An In-House RD1-Based Enzyme-Linked Immunospot–Gamma Interferon Assay Instead of the Tuberculin Skin Test for Diagnosis of Latent Mycobacterium tuberculosis Infection

Luigi Codecasa,1 Paola Mantegani,2 Laura Galli,2 Adriano Lazzarin,2,3 Paolo Scarpellini,2 and Claudio Fortis2*

Villa Marelli Institute, Lombardy Regional Reference Centre for Tuberculosis, Niguarda Hospital, Milan, Italy1; Clinic of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy2; and “Vita-Salute” San Raffaele University, Milan, Italy3

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Identification of individuals infected with Mycobacterium tuberculosis is essential for the control of tuberculosis (TB). The specificity of the currently used tuberculin skin test (TST) is poor because of the broad antigenic cross-reactivity of purified protein derivative (PPD) with BCG vaccine strains and environmental mycobacteria. Both ESAT-6 and CFP-10, two secretory proteins that are highly specific for M. tuberculosis complex, elicit strong T-cell responses in subjects with TB. Using an enzyme-linked immunospot (ELISPOT)–IFN-γ assay and a restricted pool of peptides derived from ESAT-6 and CFP-10, we have previously demonstrated a high degree of specificity and sensitivity of the test for the diagnosis of TB. Here, 119 contacts of individuals with contagious TB who underwent TST and the ELISPOT–IFN-γ assay were consecutively recruited. We compared the efficacy of the two tests in detecting latent TB infection and defined a more appropriate TST cutoff point. There was little agreement between the tests (k = 0.33, P < 0.0001): 53% of the contacts with a positive TST were ELISPOT negative, and 7% with a negative TST were ELISPOT positive. Furthermore, respectively 76 and 59% of the ELISPOT-negative contacts responded in vitro to BCG and PPD, suggesting that most of them were BCG vaccinated or infected with nontuberculous mycobacteria. The number of spot-forming cells significantly correlated with TST induration (P < 0.0001). Our in-house ELISPOT assay based on a restricted pool of highly selected peptides is more accurate than TST for identifying individuals with latent TB infection and could improve chemoprophylaxis for the control of TB.

One-third of the world’s population is believed to be latently infected with Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB), whose global resurgence, supported by the human immunodeficiency virus type 1 (HIV-1) pandemic and emerging multidrug resistance, underlines the need for new control measures and strategies to make a specific diagnosis and prevent transmission (19). Moreover, continued vigilance against active and latent TB is essential in industrialized countries because of the increased immigration from areas with endemic infection. The compulsory screening of immigrant populations for TB has been proposed as a means of controlling this communicable disease (14, 15) but, although the tuberculin skin test (TST) is the method of choice for detecting latent M. tuberculosis infection (LTBI), it cannot be considered a gold standard because of the number of false-positive and negative reactions, and the variability of their interpretation (1, 2). In particular, the purified protein derivative (PPD) antigen used for the test has broad cross-reactivity with antigens derived from various mycobacterial species, including the vaccine strains of Bacillus Calmette-Guérin (BCG) (1, 2), and false-negative results occur in patients with advanced TB or severe immunodeficiency (10, 25). Another operational drawback of TST is the need for a return visit to allow a reading of the results, and it is worth noting that the BCG vaccination of children commonly practiced in most parts of the world reduces its specificity.

New in vitro tests based on an ability to detect the gamma interferon (IFN-γ) released by activated T lymphocytes have recently been proposed (35). These assays use antigens specific for M. tuberculosis, such as early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are two low-molecular-mass secretory proteins encoded by genes located within region of difference 1 (RD1) of the M. tuberculosis genome (7, 41). This region is absent in all of the vaccine strains of BCG and in nontuberculous mycobacteria (NTM) with the exception of M. kansasiis, M. marinum, and M. szulgai (33). These proteins and the synthetic overlapping peptides corresponding to the full length of each elicit a strong T-cell response in animal models of TB (9, 43) and human with active TB infection (4, 5, 36, 38, 42, 44) or LTBI (11, 18, 29, 30, 37, 39).

