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Chimeric Synthetic Peptides Containing Two Immunodominant Epitopes from the Envelope gp46 and the Transmembrane gp21 Glycoproteins of HTLV-I Virus

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-I virus were synthesized. Monomeric peptides P7 and P8 represent sequences from transmembrane protein (gp21) and envelope protein (gp46) of the virus. The peptide P7 is a gp21 (374–400) sequence and the peptide P8 is a gp46 (190–207) sequence. Those peptides were arranged in a way that permits one to obtain different combinations of chimeric peptides (P7-GG-P8 and P8-GG-P7), separated by two glycine residues as spacer arms. The antigenic activity of these peptides were evaluated by UltramicroEnzyme-linked immunosorbent assay (UMELISA) by using panels of anti-HTLV-I-positive sera (*n* = 22), anti-HTLV-I/II-positive sera (*n* = 2), HTLV-positive (untypeable) serum samples (*n* = 2), and anti-HTLV-II-positive sera (*n* = 11), 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 monomeric peptides together. The chimeric peptide P7-GG-P8 proved to be the most reactive with anti-HTLV-I-positive sera. These 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-I diagnostics.

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

Human T-cell lymphotropic virus type I (HTLV-I) (1) is associated with adult T-cell leukemia/lymphoma (ATLL) and a degenerative neurological disease, tropical spastic paraparesis (TSP) (2–4), also called HTLV-I, associated tentatively with certain forms of polymyositis, polyarthritis, infective dermatitis (5), and uveitis (6). The virus is endemic in Japan and the Caribbean (7), and in certain risk groups in Africa (8), South America, Europe, and the United States. This virus is transmitted from mother to infant by breastfeeding (9), between male and female by sexual intercourse (10), and by blood transfusion (11).

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 (12), followed by Western blot confirmation of suspected positive specimens (13).

The synthetic peptides, derived from conserved domains of HTLV, that show high specificity and sensitivity in detecting antibodies in sera from infected patients, have been used in the immunodiagnostic (14, 15).

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 (16–19).

The HTLV-I envelope glycoprotein is synthesized as a protein precursor (gp62) (20). This protein is then cleaved by cellular protease into two proteins, a surface protein (gp46) and a transmembrane protein (gp21).

The envelope glycoproteins of HTLV-I virus are essential in the early stage of viral infections and are the most immunogenic proteins of all viral antigens.

For the purpose of detection of antibodies to HTLV-I, we have synthesized two chimeric peptides which includes sequences from the immunodominant epitopes

[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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located at the gp21 (374–400) and the gp46 (190–207) proteins. Comparative results of these 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 (21, 23) using *tert*-butoxycarbonyl (Boc) chemistry. Two of them represented immunodominant sequences from gp21(P7) and gp46 (P8) proteins of HTLV-I virus. The other two were a chimeric peptides involving sequences from P7 and P8 peptides. Two glycine residues were added separating the two immunodominant sequences.

The peptides were assembled using 4-methylbenzyhydrylamine resin (MBHA) (0.8 mmol/g; 100–200 mesh) purchased from Bachem (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 (Riedel de Haen, Germany). The coupling reactions with DIPCDI 0,2 mol/L in DCM or 1-hydroxybenzotriazole (HOBT) esters (Sigma) in DMF were monitored by a qualitative ninhydrin test (24) 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 HF" method (25, 26) 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 (27) 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 of the synthesized peptides showed a major peak along. The peptides were detected by UV at $\lambda = 226$ nm. Data were processed by the Biocrom program (CIGB, Cuba).

Mass spectrometry. Peptide molecular weights were verified by electrospray ionization–mass spectrometry (ESI-MS) (28–30) 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. Ultramicro-ELISA (UMELISA) assay was performed using 96-well 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 phosphatase (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 incubation 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

