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Pretreatment Intracerebral and Peripheral Blood Immune Responses in Vietnamese Adults with Tuberculous Meningitis: Diagnostic Value and Relationship to Disease Severity and Outcome

Cameron P. Simmons,1* Guy E. Thwaites,* Nguyen Than Ha Quyen,* Estee Torok,* Dang Minh Hoang,* Tran Thi Hong Chau, ‡ Pham Phuong Mai, ‡ Nguyen Thi Ngoc Lan, ‡ Nguyen Huy Dung, ‡ Hoang Thi Quy, ‡ Nguyen Duc Bang, ‡ Tran Tinh Hien, ‡ and Jeremy Farrar*

Tuberculous meningitis (TBM) is the most devastating form of tuberculosis. Both intracerebral and peripheral blood immune responses may be relevant to pathogenesis, diagnosis, and outcome. In this study, the relationship between pretreatment host response, disease phenotype, and outcome in Vietnamese adults with TBM was examined. Before treatment, peripheral blood IFN-γ ELISPOT responses to the Mycobacterium tuberculosis Ags ESAT-6, CFP-10, and purified protein derivative (PPD) were a poor diagnostic predictor of TBM. Cerebrospinal fluid IL-6 concentrations at presentation were independently associated with severe disease presentation, suggesting an immunological correlate of neurological damage before treatment. Surprisingly however, elevated cerebrospinal fluid inflammatory cytokines were not associated with death or disability in HIV-negative TBM patients at presentation. HIV coinfection attenuated multiple cerebrospinal fluid inflammatory indices. Low cerebrospinal fluid IL-6 concentrations were independently associated with death in HIV-positive TBM patients, implying that IFN-γ contributes to immunity and survival. Collectively, these results reveal the effect of HIV coinfection on the pathogenesis of TBM and suggest that intracerebral immune responses, at least in HIV-negative cases, may not be as intimately associated with disease outcome as previously thought. The Journal of Immunology, 2006, 176: 2007–2014.

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2 Abbreviations used in this paper: TBM, tuberculous meningitis; CSF, cerebrospinal fluid; OCS, Glasgow Cancer Score; MBG, mononoke induced by IFN-γ; IP-10, IFN-γ-inducible protein 10; PPD, purified protein derivative; SFU, spot-forming unit; CI, confidence interval. ESAT-6, 6-kDa early secretory antigenic target; CFP-10, culture filtrate protein 10.

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Immune responses to *M. tuberculosis* are not restricted to the subarachnoid space during TBM. T cell responses to mycobacterial Ags, which are crucial in mediating resistance to *M. tuberculosis* infection, are detectable in the peripheral blood of TBM patients (4). Of particular importance are T cells specific to the *M. tuberculosis*-secreted Ags 6-kDa early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These Ags are major and possibly protective targets of T cell responses in *M. tuberculosis*-infected individuals (11, 12). Epidemiological studies have indicated that detectable IFN-γ ELISPOT responses to these Ags are strongly and specifically associated with both latent *M. tuberculosis* infection and clinically apparent pulmonary and pleural tuberculosis (TB) (13–16). In the setting of TBM, a better understanding of the frequency and magnitude of ESAT-6- and CFP-10-specific T cells in peripheral blood could potentially be useful in the diagnosis, monitoring of treatment, and prediction of outcome.

The aim of the current study was 2-fold. First, to assess the utility of peripheral blood ESAT-6- and CFP-10-specific IFN-γ ELISPOT assays in the diagnosis of TBM. Second, to identify CSF immunological and biochemical correlates of disease severity and outcome in HIV-seropositive and -seronegative TBM patients. The results suggest that ESAT-6 and CFP-10 IFN-γ ELISPOT assays have limited value in the diagnosis of TBM. In addition, CSF inflammatory cytokine concentrations in HIV-negative TBM patients were not strongly associated with disease severity at presentation or with death and disability. However, an important finding was the relationship between CSF concentrations of IFN-γ and outcome in HIV-associated TBM cases.

