V3 Peptide Binding Pattern and HIV-1 Transmission Route in Rio de Janeiro

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To characterize antibody binding to a panel of V3 loop peptides representing diverse HIV-1 neutralization epitopes, 149 HIV-1 infected individuals from Rio de Janeiro (RJ) were investigated. Results were analyzed with respect to risk factors for infection and other epidemiological and clinical data. Peptide reactivity was not associated with sex, clinical status, CD4 counts, antigenemia or β2-microglobulin serum level. A segregation of peptide reactivity according to route of infection was encountered. This finding suggests that more then one viral strain may be circulating in RJ, in subjects with different risk factors for HIV-1 infection. An investigation of prevalent HIV-1 genotypes, serotypes and immunotypes may be of importance for the design and selection of potential vaccines to be used in Brazil as well as for the selection of populations to be included in future vaccine efficacy trials.

Key words: HIV - AIDS - V3 loop - antigenic variation - Brazil

Human Immunodeficiency Virus type-1 (HIV-1) shows a high degree of genetic and antigenic polymorphism. Variability has been described between isolates from different geographic areas (Cheingsong-Popov et al. 1992) and within infected individuals during the course of infection (Holmes et al. 1992, Korber et al. 1992). Genetic diversity between HIV-1 isolates is not uniformly distributed along the viral genome, being more prominent within certain regions of the external envelope glycoprotein (hypervariable regions V1-V4 of gp120). The HIV-1 principal neutralizing determinant (PND) is situated in the third hypervariable domain (V3 loop) of gp120 (Rusche et al. 1988). The V3 loop is an important epitope for neutralization, viral tropism, host range and syncytium inducing capability (Cann et al. 1992). An epitope localized at its tip acts as the major binding site for type-specific neutralizing antibodies (Gouldsmit et al. 1988), and anti-V3 loop antibodies possess protective capabilities (Neurath et al. 1991).

Enzyme immunoassays based on synthetic peptides derived from known V3 loop sequences have been developed. The existence of serologic diversity and its geographic distribution have been demonstrated (Cheingsong-Popov et al. 1992). In a study in Thailand, results obtained suggest that peptide based assays are able to discriminate by serum reactivity between two genotypes in HIV-1 infected individuals (Pau et al. 1993). In another study utilizing sera obtained in several countries, it was demonstrated that serum reactivity to different peptides correlates with major phylogenetic subgroups (Cheingsong-Popov et al. 1994). Thus, albeit subject to limitations (Moore et al. 1994), available data indicate that investigation of seroreactivity to synthetic peptides might be a rapid, easy-to-perform and relatively inexpensive epidemiologic tool for the investigation of circulating viral strains (Cheingsong-Popov et al. 1992, 1994).

To investigate and characterize serum binding to peptides representing diverse HIV-1 V3 neutralization epitopes, serum samples (n=149) were collected between August and December, 1991, from outpatients attending a prospective cohort study of HIV-infection in Rio de Janeiro (RJ). One hundred and thirty four subjects acquired HIV infection through sexual intercourse, and 15 reported parenteral risk factors (IV drug use or blood transfusion). The clinical stage for each subject was determined using the WHO staging system (WHO 1986). Lymphocyte subset determination (CD4 and CD8) was performed by FACScan (Becton-Dickinson, USA). Serum β2-microglobulin (Pharmacia Diagnostics, Sweden), and p24 antigen after acid hydrolysis (Coulter, USA) were also measured. In addition, for comparison purposes, sera from HIV-1 infected subjects from the United States of America (USA) (n=105) and the United Kingdom (UK) (n=103) were also analyzed.

Reactivity to V3 loop peptides (MN, HXB2,

Z3, Z321, Z6, and MAL) was assessed by EIA (Cheingsong-Popov et al. 1992). Of the Brazilian sera, 102 (68.5%) reacted to MN, 43 (28.9%) to Z3, 18 (12.1%) to HXB2, 19 (12.1%) to Z321, 16 (10.7%) to MAL, 10 (6.7%) to Z6, whereas 35 (23.5%) did not react with any of the peptides tested. HIV-1 Western Blot was performed with all 35 peptide-negative samples and 30/35 (87%) of the sera reacted to gp160 and/or gp120 antigens. The remaining 5 sera were HIV-1 Western Blot positive on the grounds of gp41, *pol* and/or *gag* reactivity, but did not react either with gp160 and gp120. HIV-1 PCR on the *pol* gene was undertaken, and was positive in all five patients.

Sera were then analyzed according to the subjects's probable route of infection, sexual or parenteral. The distribution of antibody reactivity to the MN V3 peptide was analyzed, and compared to the distribution in subjects from the UK and USA. Differences in MN reactivity in sera from subjects from RJ were demonstrated, in relation to route of transmission. In the sexual transmission group, 86/134 (64%) subjects had binding antibodies to the MN peptide. In the parenteral group, 14/15 (93%) sera were MN reactive (p < 0.03, 2-tailed Fisher's exact test). In contrast, 99-100% of all sera from HIV-1 infected subjects from the UK and the USA had binding antibodies to the MN V3 peptide (Cheingsong-Popov et al. 1992). Peptide reactivity was not associated with sex, clinical status, CD4 counts, antigenemia or $\beta 2$-microglobulin serum level.

In summary, 68.5% of the Brazilian sera tested reacted to the MN peptide, a distribution of binding antibodies that differs greatly from that seen with North American and European sera. Similar results have been previously described (Carrow et al. 1991, Bongertz et al. 1994). In addition, over 35% of the Brazilian sera tested (109/296) did not react to any of the peptides, although the great majority of the negative sera did contain detectable antibodies to *env* products, gp160/120. In previous studies, utilizing sera from several countries, all sera tested were found to contain antibodies to at least one of the peptides of this panel (Cheingsong-Popov et al. 1992). A segregation of peptide reactivity according to route of infection was encountered. This finding suggests that more then one viral strain may be circulating in RJ, in subjects with different risk factors for infection, similar to that which has been described in Thailand (Pau et al. 1993). In fact, sequence analysis of the V3 loop of Brazilian isolates has indicated the heterogeneity of the viral strains circulating in Brazil (Potts et al. 1993, Morgado et al. 1994, Janini et al. unpublished data). HIV-1 V3 sequences have been further analyzed in a phy-

logenetic tree analysis and some isolates have been shown to belong to a new F subgroup (Morgado et al. 1994). However, there are no substantial studies to correlate the genotypes of HIV-1 variants from Brazil, their antigenicity and antibody response. Our serological data on V3 peptide binding support the heterogeneous sequence data, further strengthening the study of genetic diversity, by providing confirmation on antigenic diversity. An investigation of HIV-1 V3 genotypes, serotypes and immunotypes in Brazil will be important for the design and selection of potential vaccines to be used in Brazil as well as for the selection of populations to be included in future vaccine efficacy trials. This type of serological screening may allow population based studies of HIV-1 diversity to be undertaken, and to point to populations where more detailed sequencing information may be of most value.

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