Revista da Sociedade Brasileira de Medicina Tropical
29(1):5-9, jan-fev, 1996.

THE INFLUENCE OF CD4+ T CELLS, HIV DISEASE STAGE AND ZIDOVUDINE ON HIV ISOLATION IN BAHIA, BRAZIL

Carlos Brites, Célia Pedroso, Nanci Silva, Warren D Johnson Jr and Roberto Badaró

HIV-1 isolation was attempted on 72 individuals, including persons with known HIV infection and five without proven HIV infection but with indeterminate Western blot patterns, as well as on low-risk HIV seronegative persons. The ability to detect HIV-1 from culture supernatant by p24 antigen capture assay was evaluated by segregating patients by absolute CD4+ cell counts, clinical stage of disease, p24 antigenemia and zidovudine use. The likelihood of a p24 positive HIV culture was highest among patients with CD4+ T-cell counts below 200/ul and patients with advanced clinical disease. Use of zidovudine did not affect the rate of HIV positivity in cultures.

Key-words: HIV. P24. Viral isolation. Brazil. CD4+ T lymphocytes.

The human immunodeficiency virus Type-1 (HIV-1) has been isolated from lymph nodes, peripheral blood mononuclear cells (PBMC), and other body fluids, from patients with HIV infection and/or the acquired immunodeficiency syndrome (AIDS)[1,8]. Different rates of virus isolation from PBMC of HIV-1 infected patients have been reported, ranging from 30-99%[4,7,10,12]. Recent modifications of standard techniques led to the ability to estimate viral load from plasma and PBMC obtained from patients with different stages of HIV-1 infection[6,9]. This method is used to increase understanding of HIV disease progression and to monitor the effect of antiviral therapy.

As of 1993 over 40,000 AIDS cases have been reported in Brazil[13]. This study was initiated to evaluate the qualitative and quantitative HIV cultivation methods and the effect of patients' CD4+ T-cell count, clinical stage of disease, p24 antigenemia and Zidovudine use on recovering HIV.

Laboratório de Retrovírus. Hospital Universitário Prof. Edgard Santos, Universidade Federal da Bahia, Salvador, BA, Brazil, and Division of International Medicine, Cornell University Medical College, New York, NY, USA.
Research supported by grants AI26506 and TW00018 from the United States Public Health Service.
Address to correspondence: Dr. C. Brites. Laboratório de Retrovírus/HUPES/UFBA. R. João das Botas s/n, Canela, 40110-160 Salvador, BA, Brazil.
Phone: (55 71) 235-4866, Fax (55-71) 245-7110.
Recebido para publicação em 08/12/94.

MATERIAL AND METHODS

Study Population. Patients attending the AIDS clinic of the Federal University of Bahia Hospital were eligible for the study. PBMC from 72 individuals were cultivated for HIV-1 isolation from the following groups: a) Fifty-one patients with HIV-1 positive Western blot (WB); b) Five low-risk HIV-1-enzyme immunoassay (EIA) reactive blood donors who had an indeterminate HIV-1 WB pattern (p24 band only); and c) Three HIV-EIA and WB-seronegative patients, who were admitted to the hospital with an infection suggesting AIDS. In addition, 13 HIV-seronegative health care workers were used as controls. All the participants of the study gave written consent. The 1988 revised criteria from CDC were used for clinical staging of the patients[14]. The study was conducted between March, 1990 and June,1993.

PBMC preparation and viral isolation procedure. Donor PBMC. Twenty milliliters of heparinized (20 U of preservative-free heparin/ml) blood were collected from healthy HIV seronegative volunteers and diluted in sterile phosphate buffered saline (1:1). Peripheral blood mononuclear cells (PBMC) separated by Ficoll-Hypaque gradient centrifugation were suspended in sterile RPMI-1640 medium (GIBCO Laboratories, Grand Island, New York, NY), containing 15% fetal calf serum (GIBCO), penicillin (100µ/ml), streptomycin (10ug/ml), and 2mM L-glutamine, and stimulated with 4µg/ml phytohemaglutinin (PHA, Sigma Co., St. Louis, MO) for three or four days at 37°C, 5% CO_2.

Qualitative and Quantitative cultures. Fifty qualitative cultures were performed in 28 HIV-1 infected individuals, 5 blood donors with indeterminate WB patterns, three HIV-seronegative patients, a child of a HIV-infected mother, and 13 seronegative controls. Patient PBMC (20 x 10^6 cells) were co-cultivated according to standard procedures described elsewhere[10]. The culture supernatants were collected at 3, 7, 10, 14, 17, and 21 days and stored at -20°C, for p24 antigen assays .

Thirty-five quantitative cultures were performed, as previously described by Ho et al[9], on 30 HIV-1 seropositive individuals (including 8 patients tested by qualitative methods).

