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ApaI polymorphism of vitamin D receptor gene is associated with susceptibility to HTLV-1-associated myelopathy/tropical spastic paraparesis in HTLV-1 infected individuals

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Abstract

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is one outcome of human T-cell lymphotropic virus type-1 (HTLV-1) infection. It remains unknown why the majority of infected people remain healthy, whereas only approximately 2–3% of infected individuals develop the disease. The active form of vitamin D has immunomodulatory effects, and allelic variants of the vitamin D receptor (VDR) appear to be associated with differential susceptibility to several infectious diseases. To investigate whether VDR single nucleotide polymorphisms (SNPs) are associated with the development of HAM/TSP, we studied four VDR SNPs in a group of 207 HAM/TSP patients and 224 asymptomatic HTLV-1 seropositive carriers (HCs) in Kagoshima, Japan, by using PCR-RFLP analysis. We found that *ApaI* polymorphism of VDR is associated with the risk of HAM/TSP, although this polymorphism did not affect the provirus load of HTLV-1 in either HAM/TSP patients or HCs.

Keywords: Vitamin D receptor; Single nucleotide polymorphism; HAM/TSP; HTLV-1; Provirus load

1. Introduction

Human T-cell lymphotropic virus type-1 (HTLV-1) [1,2] infection is closely associated with a slowly progressive neurological disease called HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [3,4]. Infection with HTLV-1 is estimated to affect 10–20 million people worldwide [5]. However, only a minority of infected individuals develops HAM/TSP, by mechanisms incompletely understood [6]. Since it has been reported that the subtype of the viral transactivator Tax is associated with the risk of developing HAM/TSP [7], many other reported findings suggest that host factors are most important to determine the risk of HAM/TSP. Our case/control studies in Kagoshima strongly support this hypothesis. In the Kagoshima population, possession of the HLA-class I genes HLA-A*02 and Cw*08 was associated with a statistically significant reduction in both HTLV-1 provirus load and the risk of HAM/TSP, whereas possession of HLA-class I HLA-B*5401 and class II HLA-DRB1*0101 predispose to HAM/TSP [8,9]. Further analysis to look at non-HLA host genetic factors revealed that the TNF-α promoter-863 A allele predisposed to HAM/TSP, whereas SDF-1 +801A 3′ UTR, and IL-15 191 C alleles conferred protection against this disease

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[10], suggesting that non-HLA gene polymorphism also affects the risk for developing HAM/TSP.

It is well known that the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 (1,25-$[OH]_2D_3$), is involved in the maintenance of mineral homeostasis [11]. The effect of 1,25-$[OH]_2D_3$ is mediated by its receptor, which is the ligand-dependent transcription factor, and the vitamin D receptor (VDR) gene consists of nine exons with a number of polymorphisms [12]. To date, over 30 studies to test an association between polymorphisms of VDR and osteoporosis were reported, with about half confirming the association and the remainder failing to confirm [13]. Not only for mineral homeostasis, 1,25-$[OH]_2D_3$ is also implicated in the regulation of the immune system [11]. The immunomodulatory actions of the 1,25-$[OH]_2D_3$ are mediated by interaction with VDR, which is expressed in resting and activated lymphocytes [14]. Therefore, VDR may also interact to determine the risk of infectious diseases. Some studies have revealed a close association between VDR polymorphisms and the outcome of certain infectious diseases such as tuberculosis [15], hepatitis B virus (HBV) [15] and leprosy [16]. Most importantly, reports by Barber et al. and Nieto et al. showed that individuals with the VDR *BsmI* BB and *FokI* heterozygotes, respectively, were associated with rapid progression to AIDS among HIV-1 seropositive intravenous drug users [17,18], suggesting that particular polymorphisms in the VDR contribute to the host immune reaction against viral infection. Since the most commonly studied VDR polymorphisms include a *Fok*I restriction fragment-length polymorphism (RFLP) in exon 2 (alleles F/f or nucleotides C/T), *Bsm*I (B/b or nucleotides T/C) and *Apa*I (A/a or nucleotides T/G) variants in intron 8, and a *Taq* I (T/t or nucleotides T/C) variant in exon 9, with lowercase alleles indicating the presence of restriction sites, we genotyped these four SNPs and analyzed the effect of each SNP on the risk of HAM/TSP.

2. Patients and methods

2.1. Study population

The study population consisted of 207 patients with HAM/TSP and 224 asymptomatic HTLV-1 seropositive carriers (HCs), all residing in HTLV-1 endemic Kagoshima Prefecture in Southern Japan. The diagnosis of HAM/TSP was done in accordance with World Health Organization criteria [19]. Clinical characteristics of the patients are shown in Table 1. All samples were taken with the consent of the patients.

2.2. Isolation and cryopreservation of PBMCs and DNA extraction

Fresh peripheral blood mononuclear cells (PBMCs) were obtained by density gradient centrifugation using a Histopaque-1077 instrument (Sigma, Tokyo, Japan) and washed three times with phosphate-buffered saline (PBS) containing 1% fetal calf serum (FCS). Isolated PBMCs were cryopreserved in liquid nitrogen until use. Genomic DNA was extracted from PMBCs using a QIAamp blood kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions.

2.3. PCR-RFLP analysis

Fifty nanograms of genomic DNA was amplified by PCR in a total volume of 25 μl using the primer and PCR conditions described by Harris et al. [20] for the *FokI*, by Wilkinson et al. [21] for the *TaqI* and *BsmI*, and Niino et al. [22] for the *ApaI* polymorphism of VDR. The primer sequences were as follows: 5′-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3′ and 5′-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3′ for *FokI*; 5′-GGG ACG ATG AGG GAT GGA CAG AGC-3′ and 5′-GGA AAG GGG TTA GGT TGG ACA GGA-3′ for *TaqI*; 5′-AAC TTG CAT GAG GAG GAG CAT GTC-3′ and 5′-GGA GAG GAG CCT CTG TCC CAT TTG-3′ for *BsmI*; 5′-GTC GCT GAG GGA TGG-3′ and 5′-GTC GGC TAG CTT CTG GAT-3′ for *ApaI*. After PCR amplification, the 15 μl of PCR product was digested for 12 h with an excess of restriction enzyme. Finally, digested PCR products were electrophoresed through a 2% agarose gel and visualized with ethidium bromide.

2.4. Quantification of HTLV-1 provirus load, CSF neopterin and anti-HTLV-1 antibody titers

To examine the HTLV-1 provirus load, we carried out a quantitative PCR method using ABI Prism 7700™ (PE-

Table 1
Clinical characteristics of HAM/TSP patients and asymptomatic HTLV-1 carriers (HCs)

	Age	Male/female	Anti-HTLV-1 antibodies[a]	HTLV-1 provirus load[b]	Neopterin in CSF[c]
HAM/TSP (n=207)	57.0±12.1[d]	62/145	×26,458±41,433	719.3±709.2	112.7±112.6
HCs (n=224)	39.8±13.1	109/115	×1478±1453	131.9±243.0	N/A

N/A: not applicable.
HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis.
HCs: asymptomatic HTLV-1 seropositive carriers.
[a] Anti-HTLV-1 antibodies were titrated by the particle agglutination method. The antibody titers were achieved by performing a serial dilution of the patient serum and noting the highest dilution at which agglutination is still present.
[b] Tax copy number per 1×10^4 PBMCs.
[c] Neopterin levels were evaluated by HPLC with fluorometric detection methods.
[d] The values are shown as the mean±S.D.

Applied Biosystems) with 100 ng of genomic DNA (roughly equivalent to 10^4 cells) from PBMC samples as reported previously [23]. Using β-actin as an internal control, the amount of HTLV-1 provirus DNA was calculated by the following formula: copy number of HTLV-1 (pX) per 1×10^4 PBMC=[(copy number of pX)/(copy number of β-actin/2)]$\times10^4$. All samples were performed in triplicate. Neopterin levels were evaluated by high-performance liquid chromatography (HPLC) with fluorimetric detection methods [24]. Serum and CSF antibody titers to HTLV-1 were determined by a particle agglutination method (Serodia-HTLV-1®, Fujirebio). The antibody titers were achieved by performing a serial dilution of the patient serum and noting the highest dilution at which agglutination is still present.

2.5. Statistical analysis

Comparisons of genotype frequency between HAM/TSP patients and HCs were calculated by the chi-squared test. For multiple comparisons of the HTLV-1 provirus load measured in HAM/TSP and HCs individuals, subdivided according to their *ApaI* genotype, we used one-factor ANOVA when variance of each group was equal by Bartlett test. If variance of each group was different, Kruskal–Wallis test was employed. Linkage disequilibrium (LD) was calculated between SNPs as previously described [25] by using SNPAlyze software ver. 3.2 (Dynacom, Mobara, Japan), which provides a *D* prime (*D'*) value between 0 and 1. A *D'* value of 0 indicates no LD between the two markers and a *D'* value of 1 indicates complete LD between two markers. Significance was considered at $p<0.05$.

3. Results

3.1. Vitamin D receptor gene polymorphism in HAM/TSP patients and asymptomatic HTLV-1 carriers

Initially, we genotyped 118 patients with HAM/TSP and 129 HCs for each SNP. There were no significant differences in the distribution of the *FokI*, *TaqI* and *BsmI* genotypes and allele frequencies between 118 HAM/TSP patients and 129 HCs (Table 2). In contrast, the *ApaI* genotypes (AA, Aa, aa) showed a significant difference in frequency ($\chi^2=8.04$ on 2 *df*, $p=0.018$). We therefore further analyzed a total of 207 cases of HAM/TSP and 224 HCs for *ApaI* polymorphism and identified a significant association between AA genotype and reduced risk of HAM/TSP ($\chi^2=10.48$ on 2 *df*, $p=0.0012$, Odds ratio=0.28, 95%CI=0.13–0.63). In both HAM/TSP patients and HCs, the genotype frequencies were distributed according to the Hardy–Weinberg equilibrium. Previously reported allele and genotype frequencies of *ApaI* polymorphism from a Japanese normal control population showed similar results with the HCs group of our present study [22].

