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Strong purified protein derivative responses are associated with poor mycobacterium inhibition in latent TB

J.S.L. Kang*, A. Cherian#, S.H. Gan#, T.H. Lee¶, K.C. Lee¶, C.B.E. Chee#, T.M. Doherty+, Y.T. Wang# and G.T. Seah*

ABSTRACT: **The tuberculin skin test (TST) using purified protein derivative (PPD) of *Mycobacterium tuberculosis* is traditionally used to diagnose latent tuberculosis (TB) infection (LTBI). However, LTBI diagnosis by peripheral blood mononuclear cell (PBMC) interferon (IFN)-γ responses to *M. tuberculosis*-specific antigens, early secreted antigenic target 6 kDa (ESAT-6) and culture filtrate protein (CFP)-10 has greater specificity. We investigated the difference in antimycobacterium cellular immunity in TB contacts who were strong TST reactors but nonresponsive to the ESAT-6/CFP-10 assay compared with those with concordant results.**

Healthy TB contacts were tested using the above two assays and mycobacterium survival was measured after co-culture of infected macrophages with their PBMCs.

Whether PPD reactivity was tested by TST or by PBMC-specific IFN-γ responses, strongly PPD-reactive TB contacts without ESAT-6/CFP-10 responsiveness showed significantly better mycobacterium inhibition activity than ESAT-6/CFP-10-responsive TB contacts with the same PPD reactivity. In the former group, stronger PPD reactivity was associated with improved mycobacterium killing, whereas ESAT-6/CFP-10 responders showed the opposite result.

PPD-reactive ESAT-6/CFP-10-nonresponsive TB contacts in our population may have had protective immunity related to prior mycobacterium exposure. ESAT-6/CFP10-responsive TB contacts are more likely to have LTBI and, in this group, strong PPD reactivity may paradoxically be associated with poor mycobactericidal activity.

KEYWORDS: **Cytokine production, immunity, tuberculosis, tuberculin skin test**

The tuberculin skin test (TST), representing delayed-type hypersensitivity to purified protein derivative (PPD) of *Mycobacterium tuberculosis*, is often used for screening tuberculosis (TB)-exposed contacts. Strong TST reactivity is usually interpreted as indicating latent TB infection (LTBI). However, PPD responses may be due to past *Mycobacterium bovis* bacille Calmette-Guérin (BCG) vaccinations [1] or immune exposure to environmental mycobacteria [2], as various *Mycobacterium* species have many genes in common. Thus, using the TST for LTBI diagnosis gives poor specificity in areas where the climate favours environmental mycobacterium proliferation [3] and where a routine BCG vaccination and revaccination programme exists.

Early secreted antigenic target 6 kDa (ESAT-6) and culture filtrate protein (CFP)-10 are immunogenic TB proteins encoded by genes absent in all BCG strains and most environmental mycobacteria [4]. The use of *in vitro* interferon (IFN)-γ release assays with ESAT-6/CPF-10 as *M. tuberculosis*-specific antigens therefore avoids cross-reactivity in BCG-vaccinated subjects, thereby overcoming some limitations of the TST. The utility of these assays has been extensively reviewed [5, 6]. Numerous human studies worldwide have shown that ESAT-6/CFP-10 is more specific than PPD in detecting LTBI [7, 8]. Although some studies suggest that the sensitivity of ESAT-based assays is lower than the TST [9], later studies show otherwise [10]. Moderately good agreement (60–80%) and positive correlation between the TST and various IFN-γ-based assays has been reported [6, 11]. However, the immune status of TB contacts with discordant results in PPD- and ESAT-based tests has yet to be fully resolved.

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AFFILIATIONS
*Dept of Microbiology and Immunology Programme, Yong Loo Lin School of Medicine, National University of Singapore,
#Tuberculosis Control Unit, Tan Tock Seng Hospital, and
¶Clifford Dispensary, Singapore.
+Dept of Infectious Disease Immunology, Statens Serum Institute, Copenhagen, Denmark.

CORRESPONDENCE
G.T. Seah
Dept of Microbiology
National University of Singapore
MD4
5 Science Drive 2
Singapore 117597
Singapore
E-mail: micsgt@nus.edu.sg

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The lack of IFN-γ activity severely compromises host immunity in human TB [12]. However, the protective implications of high TB-specific IFN-γ levels (*e.g.* IFN-γ responses of PPD-stimulated lymphocytes) are ambiguous, as IFN-γ expression does not directly correlate with protective outcomes when TB-exposed contacts are followed up for TB reactivation [13]. We postulate that this could be because people with strong PPD-specific responses attributable to different clinical situations (*e.g.* active TB infection, LTBI, BCG vaccination or exposure to environmental mycobacteria) may have different levels of protective immunity. This may also underlie discrepancies in outcomes of ESAT *versus* PPD response assays.

