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Intrathecal Synthesis of Immunoglobulin G and *Mycobacterium tuberculosis*-Specific Humoral Immune Response in Tuberculous Meningitis

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Local synthesis of immunoglobulin G (IgG) in the central nervous system was investigated in 10 patients with tuberculous meningitis (TBM), 15 patients with aseptic meningitis (AM), and 15 patients with pulmonary tuberculosis only (PTBO). The IgG synthesis rate for patients with TBM was 56.4 ± 18.9 mg/day (mean ± standard deviation), which was significantly higher than that for patients with AM (8.0 ± 6.7 mg/day, $P < 0.001$) and that for patients with PTBO (7.5 ± 4.4 mg/day, $P < 0.001$). Therefore, the increased IgG synthesis rate in the central nervous system provided supporting evidence for differentiating the diagnosis of TBM from that of AM (sensitivity, 100%; specificity, 83.3%). Simultaneous measurement by enzyme-linked immunosorbent assay of IgG seroreactivity to lipoarabinomannan and purified protein derivative antigens in cerebrospinal fluid (CSF) demonstrated seropositivity in all 6 patients with TBM, 4 of 15 patients with AM, and 4 of 10 patients with PBTO. All patients showing false-positive reactivity in CSF demonstrated seropositivity in sera and normal ranges for IgG synthesis rates in CSF. Also, the semiquantitive measurement of IgG antibody (Ab) titers in these patients demonstrated higher IgG Ab titers in serum than in CSF except for one patient with a highly elevated albumin quotient, suggesting a leaky blood-brain barrier. The results strongly suggested that the *Mycobacterium tuberculosis*-specific IgG Abs were diffusible through the blood-brain barrier, which addresses the pitfall of serological tests for the early diagnosis of TBM. The serological detection of IgG Abs to lipoarabinomannan and purified protein derivative antigens in CSF could be misleading in the presence of simultaneously elevated levels of IgG Abs in serum.

Tuberculous meningitis (TBM) is still a serious cause of morbidity and mortality in developing nations. Previous clinical studies of TBM have clearly demonstrated that the timing of treatment is the most critical factor affecting the ultimate outcome, which stresses the importance of early diagnosis of TBM (11).

Among the various methods recently developed for the early diagnosis of TBM, enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies (Ab) to *Mycobacterium tuberculosis* antigens (Ag) in cerebrospinal fluid (CSF) has been the most widely investigated (2, 4, 8, 9). ELISA is technically simple and inexpensive, and the results can be obtained on the day of testing. The central nervous system (CNS) is immunologically secured, bacterial contaminations are absent in CSF, and a nontuberculous mycobacterial meningitis is extremely rare, all of which provide further advantages for the serodiagnosis of TBM.

Previous clinical trials of *M. tuberculosis*-specific Ab detection in CSF by ELISA demonstrated its clinical usefulness for the early diagnosis of TBM; however, occasional false-positive results have been reported (3, 6, 16, 22). Although the reasons for the false-positive results have not been thoroughly investigated yet, the possibility of passive transfer of Ab from serum to CSF is critical in determining the diagnostic utility of ELISA, especially in areas of endemic tuberculosis where a large population is seropositive for *M. tuberculosis*-specific Ag.

For further evaluation of the dynamics of humoral Ab to *M. tuberculosis*-specific Ag in TBM, we investigated the status of de novo synthesis of immunoglobulin G (IgG) in the CNS and serological Ab detection by ELISA in both serum and CSF for three patient groups: those with TBM, those with aseptic meningitis (AM), and those with pulmonary tuberculosis only (PTBO).

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MATERIALS AND METHODS

Patients. All patients with meningitis admitted to the neurology ward of Yonsei University Severance Hospital from March 1992 to August 1993 had lumbar punctures for diagnostic purposes and at least one computerized tomography brain scan before the lumbar puncture. Routine CSF examinations included cell count with differential, protein measurement, glucose measurement, direct smear and culture for acid-fast bacillus, Gram stain and culture for bacteria, India ink preparation and cryptococcal antigen test, direct smear and culture for fungus, and Venereal Disease Research Laboratory test. Patients with PTBO were admitted to the pulmonary ward, and a diagnostic lumbar puncture was conducted for the evaluation of headaches or other minor neurological complaints. Blood was collected from each patient by venipuncture at the time of lumbar puncture.

