INTERNATIONAL JOURNAL OF LEPROSY

Volume 64, Number 4
Printed in the U.S.A.
(ISSN 0148-916X)

Enzyme-Linked Immunosorbent Assay (ELISA) with Mycobacterial Crude Antigens for the Sero-Epidemiological Diagnosis of Active Tuberculosis[1]

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Tuberculosis and leprosy are the major human mycobacterioses, and both remain as important public health problems, in spite of worldwide efforts to control them ([7, 30]). Consequently, it is necessary to perform large epidemiological surveys for the detection of active cases which allow the continuous transmission of both diseases, particularly when limited or no intervention takes place. Also, the determination of the extent of the corresponding endemias would provide a solid basis for better surveillance programs ([6, 34]). Nevertheless, the routine diagnosis of tuberculosis and leprosy largely relies on highly expensive and time-consuming clinical and laboratory studies, quite inadequate for epidemiological studies. The best choice for this purpose would be nonexpensive laboratory diagnostic procedures which can be set up in every public health laboratory, especially in developing countries where the magnitude of both problems is greater ([9, 19]).

The first methodological approach to the epidemiological diagnosis of tuberculosis and leprosy employed serological techniques ([10, 29]). Great difficulties with the specificity of the mycobacterial antigens and the development of the polymerase chain reaction (PCR) techniques lowered the interest in the diagnosis of mycobacterioses, especially tuberculosis, through antibody-based methods ([28]). However, the usefulness of the PCR tests for tuberculosis was not well established until now ([8, 31]) and, in any case, this procedure is too expensive and out of reach for developing countries with limited financial resources. For these reasons, this paper forms a part of our interest in re-evaluating the usefulness of serodiagnostic tests for mycobacterial diseases, identifying their limitations and the conditions necessary for the correct interpretation of the results.

Among serological techniques, the enzyme-linked immunosorbent assay (ELISA) seems to be the most appropriate for the diagnosis of mycobacterial diseases ([5, 10]). In the case of *Mycobacterium tuberculosis,* the lack of a species-specific molecule has contributed to the development of ELISAs using crude, semicrude and purified molecules. Even though there are reports about better diagnostic performances with purified molecules ([10, 28, 33]), there is no unified consensus concerning which one is the best, and consequently, there are no protocols for their pilot or industrial production and standardization of the reagents. Therefore, none is commercially available. Consequently, tuberculosis serodiagnostic techniques under use in most laboratories from developing countries usually employ crude antigens whose crossreactivity with other mycobacteria limit the accuracy of the positive predictive results. This situation is especially striking in leprosy-endemic areas because the large amounts of bacilli spread by active cases easily induce humoral anti-mycobacterial responses in households as well as in the general population ([1]).

[1] Received for publication on 12 December 1995; accepted for publication in revised form on 29 April 1996.

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In this paper, the diagnostic performances of ELISAs employing six commonly used, crude mycobacterial preparations were evaluated in confirmed active cases, with either tuberculosis or leprosy, as well as in household contacts of tuberculosis patients. Assayed antigens were intact whole bacilli, lipid-free whole bacilli, and crude, protein-enriched, soluble extracts from *M. tuberculosis* H37Rv and *M. bovis* BCG strain. The evaluation of sensitivity and specificity of each preparation was accomplished using sera from well-defined tuberculosis and leprosy patients, household contacts of tuberculosis cases, and samples from healthy individuals from a low and a high leprosy-endemic area. Cases and controls were carefully matched for their ethnic and socioeconomic backgrounds.

MATERIALS AND METHODS

Cases and controls. One-hundred-six serum samples from tuberculosis cases and their contacts were included in the study. Tuberculosis sera were obtained from 46 adult patients with nontreated pulmonary tuberculosis admitted to the Servicio de Neumologia, Hospital General de Mexico, Secretaria de Salud, Mexico City, Mexico. All cases had clinical and radiological evidence of pulmonary tuberculosis, confirmed through positive sputum smears and *M. tuberculosis* cultures. In addition, 60 household contacts from active tuberculosis cases, without any clinical feature suggesting tuberculosis, were selected among familial and nonfamilial individuals living together for more than 6 months.

