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Analysis of antibody specificity against the third variable region of the envelope glycoprotein gp120 of HIV-1 in plasma from HIV-1-positive individuals residing in Brazil

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1. Antibody specificity for the principal neutralization domain (PND) of the human immunodeficiency virus type 1 (HIV-1) was studied in plasma from 122 HIV-1-infected individuals residing in Brazil.

2. Using 8 overlapping sequential pentadecapeptides corresponding to the third variable region (V3) of 5 different HIV-1 isolates in an enzyme-linked immunosorbent assay (ELISA), a preferential recognition of the peptides with amino acid sequences corresponding to the HIV-1 isolates IIIB and MN (maximal reactivities of 60-70%) compared to the isolates SC, WMJ-2 or RF (maximal reactivities below 60%) was observed.

3. A difference was observed in the overall reactivity pattern to HIV-1 SC peptides of plasma collected from individuals residing in the Brazilian states of Rio de Janeiro and Bahia. However, a statistically significant increased recognition by Bahian plasma was only observed for the HIV-1 SC C55 peptide.

4. The mean CD4/CD8 ratio of the group of plasma with an isolate-restricted recognition of peptides (0.522 ± 0.074) was significantly lower than that of the total group of plasma (1.00 ± 0.18).

Key words: HIV-1, AIDS, antibodies, PND, V3 loop, Brazil.

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Introduction

The detection of Human Immunodeficiency Virus (HIV) neutralizing antibodies (Weiss et al., 1986) initiated the search for the antigenic epitopes capable of inducing neutralizing antibodies and the evaluation of the efficacy of HIV neutralization by these antibodies. The third variable region (V3) of the envelope glycoprotein gp120 of HIV-1 has been identified as the "principal neutralizing domain" (PND) of this virus (Goudsmit et al., 1988; La Rosa et al., 1990). As progress towards vaccines, immunoprophylactic and immunotherapeutic drugs is being reported it is necessary to analyze the potential effectiveness of such measures in countries where the study of the characteristics of the virus has only been started recently. This is the case for Brazil, where very large numbers of AIDS cases have been reported but very little is known about the HIV polymorphism. As a number of studies on V3 region sequences and its induced immune response are reported, it becomes clear that HIV-1 isolates from different geographical areas such as Thailand (Pau et al., 1993), the Commonwealth of Independent States (former Soviet Union: Cheingsong-Popov et al., 1993), Tanzania (Zwart et al., 1993), the Central African Republic (Murphy et al., 1993) and Ethiopia (Ayehunie et al., 1993) are quite different from the V3 region consensus described for the European/North-American isolates that have been used in the development of anti-HIV/AIDS vaccines.

In this report, an indirect method for evaluating HIV polymorphism at the PND has been employed, which is based on the analysis of specificity of antibodies in plasma from HIV-1-infected persons residing in Brazil against synthetic peptides.

Material and Methods

Patients

Blood was collected from HIV-1-positive patients at different hospitals in Rio de Janeiro, RJ (N = 103) (individuals enrolled in a "Multicenter study of AIDS transmission in Rio de Janeiro" financed by NIAID/PAHO/Brazilian Ministry of Health) and Salvador, BA (N = 19), between 1988 and 1992. Diagnosis was based on results obtained by enzyme immunoassay (Abbott HIV-1 recombinant EIA, Abbott Divisão Diagnóstica, São Paulo, SP) and confirmed by Western blot (HIV-1 Biotech/Dupont HIV-1 Western blot kit, Biotech Research Laboratories Inc., Rockville, MD). Only plasma samples that were positive in both tests were used in this study. All plasmas were separated into aliquotes upon arrival and

stored at -20°C. Information on age, sex and clinical classification was available in most cases. Analysis of peripheral blood cells was carried out for 93/103 patients from Rio de Janeiro using the labelled monoclonal antibody reagent T4-RD1/T8-FITC for differential labelling of CD4+ and CD8+ lymphocytes and the reagent T11-RD1/B1-FITC for the labelling of total T and B lymphocytes (Coulter Corporation, Hialeah, FL). CD4/CD8 ratios were compared by statistical analysis (Taylor et al., 1989).

