Diagnosis of Tuberculous Meningitis Due to Detection of ESAT-6-Specific Gamma Interferon Production in Cerebrospinal Fluid Enzyme-Linked Immunospot Assay

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A coma patient was diagnosed with tuberculous meningitis by the detection of ESAT-6-specific gamma interferon-secreting cells in the patient's cerebrospinal fluid by enzyme-linked immunospot assay prior to the identification of the pathogen in a culture of the cerebrospinal fluid.

CASE REPORT

In October 2007, a 31-year-old woman visited a neighborhood clinic because of persistent fever and fatigue that had lasted 2 weeks. The patient had been diagnosed with Base-dow's disease 11 years earlier, but no drugs had been prescribed over the previous 3 years. Because a thyrotoxic crisis was first suspected, treatment with methimazole, hydrocortisone sodium succinate, potassium iodide, and propranolol hydrochloride was initiated. Although the treatment gradually normalized the patient's thyroid function, fever and fatigue persisted. Moreover, headache and hypertonic polyuria (5,500 ml/day) developed. Due to these manifestations, the patient was referred to our hospital on 30 October 2007.

On admission, she was in a deep coma, with a Glasgow coma scale score of 3 to 4. Neurological examinations revealed anisocoria and the absence of papillary light reflex and corneal reflex but no meningeal signs, such as neck stiffness. Computed tomography (CT) of the brain demonstrated ventricular expansion without evidence of parenchymal lesions (Fig. 1). On the basis of a diagnosis of hydrocephalus, external ventricular drainage was performed. Examination of the cerebrospinal fluid (CSF) revealed pleocytosis, predominantly increased mononuclear cells strongly suggested tuberculous meningitis (TBM). Moreover, a further delay of treatment might have resulted in critical consequences. Therefore, antituberculous therapy was initiated prior to establishment of the diagnosis of TBM. To confirm this diagnosis, M. tuberculosis antigen-specific immune responses were evaluated by an enzyme-linked immunospot (ELISPOT) assay with peripheral mononuclear cells (PBMCs) and CSF cells. In brief, cells were collected from the peripheral blood and 15 ml of CSF and were stimulated with either M. tuberculosis-specific antigens, early secretory antigenic target 6 (ESAT-6), or culture filtrate protein 10 (CFP-10) for 2 h in RPMI 1640 culture medium containing 10% fetal calf serum (Equitech-bio, Inc., Kerrville, TX). Thereafter, the cells were transferred into an anti-gamma interferon (IFN-γ) monoclonal antibody-coated plate and cultured for another 16 h. The number of IFN-γ-secreting cells was determined with a dissection microscope (Carl Zeiss, Aalen-Oberkochen, Germany). The technique revealed significant numbers of ESAT-6-specific IFN-γ-secreting cells in PBMCs (45 spots/10^5 PBMCs) and CSF (105 spots/10^5 CSF cells). The frequency of occurrence of the antigen-specific IFN-γ-secreting cells in CSF was 2.3 times that in PBMCs. Because the diagnosis of TBM was made from these immunological findings, antituberculous drugs were continued. Later, the diagnosis was confirmed by the identification of M. tuberculosis in the 5-week CSF culture.

TBM, which accounts for approximately only 6% of all cases of extrapulmonary tuberculosis, is one of the most serious clinical forms of tuberculosis, with a high mortality rate and disabling neurological sequelae (8, 9). It is often hard to make a diagnosis of TBM because the conventional CSF examina-

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tion is not always sufficient for detection of the responsible pathogen. 

*M. tuberculosis* is reportedly identified by staining for acid-fast bacilli in a CSF smear in only 10% to 20% of TBM patients, whereas the pathogen is found by mycobacterial culture in 25% to 80% of TBM patients (8). However, it typically takes more than 4 to 6 weeks for the culture to identify the pathogen. This is a critical point, because delayed treatment of TBM is associated with a high mortality rate and irreversible neurological deficits (10). Indeed, in the present case, antimycobacterial therapy was started before the results of the culture examinations were confirmed. A meta-analysis of 14 studies of nucleic acid amplification tests for the diagnosis of TBM showed a combined sensitivity of 56% and a combined specificity of 98% (6). Thus, a positive nucleic acid amplification test result strongly supports the diagnosis of tuberculosis, whereas a negative result does not necessarily exclude the possibility. Therefore, the option of a rapid and accurate diagnostic procedure is required.

Although staining for acid-fast bacilli and nucleic acid amplification tests failed to detect the pathogen in the CSF and sputum of the present case, subacute fever, pleocytosis dominated by mononuclear cells, and a decreased glucose level in the CSF and subsequent hydrocephalus strongly suggested TBM. Evidence is accumulating that a novel immunological diagnostic assay, the T-SPOT.TB assay, which detects *M. tuberculosis* antigen-specific T cells in peripheral blood by use of the IFN-γ ELISPOT assay, is very useful for the diagnosis of tuberculosis, including latent and atypical forms (2, 4). This procedure provides results in about 20 h. The sensitivity of the ELISPOT assay-based technique is reported to range from 83 to 97% for patients with active tuberculosis (4). Unfortunately, TBM is exceptional in this regard, because the technique has been shown to detect the antigen-specific T cells in peripheral blood in only 58% of TBM patients (7).

In this study we applied the technique to the CSF of a patient diagnosed with TBM, because *M. tuberculosis*-specific T cells are known to accumulate as the site of infection. This is the case for pleural effusions from patients with tuberculous pleurisy. Losi et al. reported that the concentrations of ESAT-6- and CFP-10-specific cells were 4.2- to 7.5-fold higher in pleural mononuclear cells than in PBMCs (5). Their data also suggest that analysis for *M. tuberculosis*-specific IFN-γ-secreting cells in pleural effusion is highly specific and sensitive for the detection of active tuberculous pleurisy, leading to a rapid and accurate diagnosis (5, 11). On the basis of this background, we conducted the ELISPOT assay, which detected ESAT-6-specific IFN-γ-secreting cells but not CFP-10-specific
cells in the CSF from a patient with suspected TBM. Actually, some of nontuberculous mycobacteria, such as *M. marinum* and *M. kansasii*, cross-react with the ESAT-6 and CFP-10 antigens of *M. tuberculosis* (1, 3). These species are extremely rare in patients with meningitis, but they are sometimes isolated from pulmonary or wound specimens. Like the case described here, significant responses to *M. tuberculosis*-specific antigens in both blood and CSF rather suggest TBM. Only the positive ELISPOT assay result with blood but not CSF needs further examination, especially in regions where infections with these species are prevalent.

As expected, the frequency of the antigen-specific cells was higher in CSF than in PBMCs, indicating that *M. tuberculosis*-specific cells accumulate in the CSF of the TBM patient. We did, however, have an advantage in this study because the patient had hydrocephalus and large amounts of CSF could be obtained from the external ventricular drainage fluid. However, the ELISPOT assay showed 105 ESAT-6-specific IFN-γ-secreting cells/15 ml of CSF, suggesting that 1 to 2 ml of CSF is enough to detect significant numbers of *M. tuberculosis*-specific cells by this assay. Indeed, the ELISPOT assay successfully detected significant numbers of *M. tuberculosis* antigen-specific T cells in 2 ml of CSF from another TBM patient in our institution. Taken together, the ELISPOT assay technique is useful for the detection of antigen-specific cells in CSF from TBM patients, even from those with no evidence of *M. tuberculosis* infection by means of other conventional techniques.

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