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The Clinical Benefit of Adjunctive Dexamethasone in Tuberculous Meningitis Is Not Associated with Measurable Attenuation of Peripheral or Local Immune Responses

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Outcome from tuberculous meningitis (TBM) is believed to be dependent on the severity of the intracerebral inflammatory response. We have recently shown that dexamethasone improved survival in adults with TBM and postulated that the clinical effect would be associated with a measurable systemic and intracerebral impact on immunological markers of inflammation. Prolonged inflammatory responses were detected in all TBM patients irrespective of treatment assignment (placebo or dexamethasone). The inflammatory response in the cerebrospinal fluid was characterized by a leukocytosis (predominantly CD3+CD4+ T lymphocytes, phenotypically distinct from those in the peripheral blood), elevated concentrations of inflammatory and anti-inflammatory cytokines, chemokines, and evidence of prolonged blood-brain barrier dysfunction. Dexamethasone significantly modulated acute cerebrospinal fluid protein concentrations and marginally reduced IFN-γ inflammatory cytokines, chemokines, and evidence of prolonged blood-brain barrier dysfunction. Dexamethasone significantly modulated acute cerebrospinal fluid protein concentrations and marginally reduced IFN-γ concentrations; other immunological and routine biochemical indices of inflammation were unaffected. Peripheral blood monocyte and T cell responses to Mycobacterium tuberculosis Ags were also unaffected. Dexamethasone does not appear to improve survival from TBM by attenuating immunological mediators of inflammation in the subarachnoid space or by suppressing peripheral T cell responses to mycobacterial Ags. These findings challenge previously held theories of corticosteroid action in this disease. An understanding of how dexamethasone acts in TBM may suggest novel and more effective treatment strategies. The Journal of Immunology, 2005, 175: 579–590.

Clinical outcome from tuberculous meningitis (TBM),4 the most lethal form of infection with Mycobacterium tuberculosis, is believed to be dependent on the severity of the host intracerebral inflammatory response (1). Corticosteroids are used in a wide variety of inflammatory conditions and provide clinical benefit by apparently diverse and incompletely understood mechanisms. They have long been considered for the adjunctive treatment of TBM, although evidence of their effect on morbidity and mortality has been difficult to obtain (2). Previous studies suggested corticosteroids attenuated the inflammatory response in the subarachnoid space of those with TBM (cerebrospinal fluid concentrations of leukocytes, protein, and glucose returned to normal faster in corticosteroid-treated patients) but were too small to demonstrate a clear effect on survival, and measurement of molecular inflammatory mediators was impossible (3). Recent controlled trials provided stronger evidence that adjunctive corticosteroids improve survival in children with TBM (4). Cerebrospinal fluid concentrations of protein, globulin, and glucose normalized faster in the corticosteroid-treated children without a significant effect on cerebrospinal leukocyte counts; other immunological mediators considered important in the pathogenesis of TBM, such as TNF-α (1), were not measured (5).

We recently performed a controlled trial of adjunctive dexamethasone in 545 Vietnamese adults with TBM that demonstrated dexamethasone improved survival but did not prevent severe disability (6). We postulated that the effect of dexamethasone on survival would be associated with a measurable anti-inflammatory response in the subarachnoid space with evidence of systemic attenuation of response to M. tuberculosis Ags. Therefore, we compared the kinetics of the inflammatory response in cerebrospinal fluid and peripheral blood from 87 adults with TBM randomly assigned to treatment with adjunctive dexamethasone or placebo. Our aim was to define a mechanism of action for the effect of dexamethasone on survival and to identify molecules and cells critical to the pathogenesis of TBM that might suggest novel and more specific treatments.

