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Immune Responses to the *Mycobacterium tuberculosis*-Specific Antigen ESAT-6 Signal Subclinical Infection among Contacts of Tuberculosis Patients

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Diagnosis of latent *Mycobacterium tuberculosis* infection is considered essential for tuberculosis control but is hampered by the lack of specific reagents. We report that strong recognition of tuberculosis complex-specific antigen ESAT-6 by healthy household contacts of tuberculosis patients correlates with the subsequent development of active tuberculosis during a 2-year follow-up period.

In the next year tuberculosis (TB) is expected to be responsible for more than 2 million deaths and up to 8 million new infections (11). Latently or subclinically infected individuals continually arise as new foci of infection, but the identification of infected individuals for treatment is extremely difficult, as it is based on intradermal injection of purified protein derivative (PPD). Unfortunately, PPD contains many antigens widely shared among mycobacteria, and the specificity of this reagent is therefore low. Several studies have demonstrated that PPD cannot reliably distinguish between previous *Mycobacterium bovis* BCG vaccination, exposure to environmental mycobacteria, or infection with *M. tuberculosis* (4, 6, 7). That the PPD skin test remains in use despite these limitations reveals the urgent need for better diagnostic tests for TB.

Recently, genes which are deleted from BCG have been identified (2), and some of these also appear to be absent from most environmental mycobacteria. One such antigen, ESAT-6, has shown promising results for use as an immunodiagnostic reagent (1). Recent studies with humans have found that a test based on the detection of ESAT-6-specific T cells via their production of gamma interferon (IFN-γ) ex vivo specifically discriminates between *M. tuberculosis* infection and exposure to other mycobacteria (8, 9, 12).

In the present study, healthy household contacts of sputum-positive TB patients were recruited from Hossana Regional Hospital, Hossanna, Ethiopia. The presence of active TB in all contacts at the time of entry into the study was excluded by radiological and clinical examinations and by sputum microscopy and culture, as described previously (5). Blood samples were obtained from all contacts at the time of entry into the study and were analyzed for in vitro responses to PPD and ESAT-6. The contacts were assessed again approximately 2 years after the first visit. On the second visit, the contacts received the same clinical and laboratory examinations for TB as they had previously.

At follow-up, 12 of the 24 contacts (50%) were classified as "remaining healthy." They had no symptoms of TB and no lesions or other suspicious findings visible on X ray. Five of these contacts were sputum negative by microscopy and culture, while the remaining seven were unable to produce sputum.

Of the remaining 12 contacts, 7 (29%) were found to have developed active TB on the basis of the presence of characteristic symptoms and findings on X rays (extensive infiltration of the lungs, calcification or lesions). For two of the contacts, the diagnosis of TB was confirmed by culture and/or microscopy, for one contact the results of both microscopy and culture were negative, and for two of the contacts the microscopy results were negative but sputum was not sent for culture. Two more contacts were classified as having pulmonary TB on the basis of radiological analysis, nonresponsiveness to 2 weeks of antibiotic therapy, and the presiding clinician's decision to treat. Finally, 5 of 24 (21%) individuals were classified as having nontubercular disease, as their chest X rays were normal and a diagnosis of TB could not be supported by microbiological findings, but they nonetheless had some symptoms (for example, persistent cough) and thus could not be defined as fully healthy.

Although the prevalence of human immunodeficiency virus (HIV) infection among the study population was low (less than 5%), all participants received confidential screening for HIV. Samples from HIV-positive individuals were excluded from the study.

In vitro restimulation of peripheral blood mononuclear cells (PBMCs) was carried out as described previously (5, 12). The supernatants were harvested on day 5 after stimulation, and the IFN-γ levels in duplicate culture supernatants were assayed with a commercial double-sandwich enzyme-linked immunosorbent assay kit, in accordance with the manufacturer's instructions (Mabtech, AB, Nacka, Sweden). The difference between the duplicate wells was consistently less than 10% of the mean.

