

## RESEARCH NOTE

## Correlation Between Anti-V3 Peptide and Neutralizing Antibodies in Plasma From HIV-1 Infected Individuals Resident in Brazil

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A positive correlation between the presence of HIV-1 neutralizing antibodies and reactivity with synthetic peptides corresponding to the principal neutralization domain (PND) in sera from HIV-1 infected individuals has been reported (R Cheingsong-Popov et al. 1992 *J Inf Dis* 165: 256-261). The determination of HIV-1 neutralizing antibodies is labor intensive and requires the production of high titered infectious HIV-1 stocks, and therefore not all laboratories can perform this assay routinely. The indirect method of estimating HIV-1 neutralizing antibodies by analyzing reactivity of patient sera with synthetic peptides has been accepted by many laboratories as an easy, viable alternative. However, not only do some reports show a lack of positive correlation between reactivity with PND peptides and presence of HIV-1 neutralizing antibodies (RQ Warren et al. 1992 *J Virol* 66: 5210-5215), but a recent study comparing reactivities with identical peptides synthesized in different laboratories has shown significant differences and points out the problems encountered in the comparison of such results obtained in different laboratories (NA Halsey et al. 1992 *J AIDS* 5: 153-157).

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In a recent study of the reactivity of 85 plasma from HIV-1 seropositive individuals resident in Brazil with synthetic peptides from the V3 region of 5 HIV-1 isolates (isolates IIIB, MN, SC, WMJ-2 and RF, peptides synthesized at the Dept. of Immunology, Karolinska Institute, Stockholm, Sweden), a comparatively low reactivity (59%) with HIV-1 isolate MN derived PND peptides was reported (V Bongertz et al. 1992 VIII Intl Conf AIDS Amsterdam, The Netherlands PoA2096), confirming a previous study of Brazilian HIV-1 positive plasma with MN PND peptides (EW Carrow et al. 1991 *AIDS Res Human Retrovir* 7: 831-838). As most studies reported in the literature show a 90% or higher reactivity with MN isolate PND peptides (P Nara et al. 1991 *FASEB* 5: 2437-2455), we concluded that Brazilian HIV-1 seropositive sera reacted in a lower degree with MN isolate PND peptides than sera from other countries. To verify if the lower reactivity was related to the origin of the peptides, we confirmed the results obtained by analysis of the same plasma with HIV-1 MN isolate PND peptides obtained from other sources. A total of five different HIV-1 MN isolate derived PND peptides, with varying amino acid sequences but all comprising the GPGR top of the V3 loop were used. The V3 MN peptide was purchased from American BioTechnologies, Boston, MA, USA; the #48 MN peptide was produced by Johnson & Johnson Biotechnological Center, La Jolla, CA, USA, and the C52, C53 and C54 MN peptides were produced at the Karolinska Institute, Stockholm, Sweden (peptide sequences are indicated in Fig. 1a). Reactivity was determined in an ELISA assay, where 10 µg/ml of the respective peptide was adsorbed unto 96 well Maxisorb plates (Nunc, Denmark). After saturation with skimmed milk, plasma obtained from a NIAID/PAHO/Brazilian Ministry of Health multicenter study cohort, diluted 1:100, were added. Binding of antibodies was detected using peroxidase labelled anti-human IgG antibodies and peroxide/ortho phenylenediamine substrate.

The V3, #48, C52 and C53 peptides were recognized in similar degree by the Brazilian plasma: 52, 55, 56 and 49%, respectively; while the C54 peptide was recognized by a lower percentage of plasma (27%).

Although the percentual reactivity with each individual peptide did not vary much, an analysis of the overlapping reactivities of the plasma studied with the different peptides indicated surprising results, as shown in Fig. 1b.

For example, although the percent reactivity with the V3 MN peptide, 52%, was similar to the one with the C52 MN peptide, 56%, the percentage of the V3 MN reactive plasma that

Fig. 1a: reactivity of plasma from HIV-1 seropositive individuals resident in Brazil (n = 85) with HIV-1 MN isolate peptides comprising the top of the principal neutralization domain. Amino acid sequence of the peptides used:

V3 MN CNKRKRIHIGPGRAFYTTKN  
 #48 MN RKRIHIGPGRFY  
 C52 MN PNYNKRKRIHIGPGR  
 C53 MN RKRIHIGPGRAFYTTK  
 C54 MN IGPGRAFYTTKNIIG