TABLE 1

Synthetic Peptides Used in This Study

Code	Sequence
P7	YAAQNRRGLDLLFWEQGGLCKALQEQC
P8	LLPHSNLDHILEPSIFWK
Q3	P7-GG-P8
Q4	P8-GG-P7

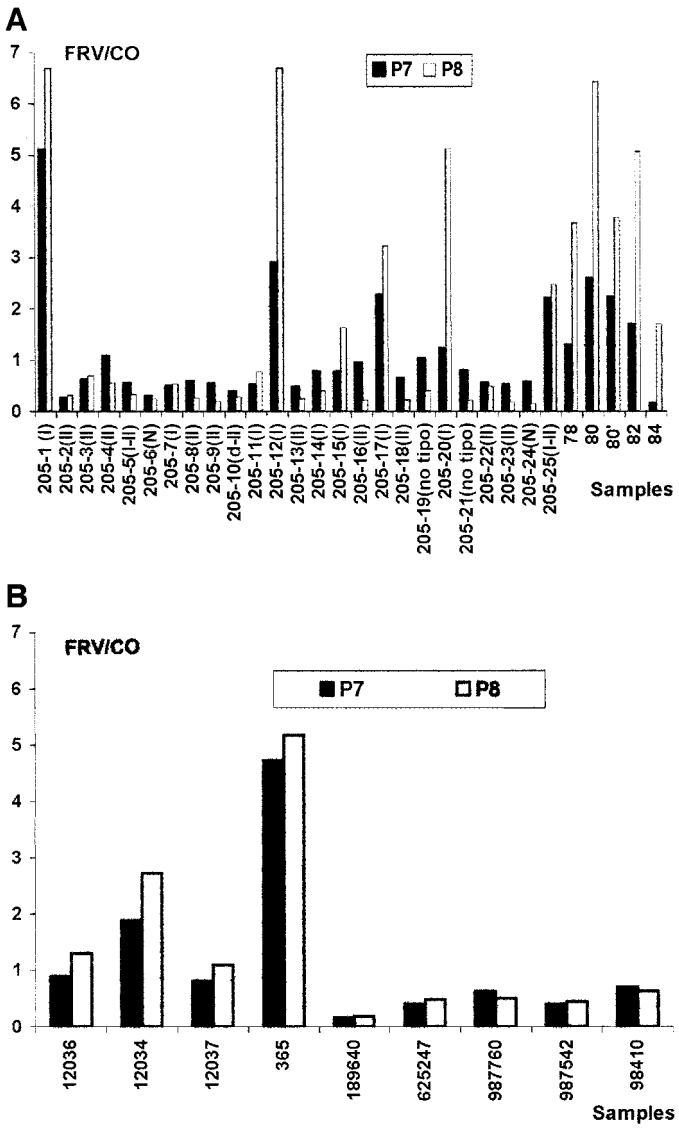

FIG. 1. UMELISA test of the synthetic peptides of the HTLV-I. (A) Reactivity of the monomeric peptides (P7 and P8) with sera of HTLV-infected individuals from anti-HTLV-I/II panel PRP-205 (Boston Biomedica Inc.) ($n = 23$) and sera from Cuban people seropositive to HTLV-I ($n = 5$). (B) Reactivity of the monomeric peptides (P7 and P8) with sera of HTLV-I-infected individuals from Colombia and Chile ($n = 9$). The samples were considered reactive when FRV/CO ≥ 1.

