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Chimeric Synthetic Peptides from the Envelope (gp46) and the Transmembrane (gp21) Glycoproteins for the Detection of Antibodies to Human T-Cell Leukemia Virus Type II

Milenen Hernández Marin,*[,1] Patricia Castellanos Pentón,* Yadaris Márquez Bocalandro,† Lilliam Pozo Peña,† Janet Díaz Navarro, and Luis Javier González López‡

**Department of Peptide Synthesis and †Department of Retroviruses, Immunoassay Center, Havana, Cuba; and ‡Physical Chemistry Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba*

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Two chimeric synthetic peptides incorporating immunodominant sequences from HTLV-II virus were synthesized. Monomeric peptides P2 and P3 represent sequences from transmembrane protein (gp21) and envelope protein (gp46) of the virus. The peptide P2 is a gp21 (370–396) sequence and the peptide P3 is a gp46 (178–205) sequence. Those peptides were arranged in a way that permits one to obtain different combinations of chimeric peptides (P2-GG-P3 and P3-GG-P2), separated by two glycine residues as spacer arms. The antigenic activity of these peptides was evaluated by Ultramicro-Enzyme-linked immunosorbent assay (UMELISA) by using panels anti-HTLV-II-positive sera (n = 11), anti-HTLV-I/II-positive sera (n = 2), HTLV-positive (untypeable) serum samples (n = 2), and anti-HTLV-I-positive sera (n = 22), while specificity was evaluated with anti-HIV-positive samples (n = 19) and samples from healthy blood donors (n = 30). The efficacy of the chimeric peptides in solid-phase immunoassays was compared with the monomeric peptides and a mixture of the monomeric peptides. Higher sensitivity was observed for chimeric peptide Q5 assay. Those results may be related to a higher peptide adsorption capacity to the solid surface and for epitope accessibility to the antibodies. This chimeric peptide would be very useful for HTLV-II diagnostic.

Key Words: **chimeric synthetic peptides; UMELISA; gp21; gp46; HTLV-II.**

Human T-lymphotropic virus type II (HTLV-II) was first isolated from two patients with hairy-cell leukemia (1) and is closely related to HTLV-I (2, 3). HTLV-II transmission occurs through the transfer of infected lymphocytes via breast-feeding, blood transfusion, sexual contact, and injecting drug (ID) use (4, 5). HTLV-II infection is endemic in some native American (6–8) and African peoples and among injecting drug users in the United States (9) and Europe.

In the routine screening of blood and by-products for transfusion, HTLV-I infection is recognized by detection of specific antibodies. Enzyme-linked immunosorbent assay (ELISA) is the recommended screening procedure (10), followed by Western blot confirmation of suspected positive specimens (11). Earlier ELISA tests used viral lysates antigens on the solid phase. Later, several recombinant antigens of the most important proteins have been identified that have serologic utility (12). The assays using recombinant proteins were more sensitive and specific because these antigens were not contaminated with human cellular proteins. However, these assays presented unspecific reactions because antibodies in the samples were able to recognize eventually host contaminants. For this reason, synthetic peptides, derived from conserved domains that show high specificity and sensitivity in detecting antibodies in sera from infected patients, have been used later on (13, 14). Although two or more antigens can be used in the assays, the sensitivity and specificity of the tests could be affected by effect of competition for binding on the solid phase and for changes in spatial distribution of antigenic determinants of bound peptides. There is a tendency toward using chimeric synthetic peptides to avoid those problems and improve the sensitivity and specificity of the assays (15–18).

For the purpose of detection of antibodies to HTLV-II, we designed, synthesized, and characterized two chimeric peptides by inserting the antigenic epitopes of

[1] To whom correspondence and reprint requests should be addressed at Immunoassay Center, Calle 134 y Avenida 25, Cubanacan Playa, Apto 6653, Ciudad de la Habana, Cuba. Fax: 0053-7-28-6514. E-mail: iqpeptidos@cie.sld.cu.