Peptides

Peptides synthetized at the Karolinska Institute, Stockholm, Sweden were used (Rossi et al., 1989). Table 1 presents the amino acid sequence of each peptide used in this study. Eight pentadecapeptides having an overlap of 10 amino acids corresponding to the V3 loop were used for each of 5 well characterized HIV-1 strains. HIV-1 IIIB, MN and SC are from North American/European isolates and WMJ-2 and RF represent Caribbean isolates.

Enzyme linked immunosorbent assay (ELISA)

Peptides (10 µg/ml in 0.1 M sodium carbonate buffer, pH 9.5) were dispensed into Maxisorb microtiter plates (Nunc Laboratories, Roskilde, Denmark), 50 µl/well. After 60-min incubation at room temperature followed by overnight (or longer) incubation at 4°C, the plates were saturated with a solution of 3% (w/v) bovine serum albumin in 0.15 M phosphate buffered saline, pH 7.4 (PBS-3% BSA), for 1 h at room temperature. After one wash with PBS, heat-denatured (56°C/1 h) plasma samples diluted 1:100 in PBS-0.3% BSA were added to each well (50 µl/well), with duplicates prepared for each sample and quadruplicates for the negative control (heat-inactivated normal human plasma pool). After 60-min incubation at room temperature, the plates were washed with PBS containing 0.05% Tween 20, and 50 µl of conjugate (horseradish peroxidase labelled goat anti-human IgG, Sigma, St. Louis, MO) diluted in PBS-0.3% BSA was added to each well. After 60 min at room temperature the plates were washed and 50 µl of the substrate/developer solution (0.01% hydrogen peroxide and 0.5 mg/ml ortho-phenylenediamine in 0.1 M sodium citrate-phosphate buffer, pH 5.0) was added. After 10 min, 50 µl of 2 M sulfuric acid was added to each well and the plates were read at 492 nm in a Titertek Multiscan reader (Flow Laboratories, McLean, VA).

The cut-off value used was the mean of the quadruplicate negative control values plus four times the standard error (mean + 4 SEM). Sample values were the mean of the duplicate sample values minus the cut-off value. Assays were

Table 1 - *Amino acid sequence of the peptides used in the present study.*

These overlapping sequential pentadecapeptides C50-C58 correspond to the third variable region (V3) of the envelope glycoprotein gp120 of five different HIV-1 isolates.

HIV-1 isolate IIIB	HIV-1 isolate MN
C50 - NTSVEINCTRPNNNT	C50 - NESVQINCTRPNYNK
C51 - INCTRPNNNTRKSIR	C51 - INCTRPNYNKRKRIH
C52 - PNNNTRKSIRIQRGP	C52 - PNYNKRKRIHIGPGR
C53 - RKSIRIQRGPGRAFV	C53 - RKRIHIGPGRAFYTK
C54 - IQRGPGRAFVTIGKI	C54 - IGPGRAFYTTKNIIG
C55 - GRAFVTIGKIGNMRQ	C55 - AFYTTKNIIGTIRQA
C56 - TIGKIGNMRQAHCNI	C56 - KNIIGTIRQAHCNIS
C57 - NMRQAHCNISRAKW	C57 - TIRQAHCNISRAKWN
C58 - AHCNISRAKWNNTLK	C58 - AHCNISRAKWNDTLR
HIV-1 isolate SC	**HIV-1 isolate WMJ-2**
C50 - KEAVEINCTRPNNNT	C50 - NESVEINCTRPYNNV
C51 - INCTRPNNNTTRSIH	C51 - INCTRPYNNVRRSLS
C52 - PNNNTTRSIHIGPGR	C52 - PYNNVRRSLSIGPGR
C53 - TRSIHIGPGRAFYAT	C53 - RRSLSIGPGRAFRTR
C54 - IGPGRAFYATGDIIG	C54 - IGPGRAFRTREIIGI
C55 - AFYATGDIIGIIGDI	C55 - AFRTREIIGIIRQAH
C56 - GDIIGIIGDIRKAHC	C56 - EIIGIIRQAHCNISR
C57 - IIGDIRKAHCNISRA	C57 - IRQAHCNISRAKWNN
C58 - AHCNISRAKWNNTLK	C58 - AHCNISRAKWNNTLK
HIV-1 isolate RF	
C50 - NASVQINCTRPNNNT	
C51 - INCTRPNNNTRKSIT	
C52 - PNNNTRKSITKGPGR	
C53 - RKSITKGPGRVIYAT	
C54 - ITKGPGRVIYATGQI	
C55 - GRVIYATGQIIGDIR	
C56 - ATGQIIGDIRKAHCN	
C57 - IGDIRKAHCNLSRAQ	
C58 - AHCNLSRAQWNNTLK	