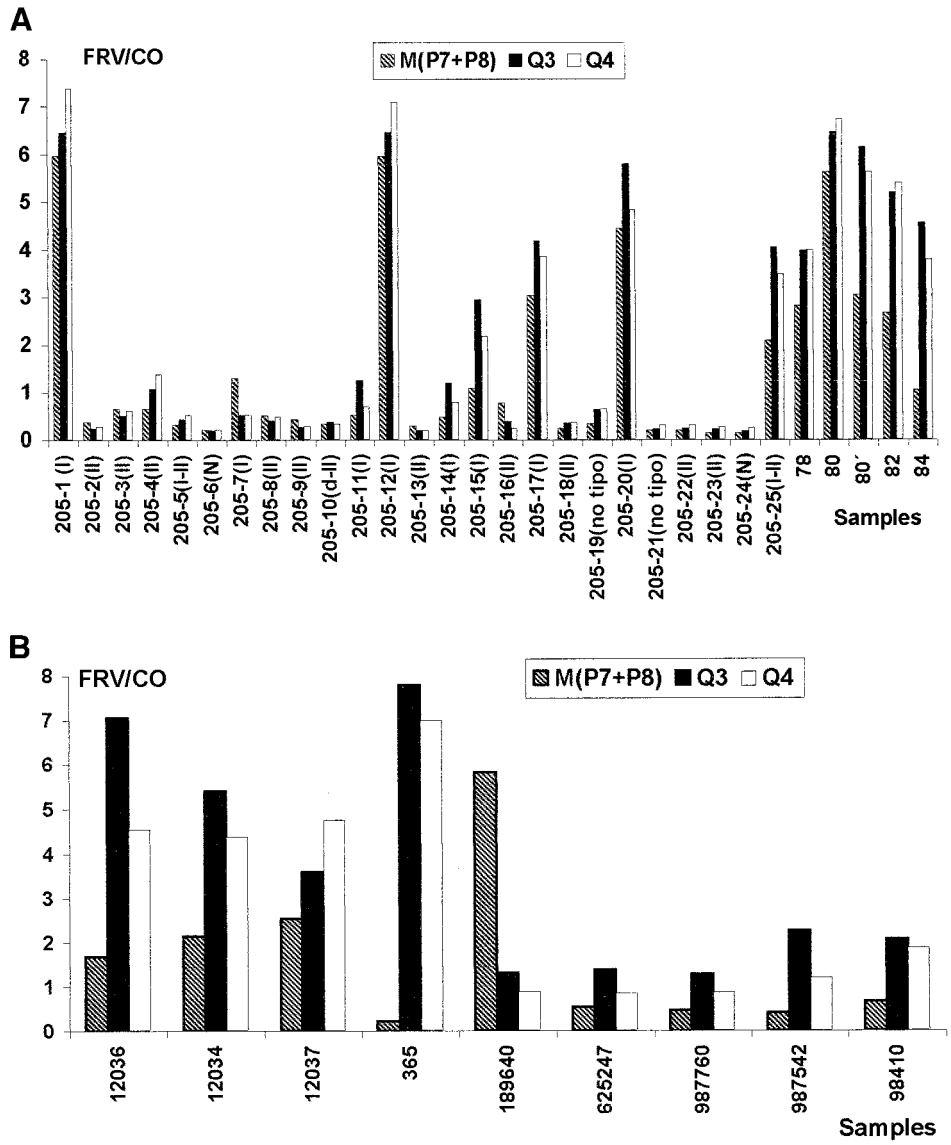

FIG. 2. UMELISA test of the synthetic peptides of the HTLV-I. (A) Reactivity of the mixture of monomeric peptides (P7 + P8) and chimeric peptides (Q3 and Q4) with sera of HTLV-infected individuals from anti-HTLV-I/II panel PRP-205 (Boston Biomedica Inc.) (n = 23) and sera from Cuban people seropositive to HTLV-I (n = 5). (B) Reactivity of the mixture of monomeric peptides (P7 + P8) and chimeric peptides (Q3 and Q4) with sera of HTLV-I-infected individuals from Colombia and Chile (n = 9). The samples were considered reactive when FRV/CO $\geq$ 1. To assess peptides specificity, samples from anti-HIV-1-positive samples (n = 19) as shown in Fig. 3 and healthy blood donors, as it is showed in Fig. 4 were tested where all specimens were finally considered as negative.

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 (CO) 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 Biomedica 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-serapositive individuals from Cuba (n = 19) were supplied by the National Reference Center for Retroviruses (Havana, Cuba).

RESULTS AND DISCUSSION

The monomeric peptides P7 (374–400) and P8 (190–207) and the chimeric peptides (Q3 and Q4) 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.

