AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 27, Number 11, 2011

DOI: 10.1089/aid.2011.0029

Short Communication
An Interferon-γ ELISPOT Assay with Two Cytotoxic T Cell Epitopes Derived from HTLV-1 Tax Region 161-233 Discriminates HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis Patients from Asymptomatic HTLV-1 Carriers in a Peruvian Population

Ivan Best,[1] Giovanni López,[1] Michael Talledo,[1] Aidan MacNamara,[2] Kristien Verdonck,[1,3] Elsa González,[1,4] Martín Tipismana,[1,4] Becca Asquith,[2] Eduardo Gotuzzo,[1,4] Guido Vanham[3,5] and Daniel Clark[1,6]

Abstract

HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic and progressive disorder caused by the human T-lymphotropic virus type 1 (HTLV-1). In HTLV-1 infection, a strong cytotoxic T cell (CTL) response is mounted against the immunodominant protein Tax. Previous studies carried out by our group reported that increased IFN-γ enzyme-linked immunospot (ELISPOT) responses against the region spanning amino acids 161 to 233 of the Tax protein were associated with HAM/TSP and increased HTLV-1 proviral load (PVL). An exploratory study was conducted on 16 subjects with HAM/TSP, 13 asymptomatic carriers (AC), and 10 HTLV-1-seronegative controls (SC) to map the HAM/TSP-associated CTL epitopes within Tax region 161–233. The PVL of the infected subjects was determined and the specific CTL response was evaluated with a 6-h incubation IFN-γ ELISPOT assay using peripheral blood mononuclear cells (PBMCs) stimulated with 16 individual overlapping peptides covering the Tax region 161–233. Other proinflammatory and Th1/Th2 cytokines were also quantified in the supernatants by a flow cytometry multiplex assay. In addition, a set of human leukocyte antigen (HLA) class I alleles that bind with high affinity to the CTL epitopes of interest was determined using computational tools. Univariate analyses identified an association between ELISPOT responses to two new CTL epitopes, Tax 173–185 and Tax 181–193, and the presence of HAM/TSP as well as an increased PVL. The *HLA-A*6801* allele, which is predicted to bind to the Tax 181–193 peptide, was overpresented in the HAM/TSP patients tested.

Introduction

Human T-lymphotropic virus type 1 (HTLV-1) is the etiological agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[1–4] One of the main mechanisms to control the number of infected cells is a strong cytotoxic T cell (CTL) response mounted against some proteins of HTLV-1.[5–12] The Tax protein has been identified as the immunodominant antigen recognized by host CTL, including the Tax 11–19 peptide, an epitope restricted by *HLA-A*0201*.[5–9] A study in *HLA-A*02*-positive subjects showed an increased frequency of Tax 11–19-specific $CD8^+$ T cells in HAM/TSP patients with respect to asymptomatic carriers (AC).[13] Other investigations have reported a protective effect of specific HLA class I alleles such as *HLA-A*02* or *HLA-Cw*08* against HAM/TSP,[14–16] as well as their association with a lower proviral load (PVL) even in AC.[14,15] Interestingly, using short-incubation interferon (IFN)-γ peripheral blood mononuclear cell (PBMC)-based enzyme-linked immunospot (ELISPOT) assays from *HLA-A*02*-positive subjects stimulated with Tax

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru.
[2]Department of Immunology, Imperial College School of Medicine, London, United Kingdom.
[3]Virology Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium.
[4]Departamento de Medicina, Facultad de Medicina, Universidad Peruana Cayetano Heredia, Lima, Peru.
[5]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.
[6]Laboratorios de Investigación y Desarrollo (LID), Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru.