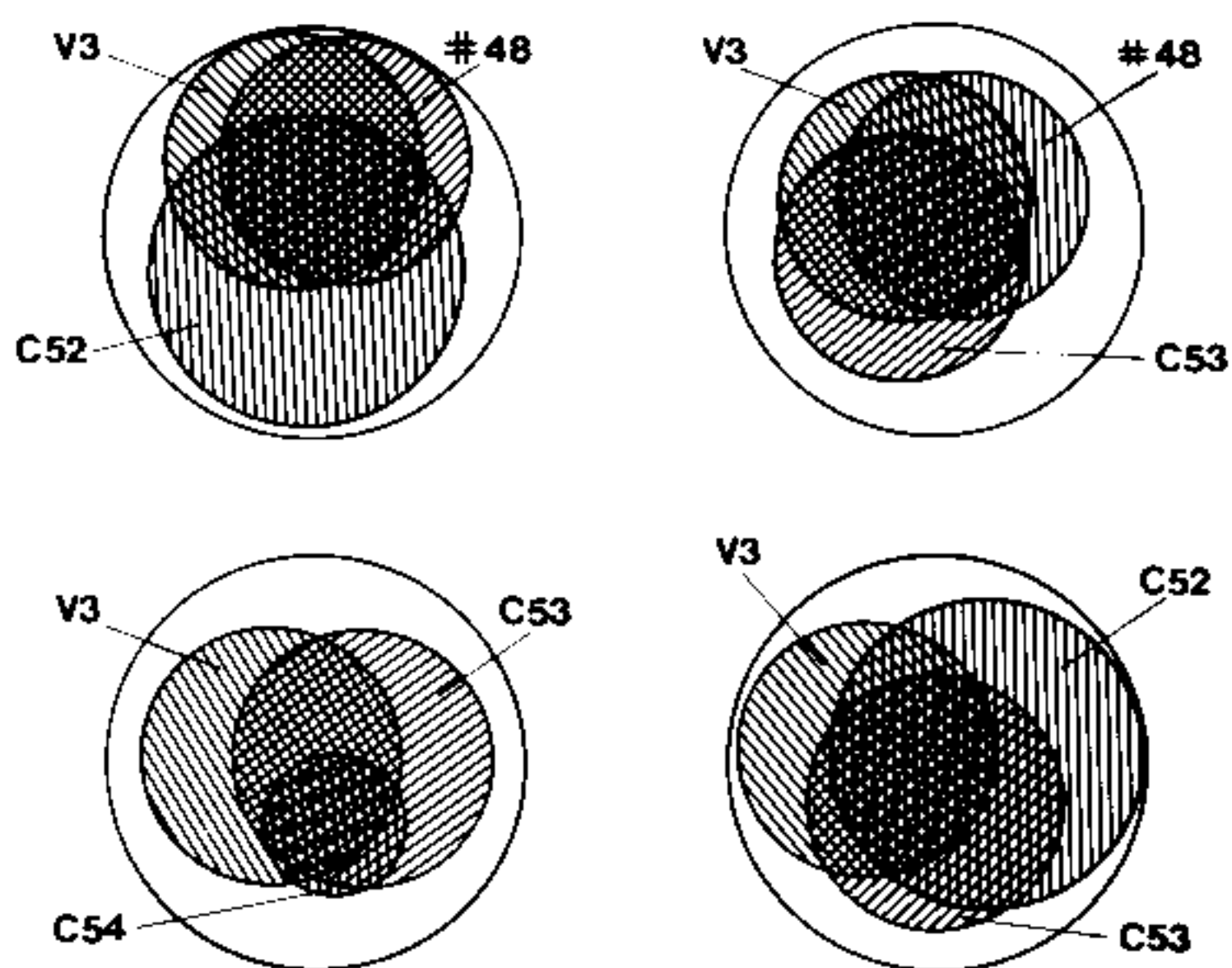


Fig. 1b: overlapping recognition of HIV-1 MN isolate PND peptides by Brazilian plasma. The radius of each circle is proportional to the percentual reactivity determined for each peptide, with outer circles proportional to the total population studied.

also recognized the C52 MN peptide was 65%. While overlapping reactivity with V3 MN and C53 MN was 65%, very similar to the one observed between V3 and C52, the overlap between C52 MN and C53 MN was only 69%. As indicated in Fig. 1b, the reactivity with C54 MN, lower than with the other peptides, almost integrally overlapped with the anti-C53 MN reactivity. We conclude that in the population studied, there are at least two different anti-MN-PND antibody specificities directed against amino acid sequences which include the GPGR top of the loop. One is a specificity directed towards an epitope that includes the PNY(NKRKRIHIGPGR) amino acids, while the other is directed towards an epitope comprising the (KRKRIHIGPGR)AFY amino acid sequence. Apparently, the amino acids KRKRIH are part of the second epitope, as the C54 MN peptide (IGPGRAFYTTKNIIG) was recognized by a lower percentage of the plasma studied (27%), unless the amino acid sequence IIG interferes with antibody binding to the V3 MN peptide sequence CNKRKRIHIGPGRAFYTTKN.