repeated when the duplicate readings did not agree within 20%. When the second evaluation of the reactivity of the plasma samples with the peptide in question produced results with an unacceptable variation between duplicates (two cases) the data for this sample/peptide combination were excluded from the analysis. Cut-off values ranged from 0.080 to 0.250 (no background values were subtracted).

Statistical analysis

The ratios of the number of CD4 to CD8 cells for the different plasma groups and reactivities with different synthetic peptides were compared using the Student *t*-test, with the statistical significance taken as $P<0.05$. The mean values and standard deviation of the means (SD) are indicated in the text.

Results

Reactivity of Brazilian plasma with PND peptides

A total of 122 plasma samples from HIV-1-infected individuals residing in Brazil were assayed by ELISA with the peptides described in Table 1. The overall percent reactivity is shown in Table 2. Two of the samples tested showed no detectable reaction with any of the peptides used in this study, even though both were diagnosed positive by EIA and Western blot assays. Eight of 122 samples (6.5%) presented low reactivity with isolated peptides. The highest reactivity (% of samples with positive reaction) was obtained with HIV-1 MN isolate-derived peptides C51 (57%), C52 (62%) and C53 (63%), the C56 peptide from HIV-1 isolate IIIB (75%) and peptide C58 (58%) from the HIV-1 RF isolate. The overall reactivity with SC-derived peptides (2-55%) was unexpectedly low. Reactivity with sequential peptides from each isolate was highest for the C51-C53 peptides, closely followed by recognition of the C56-C58 peptides (IIIB isolate) or C57-C58 peptides (all other isolates), while only the plasma samples that showed an overall high reactivity with all peptides recognized the C50-C51 or C53-C56 sequential peptides from the isolates tested. The lowest reactivity (16-30%) was observed with peptides from the WMJ-2 HIV-1 isolate, with very low reactivity even for its C53 peptide (6.5%).

Cross-reactivity with peptides from different HIV-1 isolates

Of the 122 plasma samples tested, 46 (38%) reacted with more than 3 corresponding peptides from the different isolates, the highest cross-reactivity

Table 2 - *Reactivity of Brazilian plasma samples with the sequential overlapping pentadecapeptides C50-C58 corresponding to the third variable region of the envelope glycoprotein gp120 of five HIV-1 isolates.*

Data are reported as percent of plasma containing antibodies to peptides (horizontal line) related to those present in the different isolates (vertical entries) determined with an ELISA direct binding assay. Plasma were obtained from 122 patients positive for HIV-1 by both ELISA and Western blot.

