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Transmembrane glycoprotein cross reactive HIV-1/HIV-2 epitope.

Original Article

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SUMMARY.

Introduction. A very high cross reactivity (24%) between transmembrane glycoproteins of HIV-1 and HIV-2 has been previously reported in Mexico.

Material and methods. In this study, peptide UIRH1, corresponding to the aminoterminal region of the transmembrane glycoprotein of HIV-2 (aa 629-652) was synthesized and used as antigen source for an ELISA. Specificity of the reaction was confirmed by a competition assay.

Results. Non HIV-2 gp32 cross reactive serum samples from HIV-1 infected people gave absorbance values similar to those obtained from HIV negative individuals (1.1 times) while HIV-1 positive sera with HIV-2 gp32 cross reactivity gave 2.8 times the ELISA absorbance value.

Discussion. We propose that this transmembrane region is responsible for the previously observed immunoblot -assayed cross reactivity among HIV-

1 infected individuals in Mexico.

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Key Words: Cross-reactivity, HIV-1 and HIV-2, peptide synthesis, transmembranal glycoprotein, ELISA.

RESUMEN.

Epítipo de reactividad cruzada en las glicoproteínas transmembranales de HIV-1/ HIV-2.

Introducción. En México se ha reportado una alta reactividad cruzada (24%) entre las glicoproteínas transmembranales del HIV-1 y HIV-2.

Material y métodos. En este estudio, se sintetizó y se utilizó como antígeno para un ELISA, el péptido UIRH1 que corresponde a la región aminoterminal de la glicoproteína transmembranal

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del HIV-2 (aa629-652). La especificidad de la reacción se confirmó mediante un ensayo de competencia.

Resultados. Los sueros de personas infectadas con el HIV-1 que no presentaron reacción cruzada con la gp32 del HIV-2 dieron valores de absorbancias similares a los que se obtuvieron de las personas seronegativas (1.1 veces) mientras que los sueros positivos al HIV-1 que presentaron reactividad cruzada dieron 2.8 veces la absorbancia de los negativos.

Discusión. Proponemos que esta región transmembranal es responsable de la reactividad cruzada observada en ensayos de inmunoblot en individuos infectados con el HIV-1 en México.

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Palabras Clave: Reactividad cruzada, HIV-1/HIV-2, síntesis de péptido, glicoproteína transmembranal, ELISA.

INTRODUCTION.

HIV-1 and HIV-2 have similar biological properties and genomic organization. A high degree of cross reactivity to the proteins codified by the high sequence homology gag and pol genes has been reported. Glycoproteins are less conserved and seroreactivity against them was originally thought to be the product of a double infection (1-4). More recently several countries have reported cross reactions to the retroviral glycoproteins in single-infected patients, especially with the HIV gp 110 (5-7). With respect to the transmembrane glycoprotein, very few studies report cross reactivity in individuals with single infections, except for West Africa reports where HIV-2 prevalence is one of the highest and 3 to 14% cross reactivity in sera of single-infected individuals was found (8,9).

Studies in Mexico have shown that a very high percentage of HIV-1 infected people present cross reactivity when their sera was tested by immunoblot against HIV-2 virus proteins. This

reactivity was significantly associated with the transmission route of the virus: 81% of the sexually infected individuals and 39% of the blood-infected patients were detected. Reactivity to gp32 transmembrane protein of HIV-2 was present in 24% of the HIV-1 positive patients (10).

In this paper we report a study performed with a peptide corresponding to aa 629-652 (epitope II) of the transmembrane protein. This region was first selected by theoretical analysis of the HIV-2 sequences as the probable domain responsible for the previously reported cross reactivity; it was then synthesized and tested on an ELISA with serum samples showing or lacking immunoblot gp32 cross reactivity.

MATERIALS AND METHODS.