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TABLE 1

HTLV-II Synthetic Peptides Used in the Study

Code	Sequence
P2	YAAQNRRGLDLLFWEQGGLCKAIQEQC
P3	QPPPTSPPLVHDSDLEHVLTPSTSWTTK
Q-5	P2-GG-P3
Q-6	P3-GG-P2

the gp21 transmembrane (370–396) and the gp46 envelope (178–205) glycoproteins. Comparative results of those chimeric antigens versus the monomeric peptides and a mixture of the monomeric peptides are presented in this report.

MATERIALS AND METHODS

Peptide synthesis. Chimeric and monomeric peptides (Table 1) were synthesized manually by a standard solid-phase method (19) using *tert*-butoxycarbonyl (Boc) chemistry. Two of them represented immunodominant sequences from gp21 (P2) and gp46 (P3) proteins of HTLV-II virus. The other two were a chimeric peptides involving sequences from P2 and P3 peptides. Two glycine residues were added separating the two immunodominant sequences.

The peptides were assembled using 4-methylbenzhydrylamine resin (MBHA) (0.8 mmol/g; 100–200 mesh) purchased from Bachem

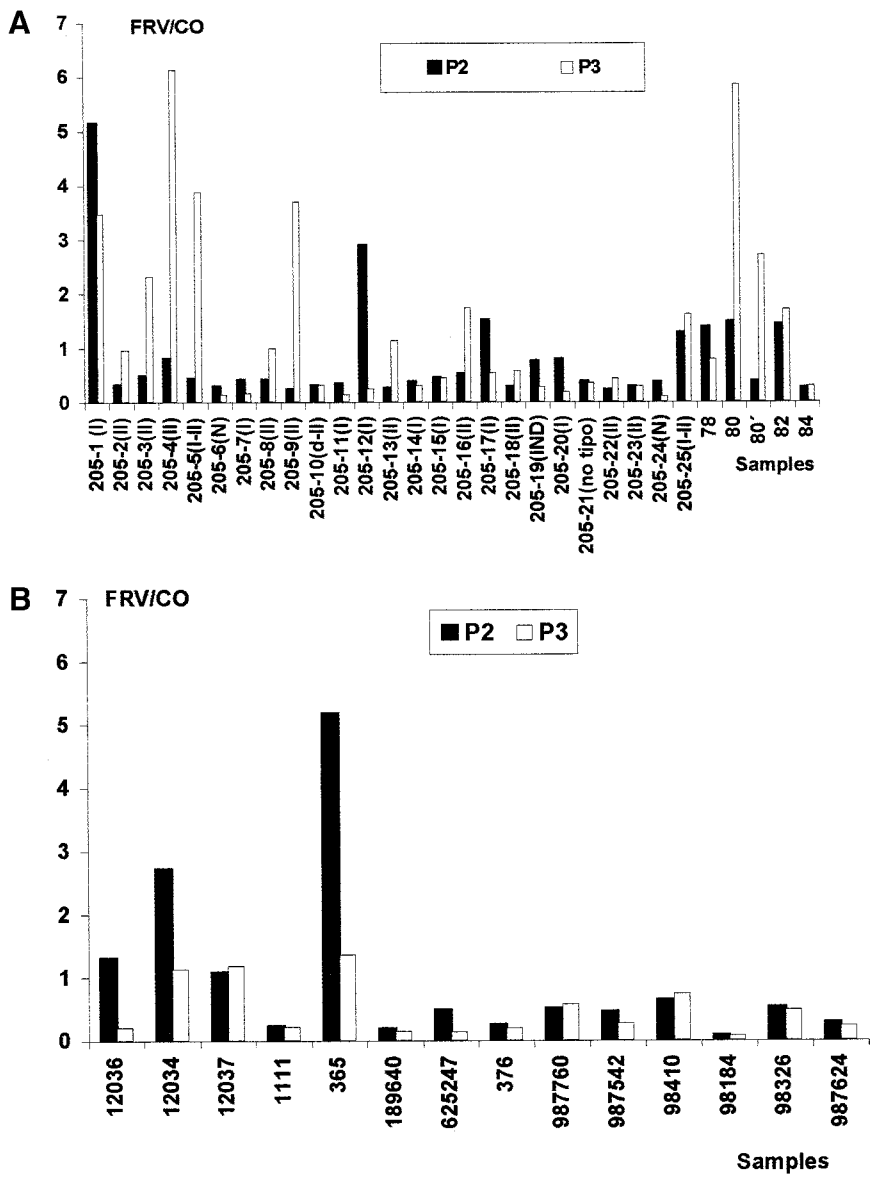

FIG. 1. UMELISA test of the synthetic peptides of the HTLV-II. (A) Reactivity of the monomeric peptides (P2 and P3) with sera of HTLV-infected individuals from anti-HTLV-I/II panel PRP-205 (Boston Biomedical Inc.) ($n = 23$) and sera from Cuban people seropositive to HTLV-I ($n = 5$). (B) Reactivity of the monomeric peptides (P2 and P3) with sera of HTLV-I-infected individuals from Colombia and Chile ($n = 9$). The samples were considered reactive when FRV/CO ≥ 1.