3.2. AA genotype is associated with a lower risk for HAM/TSP only in female gender and FokI heterozygotes

Interestingly, the protective effect of *ApaI* AA genotype was observed only in the female subjects (72 HAM/TSP patients and 59 HCs, $\chi^2=7.11$ on 2 *df*, $p=0.029$) but not in the male subjects (38 HAM/TSP patients and 50 HCs, $\chi^2=4.31$ on 2 *df*, $p=0.116$). Because a series of the three polymorphisms (*BsmI*, *ApaI* and *TaqI* SNPs) in the 3′

Table 2
Frequencies of genotypes and alleles for the different polymorphisms in the vitamin D receptor gene

SNP	Allele	HAM/TSP	HCs	*P* value[a]	Genotype	HAM/TSP	HCs	*P* value[b]
ApaI	A	105 (25.4)[c]	143 (31.9)	0.034*	AA	8 (3.9)	28 (12.5)	0.0053*
	a	309 (74.6)	305 (68.1)		Aa	89 (43.0)	87 (38.8)	
					aa	110 (53.1)	109 (48.7)	
	total	414	448		total	207	224	
FokI	F	141 (59.7)	157 (60.9)	0.80	FF	44 (37.3)	50 (38.8)	0.97
	f	95 (40.3)	101 (39.1)		Ff	53 (44.9)	57 (44.2)	
					ff	21 (17.8)	22 (17.1)	
	total	236	258		total	118	129	
BsmI	B	28 (11.9)	32 (12.4)	0.85	BB	2 (1.7)	1 (0.8)	0.70
	b	208 (88.1)	226 (87.6)		Bb	24 (20.3)	30 (23.3)	
					bb	92 (78.0)	98 (76.0)	
	total	236	258		total	118	129	
TaqI	T	208 (88.1)	228 (88.4)	0.93	TT	92 (78.0)	100 (77.5)	0.78
	t	28 (11.9)	30 (11.6)		Tt	24 (20.3)	28 (21.7)	
					tt	2 (1.7)	1 (0.8)	
	total	236	258		total	118	129	

HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis.
HCs: asymptomatic HTLV-1 seropositive carriers.
[a] *P* values are calculated by χ^2-test with 2×2 contingency table.
[b] *P* values are calculated by χ^2-test with 2×3 contingency table.
[c] Numbers in parentheses are percentage.
* $P<0.05$.

Table 3
Linkage disequilibrium (LD) between four vitamin D receptor polymorphisms in HTLV-1 infected subjects

VDR SNP	*FokI*-HAM	*BsmI*-HAM	*TaqI*-HAM
ApaI-HAM	0.23444	**0.93886**	**1.0000**
	FokI-HAM	0.58781	0.44849
		BsmI-HAM	**0.91828**
			TaqI-HAM
ApaI-HCs	0.05065	**0.87419**	**0.93298**
	FokI-HCs	0.59933	0.41685
		BsmI-HCs	**0.88339**
			TaqI-HCs
ApaI-All	0.03603	**0.90706**	**0.96805**
	FokI-All	0.59305	0.43561
		BsmI-All	**0.90042**
			TaqI-All

HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis.
HCs: asymptomatic HTLV-1 seropositive carriers.
LD was calculated between SNPs, which provides a *D* prime (*D'*) value between 0 and 1.
D' values between two SNPs in each group were shown.
No LD=0, complete LD=1, strong LD=0.7–1.0 (values in bold).

UTR of the VDR gene have been shown to be in strong LD with one another in Western countries [26], we analyzed the presence of the LD in our subjects. As a result, the *BsmI*, *ApaI* and *TaqI* polymorphisms are in strong LD whereas the *FokI* polymorphism at the translation initiation site in exon2 of the VDR gene was not in significant linkage with the other polymorphisms (Table 3). Since previous report by Nieto et al. showed clear association between combined genotypes for *FokI* and *BsmI* polymorphisms and a faster progression to AIDS among HIV-1 seropositive intravenous drug users, despite no significant linkage between two polymorphisms in their population [18], we also tested whether the *FokI* genotype affect the observed protective effect of *ApaI* genotype against HAM/TSP development. When we analyzed the distribution of the *ApaI* polymorphism in the 274 HTLV-1 infected individuals with the non-Ff genotype (homozygous FF and ff), we observed that there were no significant differences in the distribution of the *ApaI* genotypes and allele frequencies between 130 HAM/TSP patients and 144 HCs. In contrast, in individuals with the Ff genotype (heterozygous, 53 HAM/TSP patients and 57 HCs), the risk ratios for progression to HAM/TSP in HTLV-1 infected individuals without AA genotype (Aa, aa and Aa+aa) was significantly higher than HTLV-1 infected individuals with AA genotype (Table 4). Therefore, the protective effect of *ApaI* AA genotype was observed only in the *FokI* heterozygotes (Ff) but not in homozygotes (FF and ff).

3.3. *Vitamin D receptor gene ApaI polymorphism is not a significant predictor of the HTLV-1 provirus load in HAM/TSP patients and asymptomatic HTLV-1 carriers*

To test whether VDR gene *ApaI* polymorphism is a significant predictor of the HTLV-1 provirus load, we measured the provirus load of HTLV-1 and compared it with *ApaI* genotypes (AA, Aa, aa) in HAM/TSP patients and HCs. Our data indicated that there was no association between *ApaI* genotypes and HTLV-1 provirus load (Table 5), CSF neopterin levels, as well as serum HTLV-1 antibody

Table 4
Vitamin D receptor *ApaI* allele/genotype frequencies in relation to *FokI* genotypes in HTLV-1 infected individuals

FokI genotype	*ApaI* allele	HAM/TSP	HCs	OR (95% CI)	*P* value
Homozygous (FF and ff)	A	41 (31.5)	49 (34.0)	0.89 (0.54–1.48)	0.66
	a	89 (68.5)	95 (66.0)	1.12 (0.68–1.86)	0.66
	total	130	144		
Heterozygous (Ff)	A	25 (23.6)	37 (32.5)	0.64 (0.35–1.17)	0.14
	a	81 (76.4)	77 (67.5)	1.56 (0.86–2.83)	0.14
	total	106	114		
FokI genotype	*ApaI* genotype	HAM/TSP	HCs	OR (95% CI)	*P* value
Homozygous (FF and ff)	AA	3 (4.6)	7 (9.7)	1 (reference)	
	Aa	35 (53.8)	35 (48.6)	2.33 (0.56–9.76)	0.24
	aa	27 (41.5)	30 (41.7)	2.10 (0.49–8.94)	0.31
	Aa+aa	62 (95.4)	65 (90.3)	2.23 (0.55–8.99)	0.25
	total	65	72		
Heterozygous (Ff)	AA	1 (1.9)	10 (17.5)	1 (reference)	
	Aa	23 (43.4)	17 (29.8)	13.53 (1.58–116.0)	0.0044*
	aa	29 (54.7)	30 (52.6)	9.67 (1.16–80.4)	0.014*
	Aa+aa	52 (98.1)	47 (82.5)	11.06 (1.36–89.7)	0.0062*
	total	53	57		

HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis.
HCs: asymptomatic HTLV-1 seropositive carriers.
Numbers in parentheses are percentage.
OR: odds ratio; 95% CI: 95% confidence interval.
* $P<0.05$.

Table 5
Vitamin D receptor *ApaI* polymorphism and HTLV-1 provirus load

	AA	Aa	aa	*P* value
HAM (207)	541.6±222.2 (8)	745.8±79.6 (89)	710.7±65.2 (110)	0.727[a]
HCs (224)	103.8±32.4 (28)	129.7±31.5 (87)	140.9±20.4 (109)	0.799[b]
All patients combined (431)	201.1±61.4 (36)	441.3±48.9 (176)	427.1±39.3 (219)	0.718[b]

HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis.
HCs: asymptomatic HTLV-1 seropositive carriers.
The values are shown as the mean tax value (tax copies/10^4 PBMCs)±S.E.
[a] *P* value by one-factor ANOVA.
[b] *P* value by Kruskal–Wallis test.

titers (data not shown) in our population. Also, the clinical course and disability of HAM/TSP were not specifically associated with the polymorphism and serum VEGF levels in HAM/TSP patients (data not shown).

4. Discussion

HTLV-1 infection is of particular interest to the field of immunology as well as neurology because it persists at a remarkably high level despite a vigorous cellular and humoral immune response and causes inflammatory demyelinating disease only in a minority of infected people. The outcome of HTLV-1 infection depends on both host genetic and viral factors. However, although different virus strains (denoted HTLV-1 subgroups) can influence the risk of developing HAM/TSP [7], the impact of HTLV-1 viral sequence variation in determining the risk of developing HAM/TSP in Kagoshima is not sufficient to predict disease. Our recent observations, as well as many reported findings, strongly suggest that the outcome of HTLV-1 infection mainly depends upon a host of genetic factors [27].

In addition to its role in calcium and skeletal homeostasis, 1,25-$[OH]_2D_3$ plays an important role in immune system modulation [11]. The 1,25-$[OH]_2D_3$ promotes monocyte differentiation, stimulates cell-mediated immunity, and inhibits lymphocyte proliferation and secretion of cytokines such as interleukin (IL)-2, granulocyte-macrophage colony-stimulating factor and interferon-γ from T cells, and IL-12 from macrophages and B cells [28–31]. The inhibiting effect of vitamin D on the immune response appears to target Th1 cells by preventing their activation and cytokine production [28]. Interestingly, previous studies indicated that the Th1 cell response is predominant in HAM/TSP [32,33] and 1,25-$[OH]_2D_3$ has the potential to suppress cell proliferation through binding to the VDR overexpressed in HTLV-1 infected T cells [34,35]. Therefore, it is interesting to test whether VDR gene polymorphism is associated with the risk for developing HAM/TSP.

