

## Use of the 2,3-Diacyl-trehalose and the Purified Protein Derivative in the Serodiagnosis of Tuberculosis in AIDS

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*The effect of the human immunodeficiency virus (HIV) infection on IgG production against purified protein derivative (PPD) and 2,3-diacil-trehalose (SL-IV) was investigated by an enzyme-linked immunosorbent assay (ELISA) test. Comparison between the antigens showed that immunocompetent patients produce preferentially antibodies to SL-IV than to PPD (73.3% versus 63.3%). Combination of these results showed an increase of the sensitivity to 80%, which decreased over the spectrum of immunodepression caused by HIV. In the tuberculous HIV seropositive group the sensitivities of SL-IV and PPD were 36.4% versus 40% and 0% versus 22.2% in the tuberculosis/acquired immunodeficiency syndrome (TB/AIDS) group. Combination of these results gave respectively 54.5% and 20%, showing that serological tests have limited value for diagnosis of tuberculosis in HIV infected patients. High antibody levels were observed in HIV seropositive asymptomatic group, but only two individuals were positive for both antigens. In the follow up, one of them developed tuberculous lymphadenitis, indicating that further work is needed to access the value of serological tests in predicting tuberculosis in HIV infected individuals.*

Key words: serodiagnosis - tuberculosis - HIV infection - PPD - SL-IV - AIDS - ELISA

The overlap between human immunodeficiency virus (HIV) infection and tuberculosis (TB) has been shown by the high HIV seroprevalence among patients with active TB (Raviglione et al. 1992, Kritski et al. 1993). From a global perspective, TB represents one of the most common HIV-related opportunistic infections. HIV infection, by progressively impairing cell-mediated immunity, appears to be the highest risk factor for endogenous reactivation or exogenous reinfection of tuberculosis (Hopewell 1992). The diagnosis of TB in HIV infection may be difficult, specially in the acquired immunodeficiency syndrome (AIDS) phase, because of atypical presentation (for both pulmonary and extrapulmonary forms) and frequent sputum smear-negative cases. The delayed diagnosis of TB among HIV seropositive individuals contributes to high mortality rate and transmission of *Mycobacterium tuberculosis* infection, specially to contacts like other HIV-infected patients and healthcare workers (CDC 1990, Hopewell 1992). Current strategies for the TB di-

agnosis in developing countries are seriously inadequate (Raviglione et al. 1992). Therefore, a more rapid, sensitive and specific test is needed that may be used to diagnosis TB in HIV-infected patients. In the last few years the characterization and identification of new specific antigens have restimulated the interest in the serologic diagnosis of TB (Daffé et al. 1989, Laszlo et al. 1992, Papa et al. 1993). Among HIV seronegative TB patients, these serologic tests offer high sensitivity and specificity and may provide a wide clinical application (Daniel 1989, Wilkins & Ivanyi 1990, Cruaud et al. 1990). But, their possible benefits for improved diagnosis of TB in HIV seropositive patients have not been determined (Theuer et al. 1989, Hoepfner 1990, Berlie et al. 1991, Barrera et al. 1992).

The purpose of this paper is to report the results of TB serodiagnosis in patients with different levels of immune depression caused by HIV infection.

### MATERIALS AND METHODS

From July 1988 to December 1990, IgG assays were performed using an enzyme-linked immunosorbent assay (ELISA) in 224 individuals as follows: 81 healthy persons without laboratory evidence of HIV infection (controls, Group

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A); 37 asymptomatic HIV seropositive patients (Group B); 19 AIDS patients without clinical or laboratory evidence of TB (Group C); 45 TB patients without clinical or laboratory evidence of HIV infection (Group D); 22 TB patients infected by HIV without AIDS symptoms (TB/HIV) (Group E), and 20 AIDS cases (according to CDC-1987 criteria) with tuberculosis (TB/AIDS) (Group F).

All TB cases were bacteriologically confirmed by isolation and identification of *M. tuberculosis* using procedures recommended by reference laboratories. All patients had a physical examination that included an examination of extrapulmonary features possibly related to TB and/or HIV infection. The control group was selected from the population attending the Chest Service. HIV serology status was determined in all patients after they had agreed to informed consent. Serum samples were tested for antibody to HIV by ELISA (Organon Teknika, Boxtel, Netherlands). All ELISA positive samples were analyzed by the Western blot immune assay (Dupont, Wilmington, DE, USA). The samples were considered positive when they reacted to both the ELISA and the Western blot test.