**Materials and Methods**

**Patient population and setting**

Study subjects were recruited from Pham Ngoc Thach Hospital and the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam. Both hospitals serve the local community and provide tertiary care for cases of severe TB (Pham Ngoc Thach Hospital) and infectious disease (HTD) in southern Vietnam. Studies on pretreatment CSF were performed in patients (≥14 years) who were recruited into a randomized controlled trial of adjuvant dexamethasone in adults with TBM between April 2001 and April 2003 (1). Only patients over 14 years of age with clinical evidence of meningitis (defined as the combination of nuchal rigidity and CSF abnormalities) were eligible to enter the study. TBM was defined as “definite” if acid-fast bacilli were seen in the CSF. It was defined as “probable” in patients with one or more of the following: suspected active pulmonary TB on chest radiography, acid-fast bacilli found in any specimen other than the CSF, and clinical evidence of other extra pulmonary TB. TBM was defined as “possible” in patients with at least four of the following: history of TB, predominance of lymphocytes in the CSF, a duration of illness of more than 5 days, a ratio of CSF glucose to plasma glucose of >0.5, altered consciousness, yellow CSF, or focal neurological signs. Patients were reclassified on discharge as having definite TBM if acid-fast bacilli were seen or *M. tuberculosis* was cultured from the CSF or as not having TBM if another diagnosis was confirmed by microbiological or histopathological evaluation.

Patients were not eligible to enter the trial if the enrolling physician believed that corticosteroids were contraindicated, if the patient had received more than one dose of any corticosteroid or >30 days of anti-TB chemotheraphy immediately before study entry, or if the consent of either the patient or the patient’s relatives was not obtained. In total, 545 patients entered the clinical trial (73 eligible cases excluded previously (1)). Pretreatment investigations of CSF were performed in 497 of the 545 enrolled cases. However, not all of the biochemical, hematological, or immunological parameters measured as part of this study could be investigated in every sample, primarily because there was insufficient CSF volume. Similarly, some clinical parameters (e.g., opening pressure) were not recorded for all cases.

Studies of peripheral blood T cell responses ELISPOTs were performed in a separate group of 47 HIV-seropositive and -seronegative TBM patients admitted consecutively to the HTD between June 2003 and May 2005. These cases were recruited into descriptive and pharmacokinetic studies of TBM. The enrollment criteria and definition of possible, probable, and definite TBM were as described above for the steroid trial with the addition that probable TBM also included computed tomography or magnetic resonance imaging brain scan evidence of TBM. All adults were tested for Abs to HIV 1/2.

The ethical and scientific committees of both hospitals, the Health Services of Ho Chi Minh City, and the Oxford Tropical Research Ethics Committee approved the study protocols. Written informed consent to participate in the study was obtained from all patients or their relatives. In unconscious HIV-positive patients, the consent of two independent physicians was deemed acceptable.

**Treatment**

All patients in whom CSF parameters were investigated received anti-TB drugs, and were randomized to adjunctive dexamethasone according to previously described treatment regimens and protocols (1). None of the patients received antiretroviral drugs. All adults recruited to this study after April 2003 received adjunctive dexamethasone.

**Clinical investigations and assessment of outcome**

Disease severity at presentation was assessed by the British Medical Research Council (BMRC) grading system (17): Grade I had a Glasgow coma score (GCS) of 15/15 with no focal neurological signs, grade II either had a GCS 11–14 or GCS 15 with focal neurological signs, grade III had a GCS of ≤10. Serial, paired, CSF and peripheral blood samples were collected before treatment. Concentrations of total and differential CSF leukocytes, lactate, glucose, and protein were measured by standard methods. Routine clinical investigations were performed on the same day the sample was taken. Other measurements were performed according to the manufacturer’s instructions (i.e., cytokine and chemokine levels were measured in every assay to gauge the extent of variation. The maximum cytokine concentrations below the detectable limit were given the value 0.1 pg/ml. To measure interassay variation, three different control samples (derived from pooled CSF) were tested in every assay to gauge the extent of variation. The maximum coefficient of variation recorded for any one cytokine was 12.3%. Blood-brain barrier integrity was assessed by measurement of paired CSF and plasma albumin concentrations by standard methods with calculation of the albumin index using the formula (albuminCSF/albumin Plasma).