Table 1. Peptide Sequences from HTLV-1 Tax Region 161–233

Number	*Peptide*	*Sequence*
1	Tax (161–173)	PPITWPLLPHVIF
2	Tax (165–177)	WPLLPHVIFCHPG
3	Tax (169–181)	PHVIFCHPGQLGA
4	Tax (173–185)	FCHPGQLGAFLTN
5	Tax (177–189)	GQLGAFLTNVPYK
6	Tax (181–193)	AFLTNVPYKRIEE
7	Tax (185–197)	NVPYKRIEELLYK
8	Tax (189–201)	KRIEELLYKISLT
9	Tax (193–205)	ELLYKISLTTGAL
10	Tax (197–209)	KISLTTGALIILP
11	Tax (201–213)	TTGALIILPEDCL
12	Tax (205–217)	LIILPEDCLPTTL
13	Tax (209–221)	PEDCLPTTLFQPA
14	Tax (213–225)	LPTTLFQPARAPV
15	Tax (217–229)	LFQPARAPVTLTA
16	Tax (221–233)	ARAPVTLTAWQNG

11-19, our group failed to confirm significant differences between HAM/TSP patients and AC (unpublished data). This finding opens the possibility that epitopes other than Tax 11–19, possibly restricted by other alleles or derived from other viral proteins,[17] can discriminate patients with HAM/TSP from AC with regard to the specific IFN-γ production elicited.

A previous study in our laboratory using pools of overlapping peptides that covered the entire sequence of Tax strongly suggested that the specific IFN-γ CTL response to Tax region 161–233 is capable of discriminating HAM/TSP patients from AC.[18] In the present study, we confirmed these findings by mapping the CTL epitopes responsible for the discriminatory response and evaluated the association between the response elicited by these epitopes and the PVL in patients with HAM/TSP and AC.

Materials and Methods

To pinpoint the specific CTL epitopes that discriminate HAM/TSP patients from AC within Tax region 161–233, we enrolled 29 HTLV-1-infected subjects (16 patients with HAM/TSP and 13 AC) consecutively from the HTLV-1 cohort at the Instituto de Medicina Tropical Alexander von Humboldt, along with 10 HTLV-1-seronegative controls (SC). The study was approved by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia and written informed consent was obtained from all participants. HTLV-1 infection was determined by at least one positive ELISA result and at least one positive confirmatory test result (either Western blot, line immunoassay, or a detectable PVL).[19] The diagnosis of HAM/TSP was made by experienced physicians according to internationally accepted criteria.[20,21]

PBMCs were isolated by Ficoll gradient centrifugation and suspended in complete medium [RPMI 1640 medium (Gibco, Paisley, Scotland) supplemented with 5% pooled human serum from healthy donors, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Gibco, Paisley, Scotland)].[18,22] We used a short-incubation (6 h) IFN-γ ELISPOT assay[11,18] to identify the epitopes that discriminate HAM/TSP patients from AC within Tax region 161–233. Freshly isolated PBMCs were stimulated with 16 individual overlapping 13-mer peptides (offset = 4 amino acids) covering Tax region 161–233 (Table 1), as well as with the Tax 11–19 peptide (Mimotopes, Clayton Victoria, Australia). Incubation with medium alone served as negative control. Each peptide was used at a final concentration of 1 μg/ml, and a CD28/CD49d costimulatory reagent (Becton Dickinson, San Diego, CA) was included in all assays as reported previously.[18] The number of spot-forming cells (SFC) was determined with the AID software (AID ELISPOT Reader, Strassberg, Germany). The background response (unstimulated cultures) was subtracted from the values obtained with peptide-stimulated samples.

In addition, the supernatants from the ELISPOT assays were analyzed with a flow cytometry multiplex assay (Bender Medsystems, Vienna, Austria) to determine the levels of 10 cytokines [interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, tumor necrosis factor (TNF)-α, and TNF-β].

The PVL was determined in all samples by quantitative PCR (qPCR) and expressed as HTLV-1 copies per 10^4 PBMCs as previously described.[22,23]

The frequency of *HLA-A*02* was determined by flow cytometry.[18] To identify the potential HLA class I alleles capable of binding CTL epitopes within Tax region 161–233, we used an *in silico* screening based on the Metaserver algorithm for evaluating 47 HLA class I alleles (http://web.bioinformatics.ic.ac.uk/metaserver/).[24] This software uses a combination of two web-based prediction software packages: NetMHC (http://www.cbs.dtu.dk/services/NetMHC) and NetCTL (http://www.cbs.dtu.dk/services/NetCTL).[25,26] The probability of a 9-mer peptide being an epitope was evaluated using a score, which combines HLA class I molecule binding affinity, TAP, and cleavage prediction without a normalization procedure known as rescaling.[24] The higher the value of this

Table 2. Demographic Characteristics of HTLV-1-Seronegative Controls, Asymptomatic Human T-Lymphotropic Virus 1 Carriers, and Patients with HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