Twenty sera from adult lepromatous leprosy patients, all positive for antibodies against *M. leprae* by the FLA-ABS test, were randomly chosen from our laboratory collection ([2, 14]). Patients were diagnosed and classified according to the standard clinical, histopathological and bacteriological methods at Servicios Coordinados de Salud Publica in Culiacan, state of Sinaloa, Mexico.

As healthy controls, two groups of adults were selected according to the tuberculosis and leprosy morbidities in the areas where they lived. In Mexico, the tuberculosis morbidity rate for 1993 was 0.14 per 1000 inhabitants; the leprosy rate for the same year was 0.12 per 1000 inhabitants. The first control group consisted of 20 sera from the state of Sinaloa, in northwestern Mexico, where morbidity rates for tuberculosis and leprosy were: 0.265 cases per 1000 inhabitants and 0.83 cases per 1000 inhabitants, respectively. The second group was formed by 47 sera obtained from altruistic blood donors in Mexico City; here, the rate for tuberculosis reached 0.06 cases per 1000 inhabitants, and the one for leprosy was of 0.02 cases per 1000 inhabitants. None of them had any history of recent mycobacterial disease or any other infectious condition, but no attempt to assess their PPD-skin reactivity was undertaken. Both patients and controls shared similar socioeconomic status and belonged to the Mexican mestizo ethnic background. Sera was aseptically separated from blood and kept frozen at –70°C until use.

Mycobacterial strains. *M. tuberculosis* H37Rv was obtained from the collection of the Departamento de Micobacterias, INDRE, Mexico City, and the *M. bovis* Danish 1331 BCG strain used for vaccination was donated by Instituto Nacional de Higiene, Secretaria de Salud, Mexico City. In both cases, the identity and purity of the strains were confirmed using standard methods and they were grown in Sauton's medium until a luxurious growth was reached. Bacterial pellets were washed three times with pH 7.4, 0.01 M phosphate buffered saline (PBS), resuspended in the same buffer, and maintained frozen at –70°C.

Intact whole bacilli preparations. For both mycobacterial strains, frozen bacilli were thawed in a water bath at 37°C, weighed and resuspended in PBS pH 7.4. *M. tuberculosis* cells were killed by irradiation at 2.5 Mrad with a ^{60}Co source. Final concentration of this suspension was adjusted to 10 mg of moist bacilli per ml.

Lipid-free whole bacilli preparations. With both organisms, isopentanol-extracted, formalin-fixed whole cells were prepared as described by Wayne, *et al.* ([39]) as follows: 20 mg of moist mycobacteria were suspended in 1 ml of 10% formalin in PBS, pH 7.4 vol/vol. After three washes with distilled water, the cells were suspended in 200 ml of distilled water, mixed with 10 ml of isopentanol (isoamyl alcohol, ACS reagent; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) and mixed for 10 min in a vortex. Bacilli were separated by centrifugation at 900 × *g* × 30 min, washed twice with 0.02% Tween 80 in distilled wa-

ter and resuspended at a concentration of 10 mg of moist cells per ml.

Protein-enriched soluble extracts. Sonic extracts of both mycobacterial strains were prepared with an adaptation of a method published elsewhere ([14]). Briefly, bacterial pellets were suspended in 2 ml of PBS pH 7.4 and disrupted by sonication on an ice-water bath for 15 min at 2-min intervals at 250 W on a Sonifier cell disrupter (Model W-370, Heat Systems; Ultrasonics Inc., North Tonawanda, New York, U.S.A.). The sonicated material was examined for the absence of intact cells by Ziehl-Neelsen staining and centrifuged at 20,000 × *g* × 15 min in the cold. The supernatant was filtered through a 0.22-μm pore membrane filter, and the protein concentration was adjusted to 2.5 μg per ml.