(i) TBM. All patients presented with clinical symptoms and signs of meningitis. Positive acid-fast bacillus smear or culture of *M. tuberculosis* from CSF was considered definite evidence of TBM. For patients with no microbiological evidence, TBM was diagnosed when the CSF examination showed findings characteristic of TBM, such as lymphocytosis or elevated protein level and low glucose level, and at least three of following minor criteria were met: (i) subacute onset of symptoms (ii) characteristic features of computerized tomography brain scan (e.g., basal leptomeningeal enhancement, acute hydrocephalus, or a tuberculoma-like lesion), (iii) presence of systemic tuberculosis (TB), (iv) history of exposure to *M. tuberculosis*, (v) evidence of cranial nerve dysfunction, (vi) favorable clinical response to antituberculosis medications.

(ii) AM. A diagnosis of AM was made when the following criteria were

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satisfied: (i) clinical signs of meningitis, (ii) characteristic CSF features (e.g., mildly increased levels of monocytosis and protein and normal glucose level), (iii) negative microbiological studies of CSF, (iv) spontaneous improvement with only conservative management, (v) no evidence of active pulmonary or extrapulmonary TB.

(iii) PTBO. All patients with PTBO tested positive by sputum smear or culture for acid-fast bacillus, and chest X rays of these patients revealed active lesions. A few patients complained of mild dull headaches, but meningeal irritation signs were absent. CSF examination was essentially normal for all patients.

IgG synthesis rate and AQ. Quantitative immunoelectrophoresis of simultaneously collected CSF and serum was conducted with a Behring nephelometer. IgG synthesis rates were calculated by the following formula (7, 19, 20):

$$\left[\left(\mathrm{IgG_{CSF}} - \frac{\mathrm{IgG_{serum}}}{369}\right) - \left(\mathrm{albumin_{CSF}} - \frac{\mathrm{albumin_{serum}}}{230}\right)\right] \times \frac{\mathrm{IgG_{serum}}}{\mathrm{albumin_{serum}}} \times 0.43 \times 5\ (\mathrm{mg/day})$$

The albumin quotients (AQ) were calculated as ($\mathrm{albumin_{CSF}}/\mathrm{albumin_{serum}}$) × 1,000. In these formulas, $\mathrm{IgG_{CSF}}$ and $\mathrm{albumin_{CSF}}$ are levels of IgG and albumin in CSF, respectively, and $\mathrm{IgG_{serum}}$ and $\mathrm{albumin_{serum}}$ are levels of IgG and albumin in serum, respectively.

IgG reactivity to PPD and LAM Ag. For all patients, initial lumbar puncture was performed on the first day of hospitalization. Samples of serum (10 ml) and CSF (3 ml) were obtained, centrifuged at 3,000 rpm for 30 min, and stored at −20°C. The ELISA described by Voller et al. (21) was employed with minor modifications and was described in detail in our previous study (16). Briefly, 50 μl each of diluted purified protein derivative (PPD) (10 μg/ml) and lipoarabinomannan (LAM) (0.2 μg/ml) was added to the wells of U-bottom microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.) and incubated overnight at 37°C in a moist chamber. The wells were then washed with phosphate-buffered saline solution containing 0.05% Tween 20 (PBST) and blocked by the addition of 100 μl of PBST–0.05% bovine serum albumin at 37°C for 1 h. After the wells were emptied, 50 μl of serum diluted 1:300 or CSF diluted 1:5 in PBST–5% normal goat serum (Gibco Laboratories, Grand Island, N.Y.) was added to the wells, which were incubated at 37°C for 90 min. After the wells were washed, 50 μl of affinity-purified peroxidase-conjugated goat anti-human IgG (Behring Diagnostic, Inc., San Diego, Calif.) diluted 1:5,000 in PBST–5% normal goat serum was added, and incubation was continued at 37°C for 1 h. After another washing, 50 μl of the substrate solution, H_2O_2–*o*-phenylenediamine, was added to the wells, which were incubated at room temperature for about 15 min. The reaction was then stopped with 50 μl of 2.5 N H_2SO_4, and the A_{490} was read. Each test was performed in duplicate, and the mean absorbance of wells without Ag was subtracted from those of wells with PPD or LAM Ag before analysis. The cutoff point for seropositivity was determined by the following formula: upper fourth + (2 × interquartile range). The cutoff points for both PPD and LAM were 0.2 in serum and 0.15 in CSF. The degree of IgG Ab reactivity to PPD and LAM Ag was expressed according to optical density on a scale of − to +++.

Statistical analysis. We used the Mann-Whitney U Wilcoxon rank sum W test because the values in each group did not have a normal distribution.