**ELISPOT assays**

IFN-γ ELISPOT assays were used to measure the frequency of peripheral blood T cell responses inducing IFN-γ in response to PPD (Serum Staten) and overlapping 15-mer peptides (overlapping by 10 aa) spanning the *M. tuberculosis* ESAT-6 and CFP-10 Ags. The assay was performed according to the manufacturer’s instructions (Mabtech). Briefly, a total of 1–3 × 10⁶ PPD was added in 100 μl of culture medium per well along with either PPD (5 μg/ml), pooled ESAT-6, or CFP-10 peptides (final concentration per peptide: 1 μg/ml) or the positive control PHA (5 μg/ml). After overnight incubation and assay development, the number of spot forming units (SFU) in each well was counted using a dissecting microscope. The cutoff for a positive response in the ELISPOT assay was calculated using the formula: mean SFU from control Ags (3–5 SFU) to achieve a confidence interval of 99% (18). ELISPOT responses in positive cases were expressed as SFU per million PBMC after subtraction of background responses (no Ag stimulation).

**Cytokine and chemokine measurements and assessment of the blood-brain barrier**

Cytokines (IL-1, IL-10, IL-1β, TNF, IL-8, IL-12p70) and chemokines (IFN-γ-inducible protein 10 (IP-10), MCP-1, RANTES, and monokine induced by IFN-γ (MIG)) were measured using a cytometric bead array assay (BD Biosciences) according to the manufacturer’s instructions, with one modification: all samples were fixed in 4% paraformaldehyde before analysis. All measurements were performed in duplicate and the results expressed as the mean value. The mean limits of detection for the cytokines and chemokines were: IL-6, 4.3 pg/ml; IL-10, 4.8 pg/ml; IL-1β, 2.8 pg/ml; TNF, 4.2 pg/ml; IL-8, 5 pg/ml; IL-12p70, 5.1 pg/ml; IP-10, 6 pg/ml; MCP-1, 5.4 pg/ml; RANTES, 4.6 pg/ml; and MIG, 4.8 pg/ml. For the purpose of analysis, samples with cytokine concentrations below the detectable limit were given the value 0.1 pg/ml. To measure interassay variation, three different control samples (derived from pooled CSF) were tested in every assay to gauge the extent of variation. The maximum coefficient of variation recorded for any one cytokine was 12.3%.
Statistics

Immunological variables associated with particular clinical presentations or outcomes were assessed by univariate and multivariate analysis. The Mann-Whitney U test was used to compare continuous parameters between two or more groups of patients; the χ² test (or Fisher’s exact test) was used for comparison of categorical variables. Values of p were corrected for multiple testing by multiplying the p value by the number of tests performed (Bonferroni’s correction). Spearman’s rank correlation was used to measure correlations between ordinal variables. Variables identified in univariate analysis as associated (p < 0.1) with the outcome variable were then incorporated into multivariate logistic regression. Forward stepwise variable selection procedure was used to identify independent predictors of outcome (with p-to-enter 0.05, p-to-remove 0.01). All analyses were performed using SPSS version 10.0 software (Microsoft; SPSS).

Results

ESAT-6- and CFP-10-specific IFN-γ ELISPOT peripheral blood responses in the immunodiagnosis of TBM

Measurable IFN-γ ELISPOT responses to ESAT-6 are considered a sensitive and specific diagnostic measure of latent and some forms of clinically apparent M. tuberculosis infection (13–16). The relevance of ESAT-6-specific IFN-γ ELISPOT responses in the peripheral blood for the diagnosis of TBM is unknown. To address this, PPD and overlapping 15-mer peptides spanning the M. tuberculosis ESAT-6 and CFP-10 Ags were used in IFN-γ ELISPOT assays against pretreatment PBMC samples from TBM cases. Subjects in this study were recruited as part of a pharmacokinetic study and a clinical descriptive study at the Hospital for Tropical Diseases. TBM patients comprised 19 HIV-negative cases, 14 (74%) of whom had M. tuberculosis cultured from their CSF, and 28 HIV-positive cases, of whom 25 (89%) were culture confirmed. The baseline clinical details of these cases are shown in Table I. As controls, we used healthy Vietnamese blood donors (university students; n = 53).

Before treatment, <60% of HIV-negative TBM cases made IFN-γ ELISPOT responses to ESAT-6, CFP-10, or PPD alone (Fig. 1A). When the number of responders to either ESAT-6 and/or CFP-10 was combined, the sensitivity was 58% (Fig. 1A). Among culture-confirmed HIV-negative TBM cases, just 50% of cases made responses to ESAT-6 and/or CFP-10 (Fig. 1A). In HIV-positive TBM cases, <60% made IFN-γ ELISPOT responses to ESAT-6, CFP-10, or PPD alone (Fig. 1B). Among culture-confirmed HIV-positive TBM cases, 64% of cases made responses to ESAT-6 and/or CFP-10 (Fig. 1B). Among healthy blood donors, ESAT-6-, CFP-10-, and PPD-specific responses were detected in 54, 49, and 62% of donors, respectively. When the number of healthy responders to either ESAT-6 or CFP-10 was combined, the response rate was 62%.