Mycobacterium growth inhibition by peripheral blood mononuclear cells (PBMCs) is known to be one measure of protective immunity [14–17]. In the present study, we determined whether the magnitude of PPD responses by IFN-γ assays or the TST was correlated with the subject's ability to kill intracellular mycobacteria, and examined this in relation to the subject's ESAT-6/CFP-10 responsiveness. We postulated that strong PPD responders without LTBI (ESAT-6/CFP-10 nonresponders) might have distinctly different mycobacterium inhibition activity from LTBI subjects (ESAT-6/CFP-10 responders), with similar PPD reactivity. This is because we believe PPD reactivity reflects protective immunity in the former group but not the latter.

The present study was performed in Singapore where there is moderate TB endemicity and routine BCG vaccination in newborns. Between the years 1958–2001, TST-nonresponding adolescents were revaccinated. Prevalent environmental mycobacteria exposure is probable, due to the equatorial climate [3]. There are therefore a number of reasons other than latent infection for strong PPD responsiveness in the Singapore adult population. We found that, when ESAT-6/CFP-10 responses were used to define LTBI, the correlation between the magnitude of PPD-specific IFN-γ responses and mycobacterium inhibitory activity was diametrically opposite in LTBI and non-LTBI (uninfected) healthy subjects. This could underlie difficulties in attributing protective outcomes consistently with the magnitude of PPD responses.

METHODS

Study subjects

Our main study group comprised healthy household contacts (''contacts'', n=105) of culture-proven TB patients at the Tuberculosis Control Unit (Tan Tock Seng Hospital, Singapore). The contacts had no physical or radiological signs of active or past TB disease and 73% had past BCG vaccination. The TST, using 1 tuberculin unit of PPD RT23 (Mantoux method), was read at 48–72 h. Contacts were recruited from among those offered chemoprophylaxis on clinical suspicion of LTBI, *i.e.* minimum criterion of TST $\geqslant$10 mm in those with one BCG scar and $\geqslant$16 mm for those with two BCG scars. These criteria were based on a previous local study relating TST size in schoolchildren and 4-yr risk of reactivation [18]. Chest radiograph cavities and smear positivity in the index case were also considered in offering prophylaxis. Analysis for tuberculin reactivity among TB contacts was adjusted for age, sex and BCG vaccination status, of which none were found to be sources of bias.

As comparator groups for the TB contacts, we also recruited 13 culture-positive pulmonary TB patients and 152 healthy community volunteers (''community''). The TB patients were PPD- and ESAT-6/CFP-10-reactive subjects within the first month of treatment. The community subjects were local residents attending a community general practice clinic for reasons unrelated to infectious or inflammatory disorders. Those with histories of past TB exposure were specifically excluded. Of these, 96% had at least one past BCG vaccination. Subjects aged >85 yrs or <18 yrs were excluded in all groups and the age distribution was comparable in all healthy groups. HIV infection was excluded on clinical history alone as the HIV rate among TB cases in Singapore is low. Informed consent for all volunteers was obtained by the attending physicians. Ethical review and approval of the study protocol was conducted by the Tan Tock Seng Hospital and National University of Singapore (Singapore) institutional review boards.

Cell stimulation and IFN-γ release assay

IFN-γ released by 2×10^5 viable PBMCs in response to 20 μg·mL^{-1} PPD or a mixture of 15-mer overlapping synthetic peptides spanning the coding regions of ESAT-6 and CFP-10 (published previously [19]) was measured in supernatants of 5-day antigen-stimulated cultures by ELISA (OptEIA human IFN-γ; Becton Dickinson PharMingen, San Diego, CA, USA). The 5-day stimulation assay rather than overnight stimulation was used because it reflects memory response to the antigens [20] and minimises the risk of weak reactors being missed as prolonged incubation increases sensitivity for detecting LTBI [21]. IFN-γ levels 4 SD above the mean concentration in unstimulated control wells were regarded as ''positive'' based on published practice [19]. Positive ESAT-6/CFP-10 responders in contacts and community subjects were regarded in this study as having LTBI [5, 6], regardless of their TST size. Clinically healthy ESAT-6/CFP-10 nonresponders were deemed to have no LTBI.

Mycobacterium inhibition assay

The mycobacterium inhibition assay was modified from WORKU and HOFT [22]. Briefly, 2×10^5 viable PBMCs were seeded in RPMI 1640 (Sigma-Aldrich, St Louis, MO, USA) supplemented with 2 mM L-glutamine (Sigma-Aldrich) and 10% fetal calf serum within 96-well plates. A sonicated preparation of dead *M. tuberculosis* (H37Rv strain, 1 μg·mL^{-1}) or PBS (control wells) was included in the cultures to expand mycobacterium-specific lymphocytes. After 2 days, 2×10^5 live *M. bovis* BCG (Pasteur strain) was added per well to infect the macrophages. Concurrently, supplementary ferric ammonium citrate (50 μg·mL^{-1}) was added for supporting optimal mycobacterium growth [23]. 3 days later, nonadherent cells were removed before infected macrophages were lysed with 0.1% saponin (Sigma-Aldrich) to release intracellular BCG. These released BCG were resuspended in Middlebrook 7H9 broth (Difco^{TM} Middlebrook; Becton Dickinson, Sparks, NJ, USA), then pulsed with 1 μCi ^{3}H-uridine (Amersham Biosciences, Buckinghamshire, UK) for 48 h [24]. The radiolabelled BCG cultures were harvested onto membranes (Tomtec cell harvester; PerkinElmer Wallac, Boston, MA, USA) and radioactivity measured (Microbeta scintillation counter; PerkinElmer). The level of radioactivity expressed as counts per min (CPM) reflects