	HTLV-1-seronegative controls (n = *10*)	*Asymptomatic HTLV-1 carriers* (n = *13*)	*HAM/TSP patients* (n = *16*)
Age in years[a]	24 (24–26.5)	36 (25.5–56)	52 (46.3–66.3)
Male gender[b]	2 (20)	5 (38)	2 (13)
Andean origin[b]	2 (20)	6 (46)	13 (81)

[a]Age is presented as the median (Q1–Q3).
[b]Male gender and Andean origin are presented as absolute numbers and percentages (in parentheses).
HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; AC, asymptomatic carriers; SC, seronegative controls; HTLV-1, human T-lymphotropic virus type 1; Q1–Q3, first quartile–third quartile.

Table 3. Immunological Markers in HTLV-1-Seronegative Controls, Asymptomatic Human T-Lymphotropic Virus 1 carriers, and Patients with HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

	HTLV-1-seronegative controls (n = *10*)	*Asymptomatic HTLV-1 carriers* (n = *13*)	*HAM/TSP patients* (n = *16*)	p *value*[a]
IFN-γ responses to Tax peptides[b]				
Tax 161–173	0 (0–1)	8 (3–72.5)	43.5 (3.8–101.5)	0.3[c]
Tax 165–177	0 (0–0.5)	16 (2.5–28.5)	37 (5.8–115.8)	0.3[c]
Tax 169–181	0 (0–1.75)	20 (0–29.5)	23 (1.3–110)	0.4[c]
Tax 173–185	0 (0–2.25)	17 (3.5–31.5)	71 (31–98.8)	**0.007**[c]
Tax 177–189	0 (0–4)	8 (1–33)	60.5 (2.3–96.8)	0.2[c]
Tax 181–193	0 (0–0.5)	14 (0–31)	77.5 (5.3–118.5)	**0.036**[c]
Tax 185–197	0 (0–0.75)	10 (0–28.5)	83.5 (11–200.3)	**0.015**[c]
Tax 189–201	0 (0–1.75)	16 (0–33)	30.5 (13.8–95)	0.1[c]
Tax 193–205	0 (0–2)	14 (0–54)	52.5 (10–97.8)	0.1[c]
Tax 197–209	0 (0–0.5)	16 (1.5–36.5)	46 (12.8–102.8)	0.1[c]
Tax 201–213	0 (0–4)	11 (2.5–45.5)	72 (8–114.5)	0.1[c]
Tax 205–217	0 (0–4)	10 (4–42.5)	80.5 (6.3–128.3)	0.1[c]
Tax 209–221	0 (0–1.5)	26 (4.5–73)	43.5 (10.3–71)	0.5[c]
Tax 213–225	0 (0–0.5)	11 (2–32)	40.5 (4.3–96.5)	0.1[c]
Tax 217–229	0 (0–2)	16 (1–37)	55 (19.8–161.8)	0.1[c]
Tax 221–233	0 (0–0.5)	18 (4.5–87.5)	56.5 (2.5–94)	0.6[c]
Tax 11–19	0 (0–4.5)	202 (78–278)	115 (52–214.8)	0.4[c]
Other cytokine responses[d]				
IL-1β				
Tax 173–185	683 (26–2773)	2747 (1090–4023)	195 (40–1399)	**0.013**[c]
Tax 185–197	1684 (40–3247)	4061 (1760–5449)	129 (59–2710)	**0.029**[c]
IL-6				
Tax 173–185	1970 (22–4406)	3770 (2899–4684)	188 (33–3762)	0.056[c]
Tax 185–197	1848 (35–17314)	7752 (4372–9343)	121 (71–6356)	0.1[c]
TNF-α				
Tax 173–185	43.5 (0–228.1)	130.2 (86.3–325.9)	0 (0–243.3)	0.1[c]
Tax 185–197	119.1 (11.5–373.9)	191.4 (115.2–428)	18.1 (0–264.9)	0.1[c]
IL-8				
Tax 173–185	1811 (856–2198)	2150 (2019–2229)	1859 (867–2310)	0.2[c]
Tax 185–197	3694 (980–5084)	3039 (2740–3371)	1819 (1004–3416)	0.3[c]
HLA frequency[e]				
*A*02*	7 (70)	12 (92)	9 (56.3)	0.081[f]
*A*6801*	0 (0)	0 (0)	6 (37.5)	0.044[f]
*B*0702*	0 (0)	0 (0)	0 (0)	—
*A*3001*	0 (0)	0 (0)	0 (0)	—
Provirus load (PVL)[g]		310 (75–991)	3948 (2313–7093)	**<0.001**[c]