Isolates	C50	C51	C52	C53	C54	C55	C56	C57	C58
IIIB	13.2	41.3	50.0	34.7	34.2	55.5	75.4	53.7	48.4
MN	34.7	57.4	62.2	62.8	32.0	26.7	49.2	31.1	27.9
SC	21.3	22.9	21.3	16.9	17.1	11.0	2.4	54.5	52.5
WMJ-2	9.0	26.2	13.9	6.5	29.7	21.9	5.1	29.7	27.0
RF	30.3	19.8	28.9	22.5	17.3	31.9	22.5	38.8	58.3

being observed with peptides C57 and C58, followed by C51 and C52. Twenty-nine percent and 30% of the plasmas showed reactivity with 2 and 3 HIV-1 isolate-derived peptides, respectively, mainly with peptides from the HIV-1 MN, IIIB and RF isolates. Only 17.2% (21/122) of the plasmas reacted with peptides from only one HIV-1 isolate, and most of them (14/21) reacted preferentially with HIV-1 IIIB isolate-derived peptides.

Comparison of plasma from different geographical areas in Brazil

The plasma samples were divided into 2 groups, one from Rio de Janeiro and one from Salvador, Bahia. The antibody specificity of the 19 plasmas from Salvador appeared to differ from that of the Rio de Janeiro samples (N = 103), mainly in terms of reactivity to HIV-1 SC-derived peptides, as shown by the reactivity data presented in Figure 1. However, only the difference in reactivity to the HIV-1 SC C55 peptide was statistically significant ($P<0.05$) between groups. The overall degree of reactivity was similar for the two groups of plasmas, plasma from both regions presenting either little or no reactivity to any of the peptides tested, "isolate restricted" reactivity or a very high degree of cross-reactivity for peptides from different HIV-1 isolates. Most marked, however, was the almost identical reactivity to the HIV-1 isolate MN-derived peptides (Figure 1).

Correlation between ELISA reactivity and CD4/CD8 ratio

The content of CD4-positive lymphocytes was measured in blood samples from 93 of the 122 individuals. The group included all clinical stages and

