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Time Course of Anti-SL-IV Immunoglobulin G Antibodies in Patients with Tuberculosis and Tuberculosis-Associated AIDS

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Immunoglobulin G (IgG) and IgM antibodies against the SL-IV antigen of *Mycobacterium tuberculosis* in the sera of patients with tuberculosis with negative serology for human immunodeficiency virus (HIV) infection (TB group; *n* = 97), patients with tuberculosis with positive serology for HIV infection (TB-HIV group; *n* = 59), and healthy controls (*n* = 289) were determined by enzyme-linked immunosorbent assay. All sera were obtained at the onset of tuberculosis, i.e., when clinical symptoms appeared. Clinical specimens were collected and cultured for the isolation of *M. tuberculosis*, and treatment with antituberculous drugs was started. Sera were also obtained from patients in the TB group at fixed intervals during treatment; sera were available from 13 patients in the TB-HIV group before the onset of tuberculosis. The best specificity and positive predictive value were obtained with the IgG assays. In the IgG assays at specificities above 96.0%, the sensitivities of the tests were 45.3 and 72.8% for the TB and TB-HIV groups, respectively, and the sensitivity was 51.9% when data from both groups were combined for analysis. For the TB group, results of this study indicated that the levels of IgG antibodies remain high during treatment. Thus, repetitive serological assays may not be useful for treatment follow-up. In the TB-HIV group, 12 of 13 patients had IgG-specific antibodies against the SL-IV antigen between 1 and 30 months before the onset of tuberculosis, so we suggest that the IgG antibody assay against SL-IV may be helpful for identifying tuberculosis in patients infected with HIV.

It has been reported that antibodies against phenolglycolipid antigen (PGL-Tb1) (11, 12) and sulfolipid antigen (SL-IV) (4) from *Mycobacterium tuberculosis* can be detected in the sera of patients early during the course of tuberculosis, and the usefulness of these antigens for enzyme-linked immunosorbent assay (ELISA) in the identification and diagnosis of tuberculosis was evaluated (6). According to the evaluation study (4), significant titers of anti SL-IV antibodies were detected by ELISA in 75.0% of patients by using a cutoff point of 0.150 and in 51.6% of patients by using a cutoff point of 0.300 (specificities, 88.0 and 100%, respectively). Those reports indicated that kinetic studies on the production of anti-SL-IV antibodies should be performed. The purpose of this study was to examine the time course of anti SL-IV immunoglobulin G (IgG) production before the onset of clinical tuberculosis and to examine its evolution during treatment.

MATERIALS AND METHODS

Study population. For the purpose of this study, sera were collected from 465 people. The serum samples were distributed as follows: 156 were from patients with tuberculosis; of these, 97 were from patients with negative serology for human immunodeficiency virus (HIV) infection (TB group) and 59 were from patients with positive serology for HIV infection (TB-HIV group); serum samples were also obtained from 289 healthy people (control group). All individuals included in the study were Spanish nationals living in Catalonia, where *Mycobacterium bovis* BCG vaccination has not been used for the prevention of tuberculosis since 1974.

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Sera were collected from patients in the TB and TB-HIV groups at the same time that specimens were obtained for the bacteriological diagnosis of tuberculosis. Sera were collected from 69 patients in the TB group at 21 days and at 2, 4, 6, 9, and 12 months after treatment started. Sera were collected from 19 patients in the TB-HIV group before and/or after tuberculosis was diagnosed; in this group of patients, sera were not obtained at regular intervals because they were used for control of HIV infection by detection of P24 antigen and were stored at −20°C in the AIDS Serology Section. In all cases tuberculosis was confirmed by isolation and identification of *M. tuberculosis*.

In the control group, 188 people had positive and 101 people had negative tuberculin skin test reactions, as measured by the Mantoux test by using 5 IU of tuberculin. Following the recommendations of the Committee for Tuberculosis of Catalonia (8), reactions above 6-mm induration were scored as positive.