[a] *p* value for the comparison between HAM/TSP patients and asymptomatic HTLV-1 carriers (AC). Significant differences are indicated in bold.
[b] IFN-γ responses to Tax peptides are expressed as IFN-γ SFCs/250,000 PBMCs.
[c] Mann–Whitney test.
[d] Other cytokine responses are expressed in pg/ml.
[e] HLA allele is presented as absolute numbers and percentages (in parentheses).
[f] Chi-square test (continuity correction).
[g] Provirus load is expressed as the HTLV-1 copy number/10^4 PBMCs.
Data are presented as median (Q1–Q3).
HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; Q1–Q3: first quartile–third quartile; IFN-γ, interferon-γ; SFC, spot-forming cells; PBMCs, peripheral blood mononuclear cells.

score, the more likely that peptide is to be an epitope for the allele indicated. We have previously validated this software for diverse proteomes[24] including 200 HTLV-1 peptides.[17] We have also successfully applied this technique to investigate the CTL response in HTLV-1[17] and HIV-1 infection.[27]

The chi-square test was used to analyze categorical variables and the Mann–Whitney *U* test for continuous variables without a normal distribution. The correlations between the PVL and the response elicited by the CTL epitopes within Tax region 161–233 and Tax 11-19 were evaluated using the Spearman's rank correlation test.

Results

Demographic data are summarized in Table 2. HAM/TSP patients and AC were significantly older than SC ($p < 0.01$). The proportion of subjects of Andean origin and the proportion of women were higher among patients with HAM/TSP

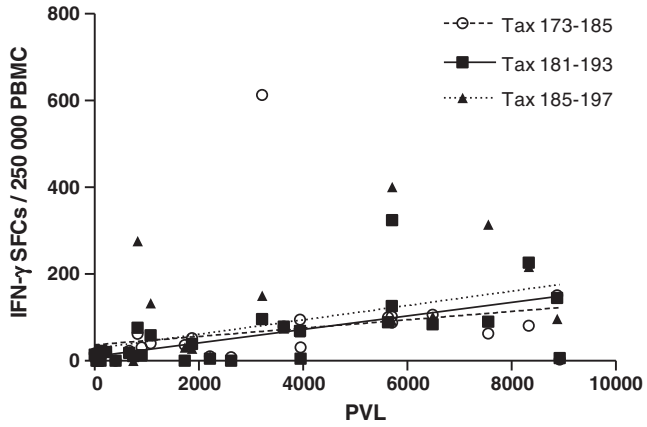

FIG. 1. Correlation between the ELISPOT IFN-γ production stimulated by Tax 173–185, Tax 181–193, and Tax 185–195 peptides, and HTLV-1 proviral load (PVL). ELISPOT, enzyme-linked immunospot; IFN-γ, interferon-γ; SFC, spot-forming cells; PBMC, peripheral blood mononuclear cells; PVL, HTLV-1 proviral load.

than among SC ($p < 0.01$). There were no significant differences in age, sex, and ethnic background between HAM/TSP patients and AC.

As shown in Table 3, PBMCs from most HTLV-1-infected subjects produced IFN-γ in response to the 16 individual overlapping peptides from Tax region 161–233 and Tax 11–19 peptide, whereas SC failed to do so ($p < 0.01$). HAM/TSP patients tended to show stronger responses than AC to the majority of individual peptides from Tax region 161–233, but only three peptides spanning amino acids 173–185 ($p < 0.01$), 181–193 ($p < 0.05$), and 185–197 ($p < 0.05$) clearly discriminated HAM/TSP patients from AC. There was a significant correlation between IFN-γ production stimulated by these peptides and PVL [Spearman's rho (ρ) 0.65, 0.62, 0.57; respectively, $p < 0.01$, $n = 29$, Fig. 1]. Therefore, two CTL epitopes were identified with this approach: one referred to as Tax 173–185 and the other located within the overlapping region of peptides Tax 181–193 and Tax 185–197. Although we confirmed a stronger IFN-γ response to Tax 11–19 in AC as compared to HAM/TSP patients, as previously reported,[18] the difference was not significant. Neither was there a significant correlation between IFN-γ production in response to Tax 11–19 and PVL [Spearman's rho (ρ) -0.19; $p = 0.3$, $n = 29$]. The fact that the IFN-γ response to the 16 peptides from Tax region 161–233 was always slightly higher in HAM/TSP than in AC, as opposed to the response to Tax 11–19, confirms the specificity of our observations.

