Sequestration of T Lymphocytes to Body Fluids in Tuberculosis: Reversal of Anergy following Chemotherapy

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The specificity of CD4 T lymphocytes was investigated in 6 patients affected by tuberculosis who had negative tuberculin purified protein derivative (PPD) skin tests at diagnosis. Polyclonal CD4 T cell lines from the peripheral blood failed to proliferate to PPD and to the 16- or 38-kDa proteins of Mycobacterium tuberculosis, while CD4 cell lines from the disease site responded to PPD and to the 16- and 38-kDa proteins and derived epitopes in vitro. Four months after chemotherapy, the patients became responsive to PPD. The proliferative response to PPD and to the 16- or 38-kDa proteins and their derived peptides decreased in CD4 T cell lines from the disease site and increased in lines from the peripheral blood. These results indicate that CD4 T cells recognizing a vast array of M. tuberculosis epitopes are compartmentalized at the site of disease in anergic patients but appear in peripheral blood after chemotherapy.

Depressed cell-mediated responses (anergy) often occur in patients affected by tuberculosis (TB). These include depressed lymphocyte proliferation to tuberculin purified protein derivative (PPD) in vitro and antigen-induced production of interferon-γ [2, 3], which is associated with negative skin test reactivity to PPD in vivo [1] and in vitro. The cause of this phenomenon is probably multifactorial, with production of suppressive cytokines, such as interleukin (IL)-10 [4] or transforming growth factor (TGF)-β [5], defective antigen presentation in acute disease [6], and compartmentalization of CD4 T lymphocytes at the site of disease [4, 7, 8] all contributing.

To investigate whether sequestration of Mycobacterium tuberculosis (MTB)—specific T lymphocytes may account for the anergy observed in TB, we compared the responses of CD4 T lymphocytes from peripheral blood and from inflammatory sites of patients with TB anergy with defined epitopes from the MTB 16- and 38-kDa antigens.

Materials and Methods

Patients. We studied 6 patients in all. We obtained peripheral blood and pleural fluid samples from 2 patients with newly diagnosed TB pleuritis; both had unilateral exudative pleural effusions. In addition, we obtained peripheral blood and ascites fluid samples from 1 patient with newly diagnosed TB ascites, peripheral blood and pericardial fluid samples from 1 patient affected by TB pericarditis, and peripheral blood and cerebrospinal fluid (CSF) samples from 2 patients with TB meningitis. A clinical diagnosis of TB was established by clinical symptoms and by chest computed tomography. MTB was detected by culture and by polymerase chain reaction in both CSF samples, in 1 pleural fluid sample, and in the ascites fluid sample. All 6 patients had negative PPD skin tests. Tuberculin PPD skin tests were considered positive when the skin induration diameter was ≥10 mm at 48–72 h after injection of 1 U of PPD (Statens Seruminstitut, Copenhagen). None of the patients had evidence of human immunodeficiency virus infection or was being treated with steroids or antitubercular drugs at the time of first sampling.

Antigens. PPD was from Statens Seruminstitut and phytohaemagglutinin (PHA; Leucoagglutinin) from Sigma (St. Louis). The recombinant 16- and 38-kDa proteins were overproduced in Escherichia coli and purified as described [9, 10]. Fourteen overlapping 20-mer peptides of the 16-kDa protein and 10 nonoverlapping 20-mer peptides of the 38-kDa protein were synthesized and purified as described elsewhere [11, 12]. For the location and sequence of the 16- and 38-kDa peptides used in this study, see [11, 12].

Preparation of polyclonal CD4 T cell lines. CD4 polyclonal T cell lines were prepared according to the method of Scotet et al. [13]. In brief, CD4 T lymphocytes were isolated from peripheral
blood mononuclear cells (PBMC) or body fluid cells by immunomagnetic sorting. Cells were cultured at $10^6$/well with $2 \times 10^4$/well irradiated PBMC (3000 rads from a cesium source), $5 \times 10^4$/well irradiated lymphoblastoid cell line (BCL), PHA (0.5 $\mu$g/mL, final concentration), and human recombinant IL-2 (200 U/mL, final concentration). After 14–21 days of culture, the cells were collected, pooled, transferred in tissue culture flasks, and further expanded in the presence of complete medium containing IL-2 (200 U/mL, final concentration). The cell lines in these studies, 92%–98% CD4 as detected by flow cytometry, were used $>3$ days after the last IL-2 addition and $>3$ weeks after initial PHA stimulation.

T lymphocyte proliferation assay. Polyclonal CD4 T cell lines were cultured in triplicate at $5 \times 10^4$/well with $2.5 \times 10^4$/well irradiated autologous BCL and antigens at the stated final concentrations: PPD (10 $\mu$g/mL), PHA (0.5 $\mu$g/mL), 16- and 38-kDa proteins (10 $\mu$g/mL), and synthetic peptides (50 $\mu$g/mL). Five days later, we added 37-kBq per well $[^{3}H]$thymidine (Amersham, Amersham, UK), and the cultures were harvested 18 h later. Results are expressed as stimulation index (SI) mean counts per minute (cpm) in the presence of antigen divided by mean cpm in the absence of antigen. Responses were considered positive if the SI was $>2.5$. SDs did not exceed 15% of the mean cpm values.