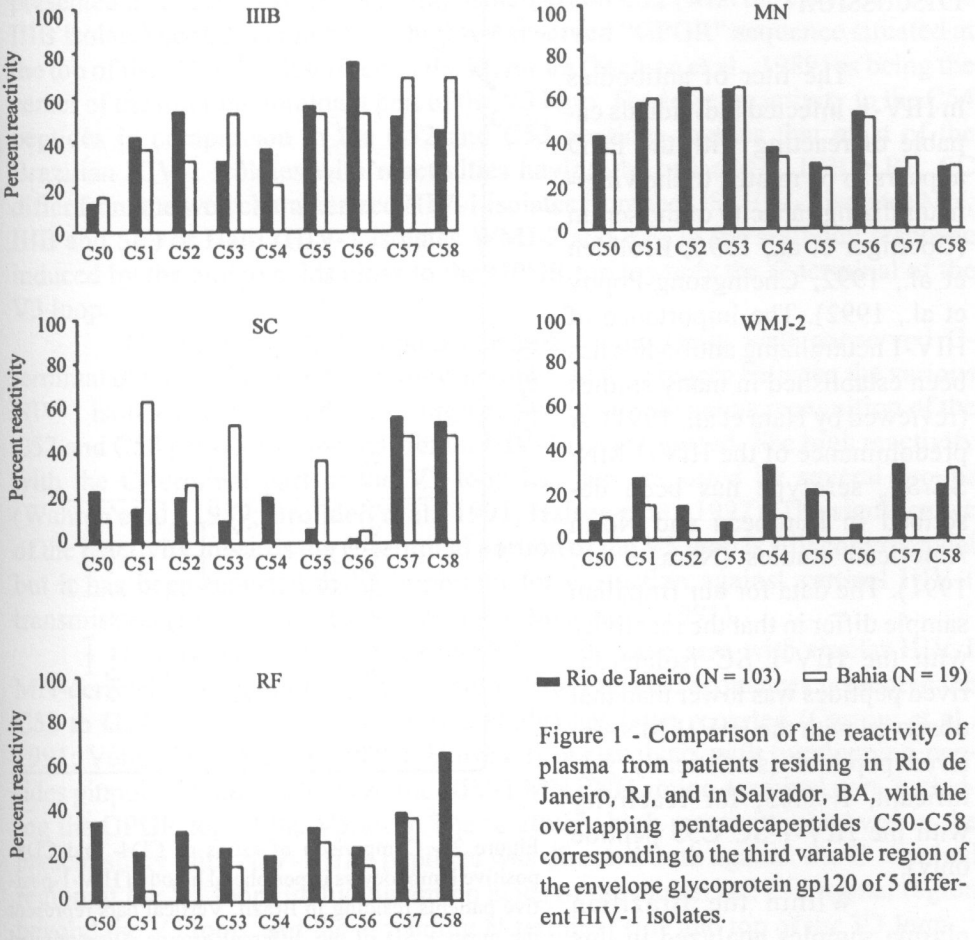

Figure 1 - Comparison of the reactivity of plasma from patients residing in Rio de Janeiro, RJ, and in Salvador, BA, with the overlapping pentadecapeptides C50-C58 corresponding to the third variable region of the envelope glycoprotein gp120 of 5 different HIV-1 isolates.

samples from both sexes (Figure 2, column A). Forty-five samples (47%) had a CD4/CD8 ratio above 0.80 (the lower limit of the CD4/CD8 ratio obtained for non-infected controls), with a mean ± SD value of 1.00 ± 0.18 (N = 40). When the ratios of the 29 most cross-reactive plasmas were analyzed, 17 (59%) showed ratios above 0.80, with mean values of 0.992 ± 0.16 (Figure 2, column B). In comparison, 13 of the 17 plasmas (77%) reacting primarily with peptides of just one isolate had ratios below the 0.80 limit, with a mean value of 0.522 ± 0.074 (Figure 2, column C). The mean CD4/CD8 ratios of the "strain specific" group of plasmas differed significantly from the total group of plasmas ($P<0.05$).

Discussion

The titer of antibodies in HIV-1-infected individuals capable of reacting with the PND appears to be related to the virus-neutralizing capacity of the serum (Boettiger et al., 1990; Broliden et al., 1992; Cheingsong-Popov et al., 1992). The importance of HIV-1 neutralizing antibodies has been established in many studies (reviewed by Nara et al., 1991). A predominance of the HIV-1 MN/SC/SF_2 serotype has been described in European and North American studies (Nara et al., 1991). The data for our Brazilian sample differ in that the reactivity with the HIV-1 SC isolate-derived peptides was lower than that observed with the HIV-1 MN-derived peptides (statistically significant, $P<0.05$, for reactivity with the HIV-1 SC C55 peptide only).

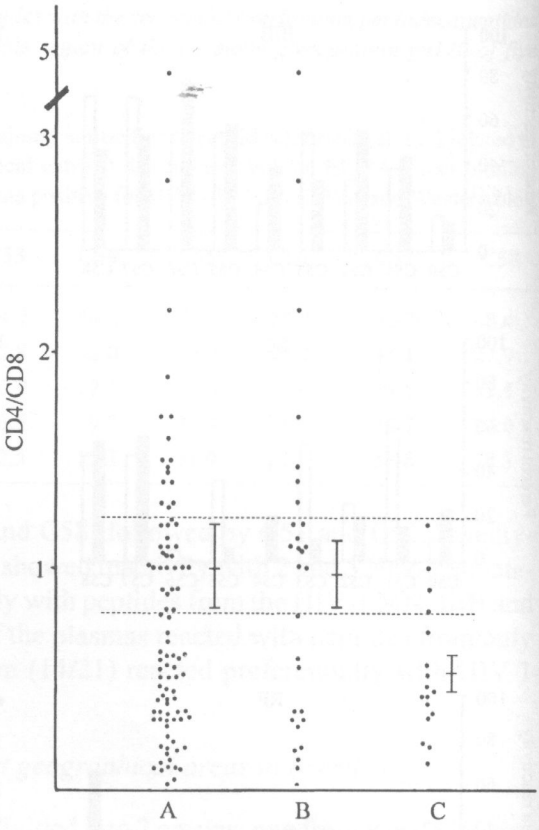

Figure 2 - Comparison of ratios of CD4- and CD8-positive lymphocytes in peripheral blood of HIV-1-positive patients residing in Brazil. Vertical bars represent the mean ± SD of the different groups. Discontinued lines indicate normal control values. *A*, Total population studied (N = 93); *B*, plasmas with high cross-reactivity with gp120 V3 loop peptides (29/93); *C*, plasmas with reactivity essentially restricted to peptides from only one HIV-1 isolate (17/93).