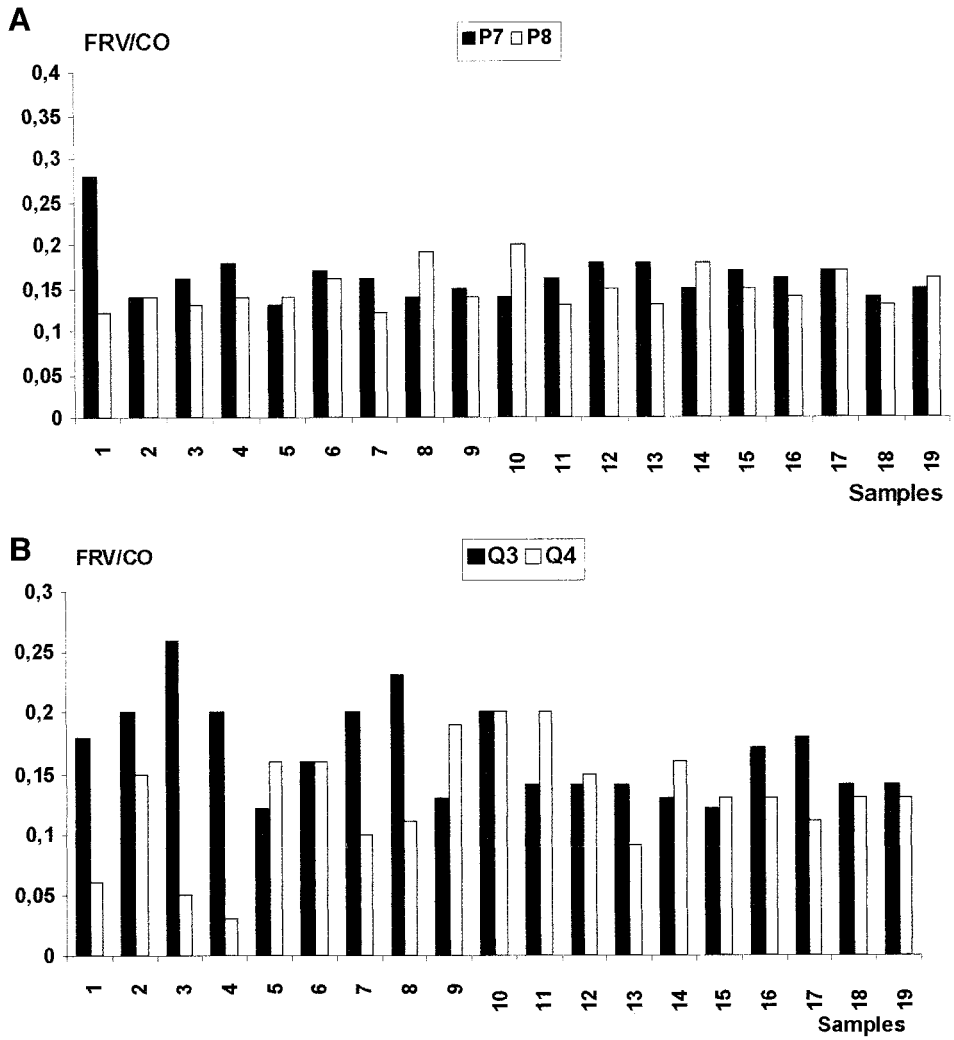

FIG. 3. UMELISA test of the synthetic peptides of the HTLV-I. (A) Reactivity of the monomeric peptides (P7 and P8) with samples from anti-HIV-1-positive samples ($n = 19$). (B) Reactivity of the chimeric peptides (Q3 and Q4) with anti-HIV-1-positive samples ($n = 19$). The samples were considered reactive when FRV/CO ≥ 1.

The sequence of monomeric peptides was deduced from (Strain ATK isolated) reported by Seiki *et al.* (1983) (31).

The monomeric peptides performance with anti-HTLV-I/II Panel PRP-205 (Boston Biomedica Inc) is showed in Fig. 1A where peptide P7 detected (4/8) HTLV-I-positive sera, one HTLV-II-positive sera and two HTLV-I/II-positive sera. Peptide P8 detected (5/8) HTLV-I-positive and one HTLV-I/II-positive sera. Negative samples (205-6 and 205-24) in this panel were negative for two synthetic peptides.

The monomeric peptide performances with sera from seropositive Cuban people to HTLV-I are shown in Fig. 1A, where peptide P7 detected (4/5) HTLV-I-positive sera, while peptide P8 detected (5/5) HTLV-I-positive sera.

The monomeric peptide performances with HTLV-I-positive samples from Colombia and Chile are shown in Fig. 1B, where peptide P7 detected (2/9)-positive samples, with one sample showing (FRV/CO) value of >2, and one sample showing moderate to low (FRV/CO). Peptide P8 detected (4/9) samples, with two samples showing (FRV/CO) values of >2. It detected two samples showing moderate to low (FRV/CO).

A mixture of monomeric peptides and chimeric peptide performances with anti-HTLV-I/II Panel PRP-205 (Boston Biomedica Inc) and with seropositive Cuban people to HTLV-I are shown in Fig. 2A, where mixture of P7 and P8 detected (5/8) HTLV-I-positive sera, with 4 samples showing (FRV/CO) values of >2 and one sample showing moderate to low (FRV/CO), detecting one HTLV-I/II-positive sample and not detecting HTLV-II-positive samples.

Chimeric peptide Q3 detected (7/8) HTLV-I-positive samples, with five samples showing (FRV/CO) values of >2, while two samples showed moderate to low