Comparison of the frequency of ESAT-6-, CFP-10-, or PPD-specific T cells in individuals who responded to these Ags did not reveal significant differences in the magnitude of responses detected in TBM patients or healthy donors (Fig. 2).

Table I. Baseline clinical variables of 47 adults with TBM in whom ELISPOT responses were measured

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV Negative (n = 19)</th>
<th>HIV Positive (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./median %/range</td>
<td>No./median %/range</td>
</tr>
<tr>
<td>Male sex</td>
<td>12/63</td>
<td>23/85</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35/16–82</td>
<td>29/20–48</td>
</tr>
<tr>
<td>Diagnosis on discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed TBM</td>
<td>14/74</td>
<td>25/89</td>
</tr>
<tr>
<td>Probable TBM</td>
<td>4/21</td>
<td>3/11</td>
</tr>
<tr>
<td>Possible TBM</td>
<td>1/5</td>
<td>0/0</td>
</tr>
<tr>
<td>Active pulmonary TB (%)</td>
<td>5b/28</td>
<td>16/59</td>
</tr>
<tr>
<td>Military TB (%)</td>
<td>2b/11</td>
<td>0/0</td>
</tr>
<tr>
<td>MRC grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5/26</td>
<td>5/19</td>
</tr>
<tr>
<td>2</td>
<td>10/53</td>
<td>9/33</td>
</tr>
<tr>
<td>3</td>
<td>4/21</td>
<td>14/52</td>
</tr>
<tr>
<td>Died</td>
<td>1/5</td>
<td>17/63</td>
</tr>
<tr>
<td>CD3⁺CD4⁺ count/μl⁺</td>
<td>Not done</td>
<td>38/2–141</td>
</tr>
<tr>
<td>CD3⁺CD8⁺ count/μl⁺</td>
<td>Not done</td>
<td>497/51–1795</td>
</tr>
</tbody>
</table>

* Missing data for one patient.

FIGURE 1. IFN-γ ELISPOT responses to ESAT-6, CFP-10, and PPD. The graphs show the percentage of (A) HIV-negative (n = 19) and (B) HIV-positive (n = 28) TBM cases with responses to ESAT-6 and/or CFP-10, ESAT-6 alone, CFP-10 alone, or PPD. In each graph, the percentage of responders is shown as a proportion of clinically defined cases (□) and microbiologically confirmed cases (■). There were 25 culture-confirmed HIV-positive cases and 14 culture-confirmed HIV-negative cases.
In particular, there were significantly more CD3+ patients that responded to ESAT-6, CFP-10, or PPD were generally IFN-γ positively associated with increasing BMRC disease grade in HIV-negative and HIV-positive TBM cases.

The collection of blood lymphocyte data in HIV-positive cases (but not HIV-negative cases since it was not clinically indicated) allowed us to relate ELISPOT responses to T cell counts. Blood CD3+CD4+ and CD3+CD8+ T cell counts in HIV-positive patients that responded to ESAT-6, CFP-10, or PPD were generally higher than in patients who did not respond to these Ags (Table II). In particular, there were significantly more CD3+CD4+ and CD3+CD8+ T cells in cases that responded to PPD and significantly more CD3+CD8+ T cells in cases that responded to CFP-10 (Table II). These data suggest profound T lymphopenia may diminish the sensitivity of the ELISPOT assay.

Relationship between clinical presentation and the acute intracerebral inflammatory response in HIV-negative TBM cases

Subjects in this study were recruited as part of a randomized controlled trial of adjunctive dexamethasone in adults with TBM (1). In this study, the severity of TBM at presentation was assessed by BMRC grade (17), which has been shown in numerous studies in different populations to be strongly predictive of outcome (1, 8, 19). We hypothesized that an excessive intracerebral inflammatory immune response would be associated with BMRC grade in both HIV-negative and HIV-positive TBM patients. To address this hypothesis, inflammatory indices in pretreatment CSF samples from 497 adults with a clinical diagnosis of TBM were measured. The baseline characteristics of the study population are shown in Table III. Immune responses in HIV-negative and HIV-positive patients were analyzed separately.