Within the Brazilian plasma samples analyzed in this study, those from Salvador, BA, did appear to present a more "European/North-American" reactivity than those from Rio de Janeiro, RJ, since they presented similarly high titers for peptides derived from both MN and SC isolates (no statistical analysis carried out).

In our Brazilian sample, highest percent reactivity was observed with peptides C51, C52 and C53 of the HIV-1 MN isolate, and with peptides C56, C57 and C58 of the HIV-1 isolate IIIB, followed by recognition of peptides C57 and C58 of the HIV-1 isolates RF and SC. As can be observed from the sequences

presented in Table 1, the C53, C54 (all isolates) and C52 (with the exception of the IIIB isolate) peptides contain the highly conserved "GPGR" sequence situated at the top of the V3 loop, described in the literature (Meloen et al., 1989) as being the center of the immunodominant part of the V3 loop. The lower reactivity to the C54 peptides in comparison to the C52 and C53 peptides implies that most of the Brazilian HIV-1 isolates with reactivities having the order MN, IIIB > RF, SC differ from the well characterized HIV-1 isolates from the US (HIV-1 isolates MN, IIIB and SC) or Haiti (HIV-1 isolates WMJ-2 and RF) in the antibody response induced by the amino acids close to the GPGR top towards the C-terminal of the V3 loop.

The C57 and C58 peptides belong to the fairly well conserved C-terminal of the V3 loop, with very few amino acid differences between the various HIV-1 isolates, as reflected by the high degree of simultaneous recognition of the C57 and C58 peptides of the 5 different HIV-1 isolates tested. The high reactivity with the C-terminal part of the V3 loop has been reported by several groups (Wahren et al., 1989; Broliden et al., 1991; Halsey et al., 1992). The significance of the reactivity towards the C-terminal portion of the V3 loop is still controversial but it has been reported to be important for protection against vertical HIV-1 transmission (Rossi et al., 1989; Rossi and Moschese, 1991).

Strong reactivity of Brazilian HIV-1 positive sera with similar HIV-1 MN-derived PND peptides (corresponding to amino acid sequences present in the C52 to C54 peptides described in this study) has been reported (Carrow et al., 1991; Vanderborght et al., 1991). However, our analysis with overlapping peptides pinpointed the reactivity to the HIV-1 MN PND amino-terminal part, including the GPGR top of the V3 loop. The relatively high reactivity observed in the present study with HIV-1 IIIB peptides was directed both against the C-terminal region of the V3 loop, including amino acids of the conserved C-terminal region beyond the V3 loop, and the ascending N-terminal side and top of the V3 loop.

High variability in the extent of reactivity of patient plasma with different PND-derived peptides of 10 to 19 amino acids, including the GPGR top of the loop with vicinal amino acids (commercial "V3 peptides") has been reported and depends on the population studied (Wahren et al., 1989; Carrow et al., 1991; Vanderborght et al., 1991; Broliden et al., 1992; Boudet et al., 1992; Cheingsong-Popov et al., 1992). Nevertheless, the higher reactivity for HIV-1 MN peptides, for example, appears to be a world-wide observation, including Africa (Nara et al., 1991), with most studies reporting reactivity exceeding 95% of the sera tested. Although in the present study reactivity with corresponding PND peptides (C52, C53) was highest with HIV-1 MN-derived peptides (62%) and our results are similar to those reported for Brazilian sera by Carrow et al. (1991), it is also clear

that fewer Brazilian sera recognize the HIV-1 MN PND than do European/North American or African sera. A study comparing ELISA data with actual neutralization of HIV-1 MN needs to be carried out to determine the significance of the lower extent of MN PND recognition by Brazilian sera.

