The immunologic paradox in the diagnosis of tuberculous meningitis

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We report a patient with microbiologically documented tuberculous meningitis showing that the therapeutic paradox, a therapy-induced switch to a neutrophil-predominant situation in the differential cell count of cerebrospinal fluid, had a correlation with an immunologic paradox, an increased *Mycobacterium tuberculosis*-specific IFN-γ-producing T cell response.
A 34-year-old female patient presented with a 1 week history of general malaise, headache, and fever of 38.5°C. Examination of the cerebrospinal fluid (CSF) on the first day revealed a lymphocytic pleocytosis (WBC 130/mm$^3$; lymphocytes 75% and polymorphonuclear cells 7%), increased protein (134 mg/dL), decreased glucose (32 mg/dL; ratio of glucose concentration in CSF to that in serum 0.3), and high adenosine deaminase levels (12 IU/L). Microscopic examination of the CSF for acid-fast bacilli was negative. Serological test for human immunodeficiency virus was negative. A brain MRI showed suspicious tuberculous granulomas. A chest X-ray was normal. A tuberculin skin test with 2 tuberculin units became negative (induration, 0 mm). From the time of admission, she was treated with isoniazid, rifampin, ethambutol, and pyrazinamide. Dexamethasone was given on the first day and tapered off over 4 weeks. After initiation of antituberculous therapy her symptoms began gradually to improve. Follow-up examinations of the CSF on day 14 and day 28 revealed a pleocytosis (WBC 120/mm$^3$; lymphocyte 49% and polymorphonuclear cells 42% and WBC 42/mm$^3$; lymphocyte 82% and polymorphonuclear cells 4%, respectively), normal protein (52 mg/dL and 43 mg/dL, respectively), and decreased glucose levels (30 mg/dL and 41 mg/dL, respectively). Later, the CSF sample taken on day 0 grew M. tuberculosis.
weeks later, and an anti-tuberculous susceptibility test revealed that the *M. tuberculosis* was susceptible to all drugs tested. On day 0, day 14, and day 28, we performed enzyme-linked immunospot (ELISpot) assays to detect interferon-γ-secreting T-cells in peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid mononuclear cells (CSF-MC), stimulated by two antigens, early secretory antigenic target-6 and culture filtrate protein-10. The ELISpot assays (T-SPOT.TB, Oxford Immunotec, Abingdon, UK) were performed as described in a previous study (6). Briefly, PBMC were immediately (within 30 minutes) separated from 8-mL samples of peripheral venous blood. Concurrent with venous sampling, 5 to 10-mL samples of CSF were obtained, and CSF-MC was separated from the CSF within 30 minutes of sampling. The cells were suspended in AIM-V media (GIBCO, Rockville, MD, USA) at a concentration of $2.5 \times 10^6$ cells/mL PBMC and $2.5 \times 10^6$ cells/mL CSF-MC. The prepared PBMC and CSF-MC were plated ($2.5 \times 10^5$ cells/well) on plates pre-coated with anti-human IFN-γ antibody and cultured for 18 hours. Spots were then counted using an automated microscope (ELiSpot 04 HR; Autoimmune Diagnostika GmbH, Strassberg, Germany).

