

## ISOLATION AND ANTIGENIC CHARACTERIZATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN BRAZIL

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*A retrovirus infecting a Brazilian AIDS patient was isolated and characterized in terms of its reactivity with sera from individuals infected with human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2). The Western blot analysis revealed that the Brazilian isolate is very similar to the well characterized HIV-1 strain. The serum of the patient from whom the virus was isolated did not react with the 140 kDa envelope glycoprotein specific for HIV-2.*

Key words: human immunodeficiency virus – isolation – antigens – Brazil

The acquired immunodeficiency syndrome (AIDS) is a disease caused by retroviruses with tropism for CD<sub>4</sub><sup>+</sup> lymphocytes (Barré-Sinoussi et al., 1983; Popovic et al., 1984; Clavel et al., 1986). AIDS was first reported in Brazil in 1982. Since then, the number of AIDS cases in this country have almost reached 2,000 cases, the majority occurring in homosexual/bisexual males, intravenous drug abusers and blood or blood-product recipients (Boletim do Ministério da Saúde, 1987).

So far, different retroviruses have been isolated from human subjects. The HIV-1 was first isolated in Europe and USA (Barré-Sinoussi et al., 1983; Popovic et al., 1984), HIV-2 in patients from West Africa (Clavel et al., 1986) and HTLV-IV in African healthy individuals (Kanki et al., 1987).

Up to now, there are no reports of the isolation of HIV in Brazil. However, serological evidence strongly suggests the presence of at least HIV-1 and HIV-2 in this country (Galvão-Castro et al., 1987; Veronesi et al., 1987). The isolation of HIV viruses in Brazil is important in order to establish the degree of antigenic and genomic homology between strains from this and other countries. The isolation and partial antigenic characterization of a HIV-1 virus from a Brazilian patient with AIDS is described in this paper.

### MATERIAL AND METHODS

**Patient** – The patient was a heterosexual, white, male, 24-year-old injured in a car accident in February, 1985, when he was given blood transfusions. He sought medical attention

in January, 1987 at the Gaffrée-Guinle Hospital, Rio de Janeiro, Brazil. Clinical and laboratory examination showed persistent fever, loss of more than 10% of body weight in one month, diarrhea, oral candidiasis, intestinal cryptosporidiosis and renal tuberculosis (CDC's classification group, 4A,C1,C2, MMWR, 1986). Skin test anergy to candidin, PPD, Streptokinase and Streptodornase and Tricophytin, leucopenia, lymphocytopenia, depletion in CD4<sup>+</sup> cells and inversion of the CD4/CD8 ratio were also observed. Serum HIV antibodies were detected by enzyme-linked sorbent assay (ELISA, Organon, Teknika, Vironostika, Holland) indirect immunofluorescence (IIF, Sandstrøm et al., 1985) and Western blot (WB, Schupbach et al., 1984).

**Origin and maintenance of cell lines and HIV** – Two neoplastic aneuploid T helper cell lines derived from individuals with lymphoid leukemia were used. HIV-1-infected and uninfected H9 cells (Popovic et al., 1984) and HIV-2-infected or uninfected CEM cells (Foley et al., 1965) were kindly provided by Dr. R.C. Gallo (National Cancer Institute, Bethesda, MD) and Dr. R. Weiss (Imperial Cancer Research Fund., London, UK), respectively. The cellular lines were maintained in RPMI-1640 medium supplemented with glutamine, penicillin, streptomycin (Gibco Diagnostics, USA) and 10% fetal bovine serum (Flow Laboratories, USA). The cells were sedimented and suspended in fresh medium every three or four days.

**Virus isolation** – Both the patient's peripheral blood lymphocytes and normal human T lymphocytes were stimulated with phytohemagglutinin-P (PHA, Sigma Chem. Co., USA) and then cocultured as described by Barré-Sinoussi et al. (1983). Interleukin-2, kindly provided by Dr. Sergio Coutinho, Instituto Oswaldo Cruz, and alfa-interferon antiserum kindly provided by J.C. Chermann, Institute Pasteur, Paris, were added to the co-culture medium. The cultures

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were monitored for viral antigen in the supernatant by ELISA (Innostest VCA HIV, Innogenetics, Belgium), kindly provided by Dr. Hugo Van Heuverswyn, following manufacturer's instructions. Once HIV antigens were detected in the culture supernatant, it was added to H9 cells. Apart from antigen detection, the infection of the cells was also evaluated by IIF and thin section electron microscopy (E.M.).