The QuantIFERON-TB assay (Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT TB assay (Oxford Immunotec, Oxford, United Kingdom) are two commercial IFN-γ assays, and a number of in-house assays have also been assessed (3, 17, 31, 35). Although the sensitivity and specificity of these assays varies across studies, a recent systemic review of the literature (35) has shown (i) that assays based on RD1 antigens are more specific than TST and PPD-based assays, (ii) that cocktails of RD1 antigens offer the best combination of specificity and sensitivity, and (ii) that RD1-based assays detect ca. 80% of individuals with suspected LTBI regardless of their previous BCG vaccination status or infection with NTM.
TABLE 1. Demographic characteristics of close contacts enrolled in the study\(^a\)

<table>
<thead>
<tr>
<th>Region of origin</th>
<th>Country (n)</th>
<th>No. of subjects</th>
<th>Median age (yr)</th>
<th>Range (yr)</th>
<th>No. of subjects aged ≤15 yr</th>
<th>Male/female ratio</th>
<th>No. of subjects BCG vaccinated</th>
<th>No. of subjects with TST ≥5 mm</th>
<th>No. of subjects receiving chemoprophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>North-West Africa</td>
<td>Ethiopia (3)</td>
<td>9</td>
<td>35.5</td>
<td>8–69</td>
<td>1</td>
<td>7/2</td>
<td>1</td>
<td>6</td>
<td>6</td>
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<td></td>
<td>Morocco (2)</td>
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<td>Senegal (2)</td>
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<td>Gambia (1)</td>
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<td>Asia</td>
<td>Philippines (18)</td>
<td>28</td>
<td>31.0</td>
<td>1–51</td>
<td>7</td>
<td>12/16</td>
<td>12</td>
<td>11</td>
<td>10</td>
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<tr>
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<td>33</td>
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<td>1–73</td>
<td>4</td>
<td>19/14</td>
<td>7</td>
<td>23</td>
<td>23</td>
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<td></td>
<td>Spain (1)</td>
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<tr>
<td>Total</td>
<td>119</td>
<td>29.5</td>
<td>1–73</td>
<td>25</td>
<td>60/59</td>
<td>67</td>
<td>77</td>
<td>62</td>
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</table>

\(^a\) Values represent totals or means for each region, except as noted. n, number of subjects by country.

We have recently validated an in-house enzyme-linked immunospot assay (ELISpot)-IFN-\(\gamma\) assay based on a restricted pool of highly selected synthetic peptides derived from ESAT-6 and CFP-10 proteins in a group of HIV-positive or HIV-negative patients with active TB (40). The test was highly specific (87%) and sensitive (93%) and made it possible to distinguish individuals infected with *M. tuberculosis* from those vaccinated with BCG. We have also compared it with the commercial T-SPOT TB assay that is currently approved for use in Europe and awaiting U.S. Food and Drug Administration approval (27). Eighty-six individuals at high risk for developing TB infection were consecutively recruited from October 2004 to January 2005 and tested under double-blind conditions in separate laboratories. The concordance between the two tests was 98.9% and the fact that only one subject resulted in-house ELISpot-positive (borderline) but T-SPOT TB negative (P. Mantegani, unpublished data) confirmed the performance of our assay.

People who have spent time in close contact with individuals with infectious TB are at the highest risk of infection and TB progression and represent a high priority for testing. For this reason, we recruited a group of household contacts who underwent TST and our in-house ELISpot assay in the context of a surveillance program.

The aims of the study were (i) to assess whether the ELISpot assay could be used to identify individuals with LTBI and to compare the in vivo (TST) and in vitro (ELISpot assay) responses to PPD, (ii) to verify whether ELISpot could help define a TST induration cutoff point that better correlates with a diagnosis of LTBI, and (iii) to verify whether positive responses to the ELISpot assay correspond to the decision to treat.

MATERIALS AND METHODS

Participants. Between November 2003 and March 2004, 119 household contacts of contagious (sputum-smear-positive) TB patients were consecutively recruited by the Villa Marelli Institute (the Lombardy Regional Reference Centre for Tuberculosis, Milan, Italy) in the context of a surveillance program, and underwent Mantoux TST and the ELISpot–IFN-\(\gamma\) assay (12, 40). Their origins and characteristics (age, gender, number of subjects with a TST-positive response or BCG-vaccinated, and number of subjects receiving chemotherapy) are shown in Table 1.