Materials and Methods

Study participants and setting

A randomized, double blind, placebo-controlled trial of adjunctive dexamethasone for the treatment of TBM was performed in 545 adults between April 2001 and April 2003 according to methods described previously (6). Study participants were recruited from two centers: Pham Ngoc Thach Hospital for tuberculosis and the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam. The immunomodulatory effects of dexamethasone were only studied in adults treated at HTD (n = 93). Adults (>14...
years of age) with definite, probable, or possible TBM were eligible to enter the trial. Definite TBM was defined as clinical meningitis (nuchal rigidity and abnormal cerebrospinal fluid parameters) and acid-fast bacilli seen, or *M. tuberculosis* cultured, from the cerebrospinal fluid. Probable TBM was defined as clinical meningitis and one or more of the following: suspected active pulmonary tuberculosis on chest radiography; acid-fast bacilli found in any other specimen; clinical evidence of other extrapulmonary tuberculosis. Possible TBM was defined as clinical meningitis and at least four of the following: history of previous tuberculosis; predominance of lymphocytes in the cerebrospinal fluid; illness duration >5 days; cerebrospinal fluid-blood glucose <0.5; altered consciousness; yellow cerebrospinal fluid; and focal neurological signs. All adults were tested for Abs to HIV 1/2.

The ethical and scientific committees of both hospitals, the Health Service of the Thai Ministry of Public Health, and the Oxford Clinical Research Ethics Committee approved the study protocol. Written informed consent to participate in the study was obtained from all patients or their relatives.

### Treatment and specimen collection

Adults were allocated randomly to start dexamethasone sodium phosphate or placebo (VIDIPHA) as soon as possible after the start of four antituberculosis drugs. Adults with grade II or III disease received an i.v. drug for 4 wk (wk 1, 0.4 mg/kg/day; wk 2, 0.3 mg/kg/day; wk 3, 0.2 mg/kg/day; wk 4, 0.1 mg/kg/day), then 4 wk of oral drug starting at 4 mg total per day, reducing each week by 1 mg until zero. Adults with grade I disease received 2 wk of an i.v. drug (wk 1, 0.3 mg/kg/day; wk 2, 0.2 mg/kg/day), then 4 wk of an oral drug (wk 3, 0.1 mg/kg/day orally; then 3 mg total per day reducing by 1 mg each week until zero).

Serial, paired cerebrospinal fluid and peripheral blood samples were collected before and after starting the study drug as part of normal clinical care. When possible, paired samples were collected on days 3, 7, 30, 60, and 270 after the commencement of treatment. In practice, there was variation in the time of sampling; details are provided for the period patients received the study drug. Over a period of 9 mo, we collected a total of 228 cerebrospinal fluid samples from 41 patients in the placebo arm and 279 cerebrospinal fluid samples from 46 patients in the dexamethasone arm. There were pretreatment samples from 41 patients in the placebo arm and 45 patients in the dexamethasone arm. In the first 4 days of treatment, there were samples from 22 patients in the placebo arm (median time, 3 days) and samples from 30 patients in the dexamethasone arm (median time, 3 days). Between days 5 and 12, there were samples from 33 patients in the placebo arm (median time, 7 days) and samples from 39 patients in the dexamethasone arm (median time, 7 days). Between days 13 and 20, there were samples from 8 patients in the placebo arm (median time, 14 days) and samples from 12 patients in the dexamethasone arm (median time, 28 days). Between days 21 and 35, there were samples from 34 patients in the placebo arm (median time, 28 days) and samples from 44 patients in the dexamethasone arm (median time, 28 days).

Routine clinical investigations, flow-cytometric analysis of cellular phenotype, and functional studies were performed on the same day the specimen was taken; all other measurements were performed on aliquots of cerebrospinal fluid supernatant frozen at −70°C. Cerebrospinal fluid supernatants were typically frozen within 3 h of collection.

### Assessment of the clinical and inflammatory response to treatment

The primary clinical outcome was death or severe disability 9 mo after randomization. Local and peripheral immune responses were assessed blind to clinical outcome and treatment allocation by the following methods.

#### Routine cerebrospinal fluid measurements

Concentrations of total and differential cerebrospinal fluid leukocytes, lactate, glucose, and protein were measured by standard methods.

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

Cytokines (IL-6, IL-10, IL-1β, TNF, IL-8, and IL-12p70) and chemokines (IP-10, MCP-1, RANTES, and Mig) were measured using a cytometric bead array assay (BD Biosciences) according to the manufacturer’s instructions. Chemokine concentrations detected in whole-blood cultures stimulated with PPD were subtracted. An assay was valid only if 1) wells containing PBMCs stimulated with PPD, mitomycin C, and IFN-γ or mitomycin C and IFN-γ had sixfold more detection than negative control wells, and 2) there were twice as many SFU in Ag-stimulated wells as negative control wells. The median background frequency of IFN-γ SFU was 6 SFU/million PBMCs (range, 0–328).