The antigens used in the ELISA were SL-IV (2,3-diacyl-trehalose), a specie-specific glycolipid from *M. tuberculosis*, kindly supplied by Dr Hugo David (Institut Pasteur, Paris, France), and PPD. The ELISA for SL-IV was performed as previously described (Cruaud et al. 1990). Briefly, the antigen was dissolved in hexane (2 µl/ml, 100 ng/well) and incubated overnight at 37°C. Blocked by the addition of 100 µl of 5% bovine serum albumin (BSA) at 37°C for 2 hr, and washed with phosphate-buffered saline (PBS) pH 7.4. Sera were diluted at 1:320 in 1% BSA and 100 µl were added to each well. After 1 hr of incubation and washing, 100 µl of goat anti human IgG β-galactosidase conjugate from Biosys (Compiègne, France) diluted at 1:2000 in PBS-BSA were added. After 1 hr of incubation at room temperature and washing, 100 µl of o-nitrophenyl-β-galactopyranoside (0.8 mg/ml, Sigma) and β-mercaptoethanol (90 µl) prepared phosphate buffer (0.1 M K<sub>2</sub>HPO<sub>4</sub>, 1 mM Mg SO<sub>4</sub>) were added. After 1 hr of incubation, 50 µl of a solution at 32% of sodium carbonate were added and A<sub>405</sub> values determined with a Titertek Multiskan apparatus (Flow Lab., USA). The incubation temperature was always 37°C. Polystyrene microtiter plates used were from Dynatech Lab. (USA) with round bottom. All sera were tested in duplicate and in each set of the experiments a reference positive and a negative pool were included. For each serum a control well was assayed in parallel without antigen. A modi-

fied procedure described by Fonseca et al. (1992) was applied in ELISA for PPD. Polystyrene microtiter plates with flat bottom were coated with 10 µg/ml of PPD RT-23 diluted in carbonate buffer pH 9.6, and incubated overnight at 4°C. Wells were washed with PBS containing 0.1% of Tween 20 (PBST) and the remain sites blocked by addition of PBST containing 1% of BSA (PBST-BSA) and incubated at 37°C for 1 hr. Sera diluted at 1:320 in PBST-BSA and 100 µl were added to each well. The following steps were performed as for SL-IV. In this study the cut off for PPD and SL-IV was established as the mean plus two standard deviations of the healthy control population sera results.

The chi-square (corrected for continuity), Fisher's exact, and Student's t-tests were used for statistical analyses. The efficiency of the serological method was evaluated by calculating the sensitivity and specificity.

## RESULTS

The distribution of ELISA OD values in the detection of antibodies to PPD and SL-IV is depicted in Fig. The cut off points obtained with PPD and SL-IV antigens in the healthy population (Group A) were similar (0.2 and 0.25, respectively), however false positive results were detected only with PPD. Considering these results the specificity required for diagnostic purposes using SL-IV antigen was 100%, while for PPD was 97.6%.

The analysis of the Group B (HIV+) and C (AIDS patients), both without TB, showed similar specificity with PPD (73% and 75%). However, a statistical significant difference was found with SL-IV (73% and 94.7%,  $p < 0.05$ ) in the same groups (Table). Only two individuals from Group B were seropositive for both antigens and they showed no cutaneous hypersensitivity to PPD (Mantoux test), which is used as a criteria to initiate chemotherapy. The HIV positive individuals without TB (Group B) were followed up for 9 to 21 months. At the end of this period, one of two positive individuals for both antigens developed tuberculous lymphadenitis and the other remained asymptomatic.

Using the SL-IV antigen, the sensitivity of the method among group D (TB), E (TB/HIV) and F (TB/AIDS) was estimated to be, respectively, 73.3% (33/45), 36.4% (8/22) and 0% (0/20). When PPD antigen was evaluated the results among Group D, E and F were, respectively 63.4% (26/41), 40.0% (8/20) and 22.2% (4/18) (Table). The specificity of the SL-IV antigen, in Group D, E and F (using the Group A, B, C as control), was respectively: 100%, 73% and 94.7%. And using

PPD antigen, these features were respectively: 97.6%, 73% and 75%.

The sensitivity obtained with both antigens (PPD and SL-IV) was 80.0% (36/45) in Group D, 54% (12/22) in Group E and 20.0% (4/20) in Group F.