Assays for HIV-1 antibodies and p24 antigens. Antibodies against HIV-1 were detected by an enzyme-linked immunosorbent assay (EIA) and confirmed by Western blot (Du Pont Corporation, and Biotech-Dupont, respectively, Willmington, DE,). The HIV-1 p24 core antigen was assayed in the serum and in culture supernatants by a antigen capture enzyme immunoassay (Abbott Laboratories, North Chicago, IL). A positive culture was defined as either a single p24 antigen concentration ≥ 1000pg/ml, 2 consecutive samples with a p24 antigen ≥ 200pg/ml, or reactive samples (at least 2 times above the cut off value) neutralized by anti-p24 monoclonal antibody (Abbott Laboratories).

CD4 cell counts. CD3+, CD4+ and CD8+ cells were counted by a standard immunofluorescent method using mouse anti-human monoclonal antibodies (Orthomune OKT_3, OKT_4, and OKT_8 monoclonal antibodies, Ortho Diagnostic Systems, Raritan, NJ). Indirect staining of the lymphocytes with labeled monoclonal antibody was performed using goat anti-mouse Ig-FITC. The lymphocyte subsets were counted using a epifluorescent microscope (Microphot - FXA - Nikon Corporation, Tokyo, Japan).

Statistical analysis. Data were entered into EPIINFO version 5.0 and analyzed by Fischer's Exact, Student's t-test, or Kruskall Wallis test.

RESULTS

Qualitative and Quantitative cultures. Forty-five of 50 (90%) HIV-1 seropositive individuals had at least one positive (qualitative or quantitative) culture for HIV-1. All patients from this group had the usual risk factors associated with AIDS, including unprotected sexual activities with high risk individuals and/or intravenous drug use. The patients consisted of 32 male homo/bisexuals, 10 intravenous drug abusers (IVDA) and 3 heterosexual females with HIV-positive sexual partners. Cultures were negative from five blood donors with indeterminate WB patterns, a child from an HIV-infected mother, and from the 3 seronegative patients. Also, all 13 seronegative healthy controls had negative HIV cultures. Six months after the initial study repeated HIV serology, in the five blood donors with indeterminate WB was negative for 3. Two remained presenting an indeterminate WB pattern for 18 months of follow-up.

Table 1 compares the recovery of HIV by qualitative and quantitative culture methods. There was no significant difference between cultures from the same individual using the qualitative versus the quantitative method. The overall rate of positive HIV cultures for the qualitative and quantitative methods was 81% and 89%, respectively, excluding the 3 seronegative AIDS patients and the individuals with indeterminate WB.

Correlation of CD4+ T-cell and clinical stage with a positive HIV-1 culture. Higher rate of viral culture positivity was associated with advanced HIV disease. The rate of viral isolation was 60% (12/20) for class II/IVA patients, in contrast to 96% (44/46) for AIDS IV-C patients. The mean CD4+ T cell count

Table1- Rates of positivity of qualitative and quantitative cultures for HIV-1.

Patients Category	Qualitative		Quantitative		Total	
	n	%	n	%	n	%
CDC Class IV-C AIDS	20/22	91	24/24	100	44/46	96*
CDC Class II-IV A	5/9	55	7/11	64	12/20	60*
HIV-1 Seronegative	0/3	-	0	-	0	-
Individuals with Indeterminate WB Pattern	0/5	-	0	-	0/5	-

* p=0.001 Student's T-test, comparing patients with CDC class IV-C AIDS and those with less advanced illness (Class II to IV-A).

was 311 ± 316 for individuals with positive cultures, and 872 ± 670 cells/µl for those with negative results (p = 0.03, Kruskall-Wallis test). When the results were analyzed by stratifying CD4+ T-cells counts of > 500, 200-500 and < 200 per µl, a significant difference was observed between cultures from patients with CD4+ T-cell count levels > 500 cells/µl and those with ≤ 500 cell/µl (Table 2).

Table 2 -Positivity of HIV-1 cultures and CD4+ cell counts.

CD4+ cell count/mm³	Cultures/Total	
	n	%
>500	9/14	65
200 - 500	12/15	80
<200	25/25	100
Total	46/54	85

There was also a significant difference in cultures positivity for patients with CD4+ cell counts above and below 500/µl (p = 0.02 Fisher exact test).

The correlation of the titer of infectious HIV-1/10^6 PBMC, and levels of CD4+ cells in the patients with positive cultures is presented in the Table 3. Previous use of Zidovudine did not appear to alter ability to isolate HIV from patients' PBMC). The rate of positivity was similar for patients using AZT (31/34) and, for individuals who did not use that drug (7/25), regardless the type of culture (p = 0.17, Fischer exact test).