Other cytokine responses to Tax 173–185 and Tax 185–197 peptides were also evaluated in the supernatants from 6-h incubation ELISPOT assays. Out of the 10 cytokines evaluated, only 4 (IL-1β, IL-6, TNF-α, and IL-8) were measurable. Whereas IL-8 production was similar in SC and both groups of HTLV-1-infected individuals, IL1-β, IL-6, and TNF-α levels were the highest in AC and the lowest in HAM/TSP patients (Table 3).

The CTL epitopes within Tax region 161–223 were divided into strong and weak binders according to their predicted binding affinity to HLA class I alleles. An *in silico* approach[17,24,27] suggested that the alleles *A*6801*, *A*3101*, *B*0702*, and *A*3001* would have a high binding affinity to the two epitopes that discriminate HAM/TSP patients from AC (Table 4). We then determined the frequency of these alleles in our population using sequence-specific PCR primers as described elsewhere.[28] Only *A*6801*, which is predicted to bind Tax 181–193, was overpresented in HAM/TSP patients as compared to AC ($p < 0.05$, Table 3). Moreover, a significant increase of the median IFN-γ response to Tax 181–193, expressed as IFN-γ SFCs/250,000 PBMCs, was observed in *HLA-A*6801*-positive subjects [median = 121; first quartile (Q1)–third quartile (Q3) = 67–251, $n = 6$] compared to *HLA-A*6801*-negative subjects (median = 14; Q1–Q3 = 2–69, $n = 23$, $p < 0.05$). In addition, the median PVL, expressed as HTLV-1 copies per 10^4 PBMCs, was significantly higher for *HLA-A*6801*-positive subjects (median = 5670; Q1–Q3 = 3063–8469) as compared to *HLA-A*6801*-negative subjects (median = 989; Q1–Q3 = 192–3945, $p < 0.05$).

Discussion

The present study was meant to fine-map CTL epitopes within Tax region 161–233, which are associated with increased IFN-γ production in HAM/TSP patients, in a follow-up of our previous work.[18] In this regard several CTL epitopes within the Tax protein have been evaluated by various research groups for their association with HTLV-1-related disease.[29–35] For instance, Tax 88–96 and Tax 272–280, restricted by *HLA-A*11*,[29] and Tax 301–309, restricted by *HLA-A*24*,[32,35] were shown to be recognized by CTL from patients with adult T-cell leukemia (ATL). Furthermore, it has been reported that

Table 4. Tax Sequences and Peptide Binding Scores to HLA Class I Alleles

		Tax peptides		
HLA allele	*Metaserver epitope*	*Sequence*[a]	*Peptide*	*Metaserver score*
*A*0211*[b]	QLGAFLTNV	G**QLGAFLTNV**PYK	Tax 177–189	1.92
*A*6801*	FLTNVPYKR	A**FLTNVPYKR**IEE	Tax 181–193	1.72
*A*3101*	FLTNVPYKR	A**FLTNVPYKR**IEE	Tax 181–193	1.61
*A*0201*[b]	QLGAFLTNV	G**QLGAFLTNV**PYK	Tax 177–189	1.53
*B*0702*	VPYKRIEEL	N**VPYKRIEEL**LYK	Tax 185–197	1.58
*A*3001*	AFLTNVPYK	**AFLTNVPYK**RIEE	Tax 181–193	1.45
*B*0702*[a]	HPGQLGAFL	FC**HPGQLGAFL**TN	Tax 173–185	1.33

[a]CTL epitopes within Tax peptides are indicated in bold.
[b]Metaserver scores for Tax 175–183 (HPGQLGAFL) binding to *HLA-B*0702*, as well as Tax 178–186 (QLGAFLTNV) binding to *HLA-A*0211* or *HLA-A* HLA-B*0702*, are included for comparison purposes.

