

PATTERNS OF SEROLOGIC RESPONSE TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) IN BRAZILIANS WITH DIFFERENT CLINICAL FORMS OF HIV INFECTION

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In order to investigate the IgG HIV-1 antibodies reactivity to structural components of the virus, 85 sera from infected Brazilians, comprising the total spectrum of HIV infection, were analysed by Western blot assay. The sera were confirmed as being positive to HIV with enzyme linked immuno assay (ELISA) and indirect immunofluorescence (IIF). Although the sera from patients reacted less intensively to the gag polypeptide of 55 KDa, no distinctive antigen reaction patterns were observed between sera patients with different clinical forms. Because of the higher frequency of reactivity to the gag p24 in AIDS patients, the patterns of anti-HIV IgG responses are similar to those observed in their African counterparts.

Key words: AIDS – HIV – serology – Western blot

Information of the frequency and intensity of serum reactivity to different Human Immunodeficiency Virus (HIV) antigens is of considerable importance for the assessment of the accuracy of confirmatory serologic diagnostic as for the identification of those viral proteins relevant in the induction of an humoral immune response in the course of the infection. Although studies have already been done on the serological response in Americans, Europeans and Africans with different clinical forms of the HIV infection (Groopman et al., 1986; Schupbach & Tanner, 1986), these have not been performed in Brazilians yet.

In this communication, we report our results on the reactivity of antibodies to different viral proteins in representative samples of sera from Brazilians infected with HIV.

MATERIAL AND METHODS

Serum samples – Sera were studied from patients with Acquired Immunodeficiency Syndrome (AIDS, 21 cases), AIDS-related complex (ARC, 17 cases), persistent generalized

lymphadenopathy (PGL, 27 cases) and 20 HIV-infected but asymptomatic persons, all from the groups at high risk for AIDS. Clinical criteria for evaluation of disease state, PGL, ARC and AIDS followed the criteria recommended by the Centers for Disease Control (CDC's, Atlanta, USA, 1986). All were found to be positive in enzyme linked immuno assay (ELISA), Weiss et al., 1985) and indirect immunofluorescence (IIF, Sandstrom et al., 1985). As negative controls, serum samples from 6 healthy individuals with no evidence of HIV infection were also tested.

ELISA – Commercially available direct binding assays using sucrose-gradient purified HIV as antigen (Organon Teknika, Boxtel, Holland and Electro-nucleonics Inc., Columbia, USA) were used to screen the sera. The performance of this assay and the interpretation of the results was done according to manufacturer's instructions.

IIF – This test was utilized for confirmation of ELISA-positive results and was carried out using both H9 infected and uninfected cells, kindly provided by Dr R. C. Gallo (National Institute of Health, Bethesda, MD, USA). The cells were apposed onto microscopic slides in such a concentration as to show an almost confluent monolayer. After room temperature drying and acetone-fixation procedures, the slides were incubated with the test sera at a

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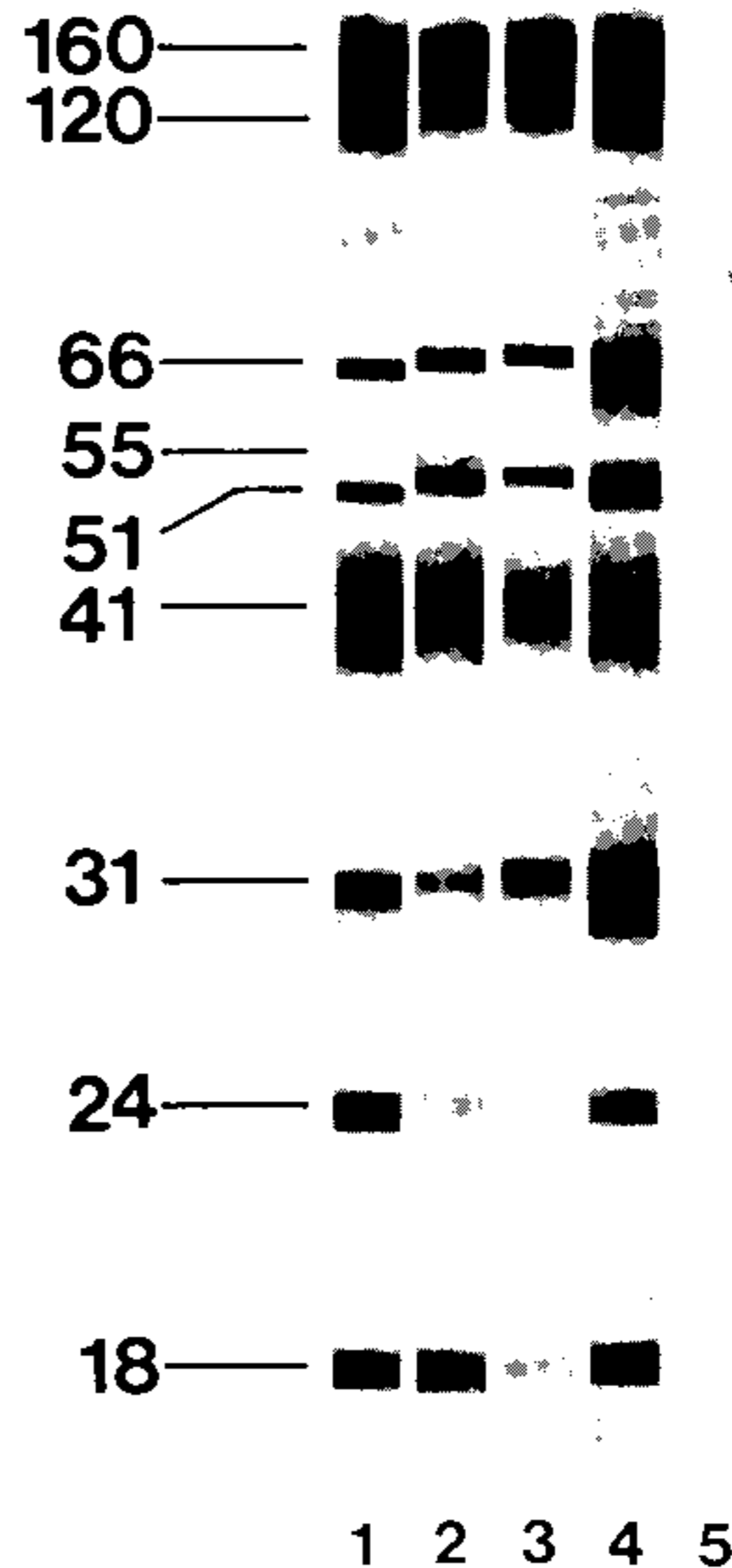
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1:8 dilution for 60 min at 37 °C. After three washings with phosphate buffered saline (PBS), the slides were incubated at room temperature with a fluorescein labelled anti-human IgG (Biolab-Merieux, Rio de Janeiro, Brazil) for 60 min. The slides were then rinsed three times five min each with PBS, and read on a Leitz epifluorescence microscope.