An attempt to correlate the serological results obtained in this study with specific clinical data collected for each patient demonstrated that a more "isolate-restricted" seroreactivity was accompanied by a lower CD4/CD8 ratio ($P<0.05$), as shown in Figure 2, column C. Page et al. (1992) reported a similar restriction of reactivity with PND in Western blots linked with progression to AIDS. These results appear to agree with data obtained recently (R.M. Hendry, personal communication) showing a positive correlation between degree of cross-neutralization of different HIV-1 isolates and a lesser reduction of CD4-positive lymphocyte numbers present in peripheral blood of HIV-1-infected individuals.

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References

Ayehunie S, Johansson B, Sönnerborg A, Zewdie DW, Britton S & Strannegard Ö (1993). Sequence analysis of selected regions of the env (V3 loop and gp41) and gag (p7) reaging frames of Ethiopian human immunodeficiency virus type 1 strains. *Archives of Virology*, 128: 229-239.

Boettiger B, Karlsson A, Andreasson PA, Naucler A, Costa CM, Norrby E & Biberfeld G (1990). Envelope cross-reactivity between human immunodeficiency virus type 1 and 2 detected by different serological methods: correlation between cross-neutralization and reactivity against the main neutralizing site. *Journal of Virology*, 64: 3493-3499.

Boudet F, Girard M, Theze J & Zouali M (1992). Antibodies of HIV-1 positive subjects and experimentally immunized primates and rodents bind to sequence divergent regions of the third variable domain (V3) of gp120. *International Immunology*, 4: 283-294.

Broliden P-A, Mäkitalo B, Akerblom L, Rosen J, Broliden K, Utter G, Jondal M, Norrby E & Wahren B (1991). Identification of amino acids in the V3 region of gp120 critical for virus neutralization by human HIV-1 specific antibodies. *Immunology*, 73: 371-376.

Broliden P-A, Von Gegerfelt A, Clapham P, Rosen J, Fenyö EM, Wahren B & Broliden K (1992). Identification of human neutralization-inducing regions of the human immunodeficiency virus type 1 envelope glycoproteins. *Proceedings of the National Academy of Sciences, USA*, 89: 461-465.

Carrow EW, Vujcic LK, Glass WL, Seamon KB, Rastogi SC, Hendry RM, Boulos R, Nzila N & Quinnan Jr GV (1991). High prevalence of antibodies to the gp120 V3 region principal neutralizing determinant of HIV-1 MN in sera from Africa and the Americas. *AIDS Research and Human Retroviruses*, 7: 831-838.

Cheingsong-Popov R, Callow D, Beddows S, Shaunak S, Wasi C, Kaleebu P, Gilks C, Petrascu IV, Garaev MM, Watts DM, Constantine NT & Weber JN (1992). Geographic diversity of Human Immunodeficiency Virus Type 1: serologic reactivity to env epitopes and relationship to neutralization. *Journal of Infectious Diseases*, 165: 256-261.

Cheingsong-Popov R, Bobkov A, Garaev MM, Kaleebu P, Callow D, Rzhimonova A, Saukhat SR, Burdajev NP, Kolomijets ND & Weber JN (1993). Identification of Human Immunodeficiency Virus Type 1 subtypes and their distribution in the Commonwealth of Independent States (Former Soviet Union) by serologic V3 peptide-binding assays and V3 sequence analysis. *Journal of Infectious Diseases*, 168: 292-297.

Goudsmit J, Debouck C, Meloen RH, Smit L, Bakker M, Asher DM, Wolff AW, Gibbs CJ & Gajdusek DC (1988). Human Immunodeficiency Virus Type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. *Proceedings of the National Academy of Sciences, USA*, 85: 4478-4482.