### Statistics

The methods for primary and stratified subgroup analysis of death and disability were reported previously (6). For the purposes of this study, adults were excluded from the analysis if they stopped the study drug early for any reason or an alternative diagnosis was assigned. Variables were compared by Student’s *t* test if normally distributed and by Mann-Whitney *U* test if not normally distributed. Categorical variables were compared by the *χ*² test (or Fisher’s exact test when appropriate). All *p* values were two-sided. The magnitude of change from baseline of inflammatory indices was assessed in patients who had paired cerebrospinal fluid samples collected <2 days before receiving the study drug and between 1 and 7 days after beginning therapy. The magnitude of change in each parameter was determined by subtracting the mean measured in the first week of therapy from the value obtained before treatment. Kinetics of serial measurements in individual patients were summarized using area under the curve, calculated per unit time of study drug administration, by the trapezoidal method. All analyses were performed using SPSS version 10 (SPSS) and STATA 5 (StataCorp).

### Results

#### Baseline variables and impact of dexamethasone on clinical outcome

Ninety-three patients were recruited to the trial of dexamethasone at the HTD (6). We excluded 6 of 93 patients from subsequent analysis of immune responses because they withdrew consent (*n* = 1), had medical contraindications that resulted in withdrawal of the
study drug earlier than prescribed (n = 4), or had a confirmed alternative diagnosis (n = 1). The baseline clinical characteristics of 87 patients randomized to dexamethasone (n = 46) or placebo (n = 41) included in this study are shown in Table 1.

Dexamethasone treatment was associated with a reduced risk of death (relative risk, 0.69; 95% confidence interval, 0.52–0.92; p = 0.01) by intention-to-treat analysis of all patients (n = 545). Treatment effect was homogenous (test of heterogeneity, p > 0.05) across subsets defined by disease severity grade (stratified relative risk of death, 0.68; 95% confidence interval, 0.52–0.91; p = 0.007), HIV infection (stratified relative risk of death, 0.78; 95% confidence interval, 0.59–1.04; p = 0.08), and hospital of admission (stratified relative risk of death, 0.70; 95% confidence interval, 0.53–0.92; p = 0.01) (6).

**Impact of dexamethasone on routine cerebrospinal fluid measurements and the integrity of the blood-brain barrier**

Collection of paired cerebrospinal fluid samples immediately before treatment and during the first week of therapy allowed us to measure the magnitude of change from baseline in a range of routine and immunological markers of inflammation. Dexamethasone elicited significantly greater improvements in cerebrospinal fluid total protein concentration during the first week of therapy (mean ± SD; net reduction with dexamethasone (n = 33) 0.64 ± 1 mg/ml vs increase with placebo (n = 39) 0.14 ± 1.4 mg/ml; p = 0.01). Dexamethasone did not significantly alter the magnitude of change from baseline of other cerebrospinal fluid parameters, including lymphocytes, neutrophils, lactate, opening pressure, the cerebrospinal fluid-plasma glucose ratio, or the albumin index during the first week of therapy compared with placebo (data not shown). Additional stratification and analysis of values collected in the first week of therapy (e.g., baseline vs days 1–3 and baseline vs days 4–7) did not reveal any additional differences between the placebo and steroid arms.

Comparison of routine variables over the 2-mo interval of study drug administration and the overall 9-mo treatment period suggested dexamethasone did not impact on the kinetics of responses (Fig. 1). Comparison of the area under the curve per unit time for each variable during the 6- to 8-wk period of study drug administration also indicated dexamethasone did not significantly modulate cerebrospinal fluid responses (data not shown).