RESULTS

Among 10 patients with TBM, three were culture positive for *M. tuberculosis*. The CSF features of the remaining seven patients were quite characteristic of TBM: lymphocytosis (range, 120 to 430), elevated protein level (180 to 1,200 mg%), and decreased glucose level (10 to 45 mg%). Chest X rays showed active pulmonary TB lesions in three patients. Computerized tomography brain scan revealed evidence of hydrocephalus in two patients and basal meningeal enhancement in another three patients. Five patients showed cranial nerve dysfunction on examination. None of the 15 patients with AM showed any abnormal neurologic deficits, and all 15 spontaneously recovered.

IgG synthesis rate. IgG synthesis rates for patients with TBM varied from 34.3 to 89.0 mg/day (mean, 56.4 mg/day), which were markedly greater than the mean IgG synthesis rate for patients with AM (7.5 mg/day; range, 1.3 to 33.3 mg/day) or PTBO (8.0 mg/day; range, 2.9 to 18.2 mg/day), which was statistically significant ($P < 0.001$). However, the IgG synthesis rate for patients with AM was not significantly different from that for patients with PTBO. When we considered the mean plus 2 standard deviations of values for PTBO patients as the upper limit of the control IgG synthesis rate, only 2 of 15 patients with AM showed abnormally increased IgG synthesis rates, compared with abnormal increases in all 10 patients with TBM (Table 1).

TABLE 1. IgG synthesis rates and AQ in three patient groups

Patient group and no.	GSR[a]	AQ
PTBO		
1	12.4	3.1
2	9.3	2.4
3	13.3	3.1
4	18.2	3.5
5	9.2	4.7
6	3.0	2.4
7	3.6	1.8
8	8.3	5.2
9	5.3	2.5
10	5.7	2.8
11	2.9	5.0
12	5.6	2.3
13	5.4	4.1
14	4.4	5.1
15	5.6	4.7
Mean ± SD	7.5 ± 4.4	3.5 ± 1.8
AM		
1	12.5	22.5[b]
2	1.3	4.2
3	1.4	5.4
4	1.8	4.7
5	2.5	2.9
6	5.6	8.2[b]
7	20.9[b]	6.7
8	10.9	5.8
9	2.4	5.4
10	2.2	14.2[b]
11	33.3[b]	6.5
12	12.7	17.1[b]
13	5.6	24.2[b]
14	2.7	8.4[b]
15	4.4	37.6[b]
Mean ± SD	8.0 ± 6.7	11.6 ± 7.7
TBM[c]		
1	69.9	60.5
2	37.1	110.8
3	50.9	27.1
4	89.0	65.6
5	42.9	7.4
6	62.5	18.2
7	34.3	7.4
8	65.8	72.9
9	36.5	9.2
10	75.8	80.3
Mean ± SD	56.4 ± 18.7[d]	40.3 ± 32.1[d]

[a] GSR, IgG synthesis rate.
[b] Value above the mean + 2 standard deviations for PTBO patients.
[c] All patients with TBM had IgG synthesis rates and AQ above the mean + 2 standard deviations for PTBO patients.
[d] $P < 0.001$ compared with values for the PTBO group.

AQ. The AQ, indicating the permeability of the blood-brain barrier (BBB), was also markedly increased for patients with TBM (mean, 40.3), compared with that of patients with AM (mean, 11.6) or PTBO (mean, 3.5), which was highly significant ($P < 0.001$). The AQ for patients with AM was slightly more elevated than that for patients with PTBO but was not statistically significant ($P > 0.05$). If we consider the mean plus 2 standard deviations of the AQ for patients with PTBO as the

TABLE 2. IgG Ab reactivities to PPD and LAM Ag in three patient groups

Patient group and no.	Reactivity[a] in:			
	Serum		CSF	
	PPD	LAM	PPD	LAM
PTBO				
1	−	+	−	−
2	+	+	−	−
3	+	+	−	−
4	+	+	−	−
5	+	+	−	−
6	+	+	−	−
7	++	++	+	−
8	++	++	+	−
9	+++	+++	++	+
10	+++	+++	+++	+++
AM				
1[b]	−	+	−	+
2	−	+	−	−
3	−	−	−	−
4	−	+	−	−
5	−	−	−	−
6[b]	−	+	−	−
7[c]	+	+	−	−
8	−	−	−	−
9	−	+	−	−
10[b]	−	−	−	−
11[c]	−	−	−	−
12[b]	+	++	−	+
13[b]	−	++	−	−
14[b]	+	+++	−	++
15[b]	+++	−	+	−
TBM[d]				
1	++	+	+++	+++
2	+++	+	+	−
4	−	−	+	−
5	++	−	−	+
9	+	+++	++	+++
10	+++	+++	+++	+++

[a] Degrees of reactivity (A_{490}) in serum: −, <0.2; +, 0.2 to 0.5; ++, 0.5 to 1.0; +++, >1.0. Degrees of reactivity (A_{490}) in CSF: −, <0.15; +, 0.15 to 0.5; ++, 0.5 to 1.0; +++, >1.0. The cutoff points for seropositivity in serum and CSF were 0.2 and 0.15, respectively, calculated as upper fourth + (2 × interquartile range).