In the present study, the *ApaI* polymorphism of VDR showed a significant difference in allele frequency, and AA genotype was associated with the reduced risk of HAM/TSP in HTLV-1 infected individuals, whereas there were no significant differences in the distribution of the *FokI*, *TaqI* and *BsmI* genotypes and allele frequencies. Interestingly, the protective effect of *ApaI* AA genotype was observed only in females, which is one of the risk factor for developing HAM/TSP. The HTLV-1 provirus load of female patients with HAM/TSP was significantly higher than that of male patients [23] and the ratio of male to female HAM/TSP patients is about 1:2, as shown in the present study. However, there was no significant difference between any VDR genotypes and HTLV-1 provirus load in either HAM/TSP patients or HCs. Also, there were no correlations between CSF neopterin levels or serum anti-HTLV-1 antibody titers. Furthermore, the clinical course and disability of HAM/TSP were not associated with any VDR polymorphisms in HAM/TSP patients. This was strikingly different from the HLA-A*02 [8] and Cw*08 [9], which were associated with both the risk of HAM/TSP and lower provirus load in HCs. Since *ApaI* polymorphism is located in intron 8 and is not affecting any splicing site and/or transcription factor binding site [36], it is unlikely that this polymorphism is directly associated with the functional difference of VDR itself. Linkage disequilibrium with truly functional polymorphism elsewhere in the VDR gene or other gene(s) may be associated with the susceptibility to HAM/TSP via gender-specific mechanism other than an apparent effect on provirus load. In our population, significant LD was found among the *BsmI*, *ApaI* and *TaqI* polymorphisms located in the 3′ UTR of the VDR gene, but *FokI* polymorphism was not in LD with these three polymorphisms. However, the *FokI* genotype affects the observed protective effect of *ApaI* genotype against HAM/TSP, as observed in HIV-1 infected intravenous drug users for progression to AIDS [18]. In HTLV-1 infected individuals with the *FokI* Ff genotype, the risk ratios for progression to HAM/TSP without *ApaI* AA genotype (Aa, aa and Aa+aa) was significantly higher than HTLV-1 infected individuals with AA genotype, whereas there were no significant differences in the distribution of the *ApaI* genotypes and allele frequencies between 130 HAM/TSP patients and 144 HCs which were non-Ff genotype (homozygous FF and ff). These findings provide strong evidence to suggest that genetic variations at the VDR locus may affect the outcome of HTLV-1 infection. Recent transmission-disequilibrium test on family-based study also showed a significant association of tuberculosis with SNP combinations *FokI–ApaI* by the increased transmission to affected offspring [37]. It is possible that the presence of unidentified, associated functional alleles that lies on this haplotype background influence the susceptibility to HAM/TSP, although further studies are needed to clarify this point.

In conclusion, our results indicate that VDR *ApaI* polymorphism is a novel non-HLA host genetic factor to evaluate the risk of HAM/TSP. The functional significance

of this observation may reveal immunotherapeutic strategies that would retard the development of HAM/TSP in the future.

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Bronchoalveolar lymphocytosis correlates with human T lymphotropic virus type I (HTLV-I) proviral DNA load in HTLV-I carriers

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AIRWAY BIOLOGY

Bronchoalveolar lymphocytosis correlates with human T lymphotropic virus type I (HTLV-I) proviral DNA load in HTLV-I carriers

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Background: A study was undertaken to investigate the pathogenesis of pulmonary involvement in human T lymphotropic virus type I (HTLV-I) carriers.

Methods: The bronchoalveolar lavage (BAL) cell profile of 30 HTLV-I carriers (15 asymptomatic HTLV-I carriers (AHCs) and 15 symptomatic HTLV-I carriers (SHCs)) with chronic inflammatory diseases of respiratory tract and eight patients with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) was investigated. The HTLV-I proviral deoxyribonucleic acid (DNA) load in peripheral blood mononuclear cells (PBMCs) and BAL fluid from HTLV-I carriers was estimated using the quantitative polymerase chain reaction method and the correlation between the lymphocyte number in BAL fluid and the HTLV-I proviral DNA load in PBMCs and BAL fluid was examined.

Results: The percentage of lymphocytes in BAL fluid was increased (>18%) in 11 of 30 HTLV-I carriers although there was no significant difference compared with control subjects. In HTLV-I carriers the lymphocyte number in BAL fluid correlated well with the copy number of HTLV-I proviral DNA in PBMCs. In addition, the copy number of HTLV-I proviral DNA in BAL fluid correlated well with the number of lymphocytes (both CD4+ and CD8+ cells) in BAL fluid.

Conclusions: These findings suggest that pulmonary lymphocytosis can occur in a subset of HTLV-I carriers without HAM/TSP and that the increased HTLV-I proviral DNA load may be implicated in the pathogenesis of pulmonary involvement in HTLV-I carriers.

Human T lymphotropic virus type I (HTLV-I) is a type C retrovirus that is aetiologically associated with adult T cell leukaemia[1 2] and with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP).[3 4] In addition to these diseases, a number of inflammatory disorders have also been described in association with HTLV-I including HTLV-I uveitis,[5] arthropathy,[6] and Sjögren's syndrome.[7] Pulmonary involvement is also associated with HTLV-I—for example, in patients with HAM/TSP and HTLV-I uveitis pulmonary involvement may be characterised by bronchoalveolar lymphocytosis.[8–11] Furthermore, a few preliminary studies have shown that similar pulmonary involvement is observed in HTLV-I carriers who have not developed HAM/TSP or HTLV-I uveitis.[10 12]

Many kinds of immunological abnormalities and an increased HTLV-I proviral deoxyribonucleic acid (DNA) load in peripheral blood, cerebrospinal fluid, and bronchoalveolar lavage (BAL) fluid from patients with HAM/TSP[13 14] and HTLV-I uveitis[11] have been reported, suggesting that immunological mechanisms related to an increased amount of HTLV-I proviral DNA may be implicated in the pathogenesis of these diseases. However, despite advances in elucidating the pathophysiology of these diseases, much of the information on the pathogenesis is confined to HAM/TSP and HTLV-I uveitis. There is little information available regarding pulmonary involvement and pathophysiology in HTLV-I carriers who have not developed HAM/TSP or HTLV-I uveitis.

To examine the incidence and pathogenesis of pulmonary lymphocytosis in HTLV-I carriers, we have analysed BAL cell profiles in HTLV-I carriers including asymptomatic HTLV-I carriers (AHCs). We also estimated the HTLV-I proviral DNA load in peripheral blood mononuclear cells (PBMCs) and BAL cells from HTLV-I carriers by the quantitative polymerase chain reaction (PCR) method and examined the correlation between the HTLV-I proviral DNA load and pulmonary lymphocytosis.

METHODS

This study was reviewed and approved by the Kagoshima University Faculty of Medicine Committee on Human Research.

Study subjects

The study subjects consisted of 30 HTLV-I carriers and eight patients with HAM/TSP consecutively presenting to our department between 1989 and 2000. The 30 HTLV-I carriers consisted of 15 AHCs (three men and 12 women) and 15 symptomatic HTLV-I carriers (SHCs; five men and 10 women) as shown in table 1. There were no significant differences in age between each of the groups and the control subjects. All subjects were seronegative for human immunodeficiency virus (HIV) 1.

To assess the cellular characteristics of BAL fluid in AHCs, the serum anti-HTLV-I antibody was checked in individuals consulting our department for an annual chest radiograph. The anti-HTLV-I antibody was measured by the gelatin particle agglutination method (Fujirebio, Tokyo, Japan). After obtaining informed consent, further examinations including fibreoptic bronchoscopy were performed on HTLV-I seropositive individuals along with careful history taking including occupational history. Individuals who

Abbreviations: AHC, asymptomatic HTLV-I carrier; BAL, bronchoalveolar lavage; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; HTLV-I, human T lymphotropic virus type I; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; SHC, symptomatic HTLV-I carrier

Table 1 Clinical background and bronchoalveolar lavage (BAL) findings of HTLV-I carriers and patients with HAM/TSP