ELISA procedure. Polystyrene plates (EIA/RIA plate, flat-bottom, high binding; Costar Corporation, Cambridge, Massachusetts, U.S.A.) were sensitized at 4°C overnight with 100 μl of the corresponding preparations described above. After blocking 90 min at room temperature with 1% bovine serum albumin (BSA grade IV; Sigma) in PBS pH 7.4, the wells were filled in duplicate with test or control sera diluted 1:100, 1:500 and 1:1000 in 2.5% normal goat sera and 0.05% Tween 20 in PBS pH 7.4, for the intact or lipid-free bacilli, and in the same solution used for blocking for the protein-enriched soluble extract. After discarding the contents, the wells were washed three times and incubated at room temperature with polyclonal goat anti-human immunoglobulin coupled to horseradish peroxidase (Sigma) at a 1:1000 dilution. After 1 hr of incubation at room temperature, the plates were washed with PBS, pH 7.2, and 100 μl of freshly prepared 0.4 mg/ml *o*-phenylenediamine with 100 μl 3% hydrogen peroxide was added as the enzyme substrate. The reaction was stopped with 4 N sulfuric acid and the absorbance (A) was read at 492 nm using an ELISA reader (Multiskan plus; Labsystems, Helsinki, Finland) against a blank without serum.

Cut-off values for each antigen preparation were calculated considering the corresponding mean absorbances obtained in the control group plus two standard deviations ([13]).

Data analysis. For each serum, the mean absorbance value for duplicate measures was calculated. When a variation larger than 10% was found, the results were discarded and the test was repeated. An intra-assay reliability test was performed considering variation coefficients of all the duplicates included in a given plate, and a <10% variation coefficient was considered optimal. In addition, an intra-assay reproducibility test was included in each plate with duplicates of known sera (high and low positive and negative controls); a <10% variation coefficient was acceptable.

We calculated the means and standard deviations of the A_{492} values for the control and study groups. The significance of the variations in antibody levels among groups was determined through Student's *t* test. Comparisons among cases and controls were made considering one or another control group separately. Specificity, sensitivity, and positive and negative predictive values for each antigen were determined using Galen and Gambino's ([17]) recommended method. After selection of the best performing antigen, we determined a coefficient of agreement for each of the other antigens as follows: 100 × number of results in agreement/total number of samples tested. To compare the selected preparation with other antigens, we used Cohen's kappa test ([15]) and a 95% confidence interval was calculated. The κ value was corrected for chance and ranged from −1 to 1, where κ = 1 corresponds to a perfect agreement, κ = −1 indicates perfect disagreement, and $\kappa = 0$ is an agreement expected by chance alone.

RESULTS

Selection of optimal amount of antigen preparations. Whole intact and whole lipid-free bacilli were only tested at 10 mg/ml as has been recommended ([39]). To find the optimal concentrations of *M. tuberculosis* and BCG soluble extract preparations, we performed ELISAs with doubling dilutions of these antigens, starting from 15 μg/ml, using randomly chosen sera, 10 from the healthy control group and 10 from the tuberculosis group. In both cases, 2.5 μg/ml was the optimal concentration found.

Comparison of the six antigen preparations. In order to determine the differences between test and control sera with each antigen, 1:100, 1:500 and 1:1000 dilutions of 10 randomly chosen tuberculosis and leprosy sera were tested. Most of the

TABLE 1. *Results of ELISA for detection of anti-mycobacterial antibodies in pulmonary tuberculosis active cases and tuberculosis household contacts using six antigens.*

Antigens	Cut-off value[a]	Tuberculosis active cases			Tuberculosis household contacts		
		No. positive/ total	% Positive	A_{492} (mean ± S.D.)	No. positive/ total	% Positive	A_{492} (mean ± S.D.)
M. tuberculosis							
Whole intact bacilli	0.614	33/47	70	1.116 ± 0.680	6/60	10	0.220 ± 0.240
Whole lipid-free bacilli	0.347	38/47	81	0.812 ± 0.596	1/60	2	0.267 ± 0.085
Soluble extract	0.281	31/47	66	0.669 ± 0.513	2/60	3	0.156 ± 0.074
M. bovis, BCG strain							
Whole intact bacilli	0.464	37/47	79	1.200 ± 0.789	6/60	10	0.350 ± 0.097
Whole lipid-free bacilli	0.313	35/47	74	0.939 ± 0.768	0/60	0	0.078 ± 0.037
Soluble extract	0.464	43/47	91	1.389 ± 0.842	6/60	10	0.283 ± 0.159

[a] Mean A_{492} in controls + 2 standard deviations (S.D.).

sera diluted 1:100 gave important background color, regardless of their origin, antigen employed and number or duration of washings. Contrastingly, consistent results were obtained with sera diluted at 1:500 and 1:1000, but some sera from tuberculosis patients were negative at the 1:1000 dilution in spite of a clear positive result at a 1:500 dilution. Therefore, only sera diluted at 1:500 were employed, and the results for controls, contacts, and patients are shown in Tables 1 and 2 as well as in Figures 1 and 2.