When percentage of plasma reactive with at least one of the five MN PND peptides is calculated, the resulting 86% appears comparable to reactivities described in previous studies (Nara

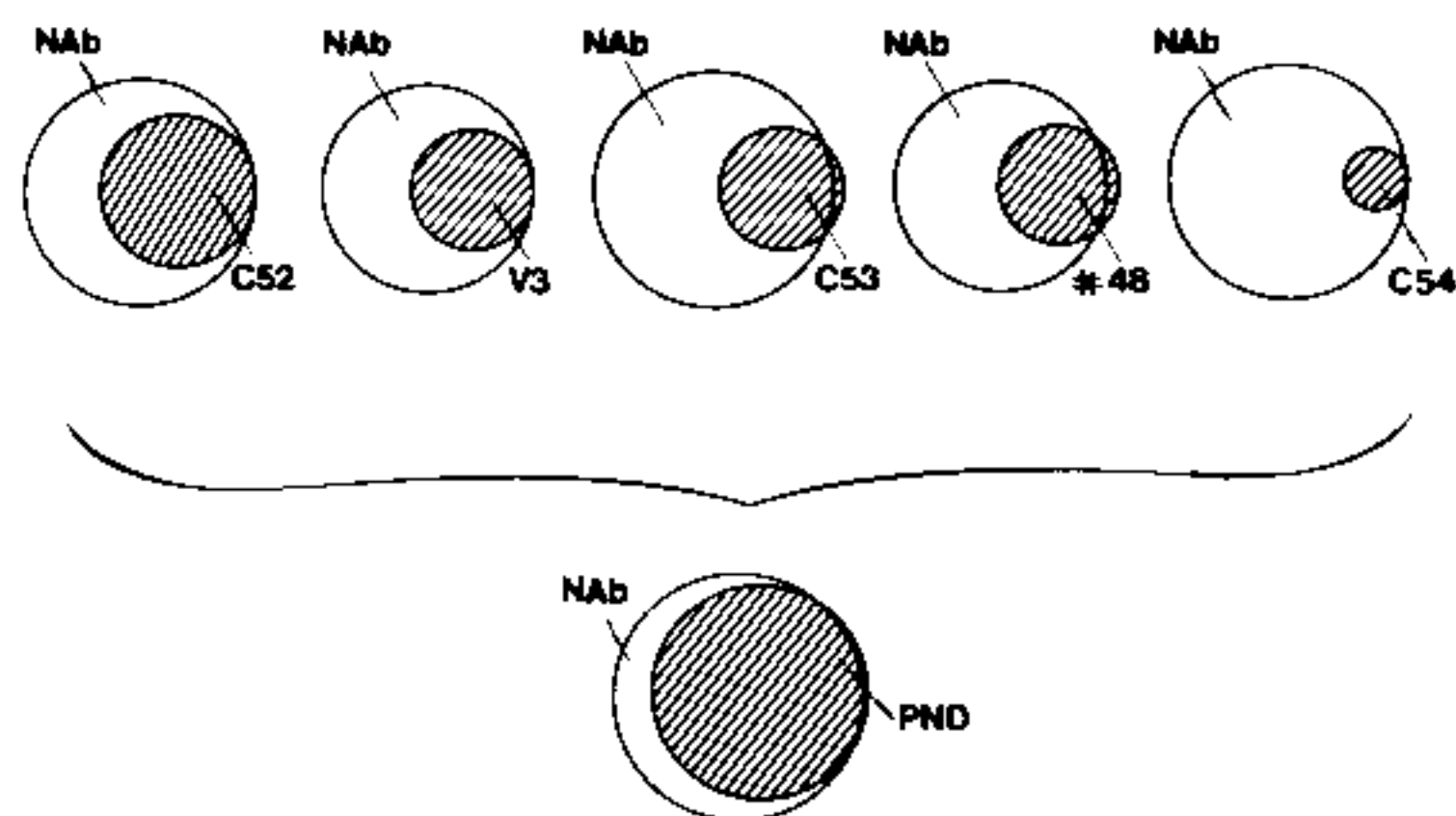


Fig. 1c: comparison of presence of HIV-1 MN isolate neutralizing antibodies (NAb) with recognition of the HIV-1 MN isolate PND peptides as determined by ELISA.

et al. *loc. cit.*). The overlap of peptide recognition and HIV-1 MN isolate neutralization by the 85 plasma was analyzed and 96% were positive for neutralizing antibodies; with titers varying from 1:100 to 1:52,600 (as determined using the MT2 microplaque assay, CV Hanson et al. 1990 *J Clin Microbiol* 28: 2030-2034). Fig. 1c indicates that a good correlation between presence of neutralizing antibodies (NAb) and reactivity with one or more of the five HIV-1 MN isolate PND peptides exists, while overlap of NAb with each individual peptide was much lower.

We conclude that recognition of the V3 loop of the HIV-1 MN isolate in sera from Brazilian HIV-1 positive individuals is comparable to reactivities reported in other countries, but is comprised of at least two distinct antibody specificities, and that a much higher percentage of Brazilian plasma recognize specifically the N terminal arm including the GPGR top of the V3 loop than reported for sera from individuals resident in other countries (Nara et al. *loc. cit.*). The detection of two different antibody specificities observed by JPM Langedijk et al. (1992 *Arch Virol* 126: 129-146), in analyses of monoclonal antibodies prepared against viral lysates, indicates that this observation may not be restricted to antibodies in plasma of HIV-1 positive individuals resident in Brazil. The lack of positive correlation between presence of neutralizing antibodies and reactivity with PND peptides reported in some studies may reflect an incomplete detection of such antibodies due to a restricted choice of peptides used.

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