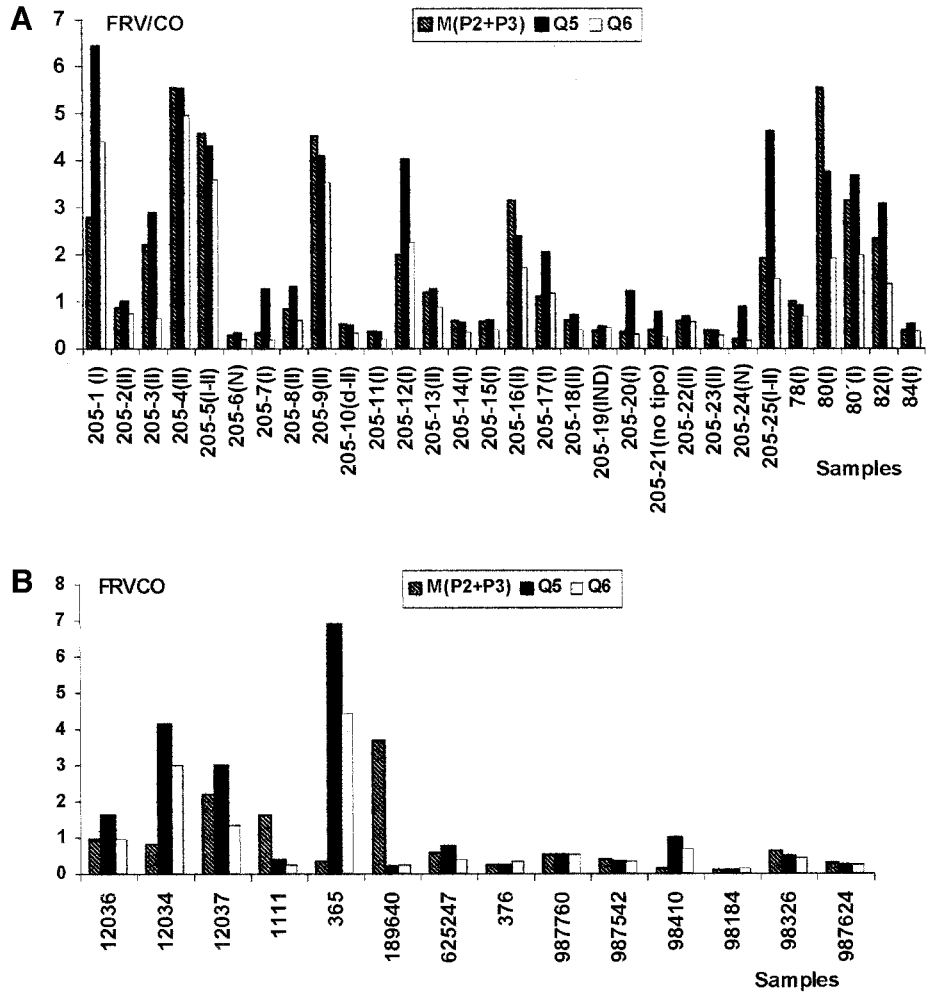

FIG. 2. UMELISA test of the synthetic peptides of the HTLV-II. (A) Reactivity of the monomeric peptides together (P2 + P3) and chimeric peptides (Q5 and Q6) with sera of HTLV-infected individuals (n = 23). (B) Reactivity of the monomeric peptides together (P2 + P3) and chimeric peptides (Q5 and Q6) with sera of HTLV-I-infected individuals (n = 9) from Colombia and Chile. The samples were considered reactive when FRV/CO ≥ 1.

(Switzerland). The appropriately protected amino acids were obtained from Bachem (Switzerland). Dichloromethane (DCM), 2-propanol (iPrOH), *N*,*N*′-dimethylformamide (DMF), trifluoroacetic acid (TFA), diisopropylethylamine (DIEA), and *N*-*N*′-ethyldiisopropylcarbodiimide (DIPCDI)) were pure for synthesis (Merck, Germany). The couplings reaction with DIPCDI 0.2 mol/L in DCM or 1-hydroxybenzotriazole (HOBT) esters (Sigma) in DMF, were monitored by a qualitative ninhydrin test (20) and usually completed within 1 h. Boc protection was removed with 37.5% TFA in DCM, followed by neutralization with 5% DIPEA in DCM. The peptides were cleaved from the resin using "Low-High" method (21, 22) with hydrogen fluoride (HF) pure for analysis (Fluka, Switzerland) in the presence of scavengers (anisole, dimethyl sulfide, 1,2-ethanedithiol and *p*-cresol) and then extracted with 30% acetic acid and the solutions were lyophilized.