Halsey NA, Markham R, Wahren B, Boulos R, Rossi P & Wigzell H (1992). Lack of association between maternal antibodies to V3 loop peptides and maternal-infant HIV-1 transmission. *Journal of the Acquired Immunodeficiency Syndrome*, 5: 153-157.

La Rosa GJ, Davide JP, Weinhold K, Waterbury JA, Profy AT, Holley LH, Karplus M, Bolognesi DP, Matthews TJ, Emini EA & Putney SD (1990). Conserved sequence and structural elements in the HIV-1 principal neutralizing determinant. *Science*, 249: 932-935.

Meloen RH, Liskamp RM & Goudsmit J (1989). Specificity and function of the individual amino acids of an important determinant of Human Immunodeficiency Virus Type 1 that induces neutralizing activity. *Journal of General Virology*, 70: 1505-1512.

Murphy E, Korber E, Georges-Courbot MC, You B, Pinter A, Cook D, Kieny M, Georges A, Mathiot C, Barré-Sinoussi & Girard M (1993). Diversity of the V3 region sequences of Human Immunodeficiency Viruses Type 1 from the Central African Republic. *AIDS Research and Human Retroviruses*, 9: 997-1006.

Nara PL, Garrity RR & Goudsmit J (1991). Neutralization of HIV-1: a paradox of humoral proportions. *FASEB Journal*, 5: 2437-2455.

Page M, Vella C, Corcoran T, Dilger P, Ling C, Heath A & Thorpe R (1992). Restriction of serum antibody reactivity to the V3 neutralizing domain of HIV gp120 with progression to AIDS. *AIDS*, 6: 441-446.

Pau CP, Lee-Thomas S, Auwanit W, George J, Ou CY, Parekh BS, Granade TC, Holloman DL, Phillips S, Schochetman G, Young NL, Takebe Y, Gayle HD & Weniger BG (1993). Highly specific V3 peptide immunoassay for serotyping HIV-1 specimens from Thailand. *AIDS*, 7: 337-340.

Rossi P & Moschese V (1991). Mother-to-child transmission of human immunodeficiency virus. *FASEB Journal*, 5: 2419-2426.

Rossi P, Moschese V, Broliden PA, Fundaro C, Quinti L, Plebani A, Giaquinto C, Tovo PA, Ljunggren K, Rosen J, Wigzell H, Jondal M & Wahren B (1989). Presence of maternal antibodies to human immunodeficiency virus type 1 envelope glycoprotein gp120 correlates with the uninfected status of children born to seropositive mothers. *Proceedings of the National Academy of Sciences, USA*, 86: 8055-8058.

Taylor JMG, Fahey JL, Detels R & Giorgi JV (1989). CD4 percentage, CD4 numbers and CD4:CD8 ratio in HIV infection: which to choose and how to use. *Journal of Acquired Immune Deficiency Syndrome*, 2: 114-124.

Vanderborght B, Santos EA, Alcantara VD, Cassalta RM, Bonecini MG, Sion FS & Morais-de-Sá CA (1991). Differences in V3 reactivities among HIV-1 infected patients: correlation with HIV-2 cross-reactivity. *First CEC-FIOCRUZ Joint Meeting, Rio de Janeiro, RJ, Brazil*, Abstract 32.

Wahren B, Rosen J, Sandstroem E, Mathiesen T, Modrow S & Wigzell H (1989). HIV-1 peptides induce a proliferative response in lymphocytes from infected persons. *Journal of Acquired Immune Deficiency Syndrome*, 4: 448-456.

Weiss RA, Clapham PR, Weber JN, Dalgleish AG, Lasky LA & Berman PW (1986). Variable and conserved neutralization antigens of human immunodeficiency virus. *Nature*, 324: 572-575.

Zwart G, Wolfs TFW, Bookelman R, Hartman S, Bakker M, Boucher CAB, Kuiken C & Goudsmit J (1993). Greater diversity of the HIV-1 V3 neutralization domain in Tanzania compared with The Netherlands: serological and genetic analysis. *AIDS*, 7: 467-474.

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