Immunoblot. Permanently infected Molt cells with HIV-2 MS strain (kindly provided by Dr. Phyllis Kanki, from the Harvard School of Public Health) and HIV-1 strain HTLVIIIb were used as antigen sources for immunoblot assays. Cultures were maintained in 1640 RPMI/10% FBS/1% 100X antibiotic-antimycotic solution (GIBCO) at 37°C, 5% CO₂ and 100% humidity, immunoblots were run as previously described (10).

Sera. Serum samples from 24 non-infected and 56 HIV-1 infected people were selected for the study. From the latter, 29 positive sera were determined to be cross reactive to gp32 HIV-2 proteins and 27 were negative in their reaction to gp32 by immunoblot. As control, an HIV-2 positive serum was used.

Sequence analysis. HIV-1 and HIV-2 sequences were obtained from the Los Alamos database (11). Sequences for HIV-2 strains ROD, NIH, ISY, BEN and GH1 were compared to HIV-1 strain XB2 with the PC Gene Version 7.0 (Intelligenetics Inc.). Antigenic profiles were carried on with the Surface Plot program from Parker Guo and Hodges (12).

Chemical peptide synthesis. The Barany and Merrifield solid phase method was used (13).

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Briefly, a chloromethylated divinylbenzene polystyrene resin (0.9 meq/Cl/g) was used as support. The blocking groups were Lys (2BrZ), Glu (Obzl), Met (O), Tyr (2Br Bzl), Trp (Form), Asp (Obzl). The peptide was freed from the support by fluorhydric acid hydrolysis and filtered by a sephadex G15 column. This peptide was synthesized following the aminoacid sequence of Myers for gp32 region 629-652 and was denominated UIRH1.

ELISA.- UIRH1 peptide was solubilized in carbonate buffer pH 9.6 and adsorbed overnight at 37°C to a maxisorp plate (Nunc) at 2 mg per well concentration. FBS was used for blocking overnight and the plate was washed 3x with PBS before use. A 1:100 serum dilution in PBS containing 0.2% Tween and 10% FBS was incubated 1h at 37°C. Plates were washed 5x and incubated with antihuman IgG conjugated with alkaline phosphatase (Sigma) at a 1:20,000 dilution for 1 hr at 37°C. Plates were washed again 5x with Tris-NaCl and the ELISA amplification system (GIBCO) was used to develop color. Absorbance values (AV) were obtained at 490 nM. Cut off point was defined as HIV-1 negative sera AV average plus 2 standard deviations.

Assay for inhibition of binding with free peptide (competition assay). A 700 mg/mL UIRH1 peptide solution was prepared by solubilization with formic acid and neutralization with Tris-NaCl pH 8. Ten serum samples at the same dilution as the ELISA were incubated with 10 mg peptide in 50 mL for 2 h at 37°C. After washing, the ELISA was performed as described above with these serum samples.

RESULTS.

Since the sequence for the strain MS-HIV-2 that we used for our immunoblots has not been reported, we used the HIV-2 strains ROD, NIH, ISY, BEN and GH1 sequences that are available in the Los Alamos database. We found a high conservation degree (approximately 90%) in the

transmembrane glycoprotein of these strains and almost 100% in the amino terminal region of these proteins, where the two main antibody induction regions have been mapped.

For HIV-1/HIV-2 comparison, we chose the GH1-HIV-2 sequence since this strain has a transmembrane glycoprotein of the same size as the MS-HIV-2 strain that we used for the immunoblots. Comparison of the amino terminal region of GH1 HIV-2 with XB2-HIV-1 showed 77.7% conservation for what has been called epitope I (aa 577-599 according to Myers) and 72% conservation in epitope II (aa 629-652).

The antigenicity profile obtained with the Surface Plot program for epitope II shows that this region has, in both HIV-1 and HIV-2 viruses, a group of exposed aminoacids in this middle part with a high degree of immunogenic potential.

Using the Liatek HIV-1/2 commercial assay (Organon Teknika) that is reported to contain a peptide with the epitope I sequence, we were able to discard the possible participation of this region in the observed cross reactivity and the region corresponding to epitope II was synthesized.