The obtained peptides were analyzed by analytical RP-HPLC (23, 24) using a RPC18 protein/peptide column (Pharmacia, LKB, Sweden) (Vydac) (4.6 × 150 mm) with a linear gradient from solution A (0.1% TFA in water) to solution B (0.05% TFA in acetonitrile) in 35 min. All the synthesized peptides showed a major peak along. The peptides were detected by UV at λ = 226 nm. Data were processed by the Biocrom program (CIGB, Cuba).

Mass spectrometry. Peptide molecular weights were verified by electrospray ionization–mass spectrometry (ESI-MS) (25–27) using a mass spectrometer with orthogonal geometry QTOF-2 (Micromass, UK). The spectrum were process with MassLinx v3.5 (Micromass, UK).

Enzyme-linked immunosorbent assay. UltramicroELISA (UMELISA) assay was performed using 96-wells white polystyrene plates (Greiner Labortechnik, Germany). Peptides were dissolved in 0.05 mol/L carbonate–bicarbonate buffer, pH 9.6. The wells were coated using 15 μL of a solution of either each monomeric peptide alone (2 μg/mL), a mixture of the monomeric peptides (each one at 2 μg/mL) or chimeric peptide (4 μg/mL). The plates were incubated for 4 h at 37°C and then washed once with 25 μL/well of 0.14 mol/L phosphate-buffered saline (PBS) with 0.05% Tween 20 (Merck). Plates were blocked with (15 μL/well) 0.1% bovine serum albumin, 0.5% Tween 20 in PBS, and incubated overnight at room temperature. Serum were diluted (1/20) in 0.015 mol/L Tris–HCl, pH 7.8, containing 0.05% Tween 20 and 5% sheep serum. The diluted samples were then added to the wells (10 μL per well), and incubated 30 min at 37°C. After washing four times with 0.015 mol/L Tris–HCl buffer, pH 7.8, 10 μL of sheep anti-human IgG conjugated with alkaline phospha-

