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# Anti-Purified Protein Derivative Cell–Enzyme-Linked Immunosorbent Assay, a Sensitive Method for Early Diagnosis of Tuberculous Meningitis

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**A cell–enzyme-linked immunosorbent assay method was developed for the diagnosis of tuberculous meningitis by determining anti-purified protein derivative antibody production by cells derived from cerebrospinal fluid and peripheral blood. Of 20 patients with clinical histories and CSF findings suggestive of tuberculous meningitis, 18 showed high production of anti-purified protein derivative immunoglobulin antibody from CSF-derived lymphocytes, while none of the controls showed such response.**

The diagnosis of tuberculous meningitis (TBM) is often difficult and time-consuming. Early diagnosis and prompt treatment are crucial, because delay in the diagnosis and initiation of treatment contributes to a high morbidity and mortality (8, 9, 14). The diagnosis of TBM is usually based on the patient's clinical history, neurological findings, and the characteristics of the cerebrospinal fluid (CSF). The neurological manifestations of central nervous system tuberculosis are highly variable (15).

The laboratory confirmation of TBM is based on the detection of acid-fast bacilli in the CSF. However, the yield of acid-fast bacilli from CSF is extremely low (9, 14). The culture of CSF for *Mycobacterium tuberculosis* is a lengthy process, which leads to a delay in diagnosis. Various immunological methods have been devised for the diagnosis of TBM (5–7); however, their sensitivity and specificity are debatable (3, 4). The antibodies may be present mainly in the diseased body compartment (1, 2, 13).

I reasoned that these problems might be circumvented, and sensitivity and specificity might be enhanced, by evaluating the antibody response at a cellular level.

In this study, purified protein derivative (PPD) was chosen as the antigen for evaluation of the antibody response of B cells derived from CSF and peripheral blood. A cell–enzyme-linked immunosorbent assay (ELISA) method was developed for the quantitative measurement of anti-PPD antibody production by these cells.

The clinical data of the 20 patients with clinical history and CSF findings suggestive of TBM are given in Table 1. The disease grading shown in Table 1 is from the British Medical Research Council trial of 1948 (12). Sixteen patients (aged 10 to 65 years) with a variety of neurological diseases were also evaluated for anti-PPD antibody production. Eight had nontuberculous meningitis, two had herpes encephalitis, four had aseptic meningitis, and two had multiple sclerosis. CSF and peripheral blood were taken at the same time, and the cells were prepared as described previously (1).

ELISA plates with a flat plastic base (Dynatech, Alexandria, Va.) were coated with 20 μg of PPD per ml diluted in phosphate-buffered saline, pH 7.4 (PBS). After overnight incubation at 4°C, the plates were washed with PBS containing 0.05% Tween 20 (PBS-T) and then aftercoated with 5% bovine serum albumin for 6 h. The plates were then washed five times with PBS-T and finally given two washings with PBS.

Two hundred microliters of the final preparation of cells (containing $4 \times 10^4$ CFU-derived cells and $2 \times 10^5$ peripheral blood lymphocytes diluted in RPMI 1640 tissue culture medium [Sigma, St. Louis, Mo.] with 7% fetal calf serum) was added in separate wells. Each sample was prepared in duplicate with two different concentrations of cells. In each plate, positive and negative controls in the form of CSF and plasma were included in separate wells. In 12 experiments, similarly prepared peripheral blood cells from a healthy volunteer were included as a negative control. The positive control CSF was obtained from a patient who had TBM and had acid-fast bacillus-positive cultures from the CSF. The negative control CSF was obtained from a patient with muscular tension headache.

Plates were kept overnight at 37°C in 5% $CO_2$ in an incubator and then washed five times with PBS-T. Alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) antiserum (Sigma) diluted 1/1,000 in PBS-T was then added. After 4 h of incubation at room temperature, the plates were again washed five times in PBS-T. Two hundred microliters of *p*-nitrophenyl phosphate (1 mg/ml; Sigma) in 1 mol of diethanolamine-HCl buffer, pH 9.8, per liter containing $0.5 \times 10^{-3}$ mol of $MgCl_2$ per liter was added. The *p*-nitrophenyl phosphate acts as the substrate for the alkaline phosphatase. The plates were then read with an ELISA reader at 405 nm. The absorbance value in a positive patient with proven TBM was up to 2.0, while negative control CSF gave an absorbance value of less than 0.05.

