Immunoglobulin A (IgA) and IgG Immune Responses against P-90 Antigen for Diagnosis of Pulmonary Tuberculosis and Screening for Mycobacterium tuberculosis Infection

Marcus B. Conde,¹* Philip Suffys,² Jose Roberto Lapa e Silva,¹ Afranio L. Kritski,¹ and Susan E. Dorman³

Unidade de Pesquisa em Tuberculose, Instituto de Doenças do Tórax, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro,¹ and Laboratório de Biologia Molecular e Diagnóstico de Doenças Infecciosas e Laboratório de Hanseníase Instituto Oswaldo Cruz, Fundação Oswaldo Cruz,² Rio de Janeiro, Brazil, and Center for Tuberculosis Research, Johns Hopkins University School of Medicine, Baltimore, Maryland³

Received 28 August 2003/Accepted 29 October 2003

The purpose of the present study was to evaluate the usefulness of detection of serum immunoglobulin A (IgA) and IgG antibodies directed against the mycobacterial P-90 antigen for the diagnosis of active pulmonary tuberculosis (PTB) among symptomatic individuals and for the detection of Mycobacterium tuberculosis infections among close contacts of PTB patients. Two commercially available enzyme immunoassay (EIA) kits (IgA EIA-TB [EIA-IgA] and IgG EIA-TB [EIA-IgG]; Kreatech Diagnostics) were evaluated in a blinded fashion by using stored serum samples from 268 individuals, including 69 patients with PTB, 41 patients with diseases other than tuberculosis (TB), 12 subjects with healed PTB, 39 close contacts of PTB patients, and 107 healthy volunteers. For the EIA-IgA, the sensitivity was 74% and the specificity was 68% when a cutoff determined by a receiver operator characteristic curve was used. For the EIA-IgG, the sensitivity was 69% and the specificity was 64%. The EIA-IgA was positive for 54% of healthy close contacts of PTB patients but only 8% of healthy controls without contact with a PTB patient or a prior personal history of TB (P < 0.001). The relatively low sensitivities and specificities of these serologic tests make them poor tools for the diagnosis of PTB among patients with suspected PTB. However, the relatively high prevalence of positive EIA-IgA results among healthy close contacts of PTB patients warrants further evaluation of this test with close contacts and other populations at risk for recent M. tuberculosis exposure and development of disease.

Pulmonary tuberculosis (PTB) remains one of the leading causes of morbidity and mortality worldwide, with approximately 8 million new cases and nearly 2 million deaths occurring each year (5). Although the examination of sputum smears for acid-fast bacilli is a rapid diagnostic method with a good sensitivity for the detection of mycobacteria among tuberculosis (TB) patients with cavitary pulmonary disease, it has a low sensitivity among patients with noncavitary pulmonary TB, those with extrapulmonary TB, and those who are unable to expectorate spontaneously (8). Because only 40 to 60% of patients with PTB are positive for acid-fast bacilli by sputum smear, culture of Mycobacterium tuberculosis is considered the “gold standard” methodology for the laboratory diagnosis of TB. However, culture may require several weeks before results are available.

There is an urgent need for rapid, cost-effective, and accurate methods for the diagnosis of TB. A serologic test is attractive because it could be relatively rapid and would not require sputum expectoration. Challenges for the development of effective serologic tests include the need to discriminate active disease from latent infection, to avoid cross-reactivity with M. bovis BCG or mycobacteria other than M. tuberculosis, and to perform consistently with genetically and immunologically diverse populations. Although most serologic tests studied to date have evaluated the immunoglobulin G (IgG)-mediated humoral immune response against mycobacterial antigens, other immunoglobulin classes may have more specificity for the diagnosis of TB, depending on the type of antigen used (4).

The purpose of the study described here was to evaluate the usefulness of detection of IgA and IgG antibodies directed against the mycobacterial P-90 antigen for the diagnosis of PTB and for the screening of close contacts of PTB patients in Rio de Janeiro, Brazil, a setting with a high prevalence of PTB and for the screening of close contacts of PTB patients in a setting with a high prevalence of PTB.

MATERIALS AND METHODS

Subject enrollment. The study setting was the Hospital Universitário Clementino Fraga Filho of the Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. Between 1 June 1997 and 31 March 1999, subjects (age, 18 years and older) with respiratory symptoms and radiographic findings consistent with PTB (TB suspects) were prospectively enrolled. Leprosy patients, asymptomatic subjects without a history of active PTB or contact with PTB patients, and asymptomatic household contacts of PTB patients were also included in the study. The study was approved by the Ethics Committee of the Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, and informed consent was obtained from all study participants.

