

RESEARCH NOTE

Prevalence of Antibodies against an Immunodominant Region of the HIV-1 Envelope Glycoprotein gp41 in Sera from HIV-1 Infected Individuals in Brazil

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As evolution in the AIDS diagnosis assay development passes from first to second and third generation assays, sensitivity has increased to approximately 100%, with very high specificities (up to 100%) (NT Constantine 1993 *AIDS* 7: 1-13). Third generation assays employ antigens containing immunodominant epitopes, in order to allow detection of antibodies significant for a specific anti-HIV response. However, even the recombinant and synthetic peptide-based assays of the second and third generation tests may not always yield 100% sensitivities and specificities in all assays, due to variabilities in HIV antigens in different geographical locations. Therefore, the frequency of antibody response to chosen epitopes has to be determined for each geographical area before introduction of such assays for routine diagnosis of HIV-1 infection.

One of the antigenic epitopes recognized as immunodominant in HIV-1 infection (JJG Wang et al. 1986 *Proc Natl Acad Sci USA* 83: 6159-6163, JW Gnann et al. 1990 *Curr Top Microbiol Immunol* 160: 131-143) is a short 7 amino acid segment of the envelope glycoprotein gp41, identified as a loop of the amino acids 603-609 CSGKLI (MBA Oldstone et al. 1991 *J Virol* 65: 1727-1734). Several research groups in North-America and in Europe have reported reac-

tivity of between 93% (H Wolf et al. 1992 *Molec Immunol* 29: 989-998) and 100% (B Wahren et al. 1989 *J AIDS* 4: 448-456) towards this epitope. However, reports of recognition of this peptide in other geographical areas are few. A study of R Kennedy (referred to in F Siegel et al. 1992 *J AIDS* 5: 583-590) reports a 63% reactivity in Africans, while P Horal et al. (1991 *J Virol* 65: 2718-2723) reports a low sensitivity for East African sera unless peptides with sequences corresponding to African isolates are employed.

Although analyses of the diversity of HIV-1 isolates circulating in Brazil are few as yet, the reports indicate that important differences between Brazilian and North-american/European isolates exist. Thus, studies analyzing the sequence of the envelope genome of Brazilian HIV-1 samples (BJ Potts et al. 1993 *AIDS* 7: 1191-1197; MG Morgado et al. 1994 *AIDS Res Human Retroviruses* 10: 569-575) indicate that Brazilian HIV-1 isolates, while belonging mainly to the subtype B prevalent in North-America and Europe, present characteristic differences at the envelope glycoprotein gp120 V3 loop and flanking regions. Also, studies on specific antibody response (EW Carrow et al. 1991 *AIDS Res Human Retrov* 7: 831-838, V Bongertz et al. 1994 *Braz J Med Biol Res* 27: 1225-1236) indicate specificities different from those found in areas where the HIV-1 type B is prevalent. However, the extension of such diversity to other immunologically important epitopes has not been studied as yet. It is, therefore, of paramount importance to test the efficacy of any assay developed for worldwide diagnosis of HIV-1 infection, based on the use of synthetic peptides, with sera collected in the geographical region where the assay will be employed.

Peptides covering the immunodominant region identified in gp41 have been synthesized and are being employed in commercial HIV-1 diagnostic kits (WJ Maskill et al. 1988 *J Virol Meth* 22: 61-73, I Van Kerkhoven et al. 1991 *Bull WHO* 69: 753-760 X Zhang et al. 1992 *J Med Virol* 38: 19-53).

To analyse whether sera from Brazilian HIV-1 infected individuals react with the gp41 immunodominant epitope to a comparable degree as do North-american/European sera, we have carried out a study with plasma from HIV-1 positive individuals resident in Brazil, selected due to their reactivity in a commercial second generation HIV-1 ELISA (Abbott HIV-1 recombinant EIA, Abbott Laboratories, Diagnostic Division, North Chicago, IL, USA).