To confirm that this antibody is being produced by the cells, in preliminary experiments, cells were incubated with cycloheximide (200 μg/ml; Sigma) for 2 h at 37°C and then washed three times in tissue culture medium. There was marked reduction (60 to 85%) in the anti-PPD absorbance value from cells preincubated with cycloheximide. Kinetic studies were done with CSF cells from three patients to determine the optimal conditions regarding the number of cells producing maximum quantity of anti-PPD IgG antibodies, and $4 \times 10^4$ cells were found to be optimal for highest absorbance value.

Of the 20 patients with clinical and routine CSF findings suggestive of TBM, 18 showed a high titer of anti-PPD IgG antibody production from the CSF-derived mononuclear cells. Ten of these patients had positive anti-PPD IgG antibody results from both CSF- and peripheral blood-derived cells, but the positive titer was higher in CSF than in peripheral blood.

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TABLE 1. Clinical findings, duration of disease, and routine CSF findings in patients with TBM

| Patient no. | Age (yr) | Sex[a] | Stage of disease | Duration of disease (days)[b] | CSF cell count/ mm$^3$ | CSF glucose (mg/dl) | Protein (mg/dl) | % Lympho-cytes |
|---|---|---|---|---|---|---|---|---|
| 1 | 68 | M | II | 6 | 50 | 39 | 49 | 95 |
| 2 | 50 | M | II | 7 | 100 | 27 | 290 | 98 |
| 3 | 20 | F | II | 10 | 120 | 30 | 119 | 82 |
| 4 | 25 | F | II | 10 | 292 | 20 | 249 | 85 |
| 5 | 25 | F | I | 14 | 274 | 15 | 162 | 95 |
| 6 | 22 | M | II | 15 | 98 | 48 | 293 | 95 |
| 7 | 30 | F | II | 20 | 680 | 35 | 327 | 95 |
| 8 | 23 | F | II | 20 | 120 | 30 | 118 | 82 |
| 9 | 15 | F | II | 21 | 72 | 33 | 110 | 70 |
| 10 | 18 | M | II | 22 | 280 | 30 | 110 | 90 |
| 11 | 30 | F | II | 30 | 140 | 26 | 169 | 75 |
| 12 | 30 | F | II | 30 | 60 | 48 | 920 | 95 |
| 13 | 19 | F | II | 30 | 482 | 23 | 3,215 | 90 |
| 14 | 40 | M | II | 30 | 120 | 38 | 348 | 99 |
| 15 | 35 | M | II | 45 | 146 | 31 | 98 | 65 |
| 16 | 14 | F | II | 45 | 82 | 53 | 132 | 98 |
| 17 | 40 | M | II | 50 | 196 | 29 | 366 | 70 |
| 18 | 32 | F | I | 60 | 232 | 37 | 145 | 70 |
| 19 | 40 | F | II | 90 | 168 | 38 | 102 | 82 |
| 20 | 70 | F | II | 180 | 104 | 47 | 135 | 92 |

[a] M, male; F, female.
[b] When CSF and blood were sampled for anti-PPD cell-ELISA evaluation.

Anti-PPD IgG antibody production, expressed as ELISA absorbance units, ranged from 0.6 to 2 (mean: 0.8) for CSF lymphocytes and from 0.2 to 1.0 (mean: 0.3) for peripheral blood lymphocytes.

None of the controls showed an anti-PPD IgG antibody response, either from CSF or from peripheral blood. No anti-PPD IgG antibody was produced by peripheral blood lymphocytes from 10 healthy blood donors.

The cell-ELISA method has been shown to be a sensitive technique in the diagnosis of TBM. This method involves the demonstration of active antibody production by cells, particularly those derived from the affected site. We have shown that anti-PPD antibody production is significantly higher in CSF-derived mononuclear cells than in those from peripheral blood, and in about a half of the cases, the specific antibody response was observed only in cells derived from the CSF. In a majority of the patients with TBM, a systemic focus of tuberculosis is lacking. This could explain the lack of an anti-PPD antibody response in the peripheral blood when it is strongly positive in the CSF.

The existence of local immunity in TBM has been suggested elsewhere (11). Kinnman et al. (10) showed that CSF-derived lymphocytes had a significantly higher proliferative response to PPD than do peripheral blood lymphocytes in patients with TBM, suggesting an intrathecal immune response.

In summary, the present method has shown high specificity and sensitivity. The method is also cost-effective and allows several samples to be analyzed simultaneously.

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