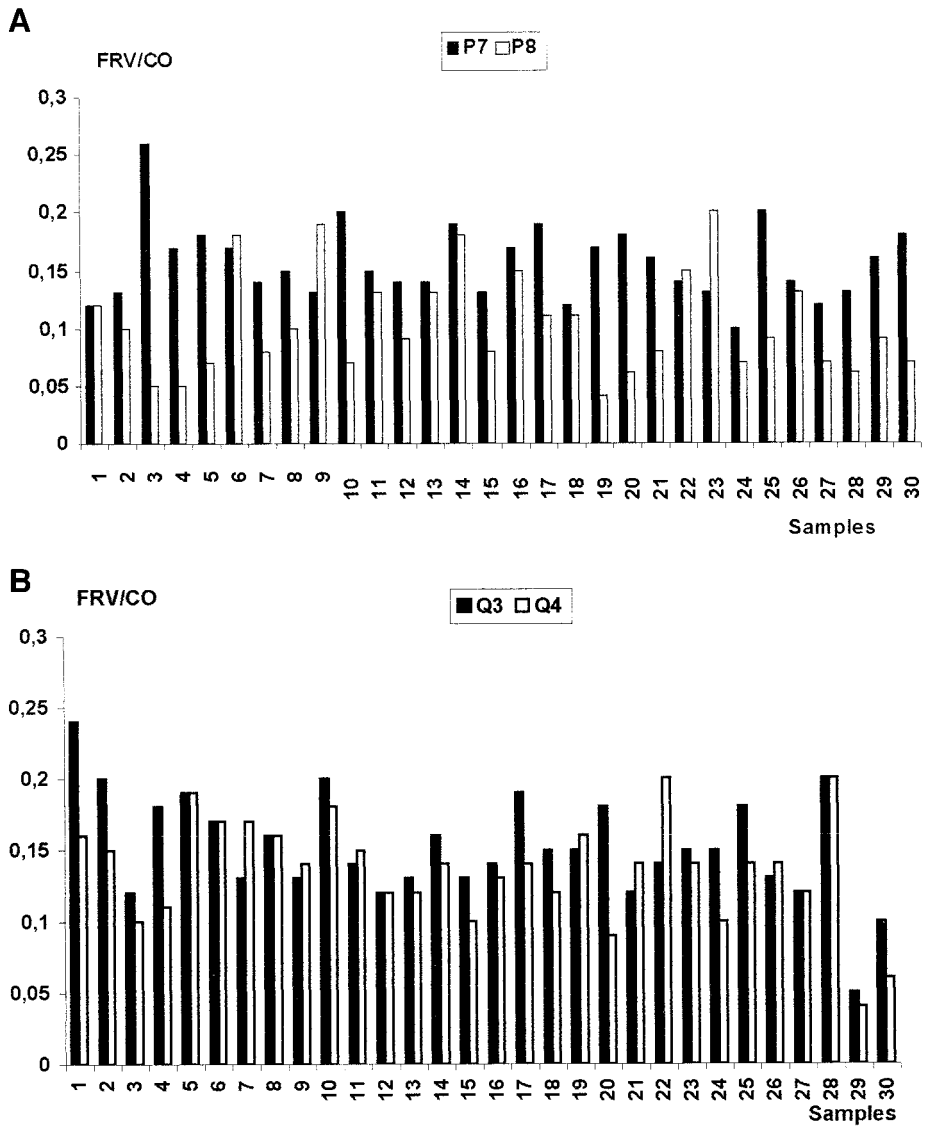

FIG. 4. UMELISA test of the synthetic peptides of the HTLV-I. (A) Reactivity of the monomeric peptides (P7 and P8) with samples from healthy blood donors ($n = 30$). (B) Reactivity of the chimeric peptides (Q3 and Q4) with samples from healthy blood donors ($n = 30$). The samples were considered reactive when FRV/CO ≥ 1.

(FRV/CO, detecting one HTLV-II-positive sample and one HTLV-I/II-positive sample. The chimeric peptide Q4 detected (6/8) with four samples showed (FRV/CO) values of >2 and two samples showed (FRV/CO) moderate to low, detecting one HTLV-II-positive sample and one HTLV-I/II-positive sample.

Chimeric peptides Q3 and Q4 detected (5/5) sera from seropositive Cuban people to HTLV-I. 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 P7 and P8 detected (4/9) HTLV-I-positive sera, with three samples showing (FRV/CO) values of >2 and one sample showing moderate to low (FRV/CO).

Chimeric peptide Q3 detected (9/9) HTLV-I-positive samples, with five samples showing (FRV/CO) values of >2, while four samples showed moderate to low (FRV/CO. Chimeric peptide Q4 detected (6/9) with four samples showing (FRV/CO) values of >2 and two samples showed (FRV/CO) moderate to low.

In order to assess peptide specificity, samples from anti-HIV-1-positive samples ($n = 19$), as shown in Fig. 3, and 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 Q3 incorporating sequences from gp21 and gp46 proteins of the HTLV-I virus was the most antigenic molecule in this study. Of the total HTLV-I-positive samples analyzed, 21/22 (95%) were detected by this peptide. Therefore this peptide represents a very promising antigen that could be used for a diagnostic system for HTLV-I detection.

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