Clinical and laboratory evaluations. A medical history was obtained from the study subjects, including information about prior BCG vaccination. For TB suspects, a physical examination (including evaluation for the presence or absence of a BCG vaccination scar), chest radiograph, and testing for human
immunodeficiency virus infection were performed. Two sputum samples from each TB suspect were stained with the hematoxylin-eosin, Papanicolaou, Ziehl-Neelsen, and Grocott's methanamine silver stains and cultured on Löwenstein-Jensen and Sabouraud media. TB suspects unable to expectorate spontaneously underwent sputum induction and/or fiberoptic bronchoscopy with bronchoalveolar lavage by a clinician not involved with this study. Asymptomatic control subjects underwent standard tuberculin skin testing, and induration equal to or greater than 5 mm was considered a positive result. Cultures positive for mycobacteria were tested by standard biochemical methods to distinguish M. tuberculosis from other mycobacteria. The criterion used for the diagnosis of active PTB was (i) positivity for M. tuberculosis by culture of a respiratory tract specimen or (ii) improvements in clinical and radiographic abnormalities after 3 months of standard anti-TB treatment in the absence of therapy directed against other pathogens and the absence of an alternative diagnosis. Patients with respiratory symptoms, radiographic findings suggestive of prior healed PTB, negative mycobacterial sputum cultures, and unchanged chest radiographs after 6 months were classified as having healed PTB.

All subjects included in the study were monitored for at least 6 months after enrollment in the study. Subjects for whom no respiratory smear or culture data were available, relevant clinical information was lacking, or the test for human immunodeficiency virus infection was positive or who had received any antibiotic treatment including treatment with anti-TB agents for more than 1 week during the 3 months prior to enrollment in the study were excluded from the study.

Serology. Serum from each subject was obtained at the time of enrollment, stored at −20°C, and thawed only once at the time of the serology assays. Serology testing was performed with commercially available enzyme immunoassay (EIA) kits (IgA EIA-TB [EIA-IgA] and IgG EIA-TB [EIA-IgG]; Kreatech Diagnostics, Amsterdam, The Netherlands). These kits detect IgA and IgG antibodies to a mycobacterial P-90 immuno-cross-reactive antigen compound, respectively. Testing was performed according to the instructions of the manufacturer. Serum from each study subject was simultaneously tested with both kits. Briefly, 100 μl of serum from each individual was diluted 1:400, added in duplicate to two microtitr well plates from each kit, and incubated for 60 min at 37°C. After four cycles of rinsing, the wells were incubated at 37°C for 60 min with 100 μl of peroxidase-labeled human anti-human IgA or peroxidase-labeled anti-human IgG conjugate, followed by four rinsing cycles. Finally, 100 μl of peroxidase substrate was added to the plates, the plates were incubated at room temperature for 30 min in the dark, and 100 μl of stop solution was added. The absorbance values at 450 nm were recorded with a spectrophotometer (Titertek Multiskan MCC/340; Flow Laboratories, McLean, Va.). As recommended by the manufacturer, the cutoff optical density (OD) was the mean value of three cutoff control OD readings. The ratio of the OD of the serum samples was defined as (mean OD for control or patient serum − OD for the blank) (mean OD for the cutoff control − OD for the blank). According to the instructions of the manufacturer, patient results were considered positive when the ratio was >1.15 and negative when the ratio was <0.85.

Statistical analysis. The sensitivities and specificities of the IgA EIA-TB and IgG EIA-TB were assessed. The positive predictive value (PPV) and the negative predictive value (NPV) were calculated by using different rates of TB prevalence. The cutoff value of the OD ratio calculated by use of a receiver operating characteristic (ROC) curve was compared to that recommended by the manufacturer. Comparisons between groups were done by the chi-square test and, for small numbers, Fisher’s exact test. Comparison between two means was done by using the t test, and comparison of the means for multiple groups was done by the one-way analysis of variance test. The McNemar test was used to evaluate the agreement.

RESULTS

Two hundred sixty-eight serum samples were evaluated, including 112 from PTB suspects, 10 from patients with leprosy, 39 from close contacts of PTB patients, and 107 from healthy volunteers. Among the 112 TB suspects, 69 were diagnosed with active PTB (63 with bacteriologic diagnosis, 6 with clinical diagnosis), 21 had nontuberculous bacterial infections, 10 had lung cancer, and 12 had healed TB. PTB patients had a mean ± standard deviation age of 39.1 ± 14 years. Compared with the PTB patients, TB suspects without PTB were older (mean age, 49 ± 14 years [P < 0.001]), and healthy controls were younger (mean age, 22 ± 4 years [P < 0.001]).