ELISA. The SL-IV antigen used in the ELISA was kindly supplied by l'Unité de la Tuberculose et des Mycobactéries, Institut Pasteur, Paris, France. Chemically, the antigen was reported to be a 2,3-diacyl-trehalose-2'-sulfate (5); however, its chemical structure was reexamined and has been established to be 2,3-diacyl-trehalose (10). The ELISA was performed by a previously described method (4). Briefly, the antigen was dissolved in hexane (2 μg/ml, 100 ng per well), dried for 2 h at 37°C, saturated with 5% bovine serum albumin overnight at 4°C, and washed with phosphate-buffered saline (PBS) (pH 7.4). We used polystyrene microtiter plates from Nunc (Roskilde, Denmark). Sera were diluted 1/250 in 0.5% bovine serum albumin, and 100 μl was added to each well. After 2 h of incubation and washing, 100 μl of goat anti-human IgG or IgM β-galactosidase conjugate (Biosis, Compiègne, France) diluted 1/1,000 in PBS was

TABLE 1. Anti-SL-IV IgG and IgM antibodies

Group[a]	OD (mean ± SE)	
	IgG	IgM
T	0.310 ± 0.023	0.103 ± 0.006
TB	0.235 ± 0.027	0.086 ± 0.005
TB-HIV	0.432 ± 0.038	0.132 ± 0.016
C	0.071 ± 0.002	0.074 ± 0.002

[a] T, total group of patients with tuberculosis (n = 156); TB, non-HIV-infected patients with tuberculosis (n = 97); TB-HIV, HIV-infected patients with tuberculosis (n = 59); C, healthy control group (n = 289).

added. After 2 h of incubation and washing with PBS, 100 μl of *o*-nitrophenyl β-galactopyranoside (0.8 mg/ml; Sigma) and β-mercaptoethanol (6 μl/ml) dissolved in a buffer (0.1 M K_2HPO_4, 1 mM $MgSO_4$, 2 mM $MnSO_4$, 2 mM Mg Tritriplex) were added. After 1 h of incubation, A_{405} values were determined with a Titertek Multiscan apparatus. The incubation temperature was always 37°C. All tests were done in duplicate by two workers on different days, and different serum samples from the same patient were tested in the same plate. In each plate, four control serum samples with previously established absorbance results were included, and when the correlation between the controls was less than 99%, the plate was discarded and the test was repeated.

Statistical analysis. The statistical analysis was performed with the BMDP program (Biomedical statistical software package) (7). Differences between continuous variables were calculated by using the t test when the variables were distributed as normal and by the Mann-Whitney U test for the other cases. In order to evaluate the capacity of antibody levels to classify patients as tuberculous or as healthy controls, we used the two-group discriminant analysis (9), with the following considerations. The Mahalanobis D^2 was applied to calculate the generalized distance between the two populations, and a 0.5 prior probability was used. The value of the independent variable (IgG or IgM antibody level) that was obtained after equating the discriminant function to zero (or after equating the two classification functions) was used as the cutoff point. Then, the allocation criterion was based on the sign of the function solved, classifying as tuberculous those patients with a positive score. The efficacy of the serological method was evaluated by calculating the sensitivity, specificity, and predictive values and the percentage of subjects correctly classified.

To assess the evolution of IgG antibodies during chemotherapy in the TB group and that of IgG antibodies in the TB-HIV group, we used the repeated measurements analysis of variance, and as a multiple comparison procedure, the Tukey's method was selected. In the latter case, P values were corrected for multiple comparisons by the procedure itself, but in all the other applications, a 0.05 significance level was used to reject the null hypothesis.

RESULTS

Preliminary observations. The mean ± standard error optical densities (ODs) are depicted in Table 1. On detecting IgG-specific antibodies, statistically significant differences were found between the mean OD of (i) the tuberculous and control groups, (ii) the TB and control groups, (iii) the TB-HIV and control groups, and (iv) the TB and TB-HIV groups ($P < 0.001$). In the detection of IgM-specific antibodies, no significant differences were found between the mean ODs of (i) the TB and control groups ($P = 0.076$) and (ii) the TB and TB-HIV groups ($P = 0.030$). Levels of IgG- or IgM-specific antibodies were higher in the patients in the TB-HIV group than they were in non-HIV-infected patients with tuberculosis.