[b] Patient with abnormally elevated AQ.

[c] Patient with abnormally elevated IgG synthesis rate.

[d] All patients with TBM had abnormally elevated IgG synthesis rates and AQ.

upper limit of normal AQ (i.e., >7.1), 7 of 15 patients with AM and all 10 patients with TBM showed evidence of abnormally increased permeability of the BBB (Table 1).

IgG Ab to *M. tuberculosis* Ag (LAM and PPD). IgG seroreactivity to PPD and LAM Ag in CSF and serum was measured by ELISA for 6 of 10 patients with TBM, all 15 patients with AM, and 10 of 15 patients with PTBO (Table 2).

In serum, IgG Ab to PPD was detected in 9 of 10 patients with PTBO, 5 of 6 patients with TBM, and 4 of 15 patients with AM. IgG Ab to LAM Ag was detected in all 10 patients with PTBO, 4 of 6 patients with TBM, and 9 of 15 patients with AM. Therefore, either PPD Ag- or LAM Ag-specific IgG Ab was detected in all (100%) patients with PTBO, 5 of 6 (83.3%) patients with TBM, and 10 of 15 (66.7%) patients with AM. IgG Ab to PPD Ag in CSF was present in 4 of 10 (40%) patients with PTBO, 1 of 15 (6.7%) patients with AM, and 5 of 6 (83.3%) patients with TBM. IgG Ab to LAM Ag was present in CSF in 2 of 10 (20%) patients with PTBO, 3 of 15 (20%) patients with AM, and 4 of 6 (66.7%) patients with TBM. Therefore, IgG Ab reactive to either PPD or LAM Ag was present in CSF in 4 of 10 (40%) patients with PTBO, 4 of 15 (26.7%) patients with AM, and all 6 (100%) patients with TBM. These figures showed that the sensitivity and specificity of assays for CSF Ab to PPD Ag were 83.3% and 80.0%, respectively, and that those of assays for CSF Ab to LAM Ag were 66.7% and 80.0%, respectively.

The semiquantitative data for IgG Ab reactivity to PPD and LAM Ag showed that all four patients (PTBO) with IgG Ab in CSF had moderately to markedly elevated IgG Ab titers in serum, which may suggest the passive transfer of IgG Ab through the intact BBB from serum to CSF. Interestingly, two patients with moderate titers of IgG Ab to both PPD and LAM Ag in serum had Ab reactivity to only PPD in CSF. On the other hand, two other patients with high titers of Ab to both LAM and PPD Ag in serum had positive reactions to both Ag in CSF. These findings may suggest that an Ab reactive to PPD Ag is more permeative than an Ab to LAM Ag. Four patients with AM who were positive for IgG Ab reactive to either PPD or LAM Ag in CSF had positive IgG Ab to corresponding Ag in serum. One patient with CSF IgG Ab to PPD Ag had highly elevated Ab titers in serum, and 3 patients with CSF IgG Ab to LAM Ag had mildly, moderately, and markedly elevated Ab titers in serum. In addition, all four patients with false-positive results for CSF had abnormally elevated AQ but normal ranges of IgG synthesis rates.

DISCUSSION

The rationale of the serological diagnosis of TBM by ELISA is based on the assumption of local synthesis of humoral Ab against *M. tuberculosis* Ag in the CNS. Malashkhia and Geladze (15) reported active PPD-induced transformation of CSF lymphocytes into B cells in patients with TBM. Plouffe (17) and Kinnman et al. (12) reported higher rates of proliferation of CSF lymphocytes than of peripheral blood lymphocytes after stimulation with PPD. Kinnman et al. (13) and Sindic et al. (18) demonstrated the presence of an oligoclonal band in the CSF of patients with TBM, which corresponded to the IgG Ab against *M. tuberculosis* Ag or bacillus Calmette-Guérin (BCG) Ag. The increased IgG synthesis rate and IgG index in CSF in patients with TBM were also demonstrated by Kinnman et al. (12) and Kalish et al. (9). In addition, previous clinical investigations clearly demonstrated the presence of Ab reactive to various *M. tuberculosis* Ag in CSF from a large percentage of patients with TBM in the absence of specific Ab in serum (16). Therefore, there is little doubt that TBM elicits local production of *M. tuberculosis* Ag-specific humoral Ab in the CNS.