Immunoblot analysis — A commercially available Western blot assay (Du Pont Company, Wilmington, Delaware, USA) was used to evaluate the sera's antibody response to the different proteins of the virus (Sarnaghadaran et al., 1984). This assay was done according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Representative immunoblot profiles of sera from Brazilians with different clinical forms of the HIV infection are shown in the Fig. The viral components that have reacted most strongly and most frequently in the sera were the env glycoproteins of 160 and 120 KDa, and the pol protein of 66 KDa (Table). Most of sera (81%) from AIDS patients did not react with the gag protein of 55 KDa. This could be explained by lower concentration of p55 in the antigen sample. Indeed, these AIDS sera reacted with gag p55 when we used our "home-made" Western blot assay (Ivo-dos-Santos & Galvão-Castro, submitted for publication). On the other hand, most of them (86%) reacted to the gag p24. With regard to reactivity to this viral component differences were observed when sera from the American and European patients, and African ones were studied. In the majority of the first ones p24 antibodies disappears from circulating blood in the terminal phase of the disease (Lange et al., 1986). In contrast, it has been reported that African AIDS patients have antibodies to p24 during almost the whole course of the disease (Barin, 1987). Despite these aspects the overall pattern of Western blot reactivity was similar to that seen in patients from Western countries. However, in order to achieve a better knowledge of the humoral responsiveness in HIV infected Brazilians, kinetic studies must be carried out to verify if there is any change in the antibody levels to the different components in the course of the infection.



Reactivity of IgG antibodies to HIV antigens electroblotted onto nitrocellulose membranes. Antigens recognized by antibodies in sera from patients with AIDS (1), ARC (2), PGL (3), asymptomatic (4) and normal human serum (5). Numbers represent molecular weights $\times 10^{-3}$

TABLE

Reactivity of sera from different clinical forms of HIV infection to different viral proteins

Bands M. W. (kDa)	Clinical forms			
	AIDS 21*	ARC 17	PGL 27	ASYMP 20
gp 160	20** (95)	17 (100)	27 (100)	20 (100)
gp 120	20 (95)	17 (100)	27 (100)	19 (95)
p 66	21 (100)	16 (94)	27 (100)	20 (100)
p 55	4 (19)	10 (59)	11 (40)	13 (65)
p 51	17 (81)	15 (88)	27 (100)	20 (100)
gp 41	20 (95)	15 (88)	27 (100)	16 (80)
p 31	18 (86)	14 (82)	26 (96)	15 (75)
p 24	17 (81)	17 (100)	26 (96)	19 (95)
p 18	15 (71)	8 (47)	22 (81)	16 (80)

* Number of sera from each group.

** Number of positive reactions.

Number in parenthesis means percent of positivity.

M. W. = molecular weight.

There is an assumption that the interpretation of results of Western blot must be scored on the number and intensity of virus specific bands, being considered positive those sera that present reactivity to gag, pol and env products (World Health Organization, 1987). Our data also indicate that the use of a licensed commercial Western blot assay such as that we have utilized in this study showed a close correlation of results with other serological tests such as IIF, as has been previously demonstrated (Gallo et al., 1986; Carlson et al., 1987). However, in order to obtain complete information of the real specificity of these assays, must be necessary to test serum samples from patients with local endemic infections, such as Chagas' disease, leishmaniasis, schistosomiasis or malaria.

RESUMO

Padrões de resposta sorológica ao vírus da imunodeficiência humana do tipo 1 (HIV-1) em brasileiros com diferentes formas clínicas da infecção — Com o objetivo de avaliar a resposta de anticorpos da classe IgG a componentes estruturais do vírus, 85 soros de pacientes brasileiros, compreendendo todo o espectro da infecção pelo HIV foram analisados pela técnica de Western blot. Os soros foram confirmados como positivos pelas técnicas de imunofluorescência indireta e imunoenzimática. Embora os soros de alguns pacientes estudados reagissem menos intensamente com o polipeptídeo de 55 KDa, não observamos nenhuma diferença de reatividade entre os soros de pacientes com as diversas formas clínicas. Entretanto, a grande frequência de reatividade ao polipeptídeo de 24 KDa nos pacientes com AIDS sugere que o padrão de resposta imune seja similar aos pacientes Africanos.

Palavras-chave: AIDS — HIV — sorologia — Western blot

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