Patient no. and clinical diagnosis/symptoms	Age/ sex	Peripheral blood WBC (/mm³)	HTLV-I Ab‡ (×)	Cell count (×10⁵/ml)	Bronchoalveolar lavage fluid AM (%)	Ly (%)	Neu (%)	Eo (%)	CD4 (%)	CD8 (%)	CD4/CD8
1 AHC	64/F	4300	2048	1.4	94.3	5.0	0.8	0.0	ND	ND	ND
2 AHC†	77/M	7500	2048	1.0	96.4	3.2	0.4	0.0	52.0	32.9	1.6
3 AHC	72/F	5800	256	2.0	90.2	8.3	1.3	0.2	47.1	27.3	1.7
4 AHC	48/F	2600	512	0.4	86.9	12.3	0.6	0.1	48.9	29.2	1.7
5 AHC	57/F	6500	1024	0.7	74.9	24.6	0.5	0.0	53.5	25.0	2.1
6 AHC	78/F	6300	8192	1.9	88.2	10.5	0.8	0.0	53.0	28.3	1.9
7 AHC	58/F	3600	8192	0.9	84.4	14.3	1.2	0.0	ND	ND	ND
8 AHC†	64/M	4300	512	0.7	96.2	3.3	0.5	0.0	ND	ND	ND
9 AHC	71/M	5400	4096	2.8	70.5	28.6	0.9	0.1	75.6	23.2	3.3
10 AHC	56/F	7000	128	1.0	80.5	18.7	0.6	0.1	44.5	39.5	1.1
11 AHC	64/F	4000	128	1.3	89.2	9.7	0.8	0.3	58.8	22.8	2.6
12 AHC	55/F	3700	2048	0.6	95.3	4.0	0.3	0.5	ND	ND	ND
13 AHC	77/F	4300	16384	1.5	76.1	23.4	0.4	0.1	75.3	13.4	5.6
14 AHC	71/F	5300	4096	0.6	84.3	13.5	1.6	0.5	64.6	26.9	2.4
15 AHC	67/F	4700	1024	0.8	91.5	5.5	1.2	0.0	42.8	17.1	2.5
16 Chronic cough	76/F	5100	256	1.0	93.6	5.7	0.7	0.0	37.1	34.8	1.1
17 Chronic cough	67/F	3900	1024	0.9	68.5	30.1	1.4	0.0	71.7	25.9	2.8
18 Chronic cough*	82/F	7000	128	1.7	95.2	4.7	0.2	0.0	ND	ND	ND
19 Middle lobe syndrome	59/F	5200	4096	0.7	61.0	34.6	3.4	0.0	55.8	21.1	2.6
20 SBS	71/F	3400	4096	0.5	92.0	7.1	0.8	0.2	48.4	31.7	1.5
21 Bronchiectasis	63/F	2800	2048	0.6	94.9	4.9	0.1	0.0	40.4	42.1	1.0
22 Inactive Tbc	57/M	3900	1024	1.3	72.7	27.3	0.0	0.0	57.1	40.5	1.4
23 Inactive Tbc†	73/M	7000	2048	3.0	94.9	4.7	0.4	0.0	60.0	30.8	2.0
24 Chronic bronchitis	47/M	4700	256	0.6	91.6	8.1	0.2	0.0	ND	ND	ND
25 Chronic bronchitis*	57/M	5200	256	1.8	87.0	11.4	1.4	0.2	ND	ND	ND
26 Bronchiectasis	72/F	5000	512	1.9	97.4	2.6	0.0	0.0	ND	ND	ND
27 Bronchiectasis	68/F	6000	128	1.5	55.1	29.6	13.1	1.2	57.5	22.9	2.5
28 SBS	46/M	6900	2048	5.8	42.5	20.3	25.0	0.6	41.1	49.7	0.8
29 Middle lobe syndrome	66/F	6300	512	2.5	44.4	55.3	0.1	0.2	61.9	29.6	2.1
30 SBS	18/F	7900	4096	2.7	47.6	45.5	7.1	0.3	41.6	55.2	0.8
31 HAM/TSP	33/F	4100	2048	2.0	42.7	56.8	0.2	0.2	43.1	51.5	0.8
32 HAM/TSP	50/F	4900	2048	2.3	65.6	21.0	13.4	0.0	46.4	49.2	0.9
33 HAM/TSP	54/F	2800	512	1.4	63.0	36.0	1.0	0.0	69.1	28.5	2.4
34 HAM/TSP	60/M	5100	512	2.4	73.4	24.5	2.1	0.0	47.1	48.2	1.0
35 HAM/TSP	65/F	5200	2048	1.7	40.8	58.9	0.3	0.0	33.1	61.0	0.5
36 HAM/TSP	59/F	4900	32768	4.0	27.0	71.9	1.1	0.0	76.2	20.4	3.7
37 HAM/TSP	34/M	5600	8192	1.6	73.2	25.7	1.2	0.0	50.2	42.1	1.2
38 HAM/TSP	50/M	6100	8192	3.9	31.2	68.3	0.0	0.5	52.0	46.9	1.1

WBC, white blood cell; M, male; F, female; HTLV-I, human T lymphotropic virus type I; AHC, asymptomatic HTLV-I carrier; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis; SBS, sinobronchial syndrome; Tbc, tuberculosis; AM, alveolar macrophage; Ly, lymphocyte; Neu, neutrophil; Eo, eosinophil; ND, not determined.
*Current smoker.
†Recent/ex-smoker.
‡HTLV-I antibody (Ab) was measured by the gelatin particle agglutination method.

worked in environments known to cause allergic lung diseases were excluded. After the diagnostic procedure, 15 AHCs were recruited to the study (table 1); the chest radiographic findings were normal in 13 individuals and two had minimal inactive tuberous lesions.

The 15 SHCs (subjects 16–30) were recruited from the outpatient clinic of our department for chronic inflammatory diseases of the respiratory tract (three sinobronchial syndrome, three bronchiectasis, two middle lobe syndrome, and two chronic bronchitis); two had an inactive tuberculous lesion and three (subjects 16–18) complained of a slight cough for 1–3 months during the study. Eight patients with HAM/TSP (three men and five women) had been diagnosed according to the criteria proposed by Osame *et al.*[15] Three HTLV-I carriers (subjects 9, 18 and 25) were current smokers, three HTLV-I carriers (subjects 2, 8 and 23) were ex-smokers with intervals ranging from 3 months to 5 years since smoking cessation, and the others had never smoked.

White blood cell counts in peripheral blood were within the normal range in all subjects. The serum anti-HTLV-I antibody titre ranged from 128 to 16384× in HTLV-I carriers and from 512 to 32768× in patients with HAM/TSP. The median (range) % vital capacity of AHCs, SHCs, and patients with HAM/TSP was 104.9% (77.2–131.8), 85.8% (75.9–131.3), and 96.7% (76.7–108.0), respectively, and the % forced expiratory volume in 1 second in the three groups was 82.2% (79.3–91.7), 70.7% (51.6–86.2), and 79.7% (70.0–88.3%), respectively. There were no significant differences in pulmonary function between the three groups.

Nine healthy individuals (three men and six women) of median age 53 years, all non-smokers, who were seronegative for HTLV-I acted as controls. They included four healthy volunteers and five healthy individuals undergoing an annual chest radiographic examination. These latter five healthy individuals were finally diagnosed as having a small solitary lung nodule without signs of pulmonary disease (n = 3) or minimal inactive tuberculous lesions (n = 2). BAL was performed to diagnose the small lung nodule and the cellular characteristics of the BAL fluid from these subjects served as

Table 2 Oligonucleotides for PCR detection of HTLV-I proviral DNA

Function	Nucleotide sequence (5′ to 3′)	Position*
Primer	GGC TCC GTT GTC TGC ATG TA	7765–7784
Primer	AAT CAT AGG CGT GCC ATC GG	8091–8072
Probe	CCT AAT AAT TCT ACC CGA AGA CTG TTT GCC	7932–7961

*GenBank Accession No. J02029.
PCR, polymerase chain reaction; HTLV-I, human T lymphotropic virus type I.

control values for this study. The chest radiographic findings of the five healthy volunteers were normal.

Bronchoalveolar lavage

Informed consent was obtained from all individuals before they underwent BAL which was performed before interventions including corticosteroid administration. Under local anaesthesia with 2% lidocaine, a fibreoptic bronchoscope was placed in the subsegment of the right middle lobe or lingua and 160 ml sterile saline was infused in four aliquots through the bronchoscope and aspirated by gentle hand suction. The lavage fluid obtained was passed through two sheets of sterile gauze and a 10 ml aliquot was centrifuged at 400*g* for 10 minutes, stained with Wright-Giemsa stain, and the cell differentials were determined (at least 500 cells were counted). The lavage fluid was then washed twice and the total number of cells counted.

An aliquot of BAL cells was used for identification of T lymphocyte subsets. The cells were washed twice with phosphate buffered saline and incubated with an optimal concentration of fluorescence conjugated monoclonal antibodies (OKT4 (CD4), OKT8 (CD8); Ortho Diagnostics, Raritan, NJ, USA). The cells were then analysed for surface fluorescence using flow cytometry (FCMID, Nihon Bunko, Tokyo, Japan).

Isolation of peripheral blood mononuclear cells (PBMCs)

The PBMCs were isolated from 30 ml heparinised peripheral blood by Ficoll-Hypaque density gradient centrifugation (Pharmatica, Uppsala, Sweden). Blood samples were obtained before performing BAL in all subjects. These samples were stored in liquid nitrogen until use.

Quantitative PCR of PBMCs and BAL cells

Quantitative PCR assay was performed as previously described.[16] The amount of HTLV-I proviral DNA was calculated using the following formula: copy number of HTLV-I (pX) per 10^4 PBMCs and per 10^4 BAL cells = [(copy number of pX)/(copy number of β-actin/2)] × 10^4

Detection of HTLV-I proviral DNA from BAL cells by PCR

To examine the presence of HTLV-I proviral DNA in BAL cells, isolated BAL cells from 10 initial AHCs (nos 1–9 and 11) were analysed. As negative and positive controls, BAL cells and HTLV-I infected TCL-Kan cells[17] from HTLV-I seronegative controls were also assayed. 1 μg of DNA extracted from BAL cells was used for the PCR. The amplification reaction of PCR

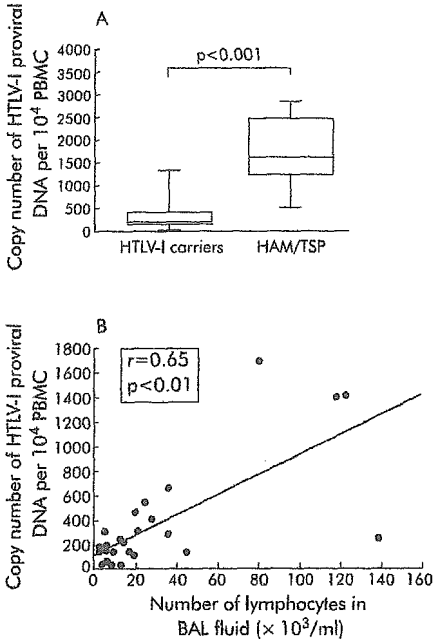

Figure 1 (A) Copy number of HTLV-I proviral DNA per 10^4 PBMCs in HTLV-I carriers and patients with HAM/TSP. The whisker box plots represent the 25–75th percentile of results inside the box, the median is shown by the horizontal bar across the box, and whiskers on the box represent the 10–90th percentiles. (B) Correlation between the number of lymphocytes in bronchoalveolar lavage (BAL) fluid and the copy number of HTLV-I proviral DNA per 10^4 PBMCs in HTLV-I carriers. HTLV-I, human T lymphotropic virus type I; PBMCs, peripheral blood mononuclear cells; HAM/TSP = HTLV-I associated myelopathy/tropical spastic paraparesis.

was performed for 35 cycles and consisted of denaturation at 94°C for 1 minute, annealing at 65°C for 2 minutes, and primer extension at 72°C for 3 minutes. 10 μl of the amplified products was blotted onto a nylon membrane and hybridised with a biotin labelled probe for HTLV-I pX. After incubation with streptavidin-alkaline phosphate conjugate, disodium 3-(4-methoxyspirol [1,2-dioxetane-3-2'-tricyclo-[3.3.1.1.3.7] decan]-4-yl)phenyl phosphate (AMPPD)[18] (Southern Light Kit, Troix) was added to the blot to a final concentration of 0.25 mM and the immersed blot was then slowly agitated for 5 minutes. Finally, the chemiluminescent signal was detected by exposing the radiographic film.