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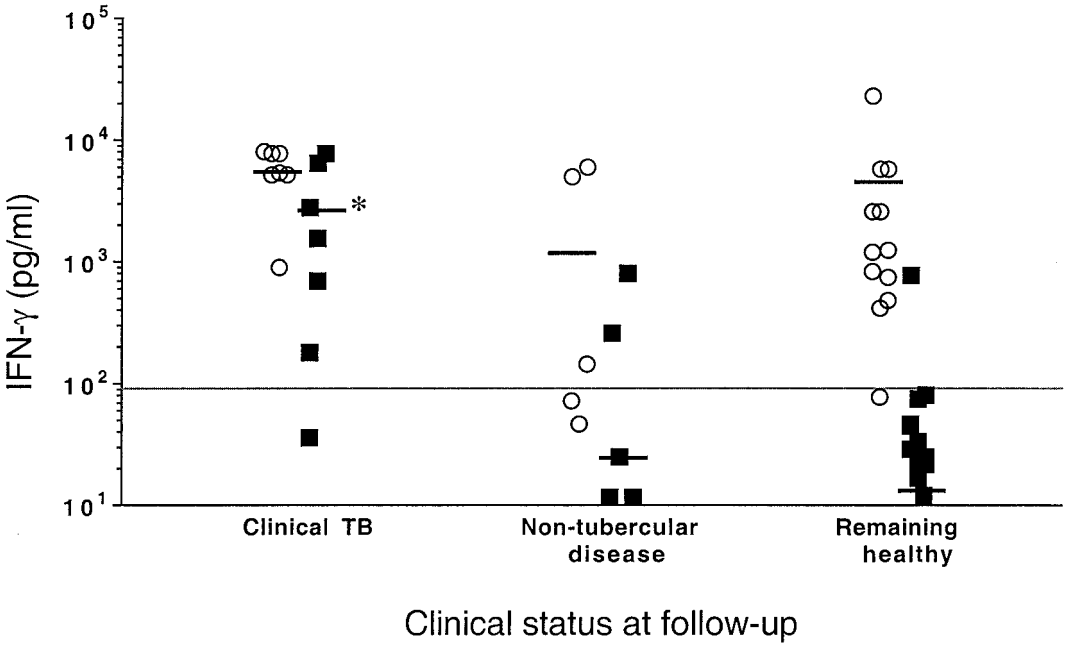

FIG. 1. In vitro IFN-γ responses of PBMCs after restimulation with PPD (○) or ESAT-6 (■) at the time of entry into the study. The results are for individual contacts segregated by their clinical status at the end of the follow-up period (2 years). The cutoff point for positivity in the assay is indicated by the solid line. Median in vitro IFN-γ responses are indicated by the heavy horizontal bar. Results significantly different from those for the contacts who remained healthy are indicated (*, $P < 0.001$).

Figure 1 shows the individual IFN-γ responses to PPD and ESAT-6 among the contacts. It can be seen that on entry into the study, the majority of contacts had significant responses to PPD, regardless of their later clinical course. Moreover, the magnitudes of these responses were not significantly different across the clinical spectrum. In contrast, responses to ESAT-6 were found mainly in the group of contacts who later developed TB, and the median response (1,553 pg/ml) was significantly higher ($P < 0.001$) than that for either the contacts who developed nontubercular disease (25 pg/ml) or those who remained healthy (18 pg/ml) (Fig. 1).

PBMCs from all seven contacts who developed TB during the follow-up period responded to PPD at the time of entry into the study (100%), as did those from the remaining 14 of the 17 (83%) contacts. Clearly, reactivity to PPD among the contacts of TB patients does not correlate with their later clinical course. In contrast, it was found that 6 of 7 (86%) of the contacts who later developed TB responded strongly to ESAT-6 at the time of entry into the study, whereas only 3 of 17 (18%) of the contacts who did not develop TB responded to ESAT-6. Interestingly, two of these three ESAT-6-responsive contacts were found among those who developed symptoms but for whom a diagnosis of TB could not be otherwise confirmed. Thus, unlike responsiveness to PPD, responses to ESAT were largely restricted to contacts who developed TB ($r < 0.0001$).

The results presented here demonstrate a strong association between in vitro responsiveness to ESAT-6 and later progression to TB among healthy contacts of TB patients. In contrast, almost all contacts whose PBMCs did not respond to this antigen, even though they were equally exposed to infection, remained healthy throughout the observation period. We conclude from this finding that immune recognition of ESAT-6 is suggestive of subclinical infection.

This conclusion is further supported by the recent observation from a study with a substantially larger cohort that healthy, unvaccinated donors in a setting of nonendemicity (in the United States) who converted to PPD skin test positivity also became responsive to ESAT-6, while those who remained PPD skin test negative did not. No data on the later occurrence of disease are available from that study since skin test converters were assumed to be infected and received prophylactic chemotherapy (13). Thus, the data suggest clinical utility for the detection of early responses to ESAT-6, and a comprehensive multicenter longitudinal study large enough to provide precise estimates of the predictive value of the test for ESAT-6 responsiveness has recently been launched. Additional *M. tuberculosis* complex-specific antigens have been identified (3). The available data suggest that the patterns of recognition of a cocktail of antigens may further improve the potential for the diagnosis of TB (10, 14). If subsequent studies confirm these findings, such antigens could be valuable new tools for the detection of TB.

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