At presentation, CSF concentrations of lactate and protein were positively associated with increasing BMRC disease grade in HIV-negative patients (Fig. 3, A and B). There was also a trend for CSF IL-6 and disease grade (Fig. 3C). Other intracerebral parameters, such as opening pressure (n = 272), CSF:blood glucose ratio (n = 405), CSF white cell count (n = 408), CSF IL-10 (n = 141), CSF IL-12p70 (n = 141), IL-1β (n = 141), IL-8 (n = 142), IFN-γ (n = 142), IP-10 (n = 24), MCP-1 (n = 23), RANTES (n = 23), and MIG (n = 24) concentrations were unrelated to disease grade. Among clinical parameters, the length of illness before presentation (Fig. 3D) and evidence of active, pulmonary TB was also significantly associated with BMRC grade III TBM (p = 0.02, χ²). Other parameters, such as radiographic evidence of milary TB, clinical extrapulmonary TB, or a history of TB therapy, were not significantly associated (by univariate analysis) with presentation in BMRC grade III.

Logistic regression was used to determine whether length of history before presentation, evidence of pulmonary TB, and CSF concentrations of IL-6 and protein were independently associated with grade III TBM. Lactate was omitted from the model because there were insufficient measurements. Logistic regression using data from 140 cases indicated only IL-6 was independently associated with grade III TBM. Lactate was omitted from the model because there were insufficient measurements. Logistic regression using data from 140 cases indicated only IL-6 was independently associated with grade III TBM. Lactate was omitted from the model because there were insufficient measurements. Logistic regression using data from 140 cases indicated only IL-6 was independently associated with grade III TBM.

Relationship between the acute intracerebral inflammatory response in HIV-negative TBM patients and death

Excessive immune activation in the subarachnoid space is thought to be associated with poor outcome in TBM; hence, the use of steroids as adjunctive therapy. Previous work from our group has documented the significant relationship between death from TBM and a relatively low CSF white cell count and a low CSF:blood glucose ratio at presentation (1). In this study, the relationship between death and other CSF inflammatory indices was investigated. The frequency with which IL-12p70 was detected in CSF at presentation was significantly greater in patients who died (4 of 33, 12%) than in those who survived (3 of 112, 2.5%; p < 0.01, χ² test), although this was not significant after correction for multiple testing. However, other parameters, such as opening pressure (n = 272), CSF protein (n = 410), CSF lactate (n = 79), IFN-γ (n = 145), CSF IL-10 (n = 145), CSF IL-12p70 (n = 145), IL-1β (n = 145), TNF (n = 145), IL-8 (n = 145), IP-10 (n = 24), MCP-1 (n = 23), RANTES (n = 24), and MIG (n = 24) concentrations were not associated with death by univariate analysis.

Differences in the intracerebral immune response between HIV-positive and HIV-negative TBM patients

There are scant data on the impact of HIV coinfection on the intracerebral immune response in TBM patients. In this study, the

![Image](http://www.jimmunol.org/DownloadedFrom/2010VIETNAMESEADULTSWITHTBM)

**FIGURE 2.** Frequency of IFN-γ ELISPOT responses to ESAT-6, CFP-10, and PPD. The graph depicts the frequency of ESAT-6, CFP-10, and PPD-specific IFN-γ SFU per million PBMC detected in individual healthy donors, HIV-negative, and HIV-positive TBM cases.

### Table II. Baseline CD4+ T cell counts (mean ±SE) in HIV-positive TBM patients stratified according to whether they responded to Ag stimulation in the ELISPOT assay

<table>
<thead>
<tr>
<th>ELISPOT Response</th>
<th>Ag</th>
<th>ESAT-6</th>
<th>CFP-10</th>
<th>PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average CD8+ cells/μl</td>
<td>+</td>
<td>564 (115)</td>
<td>563 (91)</td>
<td>713 (116)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>301 (133)</td>
<td>203 (179)</td>
<td>261 (111)</td>
</tr>
<tr>
<td>Average CD4+ cells/μl</td>
<td>+</td>
<td>42 (10)</td>
<td>41 (8)</td>
<td>52 (10)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>27 (10)</td>
<td>24 (14)</td>
<td>24 (9)</td>
</tr>
</tbody>
</table>

*CD4+ T cell counts were available from 23 of 28 HIV-positive patients.*

*p = 0.04, Mann-Whitney U test, CD8+ cells/μl in CFP-10 responders vs nonresponders.