**Antigenic characterization** – In order to obtain viral antigen, the culture fluids from H9 infected with the Brazilian HIV isolate were submitted to differential centrifugation (Ivodos-Santos, manuscript in preparation). Briefly, the infected cultures were sequentially centrifuged at 200 g for 10 min at 4°C and 5,000 g for 30 min at 4°C. The supernatant was centrifuged at 41,000 g/2 hours/4°C in order to pellet the virus. The pelleted virus was assayed by Western blot (Towbin et al., 1979): a sample of semi-purified virus was dissociated in Sodium Dodecyl Sulphate (SDS) and 2-mercaptoethanol and electrophoresed by SDS-polyacrylamide gel electrophoresis (PAGE) on a 10% acrylamide gel. The protein bands were then transferred to nitrocellulose paper (Schleicher and Schuell, Dassel, FRG) in a Transblot apparatus (BioRad Laboratories, USA). The nitrocellulose paper was blocked in a 0.3% Tween-20/PBS solution and the strips were incubated with serum samples diluted 1:100 in the same buffer, for 90 min. All incubations and washings were carried out at room temperature. The strips were then incubated with a peroxidase conjugate of goat anti-human IgG diluted 1:1,000 for 60 min. After washing, the strips were incubated with a chromogenic substrate, consisting of Diaminobenzidine 0.25 mg/ml/citrate-phosphate buffer, pH 5.0 and H<sub>2</sub>O<sub>2</sub> 0.001%. The reaction was stopped by immersion of the strips in distilled water. H9 and CEM lymphoblastoid cultures infected or uninfected with known HIV-1 and HIV-2, respectively, were tested in parallel as controls.

## RESULTS AND DISCUSSION

**Virus isolation** – As shown in Fig. 1, viral antigens were first detected on the ninth days of cocultivation. Two days later, the positive supernatants were added to uninfected H9 cells. The viral antigen concentration increased until the fifteenth day and subsequently reached a plateau, with fluctuations. On the 23rd day, the majority of the cells (9,5%) showed the characteristic membrane staining pattern of immunofluorescence shown in Fig. 2 (Sandström et al., 1985). Examination of sections of infected cells by E.M. revealed the presence of mature virions and occasional budding viral particles (Fig. 3).

**Antigenic characterization** – Western blot analysis of concentrates of the Brazilian HIV isolate revealed envelope polypeptides of the same molecular weight as those of HIV-1, but different from HIV-2. Indeed, the serum from this Brazilian patient reacted with the 41 kDa transmembrane glycoprotein present in both HIV-1 control and Brazilian isolate, but not with the approximately 140 kDa envelope glycoprotein of the HIV-2 control. On the other hand, when the Brazilian HIV isolate was tested with serum from an individual infected with HIV-2, there was reaction with the viral core polypeptides only (Fig. 4). The methods used in our study are not sufficiently sensitive to detect intra-typic differences. The present results indicate that the virus isolated from a Brazilian patient with AIDS in Rio de Janeiro belongs to HIV type 1.