tase (Boehringer Mannheim GmbH, Germany) were added to each well. The plates were then incubated for 30 min at 37°C followed by four washes with Tris–HCl buffer. The enzymatic activity was developed by adding to each well 10 μL of 0.3 mg/mL of the fluorogenic substrate 4-methylumbelliferyl phosphate (Koch Light Ltd, Haverhill, Suffolk, England) in 1 mol/L diethanolamine buffer pH 9.8 and incubating at room temperature for 30 min. The fluorescence was measured on a PR-521 plate reader (Immunoassay Center, Havana, Cuba) (Excitation at 365 nm and emission at 450 nm). All assays included positive and negative controls. The fluorescence reading from each sample was normalized as a relative value (FRV) over the fluorescence value of a positive control on the same plate. Samples were considered positive when FRV were equal or higher than the cutoff value (0.28) All numeric results are means of duplicates.

The analyzed samples were anti-HTLV-I/II panel PRP-205 (n = 25) from Boston Biomedical Inc. (BBI, Rockville, MD). HTLV-I positive specimens (n = 5) from Cuba and HTLV-I-positive individuals from Chile and Colombia. The healthy blood donors (n = 30) were from Marianao blood bank (Havana, Cuba) and HIV-seropositive individuals from Cuba (n = 19) were supplied by the National Reference Center for Retroviruses (Havana, Cuba).

RESULTS AND DISCUSSION

The monomeric peptides P2 (370–396) and P3 (178–205) and the chimeric peptides (Q5 and Q6) were synthesized by the conventional solid-phase peptide methodology using Boc chemistry. The appropriately protected amino acids were incorporated into the peptide sequence using DIPCDI/HOBt activation. The monomeric and chimeric peptides were obtained in good yield and purity.

The sequence of monomeric peptides was deduced from (HTLV-II isolated) reported by Seiki *et al.* (1983) (28).

The monomeric peptide performances with anti-HTLV-I/II panel PRP-205 (Boston Biomedical Inc.) and sera from seropositive Cuban people (n = 5) are shown in Fig. 1A, where peptide P2 not detected HTLV-II-positive samples, detected (3/8) HTLV-I-positive sera, detected (1/2) HTLV-I/II-positive sera. Besides detected (3/5) sera from Cuban people seropositive to HTLV-I. Peptide P3 detected (6/11) HTLV-II-positive sera, one HTLV-I positive sera, and detected (2/2) HTLV-I/II positive sera. Negative samples (205-6 and 205-24) in this panel were negative for two synthetic peptides. Detected (3/5) sera from Cuban people seropositive to HTLV-I.

The monomeric peptide performances with HTLV-I-positive samples from Colombia and Chile are shown in Fig. 1B where peptide P2 detected (4/9) positive samples, with two samples showing (FRV/CO) value of >2, and two samples showing moderate to low (FRV/CO). Peptide P3 detected (3/9) samples, the three samples showing moderate to low (FRV/CO).

A mixture of monomeric peptides and chimeric peptides performance with anti-HTLV-I/II Panel PRP-205 (Boston Biomedical Inc.) and with Cuban people seropositive to HTLV-I is shown in Fig. 2A where mixture of monomeric peptides detected (5/11) HTLV-II-positive sera, with four samples showing (FRV/CO) values of >2 and one sample showing moderate to low (FRV/CO), detected (3/8) HTLV-I-positive sera, detected (2/2) HTLV-I/II-positive sera, and detected (3/5) sera from Cuban people seropositive to HTLV-I.