The standard TST was performed using five tuberculin units (0.1 ml) of PPD administered intradermally in the volar surface of the forearm by an experienced person, and the size of the induration was evaluated after 48 to 72 h. An X-ray image was obtained for each subject with an induration of ≥5 mm (a positive reaction), and subjects were considered for latent TB preventive treatment according to the Lombardy regional guidelines adapted from the Centers for Disease Control and Prevention guidelines (Fig. 1) (1, 26). The subjects’ case histories and previous BCG vaccinations, proved by BCG scar or record, were investigated (bearing in mind that most of the contacts came from countries where BCG vaccination is extensively used at least at birth and that the absence of a scar does not necessarily mean lack of vaccination because not all vaccinated individuals develop a detectable scar). The subjects who were not excluded because of their individual clinical condition or because they refused preventive treatment usually received isoniazid at 5 mg/kg/day for 6 months; those aged 15 years or less were prescribed isoniazid for 2 months regardless of TST positivity and then underwent a repeat test. The subjects with TST induration of <5 mm, underwent a second test after 2 months; if the results were positive, they underwent chest X-ray and were considered for chemoprophylaxis.

Ex vivo ELISpot assay. The ELISpot–IFN-\(\gamma\) assay was performed as previously described (40). Briefly, \(2 \times 10^5\) cells/well of peripheral blood mononuclear cells (PBMC) were seeded in triplicate in 96-well plates (MAIPS4510; Millipore, Bedford, Mass.) precoated with anti-IFN-\(\gamma\) capture monoclonal antibody (B-B1; Diaclone, Besançon, France) and stimulated with the different
antigens for 24 h at 37°C in air plus 5% CO₂. Biotinylated anti-IFN-γ detection monoclonal antibody (B-G1; Diaclide) was added for 4 h, followed by the addition of streptavidin-alkaline phosphatase conjugate (Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany) for 1 h. After a washing step, the nitroblue tetrazolium-BCIP (5-bromo-4-chloro-3-indolylphosphate; Sigma, St. Louis, Mo.) chromogenic substrate was added.

The individual spots were counted by using an automated image analysis system ELISPOT reader (AID-GmbH, Strassberg, Germany). The responses were scored as positive if the test wells contained a higher mean number of spot-forming cells (SFC) than the mean number plus two standard deviations in the negative control wells, and when the number of SFC per million PBMC in the stimulated wells (minus the values of the negative control wells) was ≥25 (in our experience with the PBMC of 128 healthy subjects, the mean number of SFC per million PBMC plus two standard deviation was <25 in negative control wells). A pool of five synthetic peptides (MTP) with a length of 20 amino acids derived from the sequences of ESAT-6 and CFP-10 proteins were used at a final concentration of 2 g/ml per peptide (10 µg/ml in total) for the detection of a specific response; PPD prepared from M. tuberculosis (Statens Serum Institut, Copenhagen, Denmark) and tested at 1 µg/ml and heat-inactivated and sonicated whole-cell BCG (Organon Teknika, Bosteel, The Netherlands) were also used. PBMC in medium alone or stimulated with phytohemagglutinin (PHA-P; Sigma) at 25 µg/ml were used as negative or positive controls, respectively.

Statistical analysis. The data were analyzed by using SAS software (version 8.2; SAS Institute). All of the significance tests were two sided, and a P value of <0.05 was considered to be statistically significant.

The differences in the mean number of MTP-specific SFC between individuals classified as 1, 2, or 4 for TB infection (according to the directions of the American Thoracic Society) (2, 40) were tested by using the Kruskal-Wallis test (1 versus 2 to 4) for nonparametric data. McNemar’s test of symmetry for paired data was used to test the equality of the off-diagonal frequencies obtained when we compared two different methods for assessing TB positivity (TST, ELISPOT-MTP, ELISPOT-PPD, and ELISPOT-BCG).

The agreement of the different TB classification tests was assessed by estimating Cohen’s Kappa (k) statistic (and the corresponding 95% confidence interval [CI]). According to Fleiss, k values of >0.75 suggest excellent agreement beyond that determined by chance; values of >0.4 and <0.75 indicate fair to good agreement, and values of <0.4 indicate poor agreement (22).

The receiver-operating-characteristics curve was calculated to measure the ability of the TST to classify correctly ELISPOT-MTP-positive and -negative individuals (measured on the basis of the area under the curve [AUC]) and to identify the optimal TST cutoff point that minimized classification errors.