Results

Recognition of MTB proteins by CD4 T cells from disease sites but not from peripheral blood: reversal after therapy. We investigated the specificity of CD4 T lymphocytes from the peripheral blood and from the sites of disease in the 6 patients affected by different clinical forms of TB who had negative PPD skin tests at diagnosis. CD4 T cell lines derived at diagnosis from the peripheral blood failed to proliferate in vitro to PPD and to 16- and 38-kDa MTB proteins (figure 1). PHA responses were normal in TB patients (data not shown). However, CD4 T cell lines prepared from the site of disease showed a strong proliferative response to PPD and to 16- and 38-kDa proteins in each patient.

Four months after therapy, the patients were responsive to PPD in vivo. Antigen-specific proliferative responses by CD4 T cell lines taken from the site of disease were consistently reduced after therapy, and CD4 T cells from 2 patients did not proliferate in response to any of the tested antigens (figure 1). In contrast, 4 months after therapy, CD4 T cell lines from the

![Figure 1](https://academic.oup.com/jid/article-abstract/180/1/225/990915)
peripheral blood of the same patients proliferated in response both to PPD and to the 16- and 38-kDa proteins.

**Epitope specificity of CD4 T lymphocytes from disease sites and peripheral blood before and after therapy.** Polyclonal CD4 T cell lines taken from the site of disease at the time of diagnosis recognized a vast array of epitopes from the 16- and 38-kDa proteins (figure 2). At least 4/14 peptides from the 16-kDa protein and 3/10 peptides from the 38-kDa protein were recognized by every patient. Some of the antigenic peptides were frequently recognized by CD4 T cell lines from the site of disease, regardless of the clinical form of TB. None of the 24 peptides used induced proliferation in CD4 T cell lines obtained from the peripheral blood of the same patients.

We compared the peptide specificity of CD4 T cell lines from peripheral blood and from the site of disease 4 months after therapy. Figure 2, upper panel, shows that the proliferative response of CD4 T cells from body fluids to 16- and 38-kDa peptides consistently decreased. In contrast (figure 2, lower panels), CD4 T cells from the peripheral blood of the patients responded to all of the peptides tested, although no peptide was recognized by all patients.

**Discussion**

Understanding the fine specificity of the human immune response to TB antigens assumes a particular importance in defining the mechanisms responsible for protection and pathogenesis. One major obstacle to this approach is given by TB patients who are PPD-negative in vivo and whose PBMC show strongly depressed or absent responses to PPD in vitro. The aim of this study was to assess the fine specificity for TB epitopes between CD4 T cells from peripheral blood and from disease sites and to ascertain whether TB-specific CD4 T lymphocytes are compartmentalized at the site of disease in anergic TB patients.

Six TB patients showed anergy at the time of diagnosis to

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**Figure 2.** Proliferative response to selected synthetic peptides before and after chemotherapy. CD4 T cell lines were prepared from peripheral blood mononuclear cells and body fluids before (0) and 4 months after chemotherapy (4) and were stimulated in vitro with indicated synthetic peptides derived from 16- and 38-kDa proteins. Lines derived from each of 6 patients are indicated by different symbols. Results are expressed as stimulation index (SI).
PPD and to the 16- and 38-kDa MTB proteins. In contrast, CD4 T cells from the site of disease consistently responded to PPD and to 16- and 38-kDa proteins in vitro, thus indicating compartmentalization of MTB-specific T cells in TB anergic patients. The repertoire of CD4 T cells at the site of disease was widely heterogeneous. Irrespective of the clinical form of TB and the anatomic location of the disease, CD4 T cell lines from the site of disease recognized a significant number of epitopes from the 16- and 38-kDa proteins. Although previous studies reported limited clonal heterogeneity of T cells localizing at the inflammatory site (pleural fluid) in anergic TB patients [8, 14], our results clearly indicate that the repertoire of CD4 T cells accumulating at the site of disease in anergic TB patients is largely heterogeneous.

Four months after chemotherapy, TB patients became PPD responsive in the periphery, and polyclonal CD4 T cell lines were generated from the peripheral blood and the site of disease. The proliferative response to PPD and to 16- and 38-kDa proteins after therapy was lower in CD4 T cell lines from the site of disease than in CD4 T cell lines derived from the peripheral blood. In contrast, CD4 T cells from the peripheral blood of a variable percentage of patients responded to the tested peptides. From these studies, we conclude that CD4 T lymphocytes with the same epitope fine specificity are sequestered to the site of the disease in patients with active TB but appear in the peripheral blood after therapy.

These results are in good agreement with other studies showing reversal of anergy during drug treatment [4, 5, 8]. Moreover, these findings may provide an explanation for skin test anergy in TB and its reversal during treatment. Presumably the decline in bacterial load and inflammation during treatment diminishes in situ expansion of TB-specific CD4 T cells and restores T cell response in the peripheral blood.

Although our results clearly demonstrate sequestration of antigen-specific CD4 T lymphocytes at the site of disease in anergic TB patients, they do not exclude the role of other mechanisms, such as defective antigen presentation [6] and the production of suppressive cytokines such as TGF-β [5] and IL-10 [4]. Compartmentalization of the immune response in humans has been documented in granulomatous diseases, such as sarcoidosis and leprosy, and the study of the cells derived from disease sites has provided insight into the immunopathogenesis of these disorders. The data presented here provide information about the range of antigenic epitopes recognized by CD4 T cells derived from the site of TB disease. Further characterization of the functional attributes of these cells will yield important information about the immunology of TB.

References