All control sera showed low absorbance readings with normal distribution curves. Since the analysis of variance showed no difference between results in healthy controls from high- or low-endemic leprosy or tuberculosis areas, they were combined to provide a single, large control group for

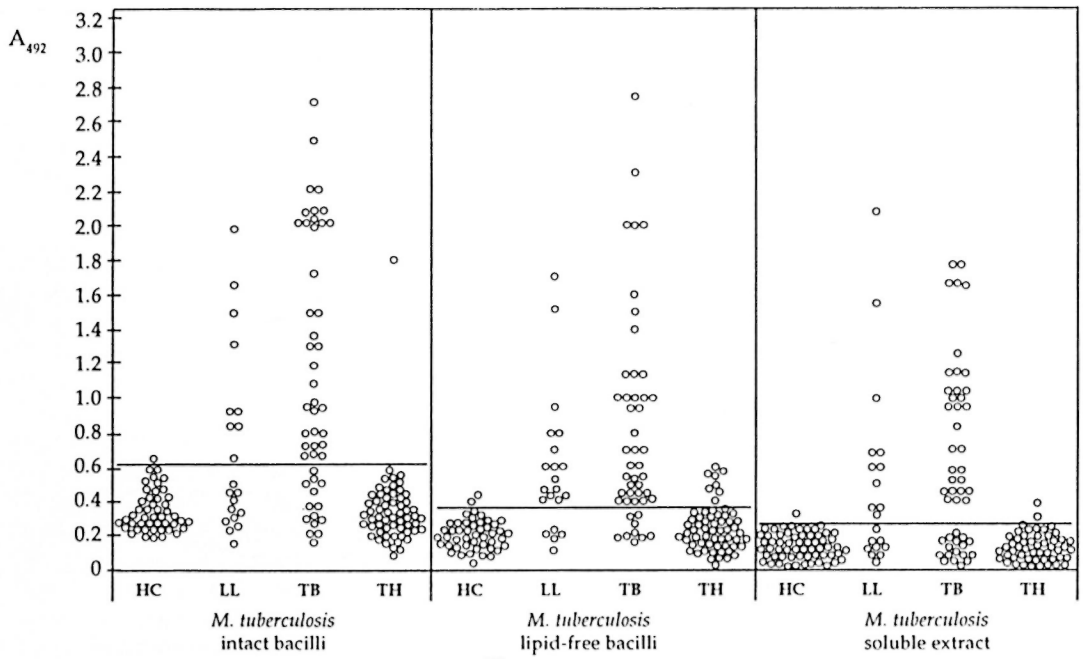

FIG. 1. Absorbance values in sera from healthy controls (HC), lepromatous leprosy cases (LL), tuberculosis patients (TB), and household contacts from tuberculosis patients (TH), using *M. tuberculosis* crude antigens in ELISA (horizontal bars show cut-off values).

TABLE 2. *Results of ELISA for detection of anti-mycobacterial antibodies in lepromatous leprosy cases using six antigens.*

Antigens	Cut-off value[a]	No. positive/ total	% Positive	A_{492} (mean ± S.D.)
M. tuberculosis				
Whole intact bacilli	0.614	9/20	45	0.694 ± 0.486
Whole lipid-free bacilli	0.347	15/20	75	0.606 ± 0.381
Soluble extract	0.281	11/20	55	0.536 ± 0.516
M. bovis, BCG strain				
Whole intact bacilli	0.464	7/20	35	0.682 ± 0.811
Whole lipid-free bacilli	0.313	12/20	60	0.678 ± 0.720
Soluble extract	0.464	3/20	15	0.270 ± 0.366

[a] Mean A_{492} in controls + 2 standard deviations (S.D.).

comparison with the test sera. As determined in the large control group, cut-off values were distinct for each preparation (Tables 1 and 2). Five out of 67 control sera gave positive results with one or another antigen, and all of them with soluble extract of BCG. In four of them absorbance was near to the respective cut-off value, and only one control serum was clearly positive to all but one of the antigens tested. Even though we tried several times, it was impossible to locate this positive responsive donor to clarify the actual individual's clinical status.