**Impact of dexamethasone on cerebrospinal fluid cytokine and chemokine concentrations**

Dexamethasone elicited a reduction in cerebrospinal fluid IFN-γ concentrations during the first week of therapy (mean ± SD; net reduction with dexamethasone (n = 27) 2.2 ± 3.3 ng/ml vs net reduction with placebo (n = 25) 2.0 ± 5.9 ng/ml), but this was marginally not significant (p = 0.06). In the first week of therapy, dexamethasone did not significantly alter the magnitude of change from baseline of the other cerebrospinal fluid cytokines or chemokines including IL-6, IL-8, IL-10, RANTES, MCP-1, Mig, and IP-10 during the first week of therapy compared with placebo (data not shown). Dexamethasone also did not significantly alter the kinetics of any of the cerebrospinal fluid cytokines or chemokines over the 2-mo period of study drug administration (Fig. 2). Concentrations of IFN-γ, IL-6, IL-8, and IL-10 fell slowly, with all cytokines remaining detectable in most patients for at least 2 mo (Fig. 2). TNF was detected in a majority of pretreatment cerebrospinal fluid samples (44 of 61 samples; range, 2.8–301 pg/ml), but its concentration fell rapidly with treatment without detectable influence of dexamethasone (Fig. 2). IL-12p70 (3 of 61) and IL-1β (7 of 61) were seldom detected in pretreatment cerebrospinal fluid samples, and dexamethasone did not significantly alter the frequency with which they were detected after initiation of therapy (data not shown). The cerebrospinal fluid contained significant concentrations of the chemokines RANTES, Mig, IP-10, and MCP-1 (Fig. 3). Their concentration was maximal before treatment, with IP-10 and Mig, in particular, present at high concentrations. Dexamethasone failed to significantly alter the kinetics of any of these molecules (Fig. 3).

**Impact of dexamethasone on the kinetics and phenotype of cerebrospinal fluid and PBLs**

Antituberculosis therapy was associated with a steady rise in the absolute number of CD3+CD4+ and CD3+CD8+ T cells and CD16+56+ NK cells in the peripheral blood of TBM patients independent of treatment assignment (Fig. 4). CD3+ T cells were the dominant lymphocyte subset present in cerebrospinal fluid (Fig. 4). Acutely (first 2 wk of therapy), CD3+ T cells were significantly overrepresented (p < 0.001) as a percentage of lymphocytes in the cerebrospinal fluid compared with the peripheral blood of the same

### Table I. Baseline clinical variables of 87 adults with TBM randomized to adjunctive dexamethasone or placebo and included in the investigation of immune responses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Allocated Dexamethasone (n = 46)</th>
<th>Allocated Placebo (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/Median</td>
<td>%/Range</td>
</tr>
<tr>
<td>Males</td>
<td>24</td>
<td>52.2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.5</td>
<td>15–68</td>
</tr>
<tr>
<td>Diagnosis on discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed TBM</td>
<td>33</td>
<td>71.7%</td>
</tr>
<tr>
<td>Probable TBM</td>
<td>5</td>
<td>10.9%</td>
</tr>
<tr>
<td>Possible TBM</td>
<td>8</td>
<td>17.4%</td>
</tr>
<tr>
<td>BCG scar*</td>
<td>3</td>
<td>10.3%</td>
</tr>
<tr>
<td>Duration of symptoms (days)</td>
<td>17</td>
<td>5–151</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47</td>
<td>30–75</td>
</tr>
<tr>
<td>Temperature on admission (°C)</td>
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<td>37–40.6</td>
</tr>
<tr>
<td>Glasgow coma score (/15)</td>
<td>14</td>
<td>6–15</td>
</tr>
<tr>
<td>MRC grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>34.8%</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>39.1%</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>26.1%</td>
</tr>
<tr>
<td>HIV infected</td>
<td>1</td>
<td>2.2%</td>
</tr>
<tr>
<td>HIV not tested</td>
<td>1</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

* Missing data from 16 in the dexamethasone group and 17 in the placebo group.
FIGURE 1. Dynamics of changes in cerebrospinal fluid leukocyte populations and cerebrospinal fluid metabolic indices in adult TBM patients randomized to dexamethasone or placebo. The data show changes in the means (±95% confidence interval) of the absolute count of cerebrospinal fluid white cells (A), percentage of neutrophils (B), percentage of lymphocytes (C), total protein concentration (D), cerebrospinal fluid albumin ratio (expressed as a percentage of the serum albumin concentration) (E), ratio of the cerebrospinal fluid-blood glucose concentration (F), cerebrospinal fluid lactate concentration (G), and opening pressure at the lumbar puncture (H).
patient (mean ± SD; 76.2 ± 12.2% in cerebrospinal fluid vs 62.8 ± 9.9% in paired blood (n = 18 patients)), suggesting these T cells were selectively recruited or retained in the cerebrospinal fluid. The absolute numbers of lymphocyte subsets in cerebrospinal fluid did not change significantly during the first 2 mo of therapy (Fig. 4), because although the total numbers of leukocytes fell, the proportion of lymphocytes increased (Fig. 1C). Dexamethasone therapy did not alter the kinetics of absolute numbers (Fig. 4) or percentages (data not shown) of any of the cerebrospinal fluid or PBL subsets.