RESULTS

Frequencies of ELISPOT-MTP responses in relation to TST reactivity. At screening, 42 of the 119 prospectively recruited close contacts (35%) had a negative TST response, and 77 (65%, including 7 with a documented previous TB infection) had a positive response.

A total of 41 (53%) of the 77 TST-positive contacts did not respond to MTP in the ELISPOT (ELISPOT-MTP) assay, and 3 of 42 (7%) TST-negative contacts were ELISPOT-MTP positive (2 with a positive and 1 with a negative response to ELISPOT-PPD; all three responded to ELISPOT-BCG) (Fig. 2). It is worth noting that a second TST repeated after 2 months in the two ELISPOT-PPD positive subjects was still negative. The test of symmetry was highly significant (P < 0.0001) and the agreement of ELISPOT-MTP assay and TST reactivity was quite small (k = 0.328; 95% CI = 0.198 to 0.459). Of the 42 TST-negative contacts (93%; 95% CI = 82 to 97), 39 were also ELISPOT-MTP negative, whereas 36 of the 77 TST-positive contacts (47%; 95% CI = 44 to 51) were also ELISPOT-MTP positive. All seven TST-positive contacts with documented previous TB infection responded to ELISPOT-MTP. When tested, the contagious index cases were positive to ELISPOT-MTP, ELISPOT-BCG, and ELISPOT-PPD (data not shown). Of the 80 ELISPOT-MTP-negative contacts, 4 were borderline (SFC × 10⁶ PBMC = 22.5); 3 with TST indurations of ≥10 mm and a positive ELISPOT-PPD and 1 with a negative response to PPD in vivo (TST) and in vitro (ELISPOT).

Frequencies of ELISPOT-MTP responses in relation to ELISPOT-BCG responses. Of the 119 contacts, 100 (84%) responded to ELISPOT-BCG (27 TST negative and 73 TST positive). All 39 ELISPOT-MTP positive contacts also responded to BCG, whereas 61 of 80 ELISPOT-MTP negative contacts (76%) were ELISPOT-BCG positive, suggesting that most of them should be vaccinated or infected with an NTM (Fig. 2 and Table 1). In fact, 44 of 67 (66%) individuals in
which a BCG scar was present did not respond to ELISPOT-MTP, but all responded to ELISPOT-BCG. The test of symmetry was highly significant ($P < 0.0001$), and the agreement between ELISPOT-MTP and ELISPOT-BCG was small ($k = 0.169$; $95\% CI = 0.088$ to $0.251$).

**Frequencies of ELISPOT-MTP responses in relation to ELISPOT-PPD responses.** When we analyzed the response frequencies to ELISPOT-PPD in 106 of 119 contacts in relation to ELISPOT-MTP, 30 of the 31 ELISPOT-MTP positive contacts (97%; $95\% CI = 86$ to $99$) also responded to PPD (the only nonresponder was TST negative but ELISPOT-BCG positive), whereas 44 of 75 ELISPOT-MTP negative contacts (59%) responded to ELISPOT-PPD (Table 2). This result corroborates the data that many contacts were vaccinated. The test of symmetry was once again highly significant ($P < 0.0001$), and the agreement between the ELISPOT-MTP response and the ELISPOT-PPD was small ($k = 0.271$; $95\% CI = 0.153$ to $0.388$).

**Frequencies of ELISPOT-PPD responses in relation to TST reactivity.** Of the 67 TST-positive contacts, 61 (91%; $95\% CI = 85$ to $95$) had a positive ELISPOT-PPD result, and 6 (9%) a negative ELISPOT-PPD result (three responders and three nonresponders to BCG; all nonresponders to MTP) (Table 3). Moreover, 13 of 39 TST-negative contacts (33%) responded to ELISPOT-PPD (11 of whom did not respond to MTP), thus demonstrating that the ELISPOT assay is more sensitive than the TST. In this case, the test of symmetry was not significant, and the agreement between the ELISPOT-PPD response and TST reactivity was quite high ($k = 0.6$; $95\% CI = 0.44$ to $0.76$).

**Correlation of ELISPOT-MTP responses with TB classification and TST induration size.** When the contacts were grouped by TB classification (2), there was a significant between-class difference in the mean number of SFC, with the highest values being observed in class 4 (Fig. 3). Likewise, there was a significant difference ($P < 0.0001$) in TST induration between the contacts with a negative or positive ELISPOT-MTP response (Fig. 4).