However, as suggested by occasional false-positive results in our previous clinical investigation (16) and others (1, 6, 10, 22), it is not necessarily true that the serological tests detect only *M. tuberculosis*-specific Ab locally produced in the CNS. Immunoglobulins in serum may diffuse into CSF through the BBB or may be actively secreted by the choroidal plexus. In fact, oligoclonal band assays of samples from patients with various systemic inflammatory illnesses have shown the presence of identical patterns in both serum and CSF, which suggested the passive penetration of immunoglobulins through the BBB from serum to CSF (5, 14, 23). In that situation, the diagnostic utility of serological tests for TBM may depend upon the presence or absence of serum Ab in the patients being tested, which has not been adequately studied in previous investigations. Especially in areas with a high prevalence of TB, the

majority of healthy patients carry high titers of humoral Ab to *M. tuberculosis* Ag (3), which may diffuse into CSF through the BBB, and the amount of diffused serum Ab may be even greater in patients with forms of active meningitis associated with the breakdown of the BBB. These assumptions suggest that the demonstration of intrathecal synthesis of IgG in the CNS and simultaneous measurement of *M. tuberculosis*-specific Ab in CSF and serum are important for the correct diagnosis of TBM. In this study, all patients with TBM showed significant local synthesis of IgG and only 2 of 15 patients with AM showed significant elevation in synthesis rates. Therefore, AM seemed relatively weak in eliciting local humoral Ab production, which was in agreement with the study of Kinnman et al. (12).

In serological testing of CSF by ELISA, all six patients with TBM showed positive reactivity to either PPD or LAM Ag. On the other hand, 4 of 10 patients with PTBO showed positive reactivity to PPD, and 2 of them were also seropositive for LAM Ag. All these patients showed high titers of corresponding Ab in their sera, while the remaining six patients, who were negative for CSF Ab, showed relatively low titers of Ab in their serum, which strongly suggested the diffusion of IgG Ab reactive to PPD or LAM Ag from serum to CSF through an intact BBB. In patients with AM, all 4 patients with reactivity to either PPD or LAM Ag in CSF had positive IgG titers in serum in the low-to-high range and also showed abnormally elevated AQ but normal ranges of IgG synthesis rates, which again strengthens the argument for passive diffusion of IgG Ab through a leaky BBB. Although our study was not designed to directly demonstrate the diffusion of serum IgG Ab to CSF, the results do clearly indicate that ELISA of CSF detects both locally synthesized and passively diffused IgG Ab.

Previous clinical investigations which employed IgG Ab assays specific for various *M. tuberculosis* Ag showed quite variable results, which was probably related to the differences in control populations. In fact, the specificity of ELISA for PPD and LAM Ag in this study was much lower than in our previous study (16), and this difference was related to the prevalence of seropositivity in patients with AM. In our previous control samples (from patients with AM), the rates of seropositivity in serum and CSF were only 28 and 6.9%, respectively, compared with 66.7 and 26.7%, respectively, in this sample. Therefore, the appropriate interpretation of serological tests for the diagnosis of TBM should require the testing of both serum and CSF. The presence of Ab in CSF without seroreactivity in serum or higher titers of Ab in CSF than in serum may be reliable evidence of TBM. On the other hand, simultaneous detection of seroreactivity in both serum and CSF, but with lower titers of Ab in CSF than in serum, should raise the possibility of passive diffusion of Ab through the BBB. In our study, the measurement of IgG synthesis rate appeared to be potentially useful in differentiating TBM from AM, because all 10 patients with TBM showed significantly elevated IgG synthesis rates, compared with only 2 of 15 patients with AM. The combination of IgG synthesis rate measurement and ELISA provided even better results, because all four AM patients with false-positive reactivity in CSF had normal IgG synthesis rates. However, an elevated IgG synthesis rate per se is not unique to TBM, and it can also be seen in patients with other meningitides, including fungal or bacterial meningitis, potentially leading to the administration of incorrect antimicrobial therapy. For these reasons, we speculate that the techniques focusing on detection of IgM Ab, which is much less diffusible than IgG Ab, or *M. tuberculosis*-specific Ag may be more reasonable alternatives for the early diagnosis of TBM, especially in areas of highly endemic TB.

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