Given the prominence of CD3+CD4+ T cells in the cerebrospinal fluid, we investigated their phenotype further. The majority of CD3+CD4+ T cells were CD45RA−CD45RO+CD95+CD38+ and expressed an αβ TCR (Fig. 5). These cells also universally

FIGURE 2. Dynamics of changes in cerebrospinal fluid inflammatory and anti-inflammatory cytokines in adult TBM patients randomized to dexamethasone or placebo. The data depict changes over time in mean (±95% confidence interval) cerebrospinal fluid concentrations of IL-8 (A), IL-10 (B), IFN-γ (C), TNF (D), and IL-6 (E). The limit of detection for each cytokine is described in Materials and Methods.
expressed the chemokine receptor CCR5 but not CCR7 (data not shown). Cerebrospinal fluid CD3^+CD4^+ T cells were phenotypically distinct from peripheral blood CD3^+CD4^+ cells from the same patient, which were rarely CD45RA^+CD45RO^+ and expressed CD95^+ at lower frequencies (Fig. 5). In cerebrospinal fluid collected during the first 2 wk of therapy from 10 patients, CD3^+CD4^+CD45RA^+CD45RO^+ cells represented, on average, 77% (range, 51–91%) of the CD3^+CD4^+ T cell population compared with 8.7% (range, 3–16%) in the paired blood sample. Thus, the majority of CD4^+ lymphocytes in the cerebrospinal fluid paradoxically coexpressed surface molecules characteristic of naive (CD45RA^+) and activated memory (CCR5^+CCR7^-CD45RO^+) T cells. The majority of cerebrospinal fluid CD3^+CD8^+ T cells also expressed an activated phenotype (CD38^+CD95^+) (Fig. 5C). Dexamethasone did not influence the absolute number or phenotypes of these cells.

**Functional phenotype of cerebrospinal fluid T cells**

Because cerebrospinal fluid T cells are in close proximity to the site of disease, we attempted to define their functional phenotype and determine whether they were a relevant source of IFN-γ in the cerebrospinal fluid. To do this, we purified cerebrospinal fluid and peripheral blood CD3^+ T cells from six TBM patients in the first 2 wk of therapy (three patients on placebo and three patients on dexamethasone; median, 7 days). Ex vivo culture of cerebrospinal fluid T cells (105 per well) for 48 h with or without mitogen activation (PMA/ionomycin), failed to elicit production of detectable levels of IFN-γ in culture supernatants. In contrast, peripheral blood CD3^+ T cells (105 per well) secreted detectable quantities of IFN-γ (mean, 623 pg/ml; range, 230-1204) after mitogen stimulation. Similar results were obtained when flow cytometry or IFN-γ ELISPOT were used to assess IFN-γ production; only mitogen-activated peripheral blood CD3^+ T cells, but not paired cerebrospinal fluid CD3^+ T cells, yielded IFN-γ-producing cells (data not shown).

To determine whether mitogen incubation induced activation-induced cell death in cerebrospinal fluid T cells, the viability of cells was measured before and after a 4-h mitogen stimulation. In response to mitogen, the viability of cerebrospinal fluid CD3^+ T cells dropped 4-fold vs a modest drop in the viability of peripheral blood CD3^+ T cells (Fig. 6).

To examine whether the cell death-signaling receptor CD95 (FAS) participated in activation-induced cell death in cerebrospinal fluid T cells, the viability of cells was measured before and after a 4-h mitogen stimulation. In response to mitogen, the viability of cerebrospinal fluid CD3^+ T cells dropped 4-fold vs a modest drop in the viability of peripheral blood CD3^+ T cells (Fig. 6).