Table 3 Bronchoalveolar lavage findings in HTLV-I carriers and HAM/TSP patients

Subjects	Cell count (×10^5/ml)	AM (%)	Ly (%)	Neu (%)	Eo (%)	CD4+ cells (%)	CD8+ cells (%)	CD4/CD8
HTLV-I carriers	1.2**	87.6	11.0	0.8	0.0	53.3	28.8	1.9
N=30 (22)†	(0.4–5.8)	(42.5–97.4)	(2.6–55.3)	(0.0–25.0)	(0.0–1.2)	(37.1–75.6)	(13.4–55.2)	(0.8–5.6)
AHCs	1.0*	88.2	10.5	0.8	0.1	53.0	26.9	2.1*
N=15 (11)†	(0.4–2.8)	(70.5–96.4)	(3.2–28.6)	(0.3–1.6)	(0.0–0.5)	(42.8–75.6)	(13.4–39.5)	(1.1–5.6)
SHCs	1.5**	87.0	11.4	0.7	0.0	55.8	31.7	1.5
N=15 (11)†	(0.5–5.8)	(42.5–97.4)	(2.6–55.3)	(0.0–25.0)	(0.0–1.2)	(37.1–71.7)	(21.1–55.2)	(0.8–2.8)
HAM/TSP	2.2**	52.9**	46.4**	1.1	0.0	48.7	47.6*	1.0
N=8	(1.4–4.0)	(27.0–73.4)	(21.0–71.9)	(0.0–13.4)	(0.0–0.5)	(33.1–76.2)	(20.4–61.0)	(0.5–3.7)
Controls	0.6	91.2	8.4	0.6	0.1	48.7	31.4	1.5
N=9	(0.3–1.0)	(87.9–95.8)	(3.8–10.5)	(0.2–1.3)	(0.0–0.4)	(33.4–59.5)	(22.7–45.1)	(0.9–2.1)

Data are shown as median (range).
N, number of subjects; AM, alveolar macrophage; Ly, lymphocyte; Neu, neutrophil; Eo, eosinophil; HTLV-I, human T lymphotropic virus type I; AHCs, asymptomatic HTLV-I carriers; SHCs, symptomatic HTLV-I carriers with chronic inflammatory diseases of respiratory tract; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis.
*p<0.05, **p<0.01 compared with control subjects.
†The cell differential in BAL fluid was determined in 30 HTLV-I carriers (15 AHCs and 15 SHCs) and T lymphocyte subsets were determined in 22 HTLV-I carriers (11 AHCs and 11 SHCs).

Table 4 Quantification of HTLV-I proviral DNA in PBMCs and BAL fluid from HTLV-I carriers and HAM/TSP patients

	PBMCs			BAL fluid		
Subjects	N	Median	Range	N	Median	Range
HTLV-I carriers	26	199	ND–1704	14	126	22–1268
AHCs	11	138	ND–1704	3	75	70–132
SHCs	15	232	30–1390	11	141	22–1268
HAM/TSP	8	1611*	261–2857	8	601**	294–3495

HTLV-I copy number per 10^4 PBMCs and per 10^4 BAL cells are presented.
HTLV-I, human T lymphotropic virus type I; AHCs, asymptomatic HTLV-I carriers; SHCs, symptomatic HTLV-I carriers with chronic inflammatory diseases of respiratory tract; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; PBMCs, peripheral blood mononuclear cells; BAL, bronchoalveolar lavage; N, number of subjects; ND, not detected.
*$p<0.01$ compared with HTLV-I carriers, AHCs and SHCs; **$p<0.01$ compared with HTLV-I carriers and SHCs.

The location and sequences of the primers and probe are summarised in table 2.

Statistical analysis

All values are shown as median (range). Statistical analysis was performed using the Mann-Whitney U test and Spearman rank correlation. p values of <0.05 were considered significant.

RESULTS

Cellular characteristics and T lymphocyte subset of BAL fluid

The recovery ratios of BAL fluid in HTLV-I carriers, AHCs, SHCs, and patients with HAM/TSP did not differ significantly from those of control subjects (data not shown). As shown in table 3, the BAL fluid cell count was increased in HTLV-I carriers, AHCs, SHCs, and patients with HAM/TSP compared with control subjects. The differential cell count showed an increased percentage of lymphocytes and a decreased percentage of macrophages in BAL fluid from patients with HAM/TSP compared with control subjects. The percentage of lymphocytes in BAL fluid was increased (>18%) in four AHCs and seven SHCs but there was no significant difference from that in control subjects.

Analysis of T lymphocyte subsets in BAL fluid showed an increased percentage of CD8+ cells in patients with HAM/TSP and an increased ratio of CD4/CD8 in AHCs.

HTLV-I proviral DNA in PBMCs and correlation with lymphocytes in BAL fluid

The copy number of HTLV-I proviral DNA in PBMCs was determined in 26 HTLV-I carriers (11 AHCs and 15 SHCs) and eight patients with HAM/TSP. As shown in table 4 and fig 1A, the copy number of HTLV-I proviral DNA per 10^4 PBMCs was significantly increased in patients with HAM/TSP compared with those of HTLV-I carriers ($p<0.001$), AHCs ($p<0.01$), and SHCs ($p<0.01$). There was no significant difference in the copy number of HTLV-I proviral DNA of PBMCs between AHCs and SHCs.

The relationship between the HTLV-I proviral load in PBMCs and the lymphocyte number in BAL fluid was examined in 26 HTLV-I carriers. As shown in fig 1B, the number of lymphocytes in BAL fluid correlated well with the copy number of HTLV-I proviral DNA in PBMCs of HTLV-I carriers ($r = 0.65$, $p<0.05$).

HTLV-I proviral DNA in BAL fluid and correlation with lymphocytes and T lymphocyte subsets in BAL fluid

HTLV-I proviral DNA was detected by PCR in BAL cells from all 10 AHCs examined and was not detected in BAL cells from HTLV-I seronegative controls.

The copy number of HTLV-I proviral DNA in BAL fluid was determined in 14 HTLV-I carriers (three AHCs and 11 SHCs) and in eight patients with HAM/TSP. As shown in table 4, the copy number of HTLV-I proviral DNA per 10^4 BAL cells was significantly increased in patients with HAM/TSP compared with those of HTLV-I carriers ($p<0.001$) and SHCs ($p<0.01$).

The correlation between HTLV-I proviral DNA load, lymphocyte number, and T cell subsets in BAL fluid was examined in 14 HTLV-I carriers and 10 HTLV-I carriers. The copy number of HTLV-I proviral DNA in BAL fluid correlated well with the number of lymphocytes in BAL fluid from HTLV-I carriers ($r = 0.58$, $p<0.05$, fig 2A), CD4+ cells ($r = 0.77$, $p<0.05$, fig 2B), and CD8+ cells ($r = 0.83$, $p<0.05$, fig 2C).

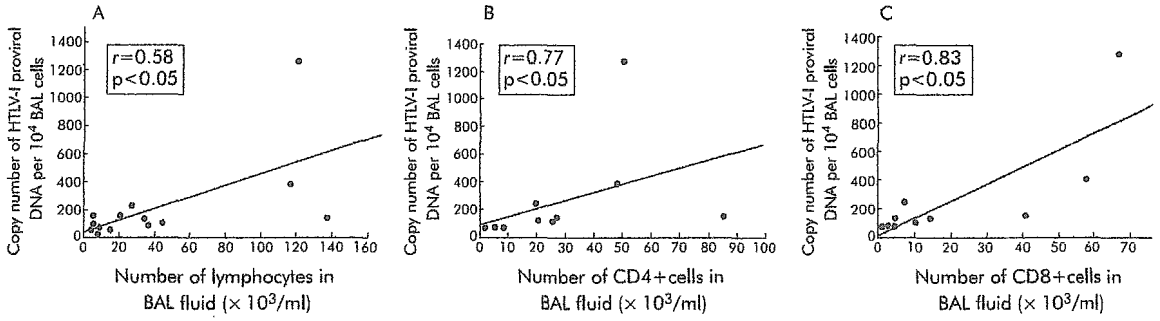

Figure 2 Correlation between (A) the number of lymphocytes in bronchoalveolar lavage (BAL) fluid and the copy number of HTLV-I proviral DNA per 10^4 BAL cells from HTLV-I carriers; (B) the number of CD4+ cells in BAL fluid and the copy number of HTLV-I proviral DNA per 10^4 BAL cells from HTLV-I carriers; and (C) the number of CD8+ cells in BAL fluid and the copy number of HTLV-I proviral DNA per 10^4 BAL cells from HTLV-I carriers. Statistical analyses were performed using Spearman rank correlation. HTLV-I, human T lymphotropic virus type I.

Follow up of asymptomatic HTLV-I carriers with bronchoalveolar lymphocytosis

Three AHCs (subjects 5, 10 and 13) with bronchoalveolar lymphocytosis (>18% lymphocytes in BAL fluid) were available for follow up evaluation of clinical and radiographic variables for 5–10 years. A further individual (subject 9) dropped out 1 year after the study. None of these four individuals showed overt respiratory illness and chest radiographic findings remained normal during the follow up period. They did not develop adult T cell leukaemia, HAM/TSP, or other HTLV-I associated disorders during this time.