Tax 11–19, Tax 31–45, and Tax 101–115, epitopes located in the Tax N-terminus and restricted by the *HLA-A*02* allele, are subject to positive selection pressures by CTL in HAM/TSP patients.[34] In addition, other alleles such as *HLA-B*07, HLA-Cw*08*, and *HLA-B*54* have been reported to be associated with HAM/TSP.[14,36] Finally, CTL responses to proteins other than Tax have also been evaluated. It was recently shown in a Japanese cohort[17] that individuals who remain asymptomatic and, independently, have a low PVL, are significantly more likely to have HLA class I alleles that bind the HBZ viral protein.

By using a specific ELISPOT assay, two CTL epitopes that had not been reported before were shown to elicit an increased IFN-γ response in PBMCs from HAM/TSP patients, which is associated with higher PVL and discriminates them from AC. Other cytokine responses were also evaluated in these subjects. Although not significant, a decrease of IL-1β, IL-6, and TNF-α production in response to Tax 175–185 and Tax 181–193 was observed in PBMCs from HAM/TSP patients as compared to AC or SC. Because IL-1β, IL-6, and TNF-α are considered typically as monokines, we hypothesize that a reduction of those cytokines in HAM/TSP might be an indirect response, as a consequence of the T cell activation. The contribution of this phenomenon to HAM/TSP pathogenesis needs to be investigated.

In addition, an *in silico* screening revealed that the *A*6801*, *A*3101*, *B*0702*, and *A*3001* alleles have a high predicted binding affinity to epitopes within Tax region 161–233 and the *A*6801* frequency seemed to discriminate between HAM/TSP and AC. Few data on the frequency of HLA alleles in Peru are available.[37–39] A study carried out in southern Peru reported allelic frequencies of 6.76% for *A*68*, 3.98% for *A*31*, 2.37% for *B*07*, and 1.69% for *A*30*.[37] As for our group of HTLV-1-infected subjects, the *A*6801* frequency was 20% in the whole group and 26.3% among individuals of Andean origin. Clearly, our finding of an increase in the frequency of the *A*6801* allele (restricting a novel Tax epitope) in HAM/TSP suggests a new CTL-mediated genetic link with HTLV-1 pathogenesis in the Peruvian population, which needs to be confirmed in larger studies.

Taken together, our results reveal two new CTL epitopes associated with HAM/TSP and increased PVL levels in a Peruvian population. The contribution of particular HLA class I alleles to the risk of HAM/TSP requires further investigation.

Acknowledgments

We thank the staff at the Instituto de Medicina Tropical Alexander von Humboldt for their valuable assistance in their attention to the HTLV-1-infected people. This work was supported by the Directorate-General for Development Cooperation of the Belgian Government through the Framework Agreement with the Institute of Tropical Medicine of Antwerp and through the Flemish Interuniversity Council (VLIR), and by the Peruvian National Board for Science and Technology (CONCYTEC).

Author Disclosure Statement

No competing financial interests exist.