A total of 120 plasma from HIV-1 seropositive (Abbott HIV-1 recombinant EIA, Cambridge Biotech HIV-1 Western blot kit, Cambridge Biotech Corp., Worcester, MA, USA) individuals in all stages of the infection collected in different hospitals in Rio de Janeiro, RJ, enrolled in a

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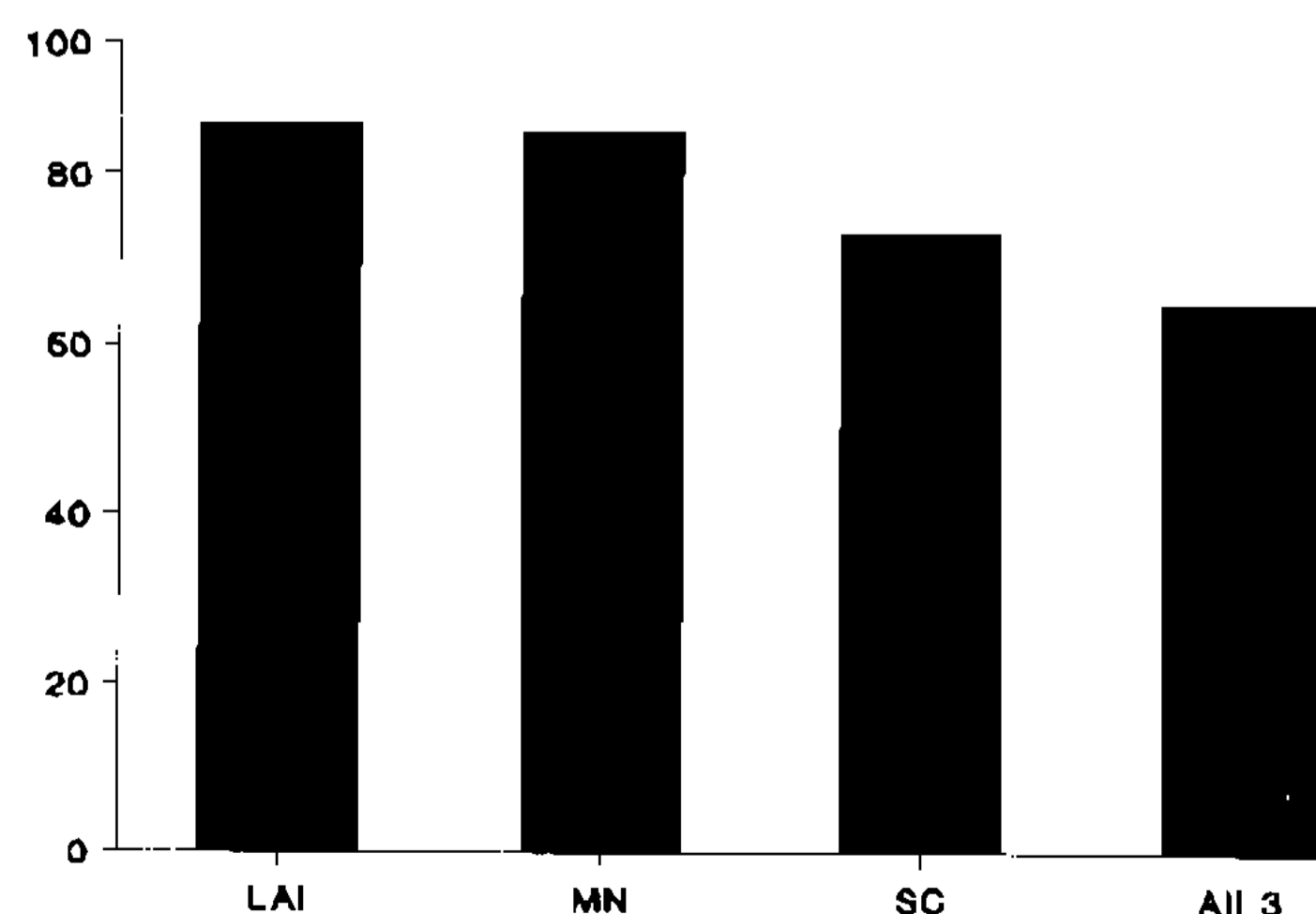
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"Multicenter study on Heterosexual HIV transmission in Rio de Janeiro" (NIAID/PAHO/Brazilian Ministry of Health), collected between 1990 and 1993, were analyzed. Both male and female individuals of varying ages and from all clinical disease stages were included. An additional 18 plasma from HIV-1 seronegative individuals from the same risk groups and three normal plasma from blood donors were used as controls. The peptides used in this study (kindly donated by the Department of Immunology of the Karolinska Institute, Stockholm, Sweden) corresponded to the HIV-1 gp41 (599-618) LAI (BH10) sequence GIWGCSGKLICTTAVPWNAS, the HIV-1 gp41 (599-618) MN sequence GFWGCSGKLICTTTVPWNAS and the HIV-1 gp41 (599-618) SC sequence GIWGCSGKLICTTTVPWNNTS. Maxisorb microtiter plates (Nunc Laboratories Inc., Roskilde, Denmark) were coated with the individual peptides (10µg/ml) and heat inactivated plasma (56°C/1h) diluted 1:100 were used (Bongertz et al. *loc. cit.*). An additional titration assay was carried out with all positive plasma using the HIV-1 MN gp41 599-618 peptide.