Peptide synthesis. A 24 amino acid peptide corresponding to aa 629 to 652 of HIV-2 was synthesized. The peptide sequence is: NH₂-ser-leu-glu-gln-ala-gln-ile-gln-gln-glu-lys-asn-met-tyr-glu-leu-gln-lys-leu-asn-ser-trp-asp-val-COOH, this UIRH1 peptide was used in an ELISA to evaluate the reactivity of all serum samples described in materials and methods.

The cut off point obtained with the negative HIV sera AV plus 2 standard deviations was 0.682. A negative HIV-1 positive HIV-2 serum gave an AV value of 3.2 times the negative sera average; the non HIV-2 cross reactive HIV-1 positive and the HIV-2 gp32 cross reactive HIV-1 positive sera gave 1.1 and 2.8 (1.8 to 5.1 range) times de value for the HIV negative sera respectively.

As shown in figure 1, ELISA performed with the UIRH1 synthetic peptide clearly differentiates between gp 32 HIV-2 cross reactive HIV-1 positive 8or HIV-2 positive control) and HIV-2 non cross

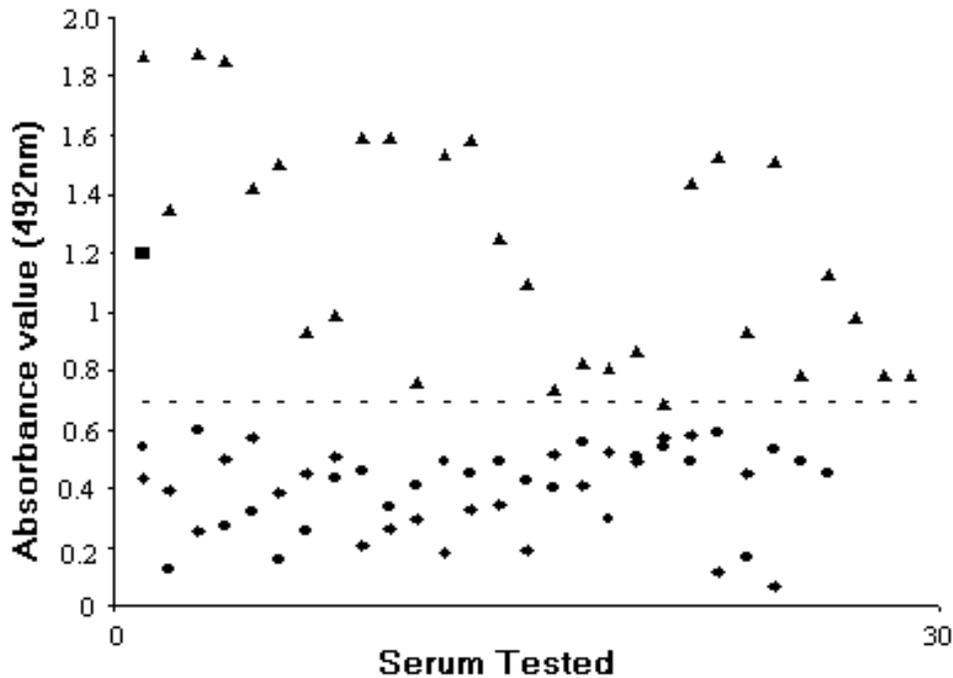


Figure 1.- Dipersion graph of HIV-2 peptide (629-652 gp 32 region) ELISA results. Two mg peptide per well were used. Results with 81 sera are shown: 24 HIV negative sera \blacklozenge ; 29 HIV 1 positive HIV 2 gp32 cross reactive sera \blacktriangle ; 27 HIV 1 positive non reactive with HIV 2 gp32 \blacksquare ; and one HIV2 positive sera \blacksquare . Cut off point was defined from the HIV negative sera AV average plus 2 standard deviations.