Table 1 shows the OD values, as well as the sensitivities and the specificities, of the EIA-IgA and EIA-IgG by using the manufacturer-recommended OD ratio cutoff values and, separately, by using the OD ratio cutoff values determined by ROC analysis. The OD values of EIA-IgA and EIA-IgG were higher for patients with PTB than for healthy controls (P < 0.001 for EIA-IgA, P < 0.001 for EIA IgG), close contacts (P = 0.003 for EIA-IgA, P < 0.001 for EIA-IgG), and patients with nontuberculous lung diseases (P < 0.001 for EIA-IgA, P < 0.001 for EIA-IgG). There were no differences between the OD values for PTB patients and individuals with healed TB. By ROC analysis, an OD ratio cutoff value of 1.4 (rather than the OD ratio of 1.15 recommended by the manufacturer) was determined for each test. By using a cutoff value of 1.4, both tests had better specificities for healthy controls, but the rate of false-positive results remained high among TB suspects without PTB. However, the proportion of subjects with a positive EIA-IgA result was significantly higher among close contacts of PTB patients than among healthy controls with no history of PTB or contact with a PTB patient, even after stratification by tuberculin skin test result and BCG vaccination (P < 0.001 for all comparisons).

To improve the diagnostic yields of the assays, we evaluated the sensitivity and specificity of the combination of EIA-IgA and EIA-IgG results, using an OD ratio cutoff value of 1.4. In a scenario in which a subject was considered to have a positive result if the results of both tests were positive, the sensitivity was 51% (95% confidence interval [CI], 45 to 57%) and the specificity was 89% (95% CI, 85 to 93%). In a scenario in which a subject was considered to have a negative result if the results of both tests were negative, the sensitivity was 92% (95% CI, 89 to 95%) and the specificity was 43% (95% CI, 37 to 49%).

By use of an OD ratio cutoff value of 1.4, the NPV calculated for TB prevalence rates of 0.054% (the mean rate for Brazil) and 0.16% (the mean rate for the city of Rio de Janeiro) were greater than 99% for both EIA-IgA and EIA-IgG. By use of TB prevalence rates for Brazil and Rio de Janeiro, the PPVs of EIA-IgA were 0.12 and 0.31%, respectively, while the PPVs of EIA-IgG were 0.10 and 0.30%, respectively.

DISCUSSION

In the present study the best results were obtained by EIA-IgA, which was more sensitive and specific than EIA-IgG. However, the performances of both tests varied according to the cutoff point used for the diagnosis of TB. By using the cutoff point suggested by the manufacturer, the sensitivities were 82 to 83% for EIA-IgA and 67 to 75% for EIA-IgG, but there were many false-positive results for non-PTB subjects. Therefore, we used a ROC curve analysis to determine a new cutoff point of 1.4 for each test (3). By use of the new experimentally derived cutoff point, the overall specificities of both tests were higher, with >90% specificity of the EIA-IgA for healthy controls. Although serum IgA directed against P-90 has been reported to occur only in individuals with active TB (11), we found many false-positive EIA-IgA results for individuals with non-TB respiratory diseases, healed TB, leprosy, and recent close contacts of TB patients. When only smear-negative PTB patients were considered, the sensitivity of EIA-
IgA was only 41%. The poor sensitivity for this important patient subgroup and the poor specificity for non-PTB patients with respiratory symptoms limit the clinical usefulness of EIA-IgA for the diagnosis of PTB among PTB suspects. Overall, our results were generally similar to those previously reported for EIA-IgA P-90-based serological tests and two different IgG-based immunochromatographic serology tests evaluated in our hospital (1, 2, 6, 7, 9, 10).

Interestingly, using a cutoff ratio of 1.4, we found that the EIA-IgA was positive for 21 of 39 (54%) healthy close contacts of PTB patients but just 9 of 107 (8%) healthy individuals without such contact (P < 0.001). The EIA-IgA therefore may be useful for detection of individuals recently infected with M. tuberculosis. To our knowledge, this is the first time that the EIA-IgA has been evaluated with close contacts of PTB patients. Our finding raises the possibility that the low specificities of some serologic tests performed in regions where TB is endemic may be the consequence of a high rate of recent M. tuberculosis infection not evident by tuberculin skin testing.

The present study has several limitations. The serologic tests described here were performed retrospectively with serum that had been stored frozen and thawed once. Although no serum had been thawed more than once and none of the samples had been frozen for more than 28 months, we cannot formally exclude the possibility that the serum storage conditions adversely affected the test performance, although we believe that this is unlikely. The use of fresh serum could have some impact on the sensitivities of the tests. In addition, we did not include sera from individuals with respiratory infections due to mycobacteria other than M. tuberculosis.

We conclude that neither EIA-IgA nor EIA-IgG is useful for the diagnosis of PTB among PTB suspects with respiratory symptoms. Additional studies are warranted to assess the utility of EIA-IgA as a screening test for M. tuberculosis infection among healthy close contacts of PTB patients.

ACKNOWLEDGMENTS

This work was supported in part by NIH grant U19AI45432 (International Collaboration in Infectious Disease Research), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-475216/03-2).

REFERENCES