DISCUSSION

The major findings of this study are: (1) bronchoalveolar lymphocytosis (>18% lymphocytes in BAL fluid) was observed in 11 of 30 HTLV-I carriers without HAM/TSP or HTLV-I uveitis (36.7%), although there was no significant difference between HTLV-I carriers and control subjects; (2) the number of lymphocytes in the BAL fluid of HTLV-I carriers was significantly correlated with the HTLV-I proviral DNA load in PBMCs; and (3) the HTLV-I proviral DNA load in the BAL fluid was significantly correlated with the number of lymphocytes, CD4+ cells and CD8+ cells in the BAL fluid of HTLV-I carriers.

Recent studies have indicated that immunological dysfunction related to the increased HTLV-I proviral load may be involved in the pathogenesis of HAM/TSP and HTLV-I uveitis.[13 16 19–23] However, some studies have shown that these immunological and virological findings are also present in some HTLV-I carriers including relatives of subjects with HAM/TSP and AHCs who have not developed HAM/TSP or HTLV-I uveitis.[13 16 20] The genetic background may be implicated in the HTLV-I proviral load and immunological dysfunction in HTLV-I carriers.[21 24] These reports suggest that HTLV-I carriers consist of groups of individuals of different genetic backgrounds with various amounts of HTLV-I proviral DNA in PBMCs. Our results showed that the HTLV-I proviral load is high in a subset of HTLV-I carriers without HAM/TSP or HTLV-I uveitis and that the increased HTLV-I proviral load correlates well with bronchoalveolar lymphocytosis in HTLV-I carriers. The present findings, together with those of previous studies, suggest that an increased HTLV-I proviral load may lead to certain systemic conditions including bronchoalveolar lymphocytosis in HTLV-I carriers.

In addition to the genetic background, direct or indirect mechanisms induced by inflammatory conditions may have influenced the HTLV-I proviral load in PBMCs of some SHCs with chronic inflammatory diseases of the respiratory tract in this study, as occurs in HIV-1 infection.[25]

The HTLV-I proviral load in BAL fluid appeared to be related to the proportion of lymphocytes in the BAL fluid of HTLV-I carriers, as reported previously.[26] Interestingly, the HTLV-I proviral load in the BAL fluid correlated with the number of CD8+ cells as well as with the number of CD4+ cells in HTLV-I carriers, even though CD4+ cells are thought to be preferentially infected by HTLV-I.[27] Our findings may be consistent with more recent observations of the tropism of HTLV-I to CD8+ lymphocytes.[28 29] Further investigations are needed to determine which T cell subsets are predominantly infected with HTLV-I in the lungs of HTLV-I carriers.

A few studies have described some AHCs with bronchoalveolar lymphocytosis who did not develop HAM/TSP and HTLV-I uveitis.[30 31] In the present study pulmonary involvement was subclinical in AHCs with bronchoalveolar lymphocytosis, similar to the findings of these previous studies. Furthermore, follow up studies suggested that the clinical development of bronchoalveolar lymphocytosis may be delayed in HTLV-I carriers.

HTLV-I infected lymphocyte(s) commonly exist in the lower respiratory tract of HTLV-I seropositive individuals, as shown in the PCR study of BAL cells. This suggests that factors other than the presence of HTLV-I in the lung—such as a systemic increase in the HTLV-I proviral load, as found in this study—may be necessary for excessive accumulation of lymphocytes in the lung. The mechanisms by which an increased HTLV-I proviral load affects pulmonary involvement in HTLV-I carriers remains to be fully clarified. One possible mechanism is that the increased number of HTLV-I infected cells enhances the probability that infected cells will enter the target organs,[31 32] resulting in a local inflammatory response. However, the frequency of HTLV-I specific cytotoxic T lymphocytes[22 23 33] related to the increased amount of the virus might be involved in the pathogenesis, as has been shown in lung disorders of patients with HIV infection.[25 34]

In conclusion, we have shown that pulmonary lymphocytic infiltration can occur in a subset of HTLV-I carriers who have not developed HAM/TSP or HTLV-I uveitis. This pulmonary involvement may be associated with an increased amount of HTLV-I proviral DNA in peripheral blood.

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LUNG ALERT

Some asthma genotypes may not respond to β_2 agonists

▲ Israel E, Chinchilli VM, Ford JG, *et al.* Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet* 2004;**364**:1505–12

This was a prospective crossover trial comparing the use of salbutamol with placebo in 78 mild asthmatics (diagnosed by chest physician, only using inhaled β agonist <56 puffs/week, FEV_1 >70%) aged 18–55 years. 50% had a genetic polymorphism resulting in homozygosity for arginine (Arg/Arg) at amino acid residue number 16 of the β_2 agonist receptor instead of glycine (Gly/Gly), as in the other half. Each patient was matched with a patient from the other group by FEV_1.

Following a 6 week run in period using a placebo metered dose inhaler (two puffs qds; rescue medication ipratropium inhaler), each pair was randomised to receive either active salbutamol (90 µg) or placebo (two puffs qds) for 16 weeks followed by an 8 week run out period using placebo and then crossed over. In the Gly/Gly group there was no change in pre-inhaler morning peak expiratory flow rate (PEFR) with placebo but an increase in PEFR with salbutamol producing a difference of 14 l/min ($p<0.05$). In the Arg/Arg group the reverse occurred with a difference of −10 l/min ($p<0.05$). This group also needed to use their ipratropium inhaler more, which did produce an increase in PEFR. Similar results were seen in FEV_1, symptom scores, and rescue inhaler use.

It appears that Gly/Gly patients respond to salbutamol while those with Arg/Arg seem to get better when salbutamol is withdrawn. It may be that the latter group actually responds to ipratropium. A longer treatment trial is needed with more patients with more severe asthma and with other genetic polymorphisms, using other β_2 agonists, to determine if reliever strategies excluding salbutamol are more suitable for Arg/Arg patients.

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Polymorphism in the Interleukin-10 Promoter Affects Both Provirus Load and the Risk of Human T Lymphotropic Virus Type I–Associated Myelopathy/Tropical Spastic Paraparesis

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To investigate non–human leukocyte antigen candidate genes that influence the outcome of human T cell lymphotropic virus (HTLV) type I infection, we analyzed 6 single-nucleotide polymorphisms in the interleukin (IL)–10 promoter region in 280 patients with HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and 255 HTLV-I–seropositive asymptomatic carriers from an area where HTLV-I is endemic. The IL-10 −592 A allele, which shows lower HTLV-I Tax–induced transcriptional activity than the C allele in the Jurkat T cell line, was associated with a >2-fold reduction in the odds of developing HAM/TSP ($P = .011$; odds ratio [OR], 0.50 [95% confidence interval, 0.30–0.86]) by reducing the provirus load in the whole cohort ($P = .009$, analysis of variance). Given the OR and the observed frequency of IL-10 −592 A, we demonstrate that this allele prevents ~44.7% (standard deviation, ±13.1%) of potential cases of HAM/TSP, which indicates that it defines one component of the genetic susceptibility to HAM/TSP in the cohort.

Human T-cell lymphotropic virus (HTLV) type I is the first characterized human retrovirus [1, 2] and is associated with adult T cell leukemia (ATL) [3, 4] and HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) [5, 6]. Unlike HIV, HTLV-I causes no disease in a majority of infected subjects (healthy carriers [HCs]). However, ~2%–3% develop ATL, and another 2%–3% develop a disabling chronic inflammatory disease involving the central nervous system (HAM/TSP), eyes, lungs, or skeletal muscles [7]. The lifetime incidence for developing HAM/TSP is only 0.25% in Japan [8]. The factors that cause these different manifestations of HTLV-I infection are not fully understood. However, our previous population association study of >200 cases of HAM/TSP and >200 HTLV-I–seropositive HCs revealed several important risk factors for HAM/TSP. One of the major risk factors is the provirus load, as has been reported elsewhere [9]. The median provirus load was 16 times higher in patients with HAM/TSP than in HCs, and a high provirus load was also associated with an increased risk of progression to disease [10]. We next investigated HLA associations and found that the HLA-A*02 and -Cw*08 genes were associated with a lower HTLV-I provirus

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Table 1. Primers and restriction enzymes used for restriction fragment-length polymorphism analysis.

Polymorphism, primer direction	Primer sequence	Restriction enzyme	Reference (accession no.)[a]
−3575 (T/A)		TSP509I	25
Forward	5′-GTTTTTCCTTCATTTGCAGC-3′		
Reverse	5′-ACACTGTGAGCTTCTTGAGG−3′		
−2849 (G/A)		*Alw*I	AF295024
Forward	5′-CTGTAATCTCAGCACTCTGG-3′		
Reverse	5′-AGTTCAAGCCATTCTCCTGC-3′		
−2763 (C/A)		*Dde*I	25
Forward	5′-GAGGACTTGCACCAGGGAACT-3′		
Reverse	5′-TCCCGAGTAGCTGGGACTACA-3′		
−1082 (A/G)		*Mnl*I	26
Forward	5′-TCTGAAGAAGTCCTGATGTCACTG-3′		
Reverse	5′-ACTTTCATCTTACCTATCCCTACTTCC-3′		
−819 (T/C)		*Mae*III	27
Forward	5′-ATCCAAGACAACACTACTAA-3′		
Reverse	5′-TAAATATCCTCAAAGTTCC-3′		
−592 (A/C)		*Rsa*I	28
Forward	5′-CCTAGGTCACAGTGACGTGG -3′		
Reverse	5′-GGTGAGCACTACCTGACTAGC-3′		

[a] Accession numbers for GenBank/EMBL/DDBJ.

load and with protection from HAM/TSP, whereas HLA-DRB1*0101 and -B*5401 were associated with susceptibility to HAM/TSP; HLA-B*5401 was also associated with a higher provirus load in patients with HAM/TSP [11, 12]. We further examined the non-HLA host genetic factors that affect the risk of HAM/TSP and reported previously [13] that the tumor necrosis factor promoter −863 A allele predisposes toward HAM/TSP, whereas the stromal cell–derived factor–1 +801A 3′ untranslated region and interleukin (IL)–15 191 C alleles confer protection. In another study [14], we reported the association between variation in the HTLV-I *tax* gene and the risk of HAM/TSP. The *tax* subgroup A was more frequently observed in patients with HAM/TSP, and this effect was independent of HLA-A*02. These findings suggest that both host genetic factors and HTLV-I subgroup play a part in determining the risk of HAM/TSP.