*p = 0.01, Mann-Whitney U test, CD8+ cells/μl in PPD responders vs nonresponders.

*p = 0.05, Mann-Whitney U test, CD4+ cells/μl in PPD responders vs nonresponders.*
mean peripheral blood CD4+ T cell count in HIV-positive cases (n = 77) was 117 per μl (range, 9–694) at presentation, and no patient received antiretrovirals during their acute illness. In accordance with their immunocompromised state, there was a trend for many, but not all markers of CSF inflammation to be attenuated in HIV-positive patients relative to HIV-negative patients. Thus, CSF opening pressures, CSF pleocytosis values, and CSF IFN-γ concentrations were all lower in HIV-positive patients relative to HIV-negative patients (Table IV). Conversely, CSF lactate concentrations were less in HIV-positive patients than in HIV-negative patients (Table IV). Interestingly, the most affected CSF parameter was the anti-inflammatory molecule IL-10, concentrations of which were ∼6-fold less in HIV-positive patients than in HIV-negative patients (Table IV). Consequently, the CSF IFN-γ:IL-10 ratio, a useful index measuring the balance of candidate proinflammatory vs anti-inflammatory cytokines, was tilted toward excess IFN-γ in HIV-positive patients relative to HIV-negative patients (Table IV). Logistic regression on 144 (112 HIV-negative and 32 HIV-positive) cases in which appropriate data were available was used to identify immunological and clinical features at presentation that independently associated with HIV infection. Included in the model were factors that by univariate analysis were associated with HIV in this patient subset. These parameters were sex, age, evidence of extrapulmonary/neural TB, CSF white blood cell count, CSF:blood glucose ratio, CSF IL-10 concentration, and CSF IFN-γ:IL-10 ratio. Logistic regression indicated male sex (p = 0.014, odds ratio (95% CI), 4.5 (1.4–15)), age (p = 0.003, odds ratio (95% CI), 0.93 (0.89–0.98)), CSF white blood cell count (p = 0.001), odds ratio (95% CI), 0.24 (0.11–0.57), CSF IFN-γ:IL-10 ratio (p = 0.009, odds ratio (95% CI), 2.47 (1.25–4.88)), and evidence of extrapulmonary/neural TB (p = 0.009, odds ratio (95% CI), 4.4 (1.1–17)) were independently associated with HIV.

**Discussion**

Understanding which aspects of the intracerebral and peripheral blood host response in TBM patients are most relevant to diagnosis, clinical presentation, and outcome could facilitate improved clinical management of this disease. In this study, we found that ESAT-6- and CFP-10-based ELISPOT assays using peripheral blood had poor sensitivity and specificity for the diagnosis of TBM in Vietnamese adults. CSF IL-6 was identified as the only inflammatory parameter that was independently associated with severe TBM disease presentation (BMRC grade III). We also found that TBM patients coinfected with HIV had an attenuated intracerebral host response and death was independently associated with lower CSF IFN-γ levels at presentation.

In some epidemiological settings, peripheral blood T cell responses to the RD1-associated Ags ESAT-6 and CFP-10 are considered useful markers of infection with *M. tuberculosis* and are probably more specific than traditional skin tests (13, 15, 20).
However, their role in the diagnosis of TB disease is less well established. Responses to these Ags have not previously been described in a large number of patients with culture-confirmed TBM. We found that measurable ESAT-6- and/or CFP-10-specific ELISPOT responses were a poor correlate of TBM in HIV-positive and HIV-negative Vietnamese adults and imply that conventional methods of microbiological diagnosis remain essential (21). In part, T cell lymphopenia and anergy resulting from disseminated TB, or advanced HIV infection, may explain the lack of peripheral blood ELISPOT responses in some TBM patients. In HIV-positive cases, there was a strong suggestion that responders to these Ags had relatively higher CD4^+^ and CD8^+^ T cell counts (Table II).