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To examine whether the cell death-signaling receptor CD95 (FAS) participated in activation-induced cell death, the anti-CD95 mAb ZB4, which inhibits CD95 signaling and apoptosis, was used in activation experiments. Cerebrospinal fluid CD3^+ T cells were isolated from six samples derived from five patients (three patients on placebo and two patients on dexamethasone) in the first 2 wk of therapy. CD3^+ T cells were cultured for 4 h with PMA/ionomycin in the presence and absence of anti-CD95 mAb ZB4 or an isotype control Ab. The mean reduction in viability of mitogen-activated

**FIGURE 3.** Dynamics of changes in cerebrospinal fluid chemokines in adult TBM patients randomized to dexamethasone or placebo. The data depict changes over time in mean (±95% confidence interval) cerebrospinal fluid concentrations of IP-10 (A), Mig (B), MCP-1 (C), and RANTES (D). The limit of detection for each chemokine is described in Materials and Methods.
FIGURE 4. Dynamics of changes in absolute counts of lymphocyte subsets in cerebrospinal fluid and peripheral blood of TBM patients randomized to dexamethasone or placebo. Shown are concentrations of CD3⁺CD4⁺ T cells, CD3⁺CD8⁺ T cells, CD16⁺CD56⁺ NK cells, and CD19⁺ B cells. Data for cerebrospinal fluid (A, C, E, and G) are shown for samples collected within the first 2 mo of therapy. Data for peripheral blood (B, D, F, and H) are shown for 9 mo of therapy. All data represent the mean (±95% confidence interval) of absolute counts at different time points.
FIGURE 5. Phenotype of cerebrospinal fluid and peripheral blood T cells in TBM patients. The phenotype of cerebrospinal fluid T cells were defined in 10 TBM cases (five from each study arm). Shown is representative flow cytometry data acquired from the cerebrospinal fluid and blood of a patient with TBM grade II 10 days after beginning therapy. In A, the percentage of CD3⁺CD4⁺ T cells in the cerebrospinal fluid and blood bearing either αβ or γδ T cell receptors or CD45RA/RO (B) is shown. In C and D, the percentage of CD3⁺CD8⁺ or CD3⁺CD4⁺ T cells, respectively, bearing CD38 or CD95 is shown. Isotype control Abs were used to establish gating parameters.
cerebrospinal fluid CD3⁺ T cells cultured with anti-CD95 was not significantly different (67 ± 16%) from T cells cocultured with an isotype control Ab (61 ± 19%). These data suggest activation-induced cell death of cerebrospinal fluid T cells occurred independently of CD95.

**Impact of dexamethasone on peripheral blood responses to M. tuberculosis Ags**

Whole-blood cultures from patients randomized to dexamethasone or placebo were used to measure ex vivo T cell and monocyte responses to mitogen and Ag stimulation. The advantage of this approach is that it measures cellular responsiveness in the presence of physiological levels of the study drug. Dexamethasone did not measurably influence the concentrations of monocyte-derived cytokines elicited by challenge of whole-blood cultures with a cell lysate of *M. tuberculosis* H37Rv (Table II). This suggests the capacity of monocytes to mount cytokine responses ex vivo to mycobacterial Ags was unimpaired in the presence of dexamethasone. Whole-blood assays were also used to detect T cell responses, measured via IFN-γ production, to rESAT-6, CFPs, and a WCL of *M. tuberculosis* (Fig. 7). The concentration of IFN-γ elicited by mitogen stimulation was suppressed early in dexamethasone-treated patients compared with placebo-treated patients (Fig. 7), although comparison of area under the curve between the two treatment arms was marginally not significant (p = 0.06). Dexamethasone did not significantly alter the T cell responses to ESAT-6, CFPs, or WCL (Fig. 7), although the number of samples examined was small.

**IFN-γ ELISPOT assays**

Responses in PBMCs were measured to PPD, rESAT-6, or overlapping peptides spanning ESAT-6 (Fig. 8). ELISPOT frequencies to all Ags were generally lowest during the acute stage of illness, particularly against PPD, but increased with time. Responses to PPD were strongest 6–10 wk after commencement of therapy but thereafter waned. Responses to rESAT-6 and to overlapping peptides spanning ESAT-6 varied, to a lesser extent, and remained detectable after 9 mo of therapy. The magnitude of the IFN-γ ELISPOT responses to these Ags was independent of treatment assignment, although the number of subjects studied was small.