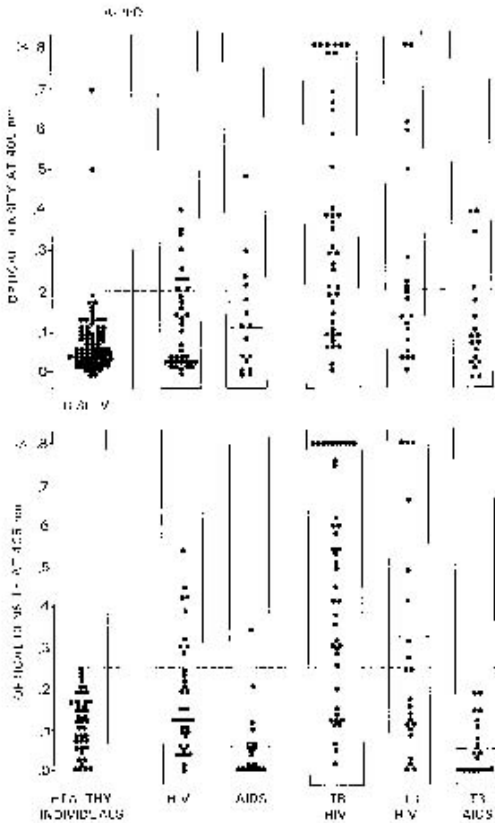
**DISCUSSION**

Among HIV seropositive TB patients, specially in the AIDS phase, failure to make the diagnosis can have serious consequences; e.g., mortality rate of 45% versus 19% when an early diagnosis is made (Hopewel 1992). Conversely, blind treatment with anti-TB exposes the patient to the risk of unnecessary toxic effects of the drugs. In our study, the performance of the antigen SL-IV and PPD was different between HIV seropositive and seronegative patients. Using the above criteria, we observed a higher sensitivity with SL-IV than with PPD in the immunocompetent group (73.3% versus 63.4%). Although immunocompetent patients produce more antibodies to SL-IV than to PPD, the differences between the two antigens were not significant ( $p>0.05$ ). Our data confirmed the results obtained by Cruaud et al. (1990) on the evaluation of SL-IV antigen for case finding of TB in non-immunocompromised patients. The high specificity and high sensitivity achieved in the ELISA test based on the SL-IV in these groups were similar to those results observed by others, using other specific antigens (Daniel 1989, Wilkins & Ivanyi 1990).

The sensitivity of the SL-IV antigen (36.4%) in the detection of HIV seropositive TB patients (Group E) and in TB patients with AIDS (0%) (Group F), was similar to that achieved by Berlie et al. (1991), using the same antigen (29.4% and 6.8%, respectively). Nevertheless, our results with Group E and F were lower than that observed by Hoepfner (1990) (78% and 67%, respectively) using TB 72 monoclonal antibody probe which binds to the *M. tuberculosis* specific 38 kD antigen and by Rigous et al. (1990) (70% and 60%) using antigen 60.

As occurred with SL-IV, the sensitivity of PPD in the detection of TB decreased from immunocompetent tuberculous patients to tuberculous AIDS patients, but some cases with AIDS and TB showed some levels of the antibody (22.2%). Using the same nonspecific antigen, Barrera et al. (1992) and Theuer et al. (1989) observed similar results. Figueiredo and Machado (1992), using a crude antigen of *M. tuberculosis*, in the same group of patients obtained higher sensitive (37.8%).

For diagnostic purposes the SL-IV antigen is of limited value in HIV seropositive patients. One way in which serologic methods might be improved is by combining the results of two or more tests. We evaluated the sensitivity with both antigens (SL-IV and PPD). The sensitivity in HIV seropositive TB patients (54.5%) and in TB patients with AIDS (20%) was slightly lower than that observed by Berlie et al. (1991), using SL-IV associated to PGL-Tb1 antigens.



Results of ELISA measurement of IgG antibodies to PPD (A) and SL-IV (B) antigens in tuberculous patients and control subjects. The optical density result obtained for each serum (mean of duplicate assays) is indicated by a single point, and group means are indicated by horizontal lines. The cut off points is indicated by the broken horizontal line.

**TABLE**  
Sensitivity and specificity of ELISA in sera from tuberculous patients

Group	Sensitivity		Specificity	
	SL-IV	PPD	SL-IV	PPD
Immunocompetent tuberculous patients	73.3%	63.4% <sup>a</sup>	100%	97.6%
HIV seropositive tuberculous patients	36.4%	40%	73%	73%
TB/AIDS patients	0%	22.2%	94.7%	75%

<sup>a</sup>: no significant difference between SL-IV and PPD ( $p>0.05$ ); TB/AIDS - AIDS patients with tuberculosis

False positive results were observed with SL-IV and PPD antigens in similar proportion in HIV seropositive asymptomatic patients, but in AIDS patients without TB, the proportion of false positive results was lower with SL-IV antigen. In asymptomatic HIV positive group, only two individuals were seropositive for both antigens and they showed no cutaneous reactivity for PPD. These results led us to follow-up these patients in order to verify the predictive value of the positive serologic test as an early diagnosis of TB, as proposed by others (van Vooren et al. 1990, Berlie et al. 1991). After a period of follow-up ranging from 9 to 21 months, one developed tuberculous lymphadenitis and the other remained asymptomatic. These observations may indicate that the method might help in deciding to use preventive anti-TB therapy in HIV infected patients.

The present study show that serological tests for the detection of anti SL-IV and anti PPD antibody in HIV seropositive patients are of limited value for TB diagnosis. The sensitivity of the immunoenzymatic test for the diagnosis of TB in patients with AIDS was lower than that observed in HIV seronegative or seropositive TB patients. These features most likely result from the underlying immune defect of HIV seropositive patients, especially in the AIDS phase. On the other hand the positivity obtained with tuberculous patients HIV positive might be due to the polyclonal hypergammaglobulinemia of IgG and IgA classes and selective defects in antigen recognitions due to intrinsic lymphocytic alterations in these patients (Lane et al. 1985).

In conclusion, our study is preliminar and further work with other specific antigens needs to be done to determine: (a) the value of serologic tests for TB diagnosis in HIV seropositive patients, and (b) the value of predicting who will develop TB to provide a basis for justifying prophylactic drug regimen.

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