MAJOR ARTICLE

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 $\times 10^4$ PBMCs = [(copy number of pX)/(copy number of β-

actin/2)] $\times 10^4$. 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-

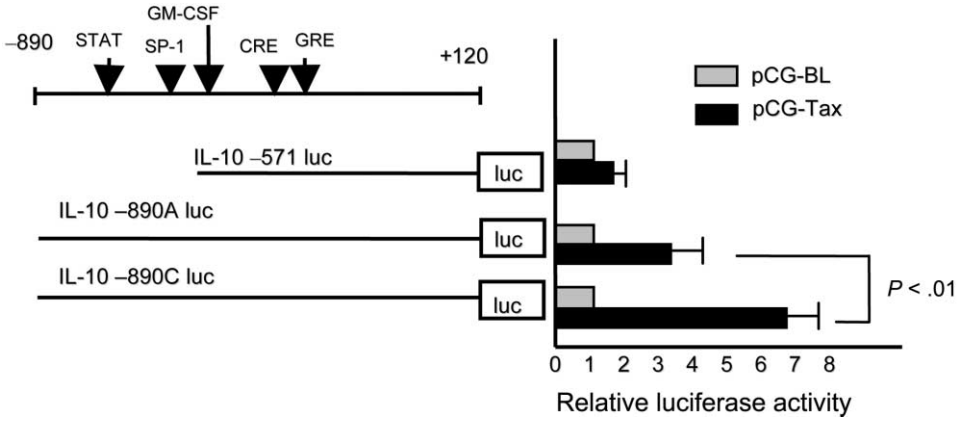

Figure 1. Interleukin (IL)–10 −592 A/C polymorphism and the Tax-mediated transcription of the IL-10 promoter. Jurkat cells were transfected with human T cell lymphotropic virus (HTLV) type I Tax expressing (pCG-Tax) or control (pCG-BL) vector and luciferase (luc) reporter constructs containing the full-length 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.

cause IL-10 mRNA expression was induced by HTLV-I Tax in both transiently and stably transfected Jurkat cells [28], it is likely that Tax directly transactivates the IL-10 promoter. The resulting overexpression of Tax in vivo may cause a Sjögren-like syndrome via an IL-10–mediated mechanism.

The implication of a heritable genetic basis for IL-10 production is supported by the concordance of IL-10 production in monozygotic twins, which suggests that genetic polymorphism could account for up to 75% of the observed variation in IL-10 production [29]. As was already mentioned, several studies have shown an association between particular polymorphisms in the human IL-10 promoter region and the outcome of certain viral infections, such as EBV [15], HBV [16], HCV [17], and HIV-1 [18]. In view of the immunomodulatory and anti-inflammatory effects of IL-10, we initially hypothesized that genetically determined lower production of IL-10 (associated with the allele −592 A) might influence disease susceptibility to HAM/TSP. This is the case for HIV-1 infection, because individuals with the IL-10 −592 AA genotype have been reported to be at higher risk of HIV-1 infection and rapid progression to AIDS [18]. In contrast, the present data show that, in HTLV-I infection, possession of the IL-10 −592 A allele prevented ~44.7% (SD, ±13.1%) of potential cases of HAM/TSP and was also a significant predictor for a lower provirus load in the entire cohort.

The −592 A/C SNP is located between the Sp1 and Ets binding site within the region between −652 and −571 nt that is necessary for IL-10 transcription [21]. It is of interest that previous reports have indicated that Tax transactivates the parathyroid hormone–related protein promoter by forming a ternary complex between Tax, Ets, and Sp-1, which acts on the promoter Sp-1 and Ets binding sites [30]. Another report showed that the HTLV-I LTR also contains a motif related to the Ets-binding sequence, named TRE-2S [31]. More important, 1 copy of the cyclic AMP response element (CRE)–like 21-bp sequence and TRE-2S in the HTLV-I LTR, contributes to the transactivation of viral gene via a ternary complex formed between Tax, Gli2 (TRE-S binding Gli oncogene family protein), and CRE-binding protein [32]. These findings suggest that a common mechanism of the HTLV-I Tax–mediated transactivation of the promoter of target genes ternary complexes formed with 2 different transcription factors. Furthermore, the results also suggest that the IL-10 promoter −592 A/C SNP, which lies between the Sp-1 and Ets binding sites, affects Tax-mediated transcription. Indeed, our cotransfection study using a Tax-expressing vector and Jurkat cells demonstrated that a IL-10 −592 luciferase vector carrying the high producer allele (C) showed higher Tax-mediated transcription than that of low producer allele (A), whereas a promoter fragment (fragment −571 to +120) that does not contain −592 SNP, as well as the neighboring Sp-1 and Ets binding site, was not transactivated by Tax. These findings suggested that HTLV-I Tax directly transactivates the IL-10 promoter and that the −592 A/C SNP affects Tax-induced transcription—that is, that the C allele is more effective than the A allele in mediating the Tax-induced transcription of IL-10. In future studies, it may be interesting to test whether Tax, Ets, and Sp-1 form a ternary complex on the IL-10 promoter and whether the −592 SNP affects this complex formation.

Among >90 non-HLA candidate gene loci that we have so far examined, the IL-10 −592 A/C SNP is the only non-HLA candidate gene locus associated with a significant reduction in both the provirus load and the risk of HAM/TSP. This observation is exactly analogous to the argument that we previously reported for HLA-A*02 and -Cw*08, where, in each case, possession of the allele was associated with both a significant reduction in provirus load in the HCs and a significant reduction in the risk of HAM/TSP [11, 12]. Thus, one possible mechanism for the observed IL-10 promoter effect is that increased the production of IL-10 reduces the efficiency of immune surveillance of HTLV-I infection—for example, by reducing the number or the activity of HTLV-I–specific cytotoxic T lymphocytes. However, the IL-10 promoter genotype remained a significant predictor of the risk of HAM/TSP even after taking the provirus load into account. This observation suggests that IL-10 increases the risk of HAM/TSP by another mechanism in addition to an apparent effect on provirus load.

In conclusion, we report that the IL-10 −592 A allele, which is associated with lower HTLV-I Tax–mediated transcriptional activity, influences both the provirus load in HTLV-I–infected individuals and the susceptibility to HAM/TSP in the Kagoshima cohort. This effect remains significant even after taking into account the other 2 known major predictors of HAM/TSP risk in this cohort—provirus load and HLA-A*02 genotype—which suggests a powerful argument in favor of a real physiological effect of this polymorphism. Further functional studies to clarify the role of IL-10 in HTLV-I infection may reveal immunotherapeutic strategies that would retard the development of HAM/TSP.

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