Viral load also correlated with the level of CD4+ T-cell counts. Individuals with CD4+ counts > 500 cells/µl had 12 ± 21 TCID while those with CD4+ counts ≤ 500 cells/µl had a 35-fold higher viral burden, 446 ± 1406 TCID. It is of note that 2 million PBMC per culture were sufficient to detect HIV by culture in 57% of individuals with CD4+ > 500/µl (Table 4).

Assays for p24 Ag detection were performed in serum samples of 35 individuals (32 seropositive and 3 seronegative for HIV 1 infection). Among the seropositive individuals, 21 (66%) had detectable levels of p24 antigen in their sera. The 3 seronegative patients tested had a negative p24 antigen test. The rate of culture positivity was similar for patients with detectable antigenemia (82%) or not (75%). The frequency of p24 antigenemia was almost two times higher among the AIDS class IV-C patients (84%) when compared with the class II/IV-A group (only 48% had detectable levels of p24 antigen, p = 0.009, Yates corrected).

DISCUSSION

In the evaluation of qualitative and quantitative culture methods, an overall HIV positivity rate of 85% was detected in HIV-seropositive individuals, with a 60% culture positivity in less ill HIV-infected individuals (asymptomatic class II/III to IV-A). We were able to detect the virus in 96% of AIDS patients with stage disease class IV-C. This is comparable to what has been reported in the literature. Quantitative or qualitative methods was not significantly different in their ability to detect HIV-1 in culture.

Table 3-Titers of infectious HIV-1 in 35 cultures of PBMC, mean of CD4+ T-Cells and use of zidovudine therapy

TCID/10^6PBMC	Number of cultures	%	Mean of CD4+ cells	Use of zidovudine
0	4	11	907 ± 542	0
0.5	14	40	222 ± 218	7
5	7	20	314 ± 340	1
50	5	14	390 ± 275	1
500	3	9	190 ± 134	0
5000	2	6	310 ± 110	1
50000	0	-	-	-
Total	35	100		10

The mean of TCID for individuals with CD4+ T-cells above 500/µl was 12 ± 21 while those patients with CD4+ T-cell counts below 500/µl had a mean of 446 ± 1379 TCID.

Table 4 - Culture isolation of HIV stratified by CD4+ T-cell counts.

CD4 T-cells per µl	Dilutions of patient PBMC									
	2 x 10^6		2 x 10^5		2 x 10^4		2 x10^3		2 x 10^2	
	n	%	n	%	n	%	n	%	n	%
50	4/7	57	1/7	29	2/7	17	0/7	0	0/7	0
200-500	11/12	92	8/12	67	3/12	25	3/12	25	1/12	8
200	13/13	100	5/13	38	2/13	15	0/13	0	1/13	8
Total	28/32	88	15/32	47	7/32	22	2/32	6	2/32	6

Foot notes: Mean (± SD) CD4+ T-cell counts of individuals with a positive and negative viral culture were 311 ± 316 /µl and 812 ± 620 /µl (P = 0.03, Kruskall Wallis test), respectively.

Patients with CD4+ T-cell counts ≤ 500/µl are more likely than those with > 500/l to have a positive viral culture. Individuals with a negative HIV culture had a mean CD4+ T-cell count that was three times higher than that of patients with positive HIV culture. Our results were similar to those reported by Spira and Burke[2,15]. The level of CD4+ T-cells in ability to culture HIV from patient PBMC probably reflects the higher viral burden in patients with more advanced HIV disease. This is suggested by our data and by others[2,9,15]. Thus, the sensitivity for detecting HIV by culture for patients with less advanced HIV infection and AIDS is 60% and 96%, respectively. Viral cultures were negative for HIV-seronegative individuals, and in five low risk blood donors with an indeterminate WB. According to other studies[5,11] this would suggest that they were not infected. The lack of false positives from cultures obtained from these HIV-seronegative or indeterminant WB individuals indicates that the specificity of culture methods is 100%.

In our experience the positivity of cultures was not affected by zidovudine use, as previously observed[3]. Among the patients using zidovudine and having positive quantitative culture, 8 had a TCID of ≤ 5, while only 2 had a higher level of infection.

In conclusion, the viral isolation from HIV-1 infected patients in our study showed a sensitivity similar to those reported in the literature. However, the higher CD4+ T-cell counts in the individuals with negative cultures explain the lower rate of viral isolation in the less ill HIV infected individuals compared to the higher rate in AIDS class IV-C patients.