To investigate further the non-HLA host genetic factors that influence the outcome of HTLV-I infection, we analyzed 6 single-nucleotide polymorphisms (SNPs) in the IL-10 promoter region and quantified the effect of each SNP on the risk of HAM/TSP, because recent studies have revealed a close association between IL-10 promoter polymorphisms and the outcome of certain viral infections, such as Epstein-Barr virus (EBV) [15], hepatitis B virus (HBV) [16], hepatitis C virus (HCV) [17], and HIV-1 [18], which suggests that particular polymorphisms in the IL-10 promoter contribute to the host immune reaction against viruses.

PATIENTS, MATERIALS, AND METHODS

Study population. Two hundred eighty patients with HAM/TSP were compared with 255 randomly selected HCs. All patients and control subjects were Japanese and resided in Kagoshima Prefecture, Japan. The diagnosis of HAM/TSP was made according to the World Health Organization diagnostic criteria [19]. All subjects provided written informed consent.

Detection of SNPs in the IL-10 promoter region. Polymerase chain reaction (PCR)–restriction fragment–length polymorphism analysis was performed for 6 SNPs. Primers and restriction enzymes used in the study are presented in table 1. A genomic PCR was performed with 50 ng of genomic DNA as template, 20 pmol of each primer, 5 mmol/L dNTP, reaction buffer provided by the manufacturer, and 1 U of Takara-Taq DNA polymerase (Takara) in a final volume of 50 μL. Fifteen microliters of the amplified PCR product was then digested for 12 h with the use of each restriction enzyme. Finally, digested PCR products were electrophoresed through a 2% agarose gel and visualized by ethidium bromide.

Provirus load measurement. To examine the HTLV-I provirus load, we performed a quantitative PCR method using an ABI Prism 7700 (PE-Applied Biosystems) with 100 ng of genomic DNA (~10^4 cells) from peripheral blood mononuclear cell (PBMC) samples, as reported elsewhere [10]. When β-actin was used as an internal control, the amount of HTLV-I provirus DNA was calculated by copy number of HTLV-I (pX) per 1 × 10^4 PBMCs = [(copy number of pX)/(copy number of β-

actin/2)] × 10^4. All samples were tested in triplicate. The lower limit of detection was 1 pX/10^4 PBMCs.

Cell line and plasmids. The human T-cell line Jurkat was maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. The expression vector pCG-Tax and the control vector pCG-BL were provided by Dr. J. Fujisawa (Kansai Medical University, Osaka, Japan). The pCG-Tax expression vector based on the human cytomegalovirus promoter for HTLV-I *tax* was constructed by inserting *tax* cDNA into the *Xba*I-*Bam*HI site of pCG-BL, as described elsewhere [20]. Human IL-10 promoter fragments (fragment −890 to +120; GenBank accession number X78437) were amplified by PCR from genomic DNA from 2 patients with HAM/TSP—one −592 AA homozygote and one CC homozygote—as described elsewhere [21]. The primers used to amplify the IL-10 region were IL-10 -890 (5′-AGC TCG AGA GTT GGC ACT GGT GTA CC-3′) and IL-10 AS (5′-ACT TCG AAG TTA GGC AGG TTG CCT G-3′). A promoter fragment that does not contain the −592 SNP, as well as the neighboring Sp-1 and Ets binding sites (fragment −571 to +120), was also amplified with the primers IL-10 −571 (5′-AAC CTC GAG GGA TAT TTA GCC CAC-3′) and IL-10 AS. The amplified products were subcloned into the pCR-Blunt II-TOPO vector (Invitrogen), and the sequences were confirmed. The correct insertions were subcloned into the *Xho*I polylinker site of the pGL2 Basic luciferase reporter vector (Promega), and sequences were confirmed again.

Transient transfection and luciferase assay. Five hundred thousand Jurkat cells were cotransfected with 2 μg of a reporter plasmid (IL-10 −592 A-Luc or IL-10 −592 C-Luc), together with 0.5 μg of either pCG-Tax or pCG-BL [20] and 300 ng of pRL-TK (Promega), to control transfection efficiency. The results of preliminary studies that measured luciferase activities from cell lysates at 24, 48, and 72 h after transfection indicated that the greatest luciferase activity was at 48 h after transfection. Therefore, after 48 h of cultivation at 37°C, cells were harvested, washed with PBS, and lysed in reporter lysis buffer (Promega). Luciferase assays were performed by use of the Dual Luciferase Assay System (Promega) and a TD-20/20 luminometer (Turner Designs). All assays were performed at least 3 times, each in duplicate.

Statistical and logistic-regression analysis. The χ^2 test was used to examine associations between HAM/TSP and the IL-10 promoter polymorphism. General linear model (GLM) analysis [22], which is a general form of multiple regression, was used to identify which factors were predictors of provirus load, in patients with HAM/TSP alone, in HCs alone, or in all subjects in the study. Logistic-regression analysis was used to identify which factors could be used to predict the odds of HAM/TSP and to fit an equation to estimate the risk in an individual of known genotype. The prevented fraction (Fp) of disease was calculated as described elsewhere [11].

RESULTS

Association of the IL-10 −592 A allele with a lower risk of HAM/TSP. The median age of patients with HAM/TSP (60.0 years; range, 12–81 years; 69.0% female) was greater than that of HCs (41 years; range, 16–65 years; 57.6% female), and there were more females in the HAM/TSP group and an absence of subjects <16 or >65 years old from the HCs; however, these factors did not affect the frequency of individual HLA alleles (data not shown). In addition, because the prevalence of HAM/TSP in Kagoshima is <1% among individuals infected with HTLV-I, very few HCs in the present cohort would be expected to develop HAM/TSP. There were no significant differences in the distribution of all genotypes and allele frequencies between 102 patients with HAM/TSP and 102 HCs in 4 SNPs tested (table 2). The nucleotide at position −2849 was nonpolymorphic in 102 patients with HAM/TSP and 102 HCs. In contrast, the IL-10 −592 A/C SNP showed a significant difference in allele frequency. We therefore analyzed further a total of 280 patients with HAM/TSP and 255 HCs (table 2; $\chi^2 = 8.48$; 2 *df*; $P = .014$) and identified a significant association between possession of an A residue in the IL-10 promoter −592 A/C SNP and a reduced risk of HAM/TSP. Possession of the IL-10 −592 A allele was associated with a >2-fold reduction in the odds of developing HAM/TSP ($P = .011$; odds ratio [OR], 0.50 [95% confidence interval, 0.30–0.86]). Given this OR and the observed frequency of the IL-10 −592 A allele in Kagoshima, we can estimate the Fp [11]. Here, Fp = 44.7% (SD, ±13.1%) when the prevalence rate of HAM/TSP is 0.01, which indicates that the IL-10 −592 A allele prevents ~44.7% (SD, ±13.1%) of potential cases of HAM/TSP in the study population.

Association of the presence of the A allele with a lower provirus load in the whole Kagoshima cohort of HTLV-I–infected individuals. We next tested the hypothesis that, if a gene is associated with a protection from HAM/TSP, it is also associated with a reduction in provirus load in HCs, given that the risk of developing HAM/TSP is dependent on the provirus load [10]. Table 3 summarizes the HTLV-I provirus load in patients with HAM/TSP and HCs, subdivided according to their IL-10 −592 A/C genotype. Because histograms of provirus load exhibited right-skewed distributions, the standard statistical technique of logarithmic transformation [22] was also used to mitigate this feature, which resulted in the data being more amenable to statistical analysis by parametric methods. To confirm whether the IL-10 −592 A/C SNP is a significant predictor of provirus load in the entire cohort, we performed multiple-regression analysis (GLMs; see Patients, Materials, and Methods). The results showed that the IL-10 −592 A/C SNP is a

Table 2. Interleukin (IL)–10 polymorphisms among patients with human T cell lymphotropic virus (HTLV) type I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and healthy HTLV-I carriers (HCs).

Polymorphism	HAM/TSP	HCs	*P*
−3575(T/A)			1.00
TT	99 (97.1)	99 (97.1)	
AT	3 (2.9)	3 (2.9)	
AA	0 (0)	0 (0)	
−2849 (G/A)			NA
GG	102 (100)	102 (100)	
GA	0 (0)	0 (0)	
AA	0 (0)	0 (0)	
−2763 (C/A)			.24
CC	95 (93.1)	89 (87.3)	
AC	7 (6.9)	13 (12.7)	
AA	0 (0)	0 (0)	
−1082 (A/G)			.38
AA	93 (91.2)	88 (86.3)	
AG	9 (8.8)	14 (13.7)	
GG	0 (0)	0 (0)	
−819 (T/C)			1.00
CC	12 (11.8)	12 (11.8)	
TC	49 (48.0)	48 (47.1)	
TT	43 (42.2)	42 (41.2)	
−592 (A/C)			**.014**[a]
AA	117 (41.8)	101 (39.6)	
AC	117 (41.8)	131 (51.4)	
CC	46 (16.4)	23 (9.0)	

NOTE. Data are no. of samples (%). The IL-10 −592 A allele was associated with a >2-fold reduction in the odds of HAM/TSP ($P = .011$; odds ratio, 0.50 [95% confidence interval, 0.30–0.86]). The proportion of potential cases of HAM/TSP that are prevented by the presence of the IL-10 −592 A allele (the prevented fraction of disease) [11] was 44.7% (SD, ±13.1%) when prevalence rate of HAM/TSP was 0.01, indicating that IL-10 −592 A allele prevents ~44.7% (SD, ±13.1%) of potential cases of HAM/TSP in the study population. NA, not applicable.