The cutoff points obtained with the discriminant functions were 0.191 for IgG antibody and 0.090 for IgM antibody. From these values (Table 2) in the IgG assays at a specificity of greater than 96.0% (only three controls with a negative skin test reaction had a serological result above the cutoff point), the sensitivities of the test were 45.3 and 72.8% for the TB and the TB-HIV groups, respectively, and 51.9% when data for both groups were analyzed together (tuberculous group). In the TB-HIV group, the positive and negative predictive values were greater than 90%. The proportion of all patients correctly classified by the test was 82.4% when IgG-specific antibodies were detected. With IgM-specific antibodies, the specificities and sensitivities were lower for all groups.

Evolution of IgG antibodies during chemotherapy (TB group). The mean ODs of IgG-specific antibodies in the TB group throughout the study period are given in Table 3. No significant differences in ODs ($P = 0.7437$) were found between the different periods of time. Also, all patients responded well to therapy and no relapses were detected during 12 months of clinical observation.

Evolution of IgG assays in HIV-positive patients (TB-HIV

TABLE 2. Sensitivities, specificities, predictive values, and cases correctly classified for the different groups of patients with tuberculosis

Antibody and group[a]	Percent				
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Cases correctly classified
IgG-specific antibodies					
T	51.9	98.9	96.4	79.2	82.4
TB	45.3	96.9	83.0	84.0	83.9
TB-HIV	72.8	99.3	95.5	94.7	94.8
IgM-specific antibodies					
T	47.4	68.7	44.7	70.9	61.3
TB	52.5	61.6	31.2	79.2	58.9
TB-HIV	47.3	76.0	28.1	87.9	71.3

[a] T, total group of patients with tuberculosis (n = 156); TB, non-HIV-infected patients with tuberculosis (n = 97); TB-HIV, HIV-infected patients with tuberculosis (n = 59); C, healthy control group (n = 289). Cutoff point for IgG, 0.191; cutoff point for IgM, 0.090.

TABLE 3. IgG-specific antibody during chemotherapy in the TB group

Time from the start of treatment	No. of serum samples	OD (mean ± SE)
Tuberculosis diagnosis	69	0.272 ± 0.037
First mo	67	0.203 ± 0.035
Second or third mo	62	0.210 ± 0.033
Second quarter (mo 4–6)	104	0.221 ± 0.029
Third quarter (mo 7–9)	13	0.204 ± 0.060
Fourth quarter (mo 10–12)	9	0.174 ± 0.088

group). The evolution of IgG antibody levels in the TB-HIV group is given in Tables 4, 5, and 6.

Table 4 shows individual test results for six patients from whom sera were available before the onset of clinical tuberculosis and during specific treatment. In all patients except one (patient 530), significant antibody titers were found in sera collected at least 30 months before tuberculosis was diagnosed, and in three patients, antibody titers remained high during chemotherapy.

Table 5 shows individual test results for seven patients from whom sera were available only before the diagnosis of tuberculosis was made. In all patients, a positive test result was detected months before tuberculosis was diagnosed. In all patients except one (patient 545), ODs above 0.300 were detected.

Table 6 shows individual test results for six patients from whom sera were available only after tuberculosis was diagnosed. All patients had positive IgG antibody test results when tuberculosis was first discovered, and in all except one patient, the IgG levels had absorbances of below 0.200 12 months after the start of treatment.

Tables 4, 5, and 6 also indicate when the diagnosis of AIDS was made (2).

DISCUSSION

The evaluation of anti-SL-IV antibody assays described here yielded data that were in agreement with those from a previous report (4), showing that for specificities above 95.0%, the sensitivity of IgG assays is above 50 to 60% and indicating that a significant number of patients with tuberculosis have low antibody titers when the disease is first discovered. The use of serology by using the SL-IV antigen in identifying and diagnosing tuberculosis has been discussed previously (4, 6). The data from this study showing that antibody titers may remain high during the course of successful treatment led to the conclusion that the level of antibodies detected at the time that the cases are first discovered does not seem to bear prognostic value as far as chemotherapy results are concerned. However, it was observed that the antibody titers in the TB-HIV group decreased earlier than they did in immunocompetent patients. For the purpose of the present discussion, we are interested in the time course of antibody production, as described below.