RESUMO

Tentativa de isolamento do vírus tipo 1 da imunodeficiência adquirida (VIH-1) foi realizada em 72 indivíduos sendo 51 pacientes com sorologia positiva para o VIH-1, confirmada por Western blot; 5 doadores de sangue com padrão indeterminado ao Western blot; 3 indivíduos com diagnóstico clínico de AIDS, porém com sorologia negativa, e 13 profissionais de saúde soronegativos. Os pacientes foram estratificados de acordo com a contagem de células CD4+, estágio clínico , antigenemia (p24) e uso de zidovudine. As culturas para o VIH-1 foram positivas em 45/50 (90%) tentativas. Houve uma correlação inversa entre o número de células CD4+ e a freqüência de isolamento do VIH-1. As culturas foram positivas em 84% dos indivíduos com CD4+ <200, contra 48% d positividade naqueles com contagem de célula CD4+ acima deste valor. O uso de zidovudine nã interferiu na positividade das culturas. Concluímo que a sensibilidade dos métodos de cultur qualitativo e quantitativo é similar para a detecçã do VIH-1. A taxa de positividade das culturas nã foi afetada pelo uso prévio de zidovudine, mas foi diretamente proporcional ao grau de imunodeficiência dos pacientes.

Palavras-chaves: VIH. p24. Isolamento viral. Brasil. CD4+ linfócitos T.

ACKNOWLEDGMENT

We thank Dr. Robert Akridge for his critical review of the paper, and Zacarias A. Santana Jr. for the computer and editorial assistence of this manuscript.

REFERENCES

1. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gurest J, Danguet C, Axler-Blin C, Brun-Vézinet F, Rozembaum W, Montagnier L. Isolation of a T-lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). Science 220:868-71, 1983
2. Gallo RC, Sarin PS, Gelmann EP, Robert-Guroff M, Richardson E, Kalyanaraman VS, Mann D, Sidhu GD, Stahl RE, Zolla-Paznes S, Leibowitch J, Popovic M. Isolation of Human T-Cell Leukemia Virus in Acquired Immune Deficiency Syndrome (AIDS). Science, 220:865-8 1983.
3. Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palkert J, Redfield R, Oleske J, Safai B, White G, Foster P, Markham PD. Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk of AIDS. Science 224:500-3, 1984.
4. Levy JA, Hoffman AD, Kramer SM, Landis JA, Schimabukuro JM, Oshiro LS. Isolation of Lymphocytopathic Retroviruses from San Francisco Patients with AIDS. Science 225:840-2, 1984,
5. Jackson JB, Coombs RW, Sannerud K, Rhame FS, Balfour HH Jr. Rapid and Sensitive Viral Culture Method for Human Immunodeficiency Virus Type 1. Journal of Clinical Microbiology 26:1416-8, 1988.
6. Castro BA, Weiss CD, Wiviott LD, Levy JA. Optimal Conditions for Recovering Human Immunodeficiency Virus from Peripheral Blood Mononuclear Cells. Journal of Clinical Microbiology 26:2371-6, 1988.

7. Coombs RW; Collier AC; Allain JP; Nikora B; Leuther M; Gjerset GF; Corey L. Plasma viremia in Human Immunodeficiency Virus Infection. New England Journal of Medicine 321:1626-31, 1989.
8. Ho DD, Moudgil T, Alam M. Quantitation of Human Immunodeficiency Virus Type 1 in the Blood of Infected Persons. New England Journal of Medicine 321: 1621-1625, 1989.
9. Ministério de Saúde. AIDS - Boletim Epidemiológico, ano VI, nº 08, 1993.
10. Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome. Mortality and Morbidity Weekly Report 36(suppl 1):3S-15S, 1987.
11. Spira TJ, Kaplan JE, Feorino PM, Warfield DT, Fishbein DB, Bozeman LH. Human Immunodeficiency Virus Viremia as a Prognostic Indicator in Homosexual Men with Lymphadenopathy Syndrome. New England Journal of Medicine 317:1093-4, 1987.
12. Burke DS, Fowler AK, Redfield RR, Dilworth S, Oster CN. Isolation of HIV-1 from the Blood of Seropositive Adults: Patients Stage of Illness and Sample Inoculum Size are Major Determinants of a Positive Culture. Journal of Acquired Immune Deficiency Syndrome 3:1159-1167, 1990.
13. Jackson JB, Mac Donald KL, Cadwell J, Sullivan C, Kline WE, Hanson M, Sannerud KJ, Stramer SL, Fildes NJ, Kwok SY. Absence of HIV Infection in Blood Donors with Indeterminate Western blot Test for Antibody to HIV1. New England Journal of Medicine 332:217-222, 1990.
14. Celum CL, Coombs RW, Lafferty W., Inui TS, Louie PH, Gates CA, McCreedy BJ, Egan R, Grove T, Alexander S. Indeterminate Human Immunodeficiency Virus Type 1 Western Blots: Seroconversion Risk, Specificity of Supplemental Test, and an Algorithm for Evaluation. Journal of Infectious Diseases 164: 656-64, 1991.
15. Burke DS, Redfield RR, Bjornson DC, Fowler AK, Oster CN. Frequent Isolation of HIV1 from the Blood of Patients Receiving Zidovudine (AZT) therapy. New England Journal of Medicine 321:1682, 1989.