[a] χ^2 for genotype, $\chi^2 = 8.48$.

significant predictor of provirus load in the entire cohort ($n = 535$; $P = .004$, Kruskal-Wallis test; $P < .01$, GLM on the log-transformed or -untransformed data). This SNP was also a significant predictor of provirus load in the HC group alone ($n = 255$; $P = .040$, Kruskal-Wallis test), but not in the HAM/TSP group ($n = 280$; $P = .243$, Kruskal-Wallis test). Also, presence or absence of the IL-10 −592 A allele was a significant predictor of the provirus load in the entire cohort ($n = 535$; $P = .001$, Mann-Whitney U test; $P < .005$, GLM), although this relationship was only marginally significant in the HC group ($n = 255$; $P = .103$; Mann-Whitney U test; $P < .13$, GLM). These analyses indicate that the IL-10 −592 A/C SNP was a significant predictor of the provirus load and that the presence of A allele was associated with a lower provirus load in the whole Kagoshima cohort of HTLV-I–infected individuals (table 3).

IL-10 −592 A/C SNP—significant predictor of HAM/TSP even after accounting for provirus load or HLA-A*02. As was already mentioned, there was a significant association between the odds of developing HAM/TSP and the IL-10 −592 A/C SNP genotype according to the results of single-factor χ^2 analysis at both the allele and the genotype level. To confirm whether the IL-10 −592 A/C SNP genotype remains a significant predictor of HAM/TSP even after taking into account the other significant predictors identified by our previous analyses, such as provirus load and HLA-A*02, we performed logistic-regression analysis. As a result, in logistic-regression analysis that included HTLV-I provirus load and IL-10 −592 A/C SNP genotype treated as a 3-level factor (i.e., AA vs. AC vs. CC), the IL-10 −592 A/C SNP remained significant as a predictor of HAM/TSP ($P = .043$). We can calculate the risk for HAM/TSP by ln(odds of HAM/TSP) = −4.1212–0.5668 (if AC)–0.0235 (if CC) + 2.0764 × $\log_{10}$ (pX/10^4 PBMCs). When we treated the IL-10 −592 A/C SNP genotype as a 2-level factor, inclusion of the absence or presence of the A allele was not significant when $\log_{10}$(pX/10^4 PBMCs) was included ($P = .399$). However, the inclusion of the absence or presence of C was significant when $\log_{10}$(pX/10^4 PBMCs) was included ($P = .047$). Therefore, we conclude that the IL-10 −592 A/C SNP genotype has predictive power for HAM/TSP even after we accounted for the HTLV-I provirus load. Next, to test whether the IL-10 −592 A/C SNP genotype remains a predictor of HAM/TSP even after we accounted for HLA-A*02, we further performed the logistic-regression analysis using samples that are available on both IL-10 −592 A/C SNP and HLA-A*02 ($n = 402$). In logistic-regression analysis that included the HLA-A*02 and IL-10 −592 A/C SNP genotype, both HLA-A*02 ($P = .001$) and IL-10 −592 A/C SNP ($P = .014$) remained significant as predictors of HAM/TSP. In this case, we can calculate the risk for HAM/TSP by the equation ln(odds of HAM/TSP) = 0.4321–0.8876 (if A*02-positive)–0.2242 (if AC) + 0.7488 (if CC). In conclusion, the IL-10 −592 A/C SNP remains as a significant predictor of HAM/TSP even after taking into account the effects of the 2 known significant predictors of the risk of HAM/TSP—provirus load and HLA-A*02.

Effect of IL-10 − 592 A/C SNP on HTLV-I Tax–mediated IL-10 promoter activity. To examine the functional significance of the −592 A/C SNP in HTLV-I infection, a 1010-bp promoter of the IL-10 gene (−890 to +120) carrying either the C or the A allele was inserted upstream of the luciferase gene in the pGL2-Basic plasmid vector, and luciferase assays were done. Because many polymorphisms in the IL-10 gene have been identified, numerous combinations of these polymorphisms may exist. Although our Kagoshima cohort of patients with HAM/TSP is the world's largest, <300 patients are available for analysis, so it would be meaningless to analyze all combinations of the IL-10 SNPs. The only sequence difference between the 2 reporter vectors was

Table 3. Interleukin (IL)–10 −592 A/C single-nucleotide polymorphism (SNP) genotype and human T cell lymphotropic virus (HTLV) type I provirus load.

Group	AA	AC	CC
HAM/TSP (280)	679.0 ± 58.2 (117)	785.8 ± 63.8 (117)	959.3 ± 139.6 (46)
HC (255)	77.2 ± 13.7 (101)	129.6 ± 15.7 (131)	194.6 ± 50.1 (23)
All patients combined (535)	400.2 ± 37.8 (218)	439.2 ± 37.5 (248)	704.4 ± 103.8 (69)

NOTE. Values are the average *tax* value (no. of *tax* copies/10^4 PBMCs) ± SE. The IL-10 −592 A/C SNP was a significant predictor of provirus load in the entire cohort ($n = 535$; $P = .004$, Kruskal-Wallis test; $P < .01$, general linear model analysis on log-transformed or -untransformed data) and of provirus load in the HTLV-I–seropositive asymptomatic carriers alone ($n = 255$; $P = .040$, Kruskal-Wallis test) but not in the HAM/TSP group ($n = 280$; $P = .243$, Kruskal-Wallis test). Values in parentheses are nos. of individuals tested. HAM/TSP, associated myelopathy/tropical spastic paraparesis; HC, healthy carrier.

the residue at position −592, which allowed us to estimate the functional differences associated with the −592 A or C residues alone. The results of the experiments showed that the functional differences were associated with the −592 A or C residues alone on HTLV-I Tax–mediated IL-10 promoter activity. These results showed that the ectopic expression of the Tax protein in Jurkat T cells increased IL-10 promoter activity by ~3 times with the A construct and 6 times with the C construct, compared with HCs ($P < .01$, Mann-Whitney *U* test) (figure 1). In contrast, the promoter fragment (fragment −571 to +120), which does not contain −592 SNP, as well as the neighboring Sp-1 and Ets binding site, was not transactivated by Tax. The basal luciferase activity without the transfecting Tax-expression vector (i.e., with transfecting empty vector, pCG-BL) did not differ between the A and C constructs. These results indicated that Tax directly transactivates the IL-10 promoter and that the C allele is more effective for Tax-mediated transcription than the A allele.

DISCUSSION

IL-10 is an important immunoregulatory cytokine that is involved in inflammatory responses, autoimmune diseases, and the response to infectious agents [23]. Although IL-10 has been reported to suppress the synthesis of proinflammatory cytokines from T cells and monocytes/macrophages, animal models have suggested that the overexpression of IL-10 in vivo can cause organ-specific autoimmune diseases, such as Sjögren syndrome [24] and type 1 diabetes [25]. Therefore, IL-10 is not regarded simply as an immunoinhibitory cytokine but also as a powerful immunostimulatory cytokine. Because transgenic mice containing the HTLV-I *tax* gene under the control of the viral long-terminal repeat (LTR) have previously been shown to develop an exocrinopathy involving the salivary and lachrymal glands that resembles Sjögren syndrome [26], which is frequently observed in patients with HAM/TSP [27], and be-

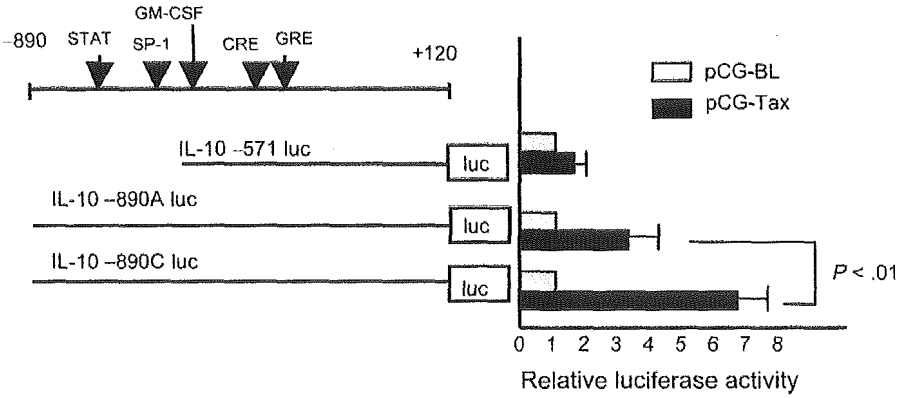

Figure 1. Interleukin (IL)–10 −592 A/C polymorphism and the Tax-mediated transcription of the IL-10 promoter. Jurkat cells were transfected with human T cell lymphotropic virus (HTLV) type I Tax expressing (pCG-Tax) or control (pCG-BL) vector and luciferase (luc) reporter constructs containing the full-length IL-10 promoter with −592 AA (−890 A-luc) or CC (−890 C-luc) or luc reporter plasmid without the specificity protein (Sp)–1 or −592 A/C SNP (−572 luc) sites. *Gray bars*, Luc activity of each reporter plasmid with control vector pCG-BL. *Black bars*, Luc activity of each reporter plasmid with Tax-expressing vector pCG-Tax. The activities are given relative to the activity of each reporter plasmid with control vector pCG-BL, which was defined as 1. The mean ± SD from 3 independent experiments is shown. The basal luciferase activity with pCG-BL was not different between −890 A-luc and −890 C-luc. The difference of luciferase activity with pCG-Tax between −890 A-luc and −890 C-luc was statistically significant ($P < .01$, Mann-Whitney *U* test). CRE, cyclic AMP response element; GM-CSF, granulocyte macrophage colony-stimulating factor; GRE, glucocorticoid response element; STAT, signal transducer and activator of transcription.