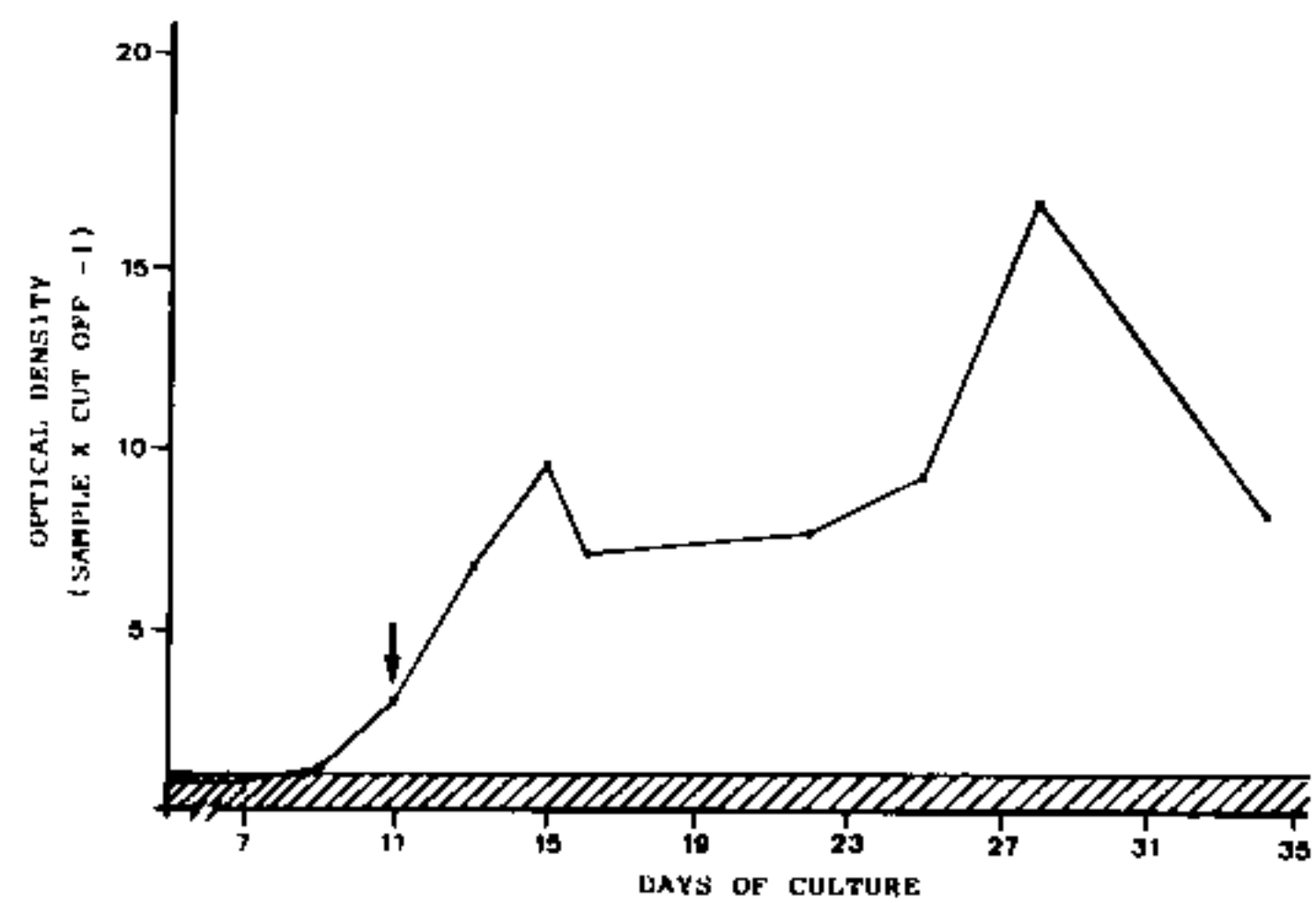


Fig. 1: Evaluation of viral antigen release in infected cell cultures by ELISA capturing test. The arrow indicates the day on which antigen positive supernatants were added to the uninfected H9 cell culture. The hatched area indicates cut off values, as calculated from the optical density produced by uninfected H9 cells.