Analysis of the ability of TST induration to discriminate negative and positive ELISPOT-MTP responses revealed an AUC of 85% ($95\% CI = 77$ to $92$; $P < 0.0001$) and showed that the best cutoff point was 12.5 mm. When TST reactivity was defined on the basis of an induration cutoff point of $\geq 10$ mm, 13 subjects changed from positive to negative, thus making the TST-positive and ELISPOT-MTP negative responses 28 of 64 (44%) instead of the initial 41 of 77 (53%; Fig. 2).

**Correspondence between ELISPOT-MTP responses and decision to treat.** The majority of the TST-positive contacts (62 of 83 [75%]) received preventive chemoprophylaxis for latent TB infection; 57 of the 119 contacts (17 with a positive TST) were not treated (Table 1 and Fig. 1). Positive ELISPOT-MTP results were significantly more frequent among the treated (27 of 62 [44%]) than the untreated contacts (12 of 57 [21%]), but

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**TABLE 2. Frequencies of ELISPOT-PPD responses in relation to ELISPOT-MTP responses**

<table>
<thead>
<tr>
<th>ELISPOT-MTP result</th>
<th>No. (%) of responses that were ELISPOT-PPD:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>31 (41)</td>
</tr>
<tr>
<td>Positive</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

**TABLE 3. Frequencies of ELISPOT-PPD responses in relation to TST reactivity**

<table>
<thead>
<tr>
<th>TST result</th>
<th>No. (%) of responses that were ELISPOT-PPD:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>26 (67)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (9)</td>
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</table>
56% (35 of 62) of the treated contacts had negative ELISPOT-MTP results.

**DISCUSSION**

The results of this study show that our in-house ELISPOT–IFN-γ/H9253 assay, which uses a restricted and highly selected pool of synthetic peptides derived from ESAT-6 and CFP-10 proteins, is more accurate in identifying subjects with suspected LTBI (close contacts of people with contagious TB) than either the TST or the ELISPOT-PPD assay. Furthermore, on the basis of ELISPOT-MTP responses, we have been able to define a TST induration cutoff point that better correlates with LTBI.

The large majority of the recruited contacts (86 of 119) came from countries where *M. tuberculosis* and NTM are endemic and BCG vaccination is a common practice (in 67 of them it was possible to document the BCG vaccination). They therefore represent a high-risk population in whom it is particularly difficult to diagnose *M. tuberculosis* infection because the specificity of the TST routinely used to detect it is low as a result of the cross-reaction of PPD with vaccine BCG strains and NTM (1, 2). For this reason, new strategies for specific diagnosis and prevention of transmission are mandatory.

The agreement between TST reactions and in-house ELISPOT-MTP responses was quite small: 53% of the TST-positive subjects did not respond to ELISPOT-MTP. The specificity of the ELISPOT-MTP assay was confirmed by the fact that 100% of the ELISPOT-MTP positive subjects were ELISPOT-BCG positive and 97% were ELISPOT-PPD positive. Conversely, the fact that 76% of the ELISPOT-MTP negative subjects were ELISPOT-BCG positive and 59% were ELISPOT-PPD positive supports the idea that most contacts may be vaccinated (Table 1) or NTM infected, thus underlining the greater sensitivity of the ELISPOT assay over the TST, as has also been reported by others (20, 28, 32, 34). Of note, 44 of the 67 individuals with a documented BCG scar were ELISPOT-MTP negative but ELISPOT-BCG positive. In a previous study, we found a smaller number of individuals who were ELISPOT-MTP negative and ELISPOT-BCG positive (63%), but most of the subjects involved were native Italians, and Italy has a low incidence of TB and BCG vaccination is not compulsory (40).

It is worth noting that 3 of the 42 TST-negative contacts were ELISPOT-MTP positive (including 2 aged less than 15 years whose second TST after 2 months of chemoprophylaxis were still negative). It has been reported that a discordance between in vivo (TST) and in vitro (ELISPOT assay) responses to PPD may be due to a specific cutaneous anergy caused by oral BCG vaccinations in spite of significant increases in mycobacterium-specific IFN-γ responses in PBMC (24); such an anergy also occurs in ca. 15% of patients with active pulmonary disease, thus giving rise to false-negative TST results (8, 24).

