To the Editor—Extrapulmonary tuberculosis (EP-TB) remains an important diagnostic and therapeutic problem. In active pulmonary TB (P-TB), clinical symptoms confirmed by a laboratory test give a relatively clear result, whereas diagnosis can be rather problematic in patients with EP-TB, children, elderly, and immunocompromised individuals. Radiographic analysis in EP-TB often is not conclusive, and the tuberculin purified protein derivative (PPD) skin test is considered by many clinicians to be unreliable. Bacteria in EP-TB cases can be present in low numbers at inaccessible sites. Therefore, invasive procedures are usually necessary to confirm the infection. Furthermore, the human immunodeficiency virus (HIV) epidemic has changed the proportion of EP-TB among TB cases, increasing the numbers of new EP-TB cases to >15% of total TB cases [1]. Thus, new early and rapid diagnostic procedures are important for TB control.

It has been shown recently that peripheral blood T lymphocytes from patients with active TB, but not unvaccinated or bacille Calmette-Guérin (BCG)-vaccinated healthy donors, produce high levels of the cytokine interferon (IFN)-γ in vitro, in response to the 2 closely related Mycobacterium tuberculosis complex-specific antigens, early secretory antigen target (ESAT-6) and culture filtrate protein-10 (CFP-10) [2–5]. Both are located in the RDI region that is lacking in BCG and in most atypical mycobacteria. Hence, these antigens are considered to be potential candidates for immunodiagnosis of TB. Recent studies have confirmed that the specificity of diagnoses of P-TB made on the basis of ESAT-6 and CFP-10 antigens is significantly higher than the specificity of those made on the basis of PPD, whereas the sensitivity of both antigens was comparable with that of PPD [2–5]. In the present study, we compared the diagnostic performance of ESAT-6 and CFP-10 antigens in HIV-negative EP-TB and P-TB patients.

Heparinized peripheral blood was obtained from 59 healthy donors (HDs), 26 of whom were unvaccinated and 36 of whom were BCG vaccinated, and from 43 untreated and culture-confirmed TB patients. TB localizations were as follows: minimal pulmonary (n = 21), lymphatic (n = 12), peritoneal (n = 4), urogenital (n = 2), skeletal (n = 3); and pleural/lymphatic/peritoneal (n = 1). Peripheral blood mononuclear cells (PBMC) were enriched by density gradient centrifugation and were stimulated (at 0.75 × 10^6 or 2.5 × 10^6/mL) in vitro with 5 μg/mL PPD or recombinant ESAT-6 or CFP-10 (all from Statens Serum Institute [3]) for 5 or 6 days. Afterwards, IFN-γ released in cell-culture supernatant was analyzed by ELISA, using commercially available reagents (PharMingen; assay sensitivity, 10 pg/mL). Values in unstimulated wells were subtracted from the value in antigen-stimulated cultures. The diagnostic performance of mycobacterial antigens was analyzed by receiver operating characteristic curves, which were based on the maximal individual responses of TB patients and HDs to ESAT-6 or CFP-10, and the cutoff value of 300 pg/mL (ESAT-6-CFP-10) or 2700 pg/mL (PPD) of IFN-γ was defined as giving the highest level of accuracy [5].

Figure 1 shows that PPD stimulation of PBMC from BCG-vaccinated HDs or TB patients (EP-TB and P-TB) induced production of IFN-γ in a large number of the individuals tested. Of importance, most patients in both TB groups responded to stimulation with ESAT-6 or CFP-10 (18 of 22 EP-TB patients and 16 of 21 P-TB patients), whereas few HDs responded (2 of 59 HDs; P < .01, Mann-Whitney U test; figure 1). The responses to ESAT-6 and CFP-10 were similar in subjects with different manifestation of EP-TB. Furthermore, PBMC from 13 of 17 EP-TB patients reacted to ESAT-6 or CFP-10 (or both) in the same
Figure 1. Interferon (IFN)-\(\gamma\) production by peripheral blood mononuclear cells from 26 unvaccinated healthy donors (BCG\(-\)), 33 bacille Calmette-Guérin (BCG)-vaccinated healthy donors (BCG\(+\)), 22 patients with extrapulmonary tuberculosis (EP-TB), and 21 patients with minimal pulmonary tuberculosis (P-TB). PBMC were stimulated with 5 \(\mu\)g/mL of tuberculin purified protein derivative (PPD; [ ] ) or early secretory antigen target (ESAT-6) or culture filtrate protein-10 (CFP-10; E6/C10; [ ]) for 5 days before testing. IFN-\(\gamma\) released in culture supernatants was analyzed by ELISA. Results are expressed as maximal individual responses of IFN-\(\gamma\) titers; dashed lines at 300 pg/mL (for ESAT-6/CFP-10) and 2700 pg/mL (for PPD) show the cutoff value used to separate positive from negative responders to each antigen.

experiment. In contrast, 2 of 17 EP-TB patients (skeletal and lymphatic forms) with advanced disease and high numbers of mycobacteria in their tissues (detected in biopsies) did not respond to ESAT-6 or CFP-10. At a cutoff value of 300 pg/mL IFN-\(\gamma\), ESAT-6 and CFP-10 had a sensitivity of 76% in EP-TB patients and 77% in P-TB patients. Sensitivity levels based on PPD at a cut-off value of 2700 pg/mL were 64% in EP-TB and 86% in P-TB patients. However, specificity levels for ESAT-6 and CFP-10 (94%) in BCG-vaccinated HDs were higher than those for PPD (55%) in the same group of donors.

Our data show that a TB immunodiagnostic blood test containing ESAT-6 and CFP-10 holds a strong potential for the diagnosis of active EP-TB and P-TB. Furthermore, although ESAT-6 is a highly sensitive reagent on its own, the combination of both antigens improved the sensitivity of a blood test without impairing specificity for the diagnosis of EP-TB.

Martin E. Munk, Sandra M. Arend, Inger Brock, Tom H. M. Ottenhoff, and Peter Andersen

References


Worldwide sources for the ESAT-6 and CFP-10 antigens can be obtained by contacting the corresponding author.

Reprints or correspondence: Dr. Martin E. Munk, Dept. of Tuberculosis Immunology, Statens Serum Institute, Copenhagen, Denmark; Departments of Infectious Diseases and Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands