Comparative Evaluation of Early Diagnosis of Tuberculous Meningitis by Different Assays

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Cerebrospinal fluid (CSF) and peripheral blood (PBL) were sampled multiple times from 25 patients with a clinical diagnosis of tuberculous meningitis (TBM) and 49 controls, including 27 patients with other infectious diseases of the central nervous system and 22 patients with other noninfectious neurological diseases. We used an enzyme-linked immunospot assay (ELISPOT) to detect anti-Mycobacterium bovis BCG antibody-secreting cells in CSF and PBL. PCR to detect a repeated insertion sequence (IS6110) specific for Mycobacterium tuberculosis in CSF, and an enzyme-linked immunosorbent assay (ELISA) to detect anti-BCG antibodies in CSF and PBL. In the meantime, culture of CSF from every TBM and control patient was done on Lowenstein-Jensen medium. ELISPOT proved to be the most valuable test, with a sensitivity of 84.0% and a specificity of 91.8%, and showed a sensitivity of 100.0% with the CSF specimens obtained within 4 weeks after the onset of TBM. The numbers of CSF anti-BCG immunoglobulin-secreting cells tested by ELISPOT were even higher in the early phase of TBM and declined while the disease was going on (P = 0.008), which allowed an early diagnosis to be made. The sensitivities of PCR and ELISA were only 75.0% and 52.3%, respectively; and the specificities were 93.7% and 91.6%, respectively. Culture of CSF on Lowenstein-Jensen medium was the least sensitive (16%) compared to the sensitivities of the other three assays. Our results demonstrate that the ELISPOT technique is worthy for routine use in the laboratory to support the clinical diagnosis of TBM.

In the past several years there has been a global increase in the incidence of tuberculosis along with the prevalence of AIDS and the emergence of multidrug-resistant strains. Tuberculosis meningitis (TBM) is a major global health problem and is the most severe form of extrapulmonary tuberculosis, with a high rate mortality. TBM is diagnosed on the basis of clinical features, cerebrospinal fluid (CSF) studies, and radiological findings. Due to the variable clinical presentations and CSF findings, which can be confused with those of other chronic infections of the central nervous system (CNS), TBM is sometimes difficult to diagnosis with certainty, especially in its early phase (about 1 to 2 weeks after onset, according to our clinical observations). During this time period, the typical clinical manifestations of TBM have not fully developed. The polymorphonuclear pleocytosis in CSF can occur early and may give an erroneous impression of bacterial meningitis. Also during this time period, the antibiotic or antituberculous treatment has lasted for just a short time, and the effect of therapy is not obvious enough to be able to make a judgment. The contrast enhancement of the basal cisterns, hydrocephalus, or lesions in the brain parenchyma on a computed tomography (CT) image or a magnetic resonance imaging image specific for TBM may not occur so early. Previous clinical studies have clearly demonstrated that the timing of the onset of chemotherapy is the most critical factor in determining the ultimate outcome, which underscores the importance of early diagnosis. The laboratory confirmation of TBM depends on the demonstration of Mycobacterium tuberculosis in CSF by culture or smear. However, smears for acid-fast bacilli exhibited a few positive results (22), with a sensitivity of about 10% (13). Culture on Lowenstein-Jensen medium takes about 8 weeks and has a limited sensitivity of about 15% (1, 19, 23). Delays in the time to diagnosis and the initiation of the correct drug treatment regimen lead to increased neurological sequelae and mortality. Therefore, a test with a good sensitivity and a good specificity for early diagnosis is greatly needed.

Kashyap et al. have demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis that a protein with a molecular mass of 30 kDa existed in the CSF of patients with TBM (9). This 30-kDa protein was later proved to be a specific antigen of M. tuberculosis and could be considered a diagnostic marker for TBM (11). The production of antibodies against the 30-kDa protein in CSF was adopted for use for the differential diagnosis of TBM in partially treated patients with pyogenic meningitis by a cell-based enzyme-linked immunosorbent assay (cell ELISA) with a sensitivity of 92% (12). However, preparation of the 30-kDa protein from the CSF of TBM patients is a prerequisite for establishment of the assay. By the dot ELISA method, polyclonal antibodies to M. tuberculosis culture filtrate protein detected M. tuberculosis antigen in 48 CSF samples (86%) obtained from all 56 patients with suspected TBM (10). In the study of Desai and Pal, the sensitivity of PCR based on the amplification of a 169-bp DNA fragment specific for M. tuberculosis was 31.4%, which is much higher than the sensitivity of culture on Lowenstein-Jensen medium (3.8%) and that of smear by the fluorochrome staining method (1.9%) (5). In another study by Brienz et al., two PCR protocols showed low sensitivities (36% and 53% for the TB AMPLICOR assay and the MPB64 nested PCR, respectively)
comparison with those of classic microbiological methods (73% and 54% for Ziehl-Neelsen staining and culture, respectively) for the diagnosis of TBM in 91 patients in southeastern Brazil (3). An immunocytopathological method with CSF-cytopsin smears was used by Sumi et al. to detect Mycobacterium antigens in the cytoplasm of CSF monocytes and showed a sensitivity of 72% (20). The overall sensitivities of dot ELISA (for the detection of Mycobacterium antigen in CSF) and PCR were 75% and 40%, respectively (21). These results may have been discrepant for two reasons. First, different assays have distinct sensitivities, even if the same antigen is used. Second, the TBM patients enrolled in those studies may have had different characteristics; for example, they may have had the disease for different periods of time and may have undergone different treatment strategies.

The early diagnosis of TBM is essential for a positive outcome; but some microbiological diagnostic techniques are insensitive, slow, or laborious. In our study, we measured anti-Mycobacterium bovis BCG immunoglobulin G (IgG)- and IgM-secreting cells in both CSF and peripheral blood (PBL) by an enzyme-linked immunosorbent assay (ELISPOT) and the anti-BCG IgG antibody titer in both CSF and PBL by ELISA. We used the BCG antigen, which was extracted from the pure BCG vaccine, for both ELISPOT and ELISA. We also performed PCR to detect the IS6110 fragment (a repeated insertion sequence specific for M. tuberculosis) in the CSF of TBM patients (6). In the meantime, culture of CSF from every TBM and control patient was done on Lowenstein-Jensen medium. Our aim was to detect a diagnostic method with a good sensitivity and a good specificity that can routinely be used in the clinical practice.

MATERIALS AND METHODS

Patients. The Institute of Neurology of Shanghai Medical College, Fudan University, is the major tertiary referral center for neurological diseases in southeastern China. During a 1-year period in 2004 and 2005, 16 female patients and 9 male patients with a mean age of 37 years (age range, 16 to 73 years) were diagnosed with TBM when at least three of the four following clinical conditions were met: (i) subacute onset of fever, headache, nausea, neck rigidity, and cranial nerve palsies responsive to antituberculous therapy; (ii) CSF abnormalities in the form of pleocytosis that, depending on the duration of the disease, was dominated by polymorphonuclear leukocytes or mononuclear cells, a reduced glucose concentration, as well as an increased total protein concentration without evidence of the presence of other etiological agents; (iii) evidence of hydrocephalus, lesions of the brain parenchyma, or basal enhancement on cranial CT or magnetic resonance imaging; and (iv) disseminated miliary tuberculosis or infiltrative pulmonary tuberculosis proved by chest X ray or CT.

Eight female patients and 19 male patients with a mean age of 33 years (age range, 10 to 79 years) with other infectious diseases (ONDs) of the CNS were enrolled as controls: 6 had suppurative meningitis with classical clinical and CSF findings and were cured after antibiotic therapy; 9 had cryptococcal meningitis verified with an India ink preparation; 1 had cryptococcosis meningitis with an elevated CSF antibody titer, as determined by the latex agglutination method; 10 had acute viral meningoencephalitis with a typical self-limited clinical course, slightly elevated CSF protein concentrations, and increased cell counts dominated by lymphocytes; and 1 had cerebral cysticercosis, as diagnosed by typical radiological presentations and an elevated specific antibody titer in CSF.

The other control group was composed of 15 female patients and 7 male patients with a mean age of 38 years (age range, 16 to 77 years). These patients had other noninfectious neurological diseases (ONDs), including polyneuritis, multiple sclerosis, motor neuron disease, cerebral infarction, etc.

All of the TBM and control patients enrolled in our study were human immunodeficiency virus negative.

About 6 ml CSF (3 ml for ELISPOT and ELISA, 2 ml for PCR, and 1 ml for culture) and 2 ml PBL were obtained from each TBM and control patient. The timing of the lumbar puncture was determined by the clinicians when they wanted to see the effect of treatment and not for experimental purposes alone. One patient (patient 8) was sampled five times, 2 patients (patients 5 and 22) were sampled four times, 4 patients (patients 1, 11, and 18, and 20) were sampled three times, 8 patients (patients 2, 4, 6, 7, 12, 14, 15, and 24) were sampled twice, and 10 patients (patients 3, 9, 10, 13, 16, 17, 19, 21, 23, and 25) were sampled only once. The detailed sampling frequency and the time period from the time of disease onset to the sampling day for each patient are listed in Table 1. Altogether 51 pairs of CSF and PBL samples were obtained from 25 TBM patients. All 51 pairs of specimens were tested by ELISPOT. Thirty-nine pairs of specimens from 21 TBM patients were tested by ELISA. Forty-three pairs of specimens from 24 TBM patients were tested by PCR (Table 1). Each control patient was sampled once. Forty-eight pairs of specimens from 48 controls were all tested by ELISPOT, PCR, and ELISA, with one exception, in which the sample was tested only by ELISPOT. All control patients did not undergo all three tests because the volume of CSF available was limited.

CSF and blood sampling. After cell counting by phase-contrast microscopy, 3 ml CSF was centrifuged at 448 × g for 15 min. The supernatant was stored for ELISA, and the cell pellet was resuspended in RPMI 1640 culture medium (GIBCO BRL containing 10% fetal calf serum (FCS; Shanghai Institute of Biological Products [SIBP], Shanghai, China). The suspension was centrifuged, washed twice in medium, and diluted to a final cell concentration of 2.5 × 10^6 to 5 × 10^6 cells per ml.

PBL was collected in heparinized glass tubes. After removing some plasma for ELISA, mononuclear cells (MNCs) were separated by density gradient centrifugation on Ficoll (Huajing Biotech, Shanghai, China), washed three times in 0.05 mol/liter of phosphate-buffered saline (PBS; pH 7.4), diluted in medium, and adjusted to a final concentration of 1 × 10^6 cells per ml.

Antigen. After the BCG vaccine was washed three times with 0.05 mol/liter of PBS, the BCG vaccine (which was obtained from Shanghai Institute of Biological Products) was put into 5 ml of distilled water. After the bacterial suspension was frozen and thawed five times, 5 ml of the bacterial suspension was sonicated at 50 W for 5 min to make the cell wall of BCG rupture and the protein antigen to be released from the cytoplasm. The suspension was then centrifuged at 4,990 × g for 20 min. The supernatant containing the BCG protein antigen was collected, adjusted to a protein concentration of 10 μg/ml, and stored at 4°C.

Enumeration of anti-BCG IgG- and IgM-secreting cells. To detect cells that secreted BCG antibodies, a solid-phase ELISPOT was performed with microwell plates with a nitrocellulose bottom (Millipore, Bedford, MA). The plates were coated with 100 μl of BCG (10 μg/ml) in coating buffer (0.05 mol/liter carbonate buffer, pH 9.6) and kept overnight at 4°C. After removal of the coating solution by suction through the nitrocellulose membranes and subsequent washings in PBS, the remaining sites were blocked with 10% FCS (SIBP) at 37°C for 1 h. The plates were then washed with PBS and dried. Samples (200 μl) containing 0.5 × 10^4 to 10^6 CSF cells or 10^5 PBL cells in 100 μl of PBS, to which 10 μl of antiprotein antigen IgG (diluted 1:1,000) or IgM (diluted 1:400; Vector, United Kingdom) was added to the appropriate wells for 4 h, were followed by the addition of avidin-biotin peroxidase (diluted 1:500; DAKO, Denmark) for 1 h. After the wells were stained with peroxidase, the red-brown immunosops that corresponded to cells with secreted anti-BCG IgG or IgM were counted and standardized to the numbers of spots per 10^6 CSF cells or peripheral MNCs (15). In the previous study (15) performed in 1990, as well as in this study, none of the OND controls had anti-BCG IgG- or IgM-secreting cells in CSF; the cutoff value for ELISPOT was determined to be 0. The detection of only one or more antibody-secreting cells was regarded as a positive result.

**Is6110 PCR.** The primers used in the Is6110 PCR (5′ CCT GCC AGC GTA GCC GTC GGG` and 5′ CTC AGC GCG GCC GTC GGG`) were reported by Eisenach et al. (6) and amplify a 123-bp fragment called Is6110. A repeated insertion sequence specific for *M. tuberculosis*. We extracted DNA from 2 ml CSF using classic proteinase K (Takara) digestion and phenol-chloroform extraction, followed by ethanol precipitation. Five microliters of the CSF DNA was amplified in a 25-μl reaction mixture containing 2.5 μl of 10× PCR buffer, 2 μl of deoxynucleoside triphosphates, 0.125 μl of Taq polymerase (Takara), 0.5 μl of each primer, and 14.375 μl of distilled water. The mixture was subjected to 35 cycles of amplification (each cycle consisted of 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s). The PCR products were analyzed on a 2.5% agarose gel. DNA was also extracted from the *M. tuberculosis* strains from the patients’ sputum samples that had been cultured on Lowenstein-Jensen medium in the laboratory. That DNA served as the template for the positive control. A negative control
to detection of growth) indicates the result of culture (positive / negative) and the sampling day. Number of treatment days indicates the amount of time between the start of antituberculous treatment and the sampling day. Culture (days to detection of growth) is as follows:

- **Patient 1:**
  - No. of treatment days: 4
  - Culture (days to detection of growth): +

- **Patient 2:**
  - No. of treatment days: 12
  - Culture (days to detection of growth): +

- **Patient 3:**
  - No. of treatment days: 4
  - Culture (days to detection of growth): ++++

- **Patient 4:**
  - No. of treatment days: 17
  - Culture (days to detection of growth): +

- **Patient 5:**
  - No. of treatment days: 4
  - Culture (days to detection of growth): +

- **Patient 6:**
  - No. of treatment days: 14
  - Culture (days to detection of growth): -

- **Patient 7:**
  - No. of treatment days: 0
  - Culture (days to detection of growth): +

- **Patient 8:**
  - No. of treatment days: 8
  - Culture (days to detection of growth): +

- **Patient 9:**
  - No. of treatment days: 14
  - Culture (days to detection of growth): -

- **Patient 10:**
  - No. of treatment days: 2
  - Culture (days to detection of growth): +

- **Patient 11:**
  - No. of treatment days: 12
  - Culture (days to detection of growth): +

- **Patient 12:**
  - No. of treatment days: 46
  - Culture (days to detection of growth): -

- **Patient 13:**
  - No. of treatment days: 6
  - Culture (days to detection of growth): +

- **Patient 14:**
  - No. of treatment days: 5
  - Culture (days to detection of growth): +

- **Patient 15:**
  - No. of treatment days: 8
  - Culture (days to detection of growth): +

- **Patient 16:**
  - No. of treatment days: 5
  - Culture (days to detection of growth): -

- **Patient 17:**
  - No. of treatment days: 14
  - Culture (days to detection of growth): +

- **Patient 18:**
  - No. of treatment days: 28
  - Culture (days to detection of growth): +

- **Patient 19:**
  - No. of treatment days: 12
  - Culture (days to detection of growth): +

- **Patient 20:**
  - No. of treatment days: 32
  - Culture (days to detection of growth): -

- **Patient 21:**
  - No. of treatment days: 14
  - Culture (days to detection of growth): +

- **Patient 22:**
  - No. of treatment days: 10
  - Culture (days to detection of growth): +

- **Patient 23:**
  - No. of treatment days: 14
  - Culture (days to detection of growth): +

- **Patient 24:**
  - No. of treatment days: 4
  - Culture (days to detection of growth): +

- **Patient 25:**
  - No. of treatment days: 144
  - Culture (days to detection of growth): +

**TABLE 1. Comparative results for TBM patients by different assays**

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<th>Assay result: ELISA</th>
<th>Assay result: PCR</th>
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*O. ICDC, IEF (ELISA) results are as follows: +, 1.09 to 1.50; ++, 1.50 to 2.00; ++++, 2.00 to 2.50. The results for CSF anti-BCG IgG-secreting cells per 10⁴ CSF cells (ELISPOT) are as follows: +, 0 to 100; ++, 100 to 200; ++++, 200 to 400; +++, 400 to 600. Duration of disease indicates the amount of time between the start of antituberculous treatment and the sampling day. Number of treatment days indicates the amount of time between the start of antituberculous treatment and the sampling day. Culture (days to detection of growth) indicates the result of culture (positive [+] or negative [-]) and the number of days taken for a positive outcome (in parentheses)."
trifuged at 1,008 g for 30 min. The sediment was then inoculated onto two slants of Lowenstein-Jensen egg medium and incubated at 37°C. The slants were inspected every day for the first week and then weekly for 14 weeks. The results for all positive cultures were confirmed by microscopy with Ziehl-Neelsen staining, and further identification was done by standard biochemical tests.

**Statistical analysis.** A multivariate regression model was used to analyze the association between the number of CSF anti-BCG IgG- or IgM-secreting cells and the following 10 factors: sex; age; duration of disease; number of treatment days; CSF cell count; the percentages of CSF mononuclear and polynuclear cells; and the number of CSF anti-BCG IgG-secreting cells, was used to disclose the association between these factors and the CSF IgG index. To ensure the reproducibility of the result, all 37 pairs of CSF and plasma were diluted and adjusted so that each pair contained the same IgG concentration. ELISA was then performed to examine the anti-BCG IgG titer in diluted CSF and plasma. Polyclonal antibody reaction reached equilibrium (after about 36 h), the diameter of the precipitin circle was measured with a spectrum plate reader (Bausch & Lomb). The mean numbers of anti-BCG IgG-secreting cells for all positive cultures were confirmed by microscopy with Ziehl-Neelsen stain and further identification was done by standard biochemical tests.

**RESULTS**

**Anti-BCG IgG- and IgM-secreting cells.** Figure 1 shows the results of anti-BCG IgG- and IgM-secreting cells detection in CSF and PBL from 25 TBM, 27 OND, and 22 OND patients. Anti-BCG IgG-secreting cells were detected in the CSF of 21 of 25 (84.0%) TBM patients, with the amounts ranging from 2.9 to 435 per 10^4 CSF cells (mean, 55.1 per 10^4 CSF cells). Eight SF specimens obtained within 4 weeks after TBM onset were all (100.0%) positive by ELISPOT. Eighteen of 20 (90.0%) CSF specimens obtained within 8 weeks after TBM onset were positive, while 22 of 31 (70.9%) CSF specimens obtained after 8 weeks were positive, revealing that the sensitivity of ELISPOT was higher in the early phase and declined while the disease was going on. Further multivariate regression analysis of 51 samples for five related factors (age, sex, sampling order, duration of disease, and number of treatment days) showed that only sex had a significant association with the number of anti-BCG IgG-secreting cells ($t = 2.263; P = 0.029$). The mean numbers of anti-BCG IgG-secreting cells for men and women were 106.5 and 31.3, respectively. From a rigorous statistical standpoint, such a study involving repeated measurements should follow the principles of a repeated-measure study design. However, that was difficult to achieve in the current study because the TBM patients were sampled when the physicians wished to see the effect of the treatment. The timing of repeated lumbar puncture could not be decided deliberately for experimental purposes. To eliminate the effect of repeated measurements, we repeated the analysis using the data for the first sample from the 25 subjects sampled (i.e., the sampling order was 1). The results for four related factors (age, sex, duration of disease, and number of treatment days) by multivariate regression showed that none of these four factors had a significant association with the number of IgG-secreting cells. We also collected biological data on the first CSF sample (CSF cell count; mononuclear cell percentage; polynuclear cell percentage; and the concentrations of protein, glucose, and chloride) obtained from the patients (Table 2). When the multivariate regression included these 6 biological indicators (of a total of 10 factors), sex, duration of disease, CSF cell count, and CSF protein concentration became significant (for sex, $t = -2.540$ and $P = 0.026$; for duration of disease, $t = -3.183$ and $P = 0.008$; for CSF cell count, $t = 2.235$ and $P = 0.045$; for CSF protein concentration, $t = 3.528$ and $P = 0.004$). This indicated that the number of CSF anti-BCG IgG-secreting cells was negatively associated with the duration of the disease. The number of CSF IgG-secreting cells was higher in the early phase of the disease and then gradually declined while the disease was going on. This trend was not affected by the artifact of therapy because the “number of treatment days” was also taken into account in the multivariate analysis.

Simultaneously, 7 of 25 TBM patients (28.0%) had positive anti-BCG IgM-secreting cells in their CSF, with the amounts ranging from 6 to 82.5 per 10^4 CSF cells (mean, 4.8 per 10^4 CSF cells). No significant association was found between the number of CSF anti-BCG IgM-secreting cells and age, sex,
sampling order, duration of disease, and number of treatment days in the 51-sample multivariate analysis, nor was any significant association found between the number of IgM-secreting cells and age, sex, duration of disease, or number of treatment days and the six CSF biological parameters in the 25-sample multivariate analysis.

Seventeen of the 25 (68.0%) TBM patients had anti-BCG IgG-secreting cells in PBL, but the numbers were much lower than the numbers of anti-BCG IgG-secreting cells in CSF and varied between 0.05 and 10.5 per 10⁴ peripheral MNCs. Five of the 25 (20.0%) TBM patients had very few anti-BCG IgM-secreting cells in their blood.

Four of the 27 OIND patients (1 with viral meningitis, 1 with cryptococcus meningitis, and 2 with suppurative meningitis) had anti-BCG IgG-secreting cells in their CSF; and the counts were 7.5, 32, 140, and 10 per 10⁴ CSF cells, respectively. No IgG-secreting cells were detected in the CSF of the 22 OND patients. The specificity of ELISPOT was 91.8%. No anti-BCG IgM-secreting cells were detected in the CSF of the control patients. Very few anti-BCG IgG- or IgM-secreting cells were detected in the PBL of OIND and OND patients.

**IS6110 PCR.** Forty-three CSF specimens from 24 TBM patients and 48 CSF specimens from 48 controls underwent PCR. Eighteen of the 24 TBM patients and 3 of the 48 nontuberculous controls (1 with multiple sclerosis, 1 with polynuerritis, and 2 with suppurative meningitis) were PCR positive. Among the four controls, two patients with suppurative meningitis were also positive by ELISPOT; the other two patients were ELISPOT and PCR negative. The sensitivity of ELISA was 52.4% and the specificity was 91.7%. No significant associations were found between QBCG IgG and disease duration or between QBCG IgG and the number of anti-BCG IgG-secreting cells in the CSF of TBM patients by multivariate regression analysis.

**Culture.** Three of 25 TBM patients (12.0%) were culture positive (Table 1). The growth of *M. tuberculosis* was detected in these three patients 30, 38, and 57 days after sampling, respectively (mean, 42 days). Two of the three TBM patients were positive by all tests (ELISPOT, ELISA, and PCR). The other one was positive only by ELISPOT and PCR. None of the 49 controls were culture positive. Taken together, Table 1 presents the comparative results obtained for 25 TBM patients by different assays.

One TBM patient (patient 6) was negative by all three tests, and so the exact diagnosis needs further validation.

**DISCUSSION**

The diagnosis of TBM is difficult because the clinical features are nonspecific and the CSF may contain so few bacilli

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<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>CSF cell count (10⁶/liter)</th>
<th>Mononuclear cells</th>
<th>Polynuclear cells</th>
<th>CSF protein concn (mg/liter)</th>
<th>CSF sugar/blood sugar ratio</th>
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* F, female; M, male.
that they are neither seen nor cultured. However, the early diagnosis and treatment of TBM can save lives and probably reduce neurological deficits; therefore, a sensitive and specific early diagnostic test is urgently required (7). In the present study, 21 of 25 patients with a clinical diagnosis of TBM had anti-BCG IgG-secreting cells in their CSF. The sensitivity of ELISPOT was 84.0%, which was higher than those of the IS6110 PCR (75.0%) and ELISA (52.4%). Only four of the control patients had anti-BCG IgG-secreting cells in their CSF. The specificity of ELISPOT was 91.8%, while the specificities of PCR and ELISA were 93.8% and 91.7%, respectively. Multivariate regression analysis with the data for the 25 subjects who were the first cases sampled showed that the number of CSF anti-BCG IgG-secreting cells in TBM patients was negatively associated with the duration of the disease (P = 0.008). The number of CSF anti-BCG IgG-secreting cells was higher in the early phase of the disease and then gradually declined, revealing that the detection of anti-BCG IgG-secreting cells is valuable for the early diagnosis of TBM. The number of CSF anti-BCG IgG-secreting cells was positively associated with the CSF cell count (P = 0.045) and the CSF protein concentration (P = 0.004). This is easy to understand, because IgG-secreting lymphocytes were a part of the CSF white cells, and these are an indicator of the intrathecal immune response as well as the CSF protein concentration. The value of anti-BCG IgM-secreting cell detection in CSF was limited because of its low sensitivity (28.0%). By parallel tests, we have demonstrated that ELISPOT is the most sensitive assay for the early diagnosis of TBM, while it maintained a high degree of specificity. Unlike other diagnostic methods for the detection of antibodies in CSF, the result of ELISPOT is not affected by damage to the BBB. The only limitation of this method is how the time period of disease involved will affect its sensitivity; thus, we suggest that CSF be sampled for ELISPOT as early as possible only if TBM is suspected.

Compared to CSF specimens, very few anti-BCG IgG-secreting cells were found in the PBL of TBM patients, indicating that a specific B-cell response mainly occurred in the CNS. Therefore, the test of CSF should not be replaced by that of PBL. Previous researchers have also demonstrated that CSF-derived cells from patients with TBM have a significantly higher proliferation response to purified protein derivative, which is suggestive of an intrathecal immune response (14). The impact of drug resistance upon bacterial clearance from CSF is unknown. However, evidence from patients with pulmonary tuberculosis suggests that streptomycin and isoniazid are responsible for the majority of bactericidal activity in the first few days of treatment (16). However, in our study multivariate regression analysis did not show any significant association between the number of treatment days and the number of CSF anti-BCG IgG- or IgM-secreting cells. The decrease in the number of CSF IgG-secreting cells later in the disease was part of the natural course of disease.

Four control patients (one with viral meningitis, one with cryptococcosis meningitis, and two with suppurative meningitis) were positive by ELISPOT. The two controls with suppurative meningitis were also positive by ELISA. In an earlier study, we also found that one control with cryptococcosis meningitis was positive by ELISPOT (15). We speculate that cross-reaction of the antigen between M. tuberculosis and other infectious organisms might be responsible for the phenomenon. A single antigen encoded by a certain fragment of the M. tuberculosis genome, such as the 38-kDa protein, Mtb11, Mtb8, and Mtb48, will cause less cross-reaction and is found to be highly specific for M. tuberculosis but lacks sensitivity (8). In a study of Houghton et al. (8), these antigens have been combined into fusion recombinants (polypeptides) by gene engineering. TbF6 is a fusion of the 38-kDa protein, Mtb11, Mtb8, and Mtb48. TbF10 is a fusion of the 8-kDa protein, Mtb11, and Mtb8. These fusion recombinants are demonstrated to have a high degree of clinical sensitivity for the detection of active tuberculosis, while they maintain a high degree of specificity (8). Such recombinant antigens may solve the problem in the future.

Antibodies to Mycobacterium can be seen in CSF from patients with various diseases that are accompanied by damage of the BBB, which will lead to false-positive results in antibody detection assays. In our study, paired CSF and plasma samples were diluted to the same IgG concentration before ELISA to diminish the effect of different permeabilities through the BBB. However, the quantification of IgG by SRID was laborious and time-consuming. At least 2 days was needed; and the outcome was poor, with a sensitivity of only about 52.4%, suggesting that ELISA is not suitable for use alone for the diagnosis of TBM. Four controls were positive by ELISA, and two of them were also positive by ELISPOT. Future studies may focus on more effective methods of diminishing the effect of BBB damage and detecting a more specific antigen with less cross-reaction with other organisms.

IS6110 PCR was not as sensitive as ELISPOT in our study. Many factors may affect the sensitivity of PCR, such as high CSF protein concentrations, low numbers of organisms in CSF, antituberculous therapy, and infection with IS6110-negative M. tuberculosis strains. (21) Expensive reagents and instruments as well as isolated laboratory areas are needed, which also limit its utility in developing countries, where the incidence of TBM is high.

Three nontuberculous controls (one with suppurative meningitis and two with noninfectious neurological diseases) were PCR positive. None of them was positive by ELISPOT or ELISA. As the diagnoses for these patients were definite, the false-positive results may have been due to contamination. In conclusion, our study has demonstrated that anti-BCG IgG-secreting cell detection in CSF by ELISPOT is the most valuable test because of its high degree of sensitivity compared to the results of ELISA and IS6110 PCR. The number of CSF anti-BCG IgG-secreting cells is higher in the early phase of TBM and then gradually declines, revealing that ELISPOT is particularly effective for the early diagnosis of TBM.

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We state that the study's authors do not have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

REFERENCES