**Discussion**

We have recently performed a randomized, controlled trial of adjunctive dexamethasone for the treatment of TBM in 545 Vietnamese adults that showed dexamethasone improved survival but failed to reduce severe disability after 9 mo of treatment (6). This study involved a representative subset of adults recruited to the trial and tested the long-standing assumption that the clinical benefits are consequent to the broad immunosuppressive actions of dexamethasone, particularly those acting on the meninges and the subarachnoid space. However, the pathogenesis of TBM is poorly understood, and the mechanism for the effect of dexamethasone on survival, in this and other infections of the CNS, notably pyogenic bacterial meninitis, remains unknown. The small numbers, the inability to analyze serial samples, and the limited availability of techniques capable of assessing the cellular and molecular immune response have limited previous studies. This study was allied to a large clinical trial and combined carefully recorded clinical data with a breadth of laboratory techniques investigating the intracerebral and extracerebral inflammatory response to *M. tuberculosis* that has never been reported previously. The results suggest that dexamethasone may deliver clinical effects via mechanisms other than generalized immunosuppression, and this has important implications for the rationale design of other adjuvant therapies.

Glucocorticoids (GCs), like dexamethasone, continue to be the major immunomodulatory agents used in clinical medicine today.
GCs act by binding to a specific but ubiquitous GC receptor that on activation translocates to the nucleus. GCs modulate cytokine expression by a combination of genomic mechanisms (reviewed in Refs. 8 and 9). Within the nucleus, the activated GC-receptor complex can 1) bind to and inactivate key proinflammatory transcription factors (e.g., AP-1, NF-κB), which takes place at the promoter-responsive elements of these factors but has also been reported without the presence of DNA; 2) via GC-responsive elements, upregulate the expression of cytokine inhibitory proteins (e.g., IκB, which inactivates the transcription factor NF-κB and thereby the secondary expression of a series of cytokines); and 3) reduce the half-life time of cytokine mRNAs. In studies with triggered human blood mononuclear cells in culture, GCs strongly diminished the production of the initial-phase cytokines IL-1β and TNF-α and the immunomodulatory cytokines IL-2, IL-3, IL-4, IL-5, IL-10, IL-12, and IFN-γ, as well as of IL-6 and IL-8 (10). Conversely, in vivo and in vitro studies have suggested GCs can also have enhancing effects on the immune response (11–14).

Surprisingly, this study found that dexamethasone did not alter the kinetics of a range of routine clinical markers of inflammation (CSF-blood glucose ratio, opening pressure, or leukocyte count) or dramatically attenuate cerebrospinal fluid cytokines or chemokines. As described previously in TBM (7, 15–18), the cerebrospinal fluid contained a rich cytokine milieu; concentrations of IFN-γ, IL-6, IL-10, and IL-8 were high and remained elevated for several months. Of note, there was a trend toward decreased cerebrospinal fluid IFN-γ concentrations in steroid-treated patients during the first week of therapy. There was also a trend toward diminished IFN-γ production when whole blood from dexamethasone-treated patients was stimulated with mitogen but not mycobacterial Ag. Collectively, these data might suggest a dexamethasone-mediated effect on IFN-γ production; additional studies will be needed to confirm this association.

Important inflammatory mediators like TNF, IL-12p70, and IL-1β were infrequently detected in acute TBM samples and dropped below detectable levels soon after the initiation of therapy. The relevance of these cytokines to host defense and disease pathogenesis during mycobacterial infection has been revealed repeatedly in animal models (19–21) but is less certain in human disease. In particular, TNF has been causally implicated in disease pathogenesis in a rabbit model of TBM (1). However, cerebrospinal fluid TNF concentrations in this study were not measurably affected by adjunctive dexamethasone. Similar observations were made by Donald et al. (16) in children with TBM and randomized

FIGURE 7. T cell responses to mycobacterial Ag or mitogen stimulation measured using whole-blood assays from adult TBM patients randomized to dexamethasone or placebo. The data depicts the mean concentration (±95% confidence interval) of IFN-γ in plasma supernatants elicited by a 48-h stimulation with a WCL of M. tuberculosis H37Rv (A), rESAT-6 (B), M. tuberculosis H37Rv CFPs (C), or PHA (D). The concentration of IFN-γ produced by PBS-stimulated wells was subtracted from Ag-stimulated wells.
to prednisone; CSF concentrations of TNF, IL-1β, and IFN-γ were unaffected by steroids. Moreover, efforts to suppress TNF expression in vivo using thalidomide have failed to improve outcome in TBM (22). Other inflammatory and vasodilatory mediators, such as prostaglandins and leukotrienes, may also be relevant to disease pathogenesis, but were beyond the scope of this study.