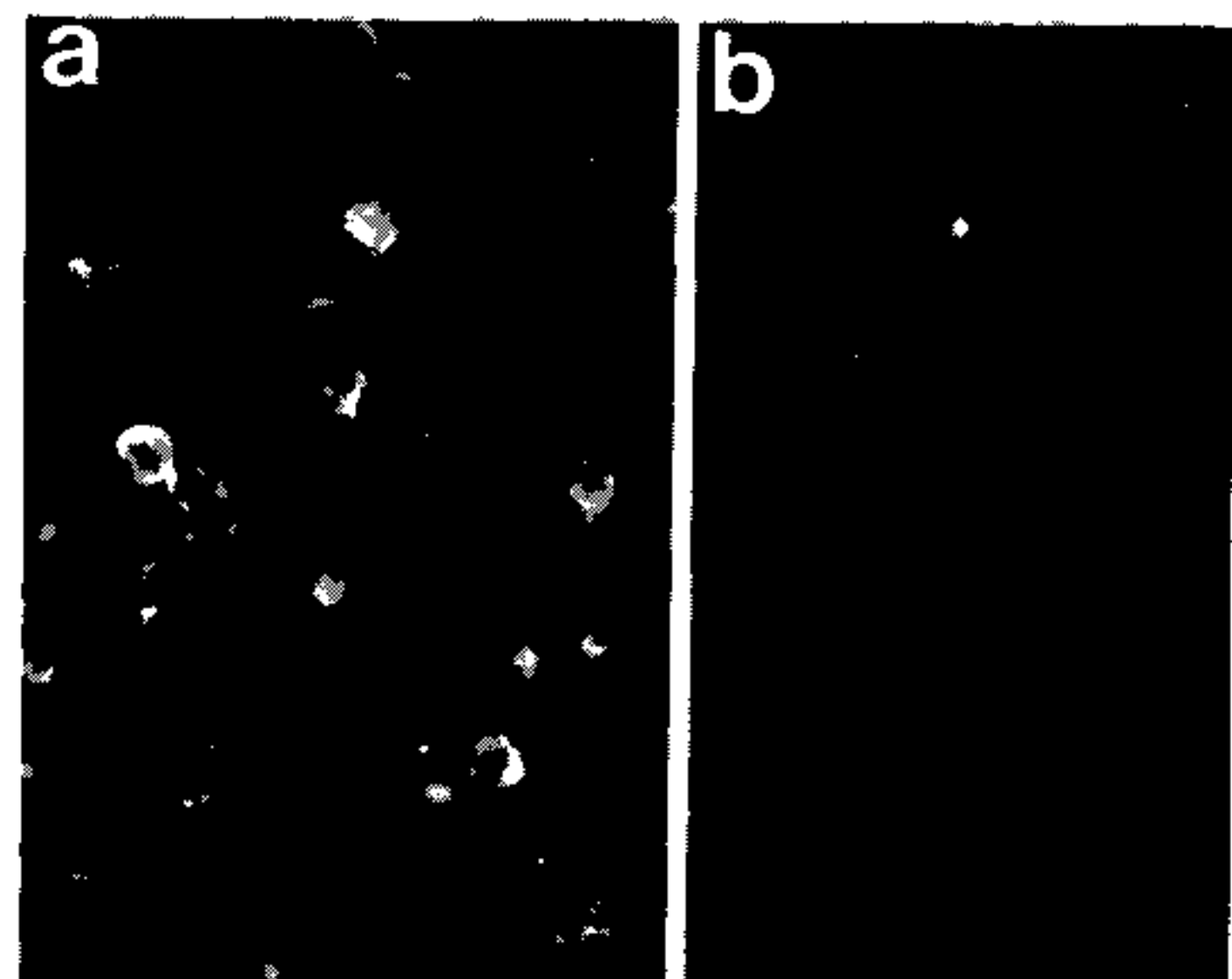


Fig. 2: Indirect immunofluorescence of H9 cells: a) infected with the Brazilian HIV isolate; b) uninfected. Serum containing HIV-1 antibodies was obtained from an asymptomatic blood donor, confirmed HIV-1 positive by ELISA and Western blot.