References

1. Osame M, Usuku K, Izumo S, *et al.*: HTLV-I associated myelopathy, a new clinical entity. Lancet 1986;1:1031–1032.
2. Gessain A, Barin F, Vernant JC, *et al.*: Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985;2:407–410.
3. Gotuzzo E, Cabrera J, Deza L *et al.*: Clinical characteristics of patients in Peru with human T cell lymphotropic virus type 1-associated tropical spastic paraparesis. Clin Infect Dis 2004;39:939–944.
4. Verdonck K, Gonzalez E, Van Dooren S, Vandamme AM, Vanham G, and Gotuzzo E: Human T-lymphotropic virus 1: recent knowledge about an ancient infection. Lancet Infect Dis 2007;7:266–281.
5. Kannagi M, Shida H, Igarashi H, *et al.*: Target epitope in the Tax protein of human T-cell leukemia virus type I recognized by class I major histocompatibility complex-restricted cytotoxic T cells. J Virol 1992;66:2928–2933.
6. Kubota R, Kawanishi T, Matsubara H, Manns A, and Jacobson S: Demonstration of human T lymphotropic virus type I (HTLV-I) tax-specific $CD8^+$ lymphocytes directly in peripheral blood of HTLV-I-associated myelopathy/tropical spastic paraparesis patients by intracellular cytokine detection. J Immunol 1998;161:482–488.
7. Bieganowska K, Höllsberg P, Buckle GJ, *et al.*: Direct analysis of viral-specific $CD8^+$ T cells with soluble HLA-A2/Tax11-19 tetramer complexes in patients with human T cell lymphotropic virus-associated myelopathy. J Immunol 1999; 162:1765–1771.
8. Kubota R, Nagai M, Kawanishi T, Osame M, and Jacobson S: Increased HTLV type 1 tax specific $CD8^+$ cells in HTLV type 1-asociated myelopathy/tropical spastic paraparesis: Correlation with HTLV type 1 proviral load. AIDS Res Hum Retroviruses 2000;16:1705–1709.
9. Nagai M, Kubota R, Greten TF, Schneck JP, Leist TP, and Jacobson S: Increased activated human T cell lymphotropic virus type I (HTLV-I) Tax11-19-specific memory and effector $CD8^+$ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: Correlation with HTLV-I provirus load. J Infect Dis 2001;18:197–205.
10. Bangham CR: The immune control and cell-to-cell spread of human-T cell lymphotropic virus type I. J Gen Virol 2003;84:3177–3189.
11. Goon PK, Biancardi A, Fast N, *et al.*: Human T cell lymphotropic virus (HTLV) type-1-specific $CD8^+$ T cells: Frequency and immunodominance hierarchy. J Infect Dis 2004;189:2294–2298.
12. Asquith B, Mosley AJ, Barfield A, *et al.*: A functional $CD8^+$ cell assay reveals individual variation in $CD8^+$ cell antiviral efficacy and explains differences in human T-lymphotropic virus type 1 proviral load. J Gen Virol 2005;86:1515–1523.
13. Kubota R, Furukawa Y, Izumo S, Usuku K, and Osame M: Degenerate specificity of HTLV-1-specific $CD8^+$ T cells during viral replication in patients with HTLV-1-associated myelopathy (HAM/TSP). Blood 2003;101:3074–3081.
14. Jeffery KJ, Usuku K, Hall SE, *et al.*: HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. Proc Natl Acad Sci USA 1999;96:3848–3853.
15. Jeffery KJ, Siddiqui AA, Bunce M, Lloyd AL, and Vine AM: The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. J Immunol 2000,165:7278–7284.
16. Talledo M, López G, Huyghe JR, *et al.*: Evaluation of host genetic and viral factors as surrogate markers for HTLV-1-associated myelopathy/tropical spastic paraparesis in Peruvian HTLV-1-infected patients. J Med Virol 2010;82:460–466.