Dexamethasone has well-documented effects on monocyte and T cell function in vitro. We therefore expected dexamethasone would modulate the ex vivo response of monocytes and T cells to antigenic stimulation. However, mycobacterial Ag-triggered whole-blood monocyte and T cell assays, plus IFN-γ ELISPOT assays, revealed no significant differences in responses between patients in the two treatment arms. These data imply that steroids, in this clinical setting and with the number of samples available for analysis, do not dramatically attenuate monocyte responses to Ag stimulation or Ag-specific, T cell-derived IFN-γ responses in vivo.

The phenotypes of cerebrospinal fluid leukocytes in patients with TBM have not been reported previously, and their role in pathogenesis is unknown. CD3⁺CD4⁺ T cells were the dominant lymphocyte subset in the cerebrospinal fluid and they coexpressed CD45RA⁺ and CD45RO⁺, a phenotype identical with alveolar CD4⁺ lymphocytes recovered from the lungs of pulmonary tuberculosis patients (23). In healthy individuals, the peripheral blood contains small numbers of these CD45RA⁺CD45RO⁺ helper T cells that primarily produce IFN-γ and are in the cell cycle (24, 25). Cerebrospinal fluid T cells also expressed the Th1 cell-associated chemokine receptor CCR5, but not CCR7, a marker of naive T cells (26). Ligands for CCR5 include RANTES and MCP-1, both of which were present in acute cerebrospinal fluid from TBM patients. Because a low cerebrospinal fluid white cell count at presentation is independently associated with poor outcome in TBM (6), the recruitment of lymphocytes, such as those described here, to the CNS appears to have a beneficial effect on disease outcome. However, there was no evidence that dexamethasone influenced the phenotype or kinetics of lymphocytes within the cerebrospinal fluid or blood, although we appreciate that the number of samples measured in this part of the study may have been inadequate to detect significant differences between treatment arms.

How many of the cerebrospinal fluid T cells were specific for mycobacterial Ags was difficult to define, because these cells underwent rapid activation-induced cell death ex vivo by a mechanism unrelated to their expression of FAS (CD95). These observations may preclude the application of in vitro mycobacterial-specific diagnostic tests on cerebrospinal fluid that rely on functional T cell responses (e.g., IFN-γ production by Ag-stimulated T cells in ELISPOT assays) (27).

Dexamethasone did not detectably modulate parameters that have been independently associated with death in TBM, such as...
the cerebrospinal fluid-blood glucose ratio and white cell count (6). However, dexamethasone significantly reduced cerebrospinal fluid total protein concentrations in the first week of therapy, consistent with previous studies in children (5). This effect on cerebrospinal fluid total protein concentration is unlikely to be due to a reduction in the leakage of serum proteins into the cerebrospinal fluid however, because the cerebrospinal fluid-blood albumin index was not significantly altered by dexamethasone. Future studies in TBM patients should aim to define the protein content of cerebrospinal fluid, the factors that influence its production and reabsorption, and their relationship to osmotic pressure.

It remains possible that dexamethasone provides clinical benefit in TBM through mechanisms unrelated to inflammation in the CNS. For example, severe adverse drug reactions (e.g., hepatitis) that result in changes or discontinuation of antituberculosis therapy are an independent risk factor for death in TBM patients (6). Because patients randomized to dexamethasone have significantly fewer severe adverse events during treatment (6), it remains possible that dexamethasone exerts some of its benefits simply by facilitating continuous antituberculosis therapy. This study characterized molecular and cellular aspects of TBM pathogenesis and suggests adjunctive dexamethasone does not improve survival from TBM by dramatically attenuating prominent markers of the immune response in the subarachnoid space or peripheral blood. Other immunological and pathophysiological mechanisms may be important; their identification would facilitate rationale drug selection for a disease that still causes significant mortality and morbidity.

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Disclosures
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References