-specific CTL proliferate rapidly in response to HTLV-I antigens and/or kill HTLV-I-infected cells rapidly (24). HAM/TSP patients, on the other hand, make a weak CTL response to HTLV-I, and the virus is allowed to reach a high equilibrium provirus load. Thus, in HCs, a high frequency of CTL is maintained by a relatively low virus load, whereas in HAM/TSP patients, a high virus load stimulates an inefficient CTL response. Consequently, at equilibrium there may be little difference in the mean frequency of HTLV-I-specific CTL between the HAM/TSP patients and the HCs, as observed in this study. These results are consistent with our previous finding (46) that the mean frequency of the Tax peptide-specific CTL, measured by limiting-dilution assays, did not differ significantly between HAM/TSP patients and HCs.

This study demonstrates that each of the major *HLA-A*02* subtypes present in the Kagoshima population is able to present an immunodominant peptide from HTLV-I Tax ($Tax_{11–19}$) to CD8$^+$ T cells, and confer protection from HAM/TSP. There may be differences between the effects of the respective *A*02* subtypes, as reflected in the pattern of proviral load reduction in the HAM/TSP and HC cohorts (Table 2). However, *HLA-A*02*$^+$ CTL responders to Tax frequently recognize more than one *A*02*-restricted epitope in Tax (49). Also, *A*02* subtypes differ significantly in their peptide-binding characteristics (50, 51). Experiments are needed to test whether the *A*02* subtypes differ in the affinity with which they bind Tax peptides.

The most probable mechanism for the pathogenesis of HAM/TSP is bystander damage to uninfected cells caused by the activated T cells found in HTLV-I infection (14, 15, 23). It is likely that CD4$^+$ cells play an important part in bystander damage in the central nervous system, because these are the predominant cells early in the active lesions of HAM/TSP (52). Moreover, it is now clear that HTLV-I in the inflammatory lesions is present only in the invading CD4$^+$ cells (53, 54). In this case, class II genes, which determine the antigen specificity of CD4$^+$ cells, could be associated with susceptibility to HAM/TSP. It is therefore possible that one of the class II alleles in the susceptibility haplotype *(HLA-B*0702-Cw*0702-DRB1*0101-DQB1*0501)* is responsible for the susceptibility effect, via an effect on CD4 T cell activation and increased bystander damage. Possession of this haplotype is associated with a lower provirus load in HAM/TSP patients. The explanation for this is that DRB1*0101$^-$ individuals only develop HAM/TSP if (on average) they have a high proviral load. DRB1*0101$^+$ individuals are, however, more susceptible and can therefore develop HAM/TSP even if they have a low proviral load. Therefore, the average proviral load of DRB1*0101$^+$ patients will be lower than that of DRB1*0101$^-$ patients.

A gene may be associated with a disease because the gene causes the disease, because it is in linkage disequilibrium with the causative gene, or because of population admixture (genetic stratification). The protection from HAM/TSP observed here is likely to be caused by *A*02* itself, not a linked gene, because (*i*) there is no evidence of linkage disequilibrium between *HLA-A*02* and any other class I or class II alleles in this population, apart from the known linkage disequilibrium between *A*0207* and *B*4601* (*B*4601* was not independently associated with disease protection; data not shown), (*ii*) there is a vigorous anti-*A*02*-restricted CTL response to HTLV-I, which is a plausible mechanism of protection, and (*iii*) the *A*02*-associated protective effect has been replicated in a small, HTLV-I-infected population in London (see *Results*). There was no evidence of population admixture in this study. It is not possible to determine which allele on the *DRB1*0101*-associated haplotype is responsible for the susceptibility effect because of the known strong linkage disequilibrium of *DRB1*0101* with other alleles in the MHC region. However, it may be possible to identify a single susceptibility allele by carrying out host genetic studies in other populations in which different alleles are linked on the susceptibility haplotype (55). The effect of *DRB1*0101* was not examined in a staged fashion because of its previously suggested association with disease susceptibility. The linked gene *B*0702* was significantly associated with disease in the first stage (uncorrected), but not in an independent population examined for *B*0702* alone in the second stage of the study, perhaps because the sample size was too small (56).

The effects of HLA class I and class II alleles (or genes in linkage disequilibrium with HLA) found in this study do not account for all of the observed difference in individual susceptibility to HAM/TSP. Previous studies have found an association between HAM/TSP and female sex (57) and early age at initial sexual activity (58). We believe that the protective effect of *A*02* is evident because *A*02* occurs at a high frequency in the population studied and because there is a highly dominant CTL target antigen which contains a dominant *A*02*-restricted epitope. It is likely that class I alleles are protective in other infectious diseases but do not occur at a sufficient frequency in the population sizes studied to reach statistical significance. Other polymorphic genes that may contribute to the different outcomes of HTLV-I infection include those that affect the efficiency of the anti-HTLV-I immune response (e.g., *TAP-1* and *-2*), or the rate of proliferation or migration of leukocytes (e.g., cytokines and their receptors, or adhesion molecules).

In conclusion, the present results indicate that MHC class I-restricted CTL reduce the proviral load of HTLV-I and consequently the risk of HAM/TSP. *DRB1*0101* predisposes to HAM/TSP in the absence of *A*02*. It is still uncertain, in both HTLV-I and HIV-1, whether antiviral class I HLA-restricted CTL benefit the host by reducing virus load or contribute to disease by damaging host tissues. Our results

strongly favor a protective role and therefore argue that an effective antiretroviral vaccine should elicit a vigorous CTL response.

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