The presence of circulating ESAT-6- and/or CFP-10-specific T cells in 62% of healthy young Vietnamese adults is consistent with a high level of exposure in this population, all of whom live in an urban setting where TB is highly prevalent. The frequency of positive ESAT-6 and/or CFP-10 responses among healthy Vietnamese adults, similar to that reported previously from healthy adults in urban India (22), underlines the difficulties in using immunodiagnostic methods to diagnose active TB in endemic settings. An alternative approach to the immunodiagnosis of TBM would entail detection of ESAT-6- and/or CFP-10-specific T cells in CSF. However, we have previously shown that CSF T cells from TBM cases fail to respond to antigenic or mitogenic stimulation ex vivo, rendering this approach inappropriate (4).

TBM patients presenting with significant neurological deficit (BMRC grade III) have a poor prognosis (1). Immune-driven inflammation in the confines of the subarachnoid space has long been considered as central to the cerebral pathology and outcome from TBM. In this study, CSF concentrations of IL-6 were independently associated with BMRC grade III. IL-6 is an acute phase cytokine with diverse actions and has been implicated in the pathophysiology of many neurological and inflammatory disorders (23). In the context of TB, murine studies suggest IL-6 is involved in stimulating early IFN-γ production, but is not essential for the development of protective immunity against M. tuberculosis (24). However, IL-6 may also play an anti-inflammatory role by suppressing gene expression of proinflammatory cytokines (25). Whether IL-6 is inflammatory, anti-inflammatory, or both, in the setting of TBM in humans is uncertain. Nevertheless, the positive association between CSF IL-6 and clinical severity is consistent with a pathogenic process in which the acute phase response to mycobacterial Ags is associated with cerebral pathology before therapy.

In agreement with previous studies (5–8), we found high baseline CSF concentrations of IL-6, IL-8, IFN-γ, and IL-10 in HIV-negative TBM patients. TNF and IL-12p70 were also detectable at baseline, but not in all patients. Significant correlations were observed between different cytokine pairs (data not shown), as might be expected in a regulated cytokine cascade, but not with cytokines and CSF cell counts, suggesting resident cells were the most likely source of these molecules. Importantly, none of the CSF cytokines or chemokines that we measured were associated with outcome.

HIV-positive individuals with TBM generally have a poor prognosis (1, 26), although the neurological features of the disease at presentation are probably not influenced by HIV infection (27). However, this study suggests HIV infection alters the intracerebral inflammatory response. Compared with HIV-negative TBM patients, there was a strong trend for HIV-positive individuals to have a reduced CSF pleocytosis and opening pressure at presentation. Most dramatically, HIV-positive individuals had diminished CSF IL-10 concentrations. Since IL-10 is an inhibitor of...
cytokine secretion, e.g., IFN-γ, by Th1 cells and NK cells, the overall effect of reduced CSF IL-10 concentrations would be a cytokine milieu skewed toward cellular activation. This was reflected in the IFN-γ:IL-10 ratio, which was heavily polarized in favor of IFN-γ in HIV-positive individuals. The basis for an attenuated CSF IL-10 response in HIV-positive individuals is uncertain, although clearly HIV infection in both the periphery and CNS could have significant modulatory effects on quantitative and qualitative aspects of the intracerebral immune response.

When HIV-positive TBM cases were stratified by outcome, death was independently associated with relatively low CSF concentrations of IFN-γ at presentation. This suggests IFN-γ, despite its inflammatory activity, plays a beneficial role in the intracerebral immune response, an observation consistent with a body of literature describing a crucial role for IFN-γ and related molecules in host resistance to mycobacterial infection (28, 29). The basis for the diminished CSF IFN-γ concentrations in HIV-positive TBM patients that subsequently died is unclear. Potentially, it reflects the underlying level of immunocompromise in these individuals; CD4 counts were significantly less in patients who died than in those who survived, although CD4 counts were not correlated with CSF IFN-γ concentrations (data not shown). Alternatively, host genetic, nutritional factors, differences in pathogen burden, or other concomitant illnesses may attenuate the IFN-γ response in these subjects. Potentially, HIV-positive TBM patients might benefit from IFN-γ as an immunoadjuvant to chemotherapy and antiretrovirals. Aerosolized rIFN-γ has been safely administered to patients with refractory pulmonary TB (30), though there are scant data on its clinical effectiveness (31).