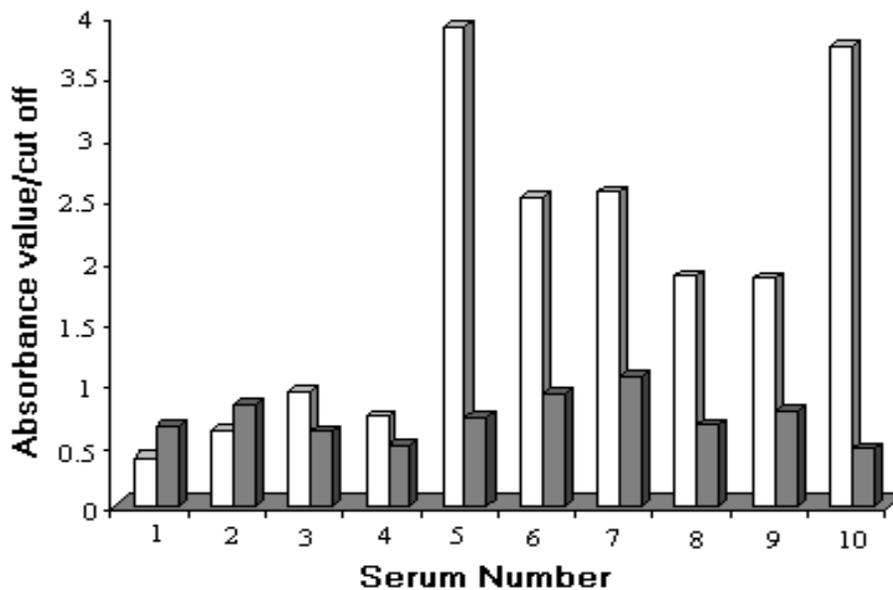


Figure 2.- Results of the competitive ELISA. Sera were pre-incubated with 10 mg of soluble synthetic UIRH1 peptide previous to the peptide ELISA performed with the same UIRH1 peptide adsorbed to the plate. 1 and 2 corresponds to HIV negative sera; 3 and 4 to HIV 1 positive non HIV 2 gp 32 cross reactive sera; 5 to HIV 2 positive sera and 6 to 10 to HIV 1 positive HIV 2 gp 32 cross reactive sera. White bars correspond to the non competitive ELISA, while black bars correspond to reactions where serum were pre incubated with soluble UIRH1 peptide.

reactive sera.

Specificity of the observed reactivity was tested by a competition assay using the soluble synthetic UIRH1 peptide with some serum samples. Results show inhibition of the reaction for gp32 HIV-2 cross reactive sera (67%) while for non-gp32 cross reactive sera only a 34% inhibition is observed (figure 2).

DISCUSSION.

Cross reactivity between human retroviruses is not a phenomenon, it has been reported especially with several gag and pol proteins as a common observation worldwide. Although before 1987 dual reactivity to glycoproteins was still considered as double infection, later on it was shown that reaction with the external glycoprotein was not necessarily an indication of infection with more than one retrovirus (4-6).

Our initial working hypothesis was that epitope II could be involved in the very high cross reactivity observed in Mexican HIV-1 positive patients with gp32 HIV-2 protein, based on this regions 72% homology between HIV-1 and HIV-2 viruses and also based on its high immunogenic potential. The results obtained with the UIRH1 peptide, which corresponds to this region, corroborate this hypothesis. Nevertheless several questions remain to be answered. Mexico, with no HIV-2 infections reported to date, presents a special phenomenon in terms that cross reactivity to the transmembrane glycoprotein of HIV-2 in HIV-1 infected individuals are even higher than in regions where HIV-2 is a prevalent infection (8-10). Another interesting observation is that HIV-2 cross reactivity is associated with sexually infected people and not with blood transfusion infected individuals, which suggests the possibility that different HIV-1 strains were introduced originally in our country and were maintained in the different risk populations for several years. Other Mexican studies regarding reactivity to gp 120 of different viruses (14) and V3 sequences of Mexican isolates

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(15) support the idea that glycoproteins of Mexican HIV-1 viruses are different in sexually versus blood infected patients; however, up to now the implications of these differences are not known.

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