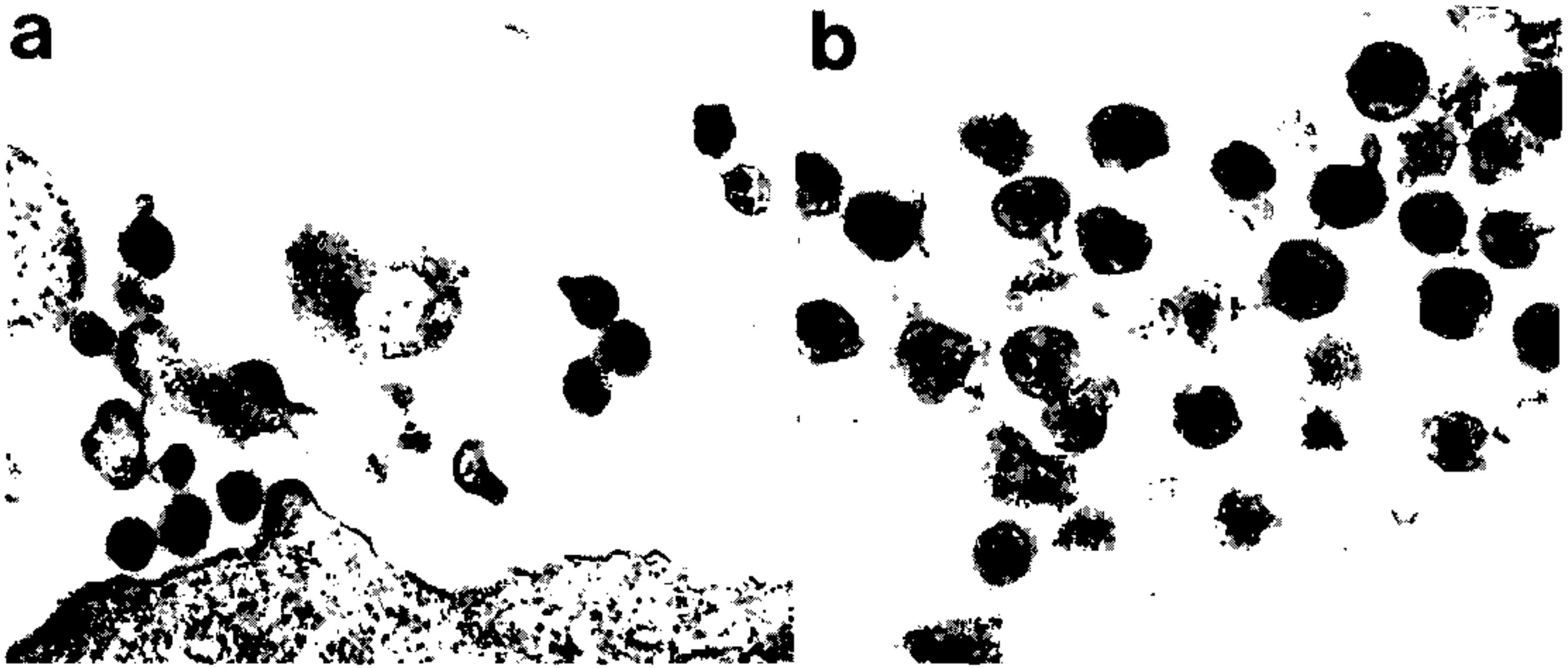


Fig. 3: Thin section of H9 cell culture infected with Brazilian HIV isolate. a) Budding particle at the cell surface and free virus particles. 49200 X. b) Group of mature virus particles with characteristic bar shaped core. 72800 X.