Mean A_{492} values for antibodies against the six antigens tested in the studied groups are presented in Tables 1 and 2. As can be seen, significant differences with controls are consistently present only in the case of tuberculosis patients ($p < 0.05$). With any of the preparations it was possible to detect as positive all of the tuberculosis sera. The highest number of positive results in the tu-

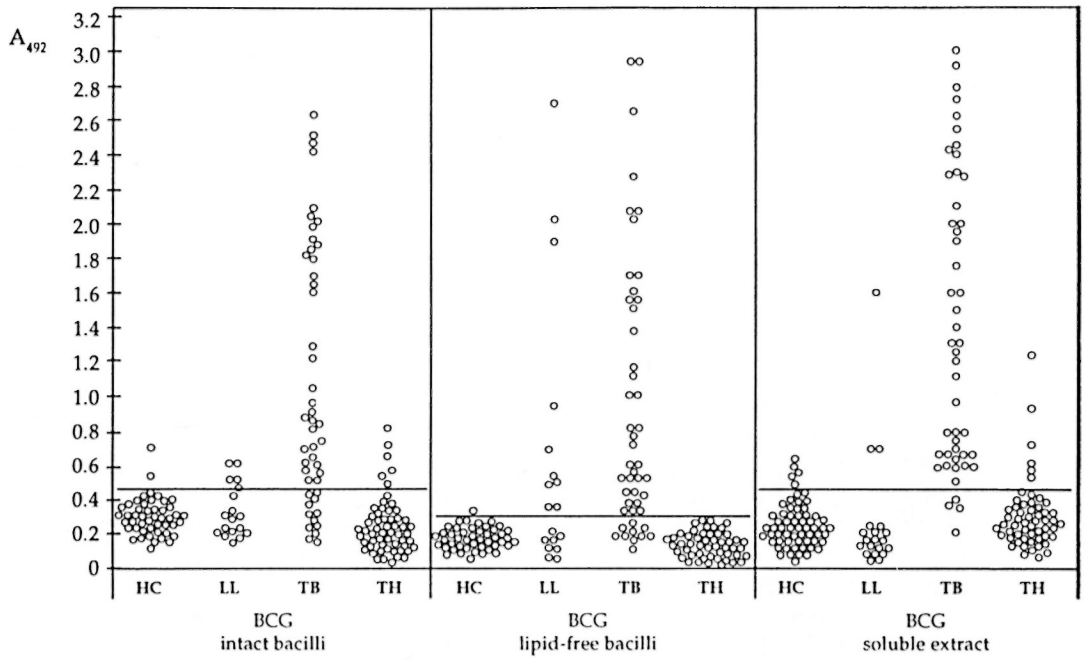

FIG. 2. Absorbance values in sera from healthy controls (HC), lepromatous leprosy cases (LL), tuberculosis patients (TB), and household contacts from tuberculosis patients (TH), using BCG crude antigens in ELISA (horizontal bars show cut-off values).

TABLE 3. *Performance of six antigens in ELISA for the serological detection of anti-mycobacterial antibodies in tuberculosis-active patients.*

Antigen	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)
M. tuberculosis				
Whole intact bacilli	74.5	97.0	94.6	84.6
Whole lipid-free bacilli	80.8	94.1	90.5	87.7
Soluble extract	70.2	94.3	89.2	82.5
M. bovis, BCG strain				
Whole intact bacilli	78.7	97.0	94.9	86.6
Whole lipid-free bacilli	74.5	98.5	97.2	84.6
Soluble extract	91.5	92.5	89.5	93.9

[a] Positive predictive value.
[b] Negative predictive value.

berculosis patients' group was obtained with the use of soluble extract of BCG (91%) and, interestingly, this preparation identified the lowest number of leprosy cases. Also, with this preparation the best value for sensitivity was achieved (91.5%) (Table 3). Even in this group, however, several serum samples had lower absorbances than the cut-off value, regardless of the fact that they came from confirmed, active tuberculosis patients (Figs. 1 and 2). As also can be seen in Table 1, most of the sera from the household contacts gave negative results with all of the preparations, and their mean antibody level for each one was very similar to the healthy controls. After obtaining these results, the six positive contacts, regardless of the preparation to which they reacted, were submitted to clinical studies. Three of them showed suggestive chest radiographs and, therefore, standard treatment was started. The remaining positive contacts are now under permanent surveillance.