Collectively, these data on TBM cases highlight a conceptual paradox in our understanding of TBM. On the one hand, prevailing dogma suggests that a strong intracerebral immune response is associated with disease severity and is also deleterious to outcome. Consistent with this argument is the observation that disease severity at presentation was positively correlated with CSF IL-6 concentrations (this study) and that adjunctive corticosteroids provide clinical benefit to HIV-negative TBM cases (1). In contrast, poor outcome from TBM in HIV-negative patients is not significantly associated with elevated CSF inflammatory cytokine concentrations at presentation (this study). In addition, our earlier work revealed that aggressive adjunctive corticosteroid therapy does not profoundly attenuate markers of CSF inflammation, despite improving outcome (4). Furthermore, a poor outcome from TBM in HIV-negative cases is associated with an attenuated CSF pleocytosis (1), and, in HIV-positive cases, a weak CSF IFN-γ response (this study). Collectively, these data serve to highlight the complexity of TBM and suggest the immunopathogenesis model, whereby intracerebral immune responses are pivotal to presentation and outcome, may be overly simplistic. Clearly, other factors contribute to TBM disease pathogenesis and outcome. The anatomical location in the cerebral tissue of tuberculous lesions is likely to be important, as probably are host and pathogen genetic determinants. For example, TBM associated with rifampin and isoniazid-resistant M. tuberculosis strains is clinically devastating (32). Concomitant infections, such as hepatitis B, may also complicate treatment and affect outcome (1).

Collectively, the results described here represent the most comprehensive description of the peripheral and intracerebral immune response in HIV-positive TBM patients at presentation.

Table IV.  Attenuated intracerebral immune responses in HIV-positive TBM patients at presentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV Negative Mean (SD)</th>
<th>HIV Positive Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log opening pressure (cm H2O)</td>
<td>1.31 (0.25)</td>
<td>1.25 (0.24)</td>
<td>0.07</td>
</tr>
<tr>
<td>Log total white blood cells in CSF (cells/µl)</td>
<td>1.90 (0.67)</td>
<td>1.76 (0.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Log CSF protein (mg/dl)</td>
<td>2.14 (0.37)</td>
<td>2.11 (0.35)</td>
<td>0.70</td>
</tr>
<tr>
<td>Log CSF lactate (mg/dl)</td>
<td>0.72 (0.22)</td>
<td>0.70 (0.11)</td>
<td>0.05</td>
</tr>
<tr>
<td>Log CSF glucose</td>
<td>−0.57 (0.23)</td>
<td>−0.59 (0.25)</td>
<td>0.53</td>
</tr>
<tr>
<td>Log CSF IFN-γ (pg/ml)</td>
<td>2.45 (1.16)</td>
<td>2.29 (1.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Log CSF IL-10 (pg/ml)</td>
<td>1.47 (0.79)</td>
<td>0.71 (1.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log CSF IL-6 (pg/ml)</td>
<td>3.61 (0.9)</td>
<td>3.7 (1.24)</td>
<td>0.24</td>
</tr>
<tr>
<td>Log CSF IFN-γ:IL-10 ratio</td>
<td>0.973 (0.990)</td>
<td>1.550 (0.697)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Log CSF IL-8 (pg/ml)</td>
<td>3.25 (0.58)</td>
<td>3.17 (0.63)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table V.  Presentation CSF indices associated with outcome in HIV-positive TBM patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Survived Mean [SD]</th>
<th>Died Mean [SD]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log CSF IFN-γ (pg/ml)</td>
<td>2.83 [0.55]</td>
<td>1.99 [1.2]</td>
<td>0.004</td>
</tr>
<tr>
<td>Log CSF IL-10 (pg/ml)</td>
<td>1.07 [0.65]</td>
<td>0.5 [1.15]</td>
<td>0.05</td>
</tr>
<tr>
<td>Log CSF IL-8 (pg/ml)</td>
<td>3.39 [0.35]</td>
<td>3.05 [0.72]</td>
<td>0.04</td>
</tr>
<tr>
<td>Blood CD4 count/µl</td>
<td>2.06 [0.348]</td>
<td>1.72 [0.437]</td>
<td>0.001</td>
</tr>
</tbody>
</table>

a Values of p derived from Mann-Whitney U test.
response in either HIV-negative or HIV-positive TBM patients. Future studies that define more thoroughly the nature of the intra-cerebral host response and its relationship to outcome are warranted. Ultimately, a better understanding of TBM pathogenesis should help improve clinical management in one of the most devastating forms of TB.

Disclosures

The authors have no financial conflict of interest.

References