17. Macnamara A, Rowan A, Hilburn S, *et al*: HLA class I binding of HBZ determines outcome in HTLV-1 infection. PLoS Pathog 2010;6:e1001117.
18. Best I, López G, Verdonck K, *et al*.: IFN-gamma production in response to Tax 161-233, and frequency of $CD4^+Foxp3^+$ and $Lin^-HLA\text{-}DR^{high}CD123^+$ cells, discriminate HAM/TSP patients from asymptomatic HTLV-1-carriers in a Peruvian population. Immunology 2009;128:e777–e786.
19. Verdonck K, González E, Maldonado F, *et al*.: Comparison of three ELISAs for the routine diagnosis of human T-lymphotropic virus infection in a high-prevalence setting in Peru. Trans R Soc Trop Med Hyg 2009;103:420–422.
20. Osame M: Review of WHO Kagoshima meeting and diagnostic guidelines for HAM/TSP. In: *Human Retrovirology: HTLV* (Blattner WA, ed.). Raven Press, New York, 1990, pp. 191–197.
21. De Castro-Costa CM, Araujo AQ, Barreto MM, *et al*.: Proposal for diagnostic criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). AIDS Res Hum Retroviruses 2006; 22:931–935.
22. Best I, Adaui V, Verdonck K, *et al*.: Proviral load and immune markers associated with human T lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in Peru. Clin Exp Immunol 2006; 146:226–233.
23. Adaui V, Verdonck K, Best I, *et al*.: SYBR Green-based quantitation of human T-lymphotropic virus type 1 proviral load in Peruvian patients with neurological disease and asymptomatic carriers: Influence of clinical status, sex, and familial relatedness. J Neurovirol 2006;12:456–465.
24. MacNamara A, Kadolsky U, Bangham CR, and Asquith B: T-cell epitope prediction: Rescaling can mask biological variation between MHC molecules. PLoS Comput Biol 2009;5:e1000327.
25. Larsen MV, Lundegaard C, Lamberth K, *et al*.: An integrative approach to CTL epitope prediction: A combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. Eur J Immunol 2005;35:2295–2303.
26. Buus S, Lauemøller SL, Worning P, *et al*.: Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach. Tissue Antigens 2003;62:378–384.
27. Kadolsky UD and Asquith B: Quantifying the impact of human immunodeficiency virus-1 escape from cytotoxic T-lymphocytes. PLoS Comput Biol 2010;6:e1000981.
28. Bunce M, O'Neill CM, Barnardo MC, *et al*.: Phototyping: Comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 1995;46:355–367.
29. Parker CE, Nightingale S, Taylor GP, Weber J, and Bangham CR: Circulating anti-Tax cytotoxic T lymphocytes from human T-cell leukemia virus type I-infected people, with and without tropical spastic paraparesis, recognize multiple epitopes simultaneously. J Virol 1994;68:2860–2868.
30. Yashiki S, Fujiyoshi T, Arima N, *et al*.: HLA-A*26, HLA-B*4002, HLA-B*4006, and HLA-B*4801 alleles predispose to adult T cell leukemia: The limited recognition of HTLV type 1 tax peptide anchor motifs and epitopes to generate anti-HTLV type 1 tax CD8(+) cytotoxic T lymphocytes. AIDS Res Hum Retroviruses 2001;17:1047–1061.
31. Harashima N, Tanosaki R, Shimizu Y, *et al*.: Identification of two new HLA-A*1101-restricted tax epitopes recognized by cytotoxic T lymphocytes in an adult T-cell leukemia patient after hematopoietic stem cell transplantation. J Virol 2005; 79:10088–10092.
32. Kozako T, Arima N, Toji S, *et al*.: Reduced frequency, diversity, and function of human T cell leukemia virus type 1-specific $CD8^+$ T cell in adult T cell leukemia patients. J Immunol 2006;177:5718–5726.
33. Kurihara K, Shimizu Y, Takamori A, *et al*.: Human T-cell leukemia virus type-I (HTLV-I)-specific T cell responses detected using three-divided glutathione-S-transferase (GST)-Tax fusion proteins. J Immunol Methods 2006;313: 61–73.
34. Kubota R, Hanada K, Furukawa Y, *et al*.: Genetic stability of human T lymphotropic virus type I despite antiviral pressures by CTLs. J Immunol 2007;178:5966–5972.
35. Tanaka Y, Nakasone H, Yamazaki R, *et al*.: Single-cell analysis of T-cell receptor repertoire of HTLV-1 Tax-specific cytotoxic T cells in allogeneic transplant recipients with adult T-cell leukemia/lymphoma. Cancer Res 2010;70:6181–6192.
36. Catalan-Soares BC, Carneiro-Proietti AB, Da Fonseca FG, *et al*.: HLA class I alleles in HTLV-1-associated myelopathy and asymptomatic carriers from the Brazilian cohort GIPH. Med Microbiol Immunol 2009;198:1–3.
37. de Pablo R, Beraún Y, Nieto A, *et al*.: HLA class I and class II allele distribution in the Peruvian population. Tissue Antigens 2000;56:507–514.
38. Moscoso J, Seclen S, Serrano-Vela JI, *et al*.: HLA genes in Lamas Peruvian-Amazonian Amerindians. Mol Immunol 2006; 43:1881–1889.
39. Arnaiz-Villena A, Gonzalez-Alcos V, Serrano-Vela JI, *et al*.: HLA genes in Uros from Titikaka Lake, Peru: Origin and relationship with other Amerindians and worldwide populations. Int J Immunogenet 2009;36:159–167.

Address correspondence to:
Ivan Best
Instituto de Medicina Tropical Alexander von Humboldt
Universidad Peruana Cayetano Heredia
Av. Honorio Delgado 430
Lima 31
Peru

E-mail: ivan.best@upch.pe