Considering the results in the pulmonary tuberculosis group, specificities were up to 92% for all antigens tested, but their sensitivities showed varying levels (Table 3). Because sensitivity is the parameter of choice for our epidemiological studies, the results with BCG protein-enriched soluble extract were considered as standard for the analysis of the remaining results. Figure 3 shows the contingency tables used for such comparisons.

DISCUSSION

Interest in the serodiagnosis of mycobacterioses, particularly of tuberculosis, has been dampened somewhat because most of *M. tuberculosis* antigens show crossreativity with other mycobacterial species, both environmental and pathogenic. Nevertheless, inexpensive and efficient methods such as ELISAs remain the best choice for the epidemiological studies especially needed in developing countries ([10]). In a previous study, efficiency in the serodiagnosis for leprosy was explored ([14]). Therefore, this work was designed to try several whole mycobacterial preparations in ELISAs for tuberculosis serodiagnosis applicable to population surveys. Consequently, special care was taken in the meticulous search for active tuberculosis patients confirmed through positive bacilloscopy and *M. tuberculosis* culture. In order to establish the level of false-positive results, sera from individuals living in the same house as a confirmed tuberculosis case, who did not show any symptoms of the disease, were included. Also, a group of well-defined sera from leprosy cases was studied in order to identify the preparation with the lowest crossreactive reactions. Control groups were checked for the absence of any suggestive history of mycobacterial infection other than BCG vaccination (which is compulsory in Mexico). According to the age of the individuals included in our control groups, most of them should have been BCG vaccinated and the low anti-mycobacterial antibody levels observed in all of them are in agreement with other reports ([3, 4, 24–26]) of a nonsignificant effect of the BCG vaccination on the number of false-positive results in antibody-based diagnostic tests for tuberculosis. It is known that

		BCG strain soluble extract +	-	
M. tuberculosis	+	36	2	
lipid-free bacilli	-	6	3	Agreement = 73.47% κ = 0.34 (0.07 - 0.61)

		BCG strain soluble extract +	-	
BCG strain	+	32	2	
lipid-free bacilli	-	10	3	Agreement = 65.31% $\kappa = \kappa$ = 0.21 (-0.03 - 0.46)

		BCG strain soluble extract +	-	
M. tuberculosis	+	31	2	
intact bacilli	-	11	3	Agreement = 63.26% κ = 0.19 (-0.05 - 0.43)

		BCG strain soluble extract +	-	
BCG strain	+	30	1	
intact bacilli	-	12	4	Agreement = 61.22% κ = 0.26 (0.04 - 0.48)

		BCG strain soluble extract +	-	
M. tuberculosis	+	28	1	
soluble extract	-	14	4	Agreement = 57.14% κ = 0.22 (0.01 - 0.43)

FIG. 3. Contingency tables of results from comparisons of ELISAs using six antigens for the serodiagnosis of tuberculosis cases (numbers in parentheses after κ values are 90% confidence intervals).

BCG vaccination provides a very limited antigenic stimulus compared to that of an active infection ([38]) and its antibody response could interfere with the serodiagnosis only when the vaccination was recently performed ([32, 36]).

Systematic statistical analysis of the results for each serum duplicate and the routine repetition of all the samples with differences >10% in absorbance, as practiced in this work, greatly reduced the variability in reactivity commonly seen in ELISA systems. Nevertheless, it must be stressed that the specificity and sensitivity of any ELISA depends not only on variables related to the method itself but also on the antigen selected and the populations under study. An example of this variability in the tuberculosis serodiagnosis can be seen in the wide differences obtained with the use of purified protein derivative (PPD) by different authors ([3, 4, 11, 23, 24, 27, 35, 37, 41]). As a result of this situation, laboratory personnel working with ELISA for tuberculosis have to deal with several options when choosing the antigen preparation for routine screening, always taking into account the epidemiological background of the population to be

screened. In this work, whole intact bacilli and soluble extracts were chosen because they are frequently used in some laboratories; the inclusion of mycobacteria depleted of external lipids (lipid-free whole bacilli) was included because such treatment could eliminate masked, relevant, antigenic surface molecules. Lipid-free bacilli, from either *M. tuberculosis* or BCG, reacted more frequently with the tuberculosis and leprosy sera than did the respective intact preparations.