In developed countries, the vast majority of new cases of pulmonary tuberculosis become the source of new infections, and thus, the pool of infected individuals is maintained in the community. Transmission of tuberculosis was successfully reduced by diagnosing and effectively treating infected patients, and consequently, a progressive reduction

TABLE 4. Evolution of IgG antibodies before and after tuberculosis diagnosis in the TB-HIV group

Time	OD of the following serum samples[a]					
	469	529	530	531	533	550
Mo before tuberculosis diagnosis						
19					0.145	
18			0.095			0.500
17	0.630					
15					0.245	
12				0.545		
9		0.846	0.190		0.100	0.618
7						0.150
3		0.750	0.140			
2	0.535					
Tuberculosis diagnosis	0.525[b]	0.400[b]	0.080[b]		0.030	[b]
Mo. after tuberculosis diagnosis						
1		0.430				
2			0.020			0.076
3			0.040			0.100
4	0.275					
5	0.390		0.045			
6	0.530			0.500	0.085	
8	0.190					
11						0.020
12					0.080	
14		0.195		0.350		
15		0.180				
21		0.185			[b]	
22		0.160				

[a] Number of the individually stored serum sample.
[b] Time of diagnosis of AIDS.

TABLE 5. Evolution of IgG antibodies before tuberculosis diagnosis in the TB-HIV group

Time	OD of the following serum samples[a]:						
	471	540	543	544	545	546	549
Mo before tuberculosis diagnosis							
30		1.000					0.340
18						0.510	[b]
15			0.360				0.084
13							0.150
12			0.430				
11							0.145
10			0.320	0.470[b]			
9	0.590	0.120	[b]				
8							0.020
7					0.200		
6							0.120
5				0.225			
4				0.511			0.130
2							0.340
1				0.170			
Tuberculosis diagnosis	1.210	0.760	0.360		0.160[b]	0.190	

[a] Number of the individually stored serum sample.
[b] Time of diagnosis of AIDS.

in the pool of infected individuals can be expected. In individuals infected with HIV, the incidence of tuberculosis is 500 times that in the general population (1). As the current epidemic of HIV infection expands, not sparing those infected by the tubercle bacilli, tuberculosis disease and transmission of tubercle bacilli should be anticipated unless specific measures to counteract it are applied. The Centers for Disease Control of the U.S. Public Health Service recommends (3) that chemoprophylaxis should be offered to people with positive HIV serology and a positive tuberculin skin reaction. The finding of a simple and reliable procedure to discriminate among people in whom the reactivation process has already started but has not yet revealed the signs and symptoms of tuberculosis would contribute to the solution of this problem. In this study, significant levels of anti SL-IV antibodies were detected in the sera of 12 of 13 individuals with positive HIV serology between 1 and 30 months before the onset of clinical signs and symptoms of tuberculosis. From the results given in Tables 4 and 5, we concluded that anti-SL-IV antibody assays may be satisfac-

TABLE 6. Evolution of IgG-specific antibodies during treatment of the TB-HIV group

Time	OD of the following serum samples[a]:					
	490	535	536	537	539	542
Tuberculosis diagnosis	0.270	0.430[b]	0.975[b]	0.510	0.315	0.350[b]
Mo after starting treatment						
2	0.140				[b]	
5	0.090					0.410
6	0.065					
7			0.265			
8			0.160			0.340
9			0.175			
10	0.050		0.230			
11			0.195		0.060	
12					0.090	0.185
13					0.080	
14			0.200	0.030	0.050	0.185
15					0.050	
16			0.115			
18			0.180			
19		0.480				
20					0.040	
21			0.185			
22		0.320			0.050	
24		0.300				
26		0.355		0.080	0.050	
30		0.390				

[a] Number of the individually stored serum sample.
[b] Time of diagnosis of AIDS.

tory for identifying tuberculosis in HIV-infected individuals and may allow the diagnosis of tuberculosis before the onset of clinical symptoms. We consider these findings to be highly significant, because only three positive serology test results were found among the control group of 289 individuals examined, regardless of whether they had positive or negative tuberculin skin reactions.

It is not yet clear whether a full course of antituberculosis treatment may be justified from serology data like those presented here can be recommended, because the number of HIV-positive patients examined in this study was not sufficiently large to make such recommendations; and it is not known whether the same results could be expected for immunocompetent people. In dealing with healthy human populations (even though they are at high risk of getting tuberculosis), it is difficult to collect all pertinent information and the necessary clinical specimens; however, further studies would contribute to more definitive conclusions about this important issue.

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