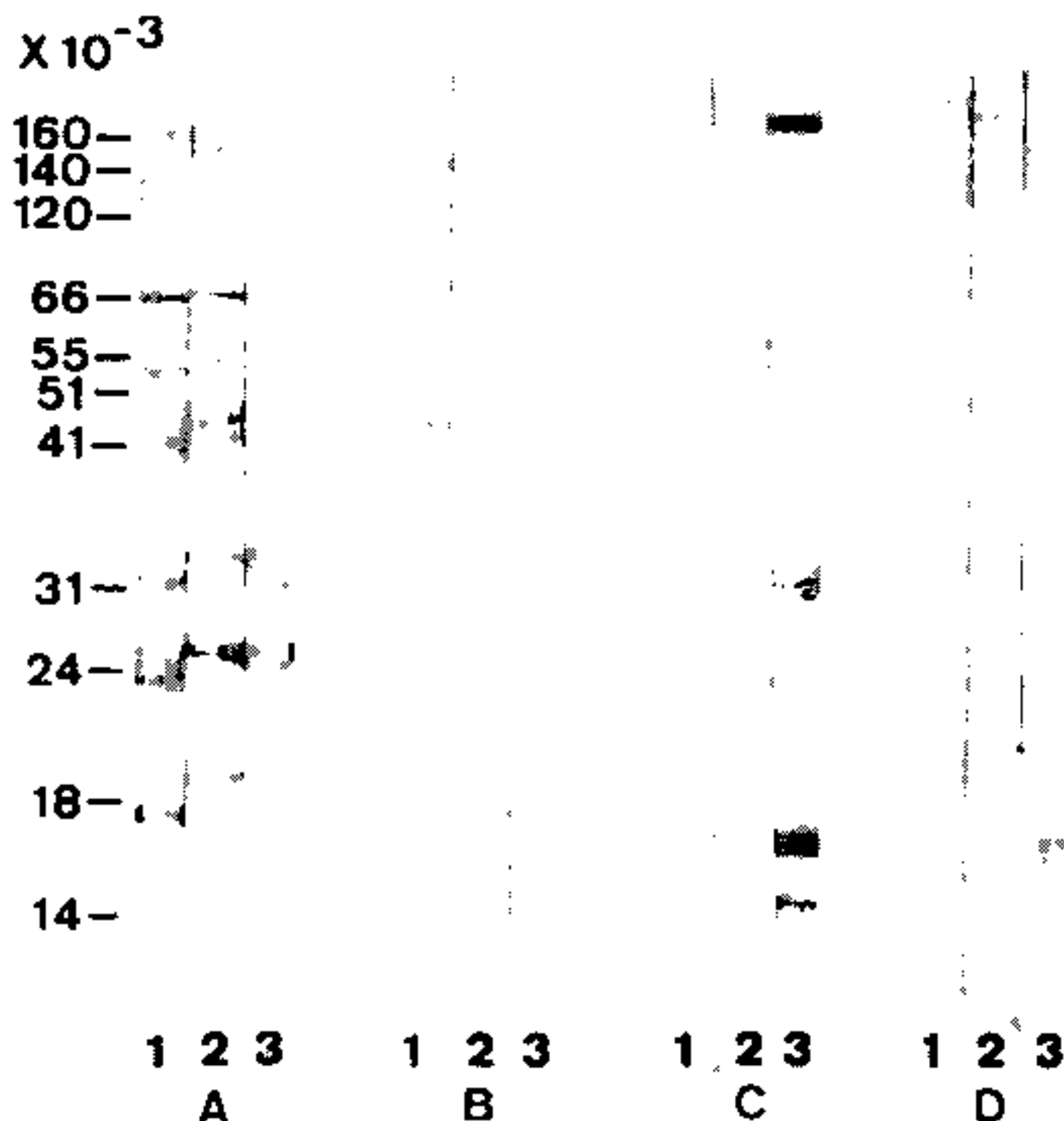


Fig. 4: Western blot of HIV-1 and HIV-2 antigens, (1) HIV-1 sucrose gradient purified ( $3 \mu\text{g}$  protein/strip); (2) Brazilian HIV and (3) HIV-2, both partially purified through differential centrifugation ( $5 \mu\text{g}$  protein/strip). A) serum from asymptomatic individual, B) serum from the patient whom the Brazilian HIV was isolated, C) serum from a HIV-2 infected person and, D) serum from uninfected individual.

To establish the prevalence of different retroviruses associated with AIDS in Brazil and to confirm the occurrence of HIV-2 suggested by the finding of Veronesi et al. (1987), characterization of isolates from different geographical areas and in a significant number will be necessary.

#### RESUMO

**Isolamento e caracterização antigênica do vírus da imunodeficiência humana (VIH/HIV) no**

**Brasil** — Um retrovírus foi isolado de um paciente brasileiro com "Síndrome de Imunodeficiência Adquirida" (SIDA/AIDS) e caracterizado em termos de sua reatividade com soros de indivíduos infectados com vírus da imunodeficiência humana dos tipos 1 e 2 (HIV-1 e HIV-2). A análise antigênica por "Western blot" revelou que o isolado brasileiro é bastante similar a uma cepa de HIV-1 bem caracterizada. A identificação do retrovírus como HIV-1 e não HIV-2 é reforçada pelo fato dos anticorpos do paciente do qual foi isolado o vírus não terem reagido com a glicoproteína de envelope de 140 kDa, específica para HIV-2.

**Palavras-chave:** vírus da imunodeficiência humana — isolamento — antígenos — Brasil

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