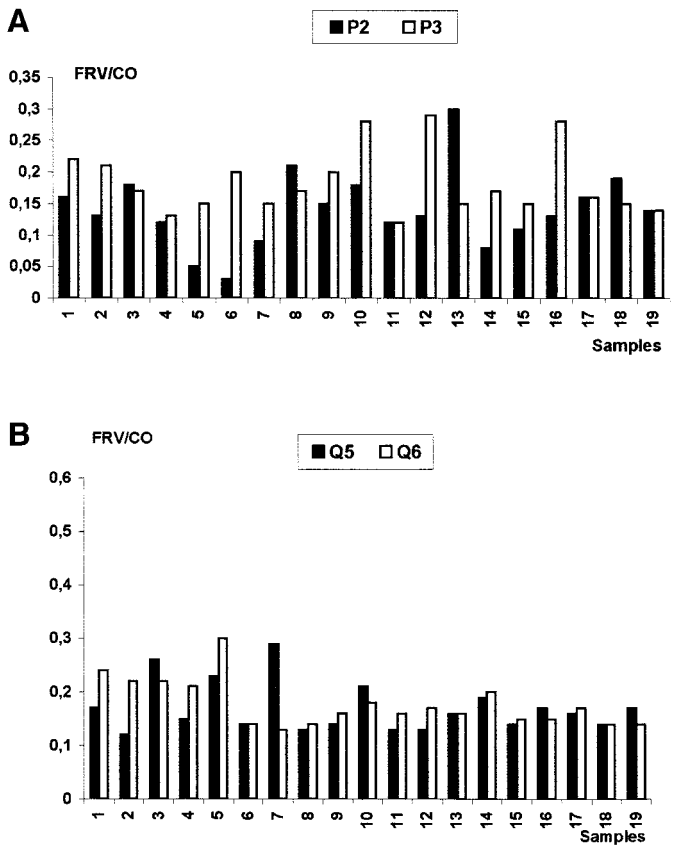

FIG. 3. UMELISA test of the synthetic peptides of the HTLV-II. (A) Reactivity of the monomeric peptides (P2 and P3) with samples from anti-HIV-1-positive samples (n = 19). (B) Reactivity of the chimeric peptides (Q5 and Q6) with anti-HIV-1-positive samples (n = 19). The samples were considered reactive when FRV/CO $\geq$ 1.

Chimeric peptide Q5 detected (7/11) HTLV-II-positive samples, with four samples showing (FRV/CO) values of >2, while three samples showing moderate to low (FRV/CO, detected (5/8) HTLV-I-positive samples, and (2/2) HTLV-I/II-positive samples. While the chimeric peptide Q6 detected (3/11) HTLV-II-positive samples, with two samples showing (FRV/CO) values of >2 and one sample showing (FRV/CO) moderate to low, detected (3/8) HTLV-I-positive samples, and (2/2) HTLV-I/II-positive samples.

Chimeric peptide Q5 and Q6 detected (3/5) sera from Cuban people seropositive to HTLV-I. A mixture of monomeric peptides and chimeric peptides performance with HTLV-I positive samples from Colombia and Chile is showed in Fig. 2B, where a mixture of monomeric peptides detected (3/9) HTLV-I-positive sera, with two samples showing (FRV/CO) values of >2 and one sample showing moderate to low (FRV/CO).