The agreement between TST and ELISPOT-PPD responses was quite high: 91% of the subjects were positive in both tests, and only 9% were TST positive and ELISPOT-PPD negative. However, 33% of the TST-negative contacts responded in vitro to PPD. These results are consistent with the greater sensitivity of ELISPOT-PPD over the TST, as previously reported (21, 23, 35).

One advantage of the ELISPOT assay is the possibility of evaluating IFN-γ production at the single-cell level (expressed as the number of SFC). In this regard, we observed a close relationship between the number of SFC and the TB classification, with the highest values being found in individuals with a documented history of TB infection (class 4). This substantially shows that the greater the exposure to *M. tuberculosis*, the greater the response to ELISPOT-MTP (20, 39). It is possible that this quantitative parameter could be used to discriminate acute infection from LTBI, but this needs to be demonstrated in larger-scale longitudinal studies.

When the ability of the TST to discriminate negative and
positive responses to ELISPOT-MTP was tested by means of the receiver-operating-characteristics curve, the AUC was 85% and the best cutoff point about 12.5 mm of induration (near the conventional 10-mm threshold). This suggests that a TST reaction should be considered positive when the induration is ≥10 mm (and not ≥5 mm as is now done) also in close contacts originating from countries with a high prevalence of TB (1, 26). Using this area of induration as a new cutoff point, 44% of the contacts (as against the initial 53%) were determined to be TST positive and ELISPOT-MTP negative. However, even after this correction, the diagnostic sensitivity of our in-house ELISPOT assay was greater than that of the TST.

The definition of positive or negative responses to ELISPOT assays is still empirical, even if a general consent on how to consider positive results exists in the literature. On the basis of our previously reported criteria (40), 4 of 80 contacts with a negative ELISPOT-MTP response were borderline (22.5 SFC per million PBMC, minus the values of the negative control wells); since 3 of these had a TST induration of ≥10 mm and were ELISPOT-PPD positive, a cutoff point of ≥20 SFC per 10^6 PBMC may better define positivity at least in close contacts.

Finally, 75% of the contacts with a positive TST reaction (after the first and second test) received preventive treatment; 20% refused or were ruled out because of their individual clinical condition. This is a real problem for any program aimed at preventing and/or controlling the spread of TB. It is interesting that 56% of the treated individuals were ELISPOT-MTP negative and that 21% of the untreated individuals were ELISPOT-MTP positive. If we also consider the technical problems relating to the execution and reading of the TST, and the need for a return visit, it can be said that the new generation of ELISPOT-IFN-γ assays based on a cocktail of RD1-restricted peptides offer a real advantage over TST by better targeting interventions against latent TB to truly infected individuals and by helping to avoid unnecessary treatment. Cost may be a limiting factor (we have calculated a cost of approximately US$6) on a per-patient basis for disposables and reagents) but, given the improved targeting of chemoprophylaxis, the ELISPOT assay could be very cost-effective in the long term. Moreover, the restricted number of highly selected peptides used in our assay further reduces its cost.

The possibility of false-negative ELISPOT-IFN-γ results and the consequent underestimate of LTBI cannot be excluded mainly because of human leukocyte antigen restrictions, especially when a limited number of peptides are used (28). However, this possibility can be at least partially excluded because of the highly sensitive nature of our test (40) and by the comparison with the commercial T-SPOT TB assay. In future studies, we plan to test individuals with acute or LTBI, either HIV positive or negative, from South Africa and Uganda countries.

At the moment, it is difficult to envisage a wide-scale application of the ELISPOT methodology in low-resource countries where TB infection is endemic due to ELISPOT’s difficulties in discriminating acute from latent infection. However, due to the increasing migration of peoples from these countries to industrialized ones, this methodology may be better designed for screening immigrants (especially close contacts of individuals with acute TB infection) (13, 16). The ELISPOT assay may also allow a more sensitive testing of healthcare workers discriminating truly infected people from BCG-vaccinated subjects or from subjects with nonspecific TST reactions (e.g., hypersensitivity to heterologous proteins) (6). However, before abandoning TST as the reference standard, long-term longitudinal studies are required in order to determine the association between positive ELISPOT responses and the subsequent risk of active TB.

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