The positive reactions of BCG preparations with sera from tuberculosis and leprosy cases, as well as those between BCG and *M. tuberculosis* preparations with leprosy sera, are the obligate consequence of the strong molecular similarity among mycobacteria ([12, 40]). Even though BCG and *M. tuberculosis* crude preparations showed better reactivity with tuberculosis samples than with leprosy sera, such crosspositivity made it inadequate to use these antigens in areas where both mycobacteria are prevalent in the population. Thus, crude preparations for seroepidemiological studies of tuberculosis can be used only in leprosy-free areas. Under these circumstances, results presented here point out that the crude preparation of choice for tuberculosis serodiagnosis is the BCG soluble extract, as has been reported by other authors ([18, 21, 24]).

The occurrence of false-negative results in some tuberculosis patients was not unexpected because this situation is fairly common in the serodiagnosis of the disease, even with methods other than ELISA or when more purified antigens are used. The available clinical and laboratory data of the seronegative patients were not sufficient to clarify the origin of such situations and specific investigations in this direction will have to be done.

It must be stressed that we suggest the use of crude mycobacterial preparations for epidemiological studies only. Moreover, in population surveys this preparation can be used confidently because it can readily detect most of the active tuberculosis cases and even in the close contacts of active cases, false-positive results seem to be not important. Studies to confirm this assumption are now in progress in our laboratory. On the other hand, for individual diagnosis of pulmonary tuberculoid cases, the use of serological methods for the routine work are excluded because results of sputum examination by microscopy, in combination or not with sputum culture, yield an accurate and highly efficient diagnosis of the condition ([20, 22]). An attractive possibility, which now is under study in our laboratory, is the employment of serological tests for the identification of extrapulmonary tuberculosis cases only where radiography is not useful and bacilloscopy and/or culture are difficult to carry out. The preliminary results are encouraging and indicate that a negative result with a BCG protein-enriched soluble extract ELISA does not rule out tuberculosis, but a positive result could be a highly reliable indicator of the disease because the method seems to be specific for mycobacterial infection.

In conclusion, the results of our present study strongly support an acceptable diagnostic value for BCG soluble extract as an antigen in an ELISA to be used for tuberculosis epidemiological surveys in leprosy-free areas when purified antigens are not available. However, the use of serological tests for individual case diagnosis of extrapulmonary tuberculosis must be regarded as a complementary procedure and the results must be cautiously interpreted, always taking into account compatible clinical data.

SUMMARY

In search for reliable, nonexpensive procedures for tuberculosis diagnosis suitable for seroepidemiological studies in leprosy-endemic areas, enzyme-linked immunosorbent assays (ELISAs) with whole intact bacilli, whole lipid-free bacilli and protein-enriched soluble extracts from the H37Rv *Mycobacterium tuberculosis* strain were evaluated. Sera tested came from 47 active, pulmonary tuberculosis adult cases, 60 household contacts of active tuberculosis cases, 20 lepromatous leprosy adult patients, and 67 healthy adult controls obtained from low and high leprosy and tuberculosis endemicity areas. There was no influence of such endemicity levels in the number of positive results in control sera. Antibody levels obtained with each of the antigens in ELISAs were significantly different in tuberculosis patients and the control groups. Ten percent of tuberculosis contacts were positive with some of the antigens and three of them showed sugges-

tive chest radiographs. The best combination for a high number of positive results with tuberculosis sera and low positive results with leprosy sera was the BCG soluble extract (91% and 15%, respectively). This preparation also yielded excellent sensitivity and specificity values for tuberculosis (91.5% and 92.5%, respectively). These data suggest that BCG soluble extract ELISAs could provide helpful information to estimate tuberculosis prevalence only in leprosy-free areas, under a situation of unavailability of purified antigens. In pulmonary cases, sputum microscopic examination and culture have higher sensibility than serodiagnosis; therefore, the utilization of BCG soluble extract ELISAs as a diagnostic aid in individual patients with suspected active tuberculosis only can be useful in extrapulmonary cases.

RESUMEN

Se evaluaron procedimientos inmunoenzimáticos (ELISAs) con bacilos intactos, bacilos deslipidizados y extractos solubles de *Mycobacterium tuberculosis* H37Rv, con el fin de encontrar un método confiable y barato para el diagnóstico de la tuberculosis en áreas endémicas de lepra. Los sueros probados correspondieron a 47 adultos con tuberculosis pulmonar activa, 60 contactos convivientes de los pacientes con tuberculosis, 20 pacientes con lepra lepromatosa y 67 controles sanos de áreas de alta y baja endemicidad para lepra y tuberculosis. El grado de endemicidad no influyó en los resultados encontrados en los sueros control. Los niveles de anticuerpo con cada uno de los antígenos fueron significativamente diferentes entre los pacientes con tuberculosis y el grupo control. Diez porciento de los contactos de los pacientes fueron positivos con alguno de los antígenos y 3 de ellos mostraron radiografías de tórax sugestivas de enfermedad. Con el extracto soluble de BCG se logró una alta reactividad con los sueros de tuberculosis y una baja positividad con los sueros de lepra (91% y 15% respectivamente). Estos datos sugieren que en situaciones donde no se cuenta con antígenos purificados, los ELISAs con extractos solubles de BCG podrían servir para estimar la prevalencia de tuberculosis en las áreas libres de lepra. Ya que en los casos pulmonares el examen microscópico del esputo y el cultivo tienen mayor sensibilidad que los métodos serológicos, la utilización de los ELISAs con extractos solubles de BCG para el diagnóstico de tuberculosis, sólo podría ser útil en los casos extrapulmonares.

RÉSUMÉ

A la recherche de tests fiables, bon marché et appropriés pour le diagnostice de la tuberculose pour des études séro-épidémiologiques dans des régions où la lèpre est endémique, nous avons évalué des tests enzymatiques (ELISA) avec des bacilles entiers intacts, des bacilles entiers sans lipides et des extraits solubles enrichis en protéines provenant de la souche H37Rv de *Mycobacterium tuberculosis.* Les serums testés provenaient de 47 cas de tuberculose pulmonaire active, 60 contacts domiciliaires de cas actifs de tuberculose, 20 cas adultes de lèpre lépromateuse et 67 témoins adultes en bonne santé venant de régions à endémicités faibles et élevées pour la lèpre et la tuberculose. Il n'y avait pas d'influence des taux d'endémicité sur le nombre de résultats positifs parmi les serums des témoins. Les taux d'anticorps obtenus avec chacun des antigènes étaient significativement différents chez les patients tuberculeux et les groupes de contrôle. Dix pourcents des contacts de tuberculeux étaient positifs pour certains des antigènes et trois d'entre eux ont montré des radiographies suggestives. La meilleure combinaison pour un nombre élevé de résultats positifs parmi les serums de tuberculeux et un nombre faible de positifs parmi les serums de lépreux était l'extrait soluble de BCG (respectivement 91% et 15%). Cette préparation donnait également d'excellentes valeurs de sensibilité et de spécificité pour la tuberculose (respectivement 91.5% et 92.5%). Ces données suggèrent que les ELISA à l'extrait soluble de BCG pourraient donner des informations utiles pour estimer la prévalence de la tuberculose dans des régions où la lèpre n'existe pas, dans des situations où les antigènes purifiés ne sont pas disponibles. Dans les cas pulmonaires, l'examen microscopique des expectorations et la culture ont une sensibilité plus élevée que le sérodiagnostic; c'est pourquoi l'utilisation d'ELISA aux extraits solubles de BCG comme aide au diagnostic chez des patients individuels suspects de tuberculose active peuvent seulement être utiles dans les cas de tuberculose extra-pulmonaire.

Acknowledgment. The authors wish to express their gratitude to María Teresa Garrido and Lamberto Blancarte from the Instituto Nacional de Diagnóstico y Referencia Epidemiológicos; María Elena Juárez from the Hospital General de México; Juvencio Ruíz-Puente from the Instituto Nacional de Higiene, and Enrique Domínguez from the Instituto Nacional de Investigaciones Nucleares for their valuable help in the accomplishment of this project. We are also indebted to Ethel García and Aurora del Río for their excellent critical review of this manuscript.

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