The detailed results of the ELISpot assays are shown in the Figure. The frequencies of IFN-γ-secreting T-cells in CSF-MC increased 2 weeks after anti-tuberculous therapy despite of clinical improvement and then slightly decreased 4 weeks after anti-
The diagnosis of tuberculous meningitis (TBM) is challenging. Therefore, if TBM is seriously suspected, many physicians usually begin empirical anti-tuberculous therapy and reconsider the diagnosis a few weeks after treatment commences (3). In this problematic clinical situation, a phenomenon known as the “therapeutic paradox”, revealing a therapy-induced switch to a neutrophil-predominant situation in the differential cell count of CSF, has been regarded by some authors as pathognomonic of TBM (5,12). It has been postulated that this phenomenon arises because of a hypersensitivity reaction related to the release of tubercular proteins during anti-tuberculous therapy (2,4). However, to our knowledge, there has been no report showing that this hypersensitive reaction has a correlation with *in vitro* cell-mediated immunity such as a *Mycobacterium tuberculosis*-specific IFN-γ-producing T cell response. In this report we characterize an “immunologic paradox” in a patient with microbiologically documented TBM.
We used the term of “immunologic paradox” as a phenomenon revealing a therapy-induced increase of *M. tuberculosis*-specific T cell response in the CSF or peripheral blood. Arias-Bouda et al. reported that an initial increase in antibody levels was observed in the early phase of treatment for 36% of all tuberculosis patients (1). Nicol et al. also showed an initial increased ELISPOT response to ESAT-6 during the first month of treatment, followed by a progressive decreased ELISPOT response to both ESAT-6 and CFP-10 (10). We assume that this is another representation of the immunologic paradox. These phenomena could be explained by an intense stimulation of the humoral and cell-mediated immune responses by antigens released from killed bacteria (1,2).

Several reports on the therapeutic paradox in TBM have been described. Sütalas et al. showed that the therapeutic paradox developed in one-third of patients with TBM, and clinical deterioration was found in half of such patients (12). Garcia-Monco et al. also reported a patient who developed the therapeutic paradox without clinical deterioration (5). However, they did not characterize any association between shifted responses of polymorphonuclear dominance and increased cell-mediated immune responses to *M. tuberculosis* antigens. In this case report, we clearly show that the therapeutic paradox was associated with the immunologic paradox of increased cell-mediated immune responses to *M. tuberculosis*-specific antigens. Interestingly, the immunologic paradox
shown by CSF-MC preceded that exhibited by PBMC in our patient. This is plausible in view of our previous finding that *M. tuberculosis*-specific T-cells are more compartmentalized to the CSF or peritoneal fluid than to the circulating blood in patients with TBM or TB peritonitis (6,7). However, further studies are needed to determine the proportion of patients with TBM who show immunologic paradoxes in CSF-MC or PBMC. It also remains to be determined whether these immunologic responses a few weeks after commencement of treatment could assist in differentiating TBM from other viral or bacterial meningitides.

Interleukin (IL)-8, a neutrophil attracting chemokine, is known to be made by a variety of leukocyte populations following stimulation by *M. tuberculosis* (9). It is interesting issue that biomarkers such as IL-8 can predict patients with TB meningitis who will subsequently develop the therapeutic paradox after anti-tuberculous therapy. Indeed, one study reported that IL-8 is elevated in tuberculous pleural effusions (11). NK cells provide a first line defense against many infections by lysis of infected cells as well as by secretion of antiviral cytokines such as IFN-\(\gamma\) (8). So, IFN-\(\gamma\)-producing spots in the ELISPOT assay do not measure CD4+ or CD8+ T cells directly since other IFN-\(\gamma\) cells, such as NK or non-cytotoxic cells, also contribute to the IFN-\(\gamma\)-producing spots. So, further studies are needed on these issues.
In conclusion, our study suggests that appearance of the immunologic paradox in repeated spinal punctures or serial blood samples in a patient with suspected TBM may give a promising clue to the presence of the most diagnostically difficult form of tuberculosis.
References


**Figure legend.** Evolution of *M. tuberculosis*-specific T-cell responses in a patient with tuberculous meningitis. The enzyme-linked immunospot assays were performed using 2.5 × 10^5 peripheral mononuclear cells (PBMC) or 2.5 × 10^5 mononuclear cells from cerebrospinal fluid (CSF-MC) on day 0, day 14, and day 28 after anti-tuberculous therapy. The diagnosis was confirmed by isolating *M. tuberculosis* from a culture of cerebrospinal fluid. Data are presented as spot forming cells/2.5 × 10^5 PBMC or CSF-MC. TNTC, too numerous to count accurately.