The sera selected by positive reactivity with the commercial kit recognized the gp41 599-618 peptides in percentages varying from 73 to 86% (Fig.). Of the 120 plasma tested, only 65% (n=78) recognized all three peptides tested; an additional 12% recognized the HIV-1 LAI and MN derived peptides only and did not react with the HIV-1 SC derived peptide, while recognition of only the HIV-1 MN derived peptide (7%) was higher than the specific recognition of either the HIV-1 LAI (3%) or HIV-1 SC (0.8%) peptides only. Six plasma failed to recognize any of the peptides used, indicating a sensitivity of 94.2% for the study here presented (Table).

Of the 18 plasma obtained from individuals of the same AIDS-risk group with negative diagnosis, two plasma did recognize gp41 599-618 peptides. One of these plasma, tested negative both by ELISA and by Western blot, recognized



Reactivity of Brazilian HIV-1 seropositive plasma with gp41 599-618 synthetic peptides corresponding to amino acid sequences of the HIV-1 isolates LAI, MN and SC.

TABLE
Sensitivity and specificity determination

	Sensitivity ^a	Specificity ^b
HIV-1 LAI peptide GIWGCSGKLICTTAVPWNAS	82.5%	95.2%
HIV-1 MN peptide GFWGCSGKLICTTTVPWNAS	86.7%	90.5%
HIV-1 SC peptide GIWGCSGKLICTTTVPWNNTS	72.5%	90.5%
Any of the three peptides	95.0%	90.5%

^a: sensitivity was calculated as the ratio between the number of true positives and the sum of true positives and false negatives, multiplied by 100. ^b: specificity was calculated as the ratio between true negative results and the sum of true negative and false positive results multiplied by 100. A total of 120 sera from HIV-1 seropositive and 21 sera from HIV-1 seronegative individuals were studied.

all three gp41 599-618 peptides used in this study. The second plasma, tested within the group of "HIV-1 negative" risk group, non-reactive in the EIA but showing reactivity with gp160 (no reactivity with gp41) in the Western blot assay, recognized the HIV-1 MN and the HIV-1 SC derived gp41 599-618 peptides. These results indicate a lower specificity of this assay in comparison to the commercial assay used in this study (Abbott HIV-1 recombinant EIA) (see Table). However, if the two plasma in question were to be considered "false negative", it might indicate a different (neither higher nor lower) specificity for the assay based on detection of anti-gp41-immunodominant epitope-antibodies than for the assay detecting reactivity against the whole viral lysate or complete envelope glycoprotein, as several sera diagnosed "positive" by western blot did not react with the peptides studied. The human plasma obtained from normal persons (outside AIDS risk groups) were negative (3/3) for all three peptides.

From these results it can be concluded that a diagnostic assay based on utilization of a synthetic peptide corresponding to the envelope gp41 immunodominant epitope has to be carefully screened with local HIV-1 positive plasma banks before introduction to commercial use. However, the use of a shorter peptide, comprising the sequence CSGKLI identified as the minimum epitope (Oldstone et al. *loc. cit.*) may increase sensitivity of the assay, as it appears to be most conserved between different HIV-1 isolates. Brazilian HIV-1 isolates should be analysed to verify if they conform to this rule.

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