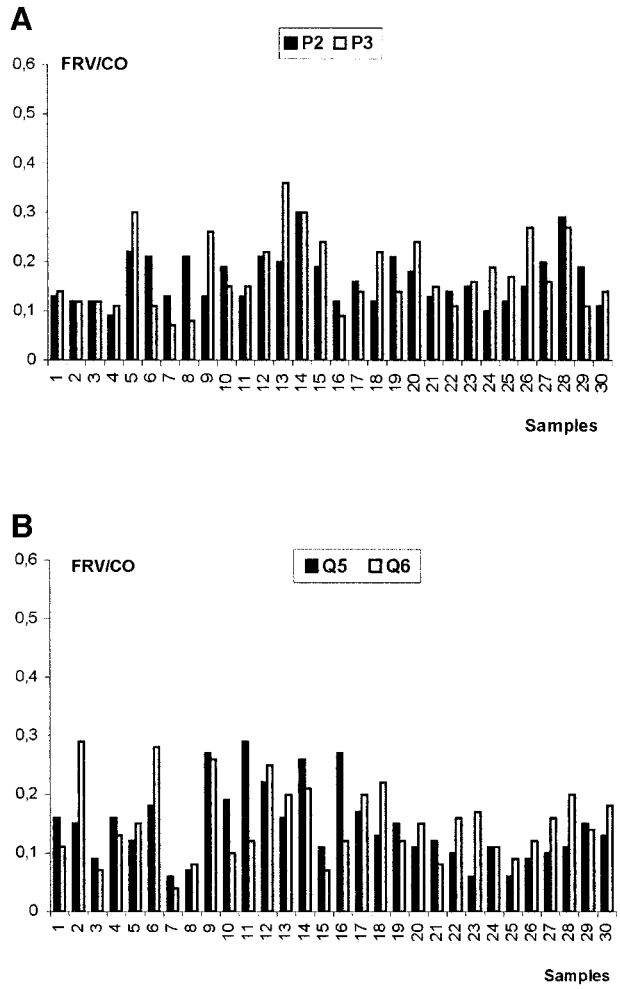

FIG. 4. UMELISA test of the synthetic peptides of the HTLV-II. (A) Reactivity of the monomeric peptides (P2 and P3) with samples from healthy blood donors ($n = 30$). (B) Reactivity of the chimeric peptides (Q5 and Q6) with samples from healthy blood donors ($n = 30$). The samples were considered reactive when FRV/CO ≥ 1.

Chimeric peptide Q5 detected (4/9) HTLV-I-positive samples, three samples showing (FRV/CO) values of >2 and one sample showing moderate to low (FRV/CO. Chimeric peptide Q6 detected (3/9), with two samples showing (FRV/CO) values of >2 and one sample showing (FRV/CO) moderate to low.

The results show that the peptides were reacted with some HTLV-I-positive sera. As expected, peptide P2, having only one amino acid substitution (I for L), emitted high fluorescence values with (10/22) HTLV-I sera. The chimeric peptide Q5 detected (12/22) because it combines peptide P2 and peptide P3 sequences.

Chimeric peptide Q5 did not detect four positive HTLV-II specimens of anti-HTLV I/II panel PRP-205. These four samples (205-10, 205-18, 205-22, and 205-23) were tested on Genelabs Diagnostics HTLV Western blot 2.4 BBI and were found they had following bands by Western blot: 205-10 (gp21, p24) (faint bands), 205-18 (gp21, p24), 205-22 (gp21, p19, p24), and 205-23 (gp21, p24). We suppose that it was possible due to: (i) the represented sequence by P2 peptide does not present the specific epitope for the detection of antibodies in the samples; (ii) the antibodies are directed to conformational epitope; and (iii) the peptide adsorption to the solid phase and epitope accessibility to the antibodies.

To assess peptides specificity, samples from anti-HIV-1-positive samples ($n = 19$), as shown in Fig. 3 and from healthy blood donors, as shown in Fig. 4, were tested where all specimens were finally considered as negative.

Our results have demonstrated that the chimeric peptides can be used to detect antibodies to more than one protein simultaneously and the efficiency of detection is dependent upon the order of the sequence.

The epitope orientation from the chimeric peptides was very determinant in the chimeric peptides antigenicity. This may be related to peptide adsorption to the solid surface and epitope accessibility to the antibodies.

The chimeric peptide Q5 incorporating sequences from gp21 and gp46 proteins of the HTLV-II virus was the most antigenic molecule in this study, since it was interactive with majority of HTLV-II sera. Therefore, this peptide should prove to be useful as antigen for a diagnostic assay for HTLV-II detection.

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