the number of viable (actively replicating) bacteria in each well. Due to insufficient cell numbers for certain subjects after completion of various assays, only a subset of each study group was assessed in this assay (13 TB subjects, eight ESAT-6/CFP-10-responsive contacts, eight ESAT-6/CFP-10-responsive community subjects, 27 ESAT-6/CFP-10-nonresponsive contacts and 17 ESAT-6/CFP-10-nonresponsive community subjects (total n=73)).

Statistics

The Kruskal–Wallis H-test was used for multigroup comparisons to determine whether the difference between groups was statistically significant. If this was significant, it was followed up with Dunn's multiple comparison *post hoc* test for pairwise comparison between two groups of interest. Spearman rank sum correlation and associated regression coefficients were used to analyse correlations between two independent variables. SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical computations.

RESULTS

PPD, ESAT-6/CFP-10 and TST responses in different clinical groups

Immune recognition of ESAT-6/CFP-10 and PPD was found in 18 and 72% of the contacts, respectively (n=105) (table 1). As these were ''high risk'' TB contacts with TST readings warranting prophylaxis for suspected LTBI, there was clearly discordance between the detection of LTBI by TST and ESAT-6/CFP-10 criteria. Response rates were 13 and 66%, respectively, in the community group (n=152). All ESAT-6/CFP-10-responsive subjects responded to PPD.

Median TST responses were 16 and 14.5 mm, respectively, in LTBI (ESAT-6/CFP-10-responsive) contacts and ESAT-6/CFP-10-nonresponsive contacts (not shown); this difference was not statistically significant. Among all contacts, the correlation of TST size with PPD IFN-γ responses, but not with ESAT-6/CFP-10 IFN-γ responses, was significant (r=0.21, p=0.03 and r=0.14, p=0.16, respectively; data not shown).

Strong TST responders in ESAT-6/CFP-10-nonresponsive contacts show better in vitro mycobacterium inhibition than LTBI contacts

Since a substantial proportion of contacts were strong TST reactors but ESAT-6/CFP-10-nonresponsive, we used the mycobacterium inhibition assay to study whether such discordant cases differed from the concordant (ESAT-6/CFP-10 responders or LTBI) cases in killing activity.

First, we investigated how the magnitude of the TST response related to *in vitro* mycobacterium inhibition in contacts. Higher viable BCG CPM were interpreted as signifying poorer mycobacterium inhibition activity. LTBI contacts with TST ⩾16 mm had significantly poorer mycobacterium killing activity than the corresponding ESAT-6/CFP-10-nonresponsive contacts (fig. 1a). Indeed, the ESAT-6/CFP-10-nonresponsive group with the strongest TST responses (⩾16 mm) showed the best *in vitro* mycobacterium inhibition, better than all LTBI groups. Among ESAT-6/CFP-10-nonresponsive contacts, the TST ⩾16 mm group also showed better mycobacterium killing than the TST 10–15-mm group. However, among those with LTBI there was no difference in mycobacterium inhibitory activity between subjects with different TST readings.

Although we knew that TST sizes correlated with the magnitude of *in vitro* PPD-specific IFN-γ responses, it was not clear whether using the latter assay to determine PPD responsiveness (instead of the *in vivo* TST) would alter the relationship with mycobacterium killing. Additionally, we wanted to compare the contacts with the community subjects. Contacts and community subjects were therefore divided into PPD_{High} and PPD_{Low} groups based on IFN-γ responses (⩾900 pg·mL^{-1} for PPD_{High}, <900 pg·mL^{-1} for PPD_{Low}) and mycobacterium inhibition was compared. The cut-off value was based on 70% of all LTBI subjects having at least this level

TABLE 1 *In vitro* responses to purified protein derivative (PPD)-based and early secreted antigenic target 6 kDa/culture filtrate protein 10 (ESAT-6/CFP-10)-based assays in different clinical groups

Group	Subjects n	Positive tests#		TST¶	
		PPD	ESAT-6/CFP-10	10–15 mm	⩾16 mm
Contacts	105	76 (72)	19 (18)	69 (66)	36 (34)
LTBI contacts+	19	19 (100)	19 (100)	9 (47)	10 (53)
ESAT-6/CFP-10 nonresponsive contacts+	86	57 (66)	0 (0)	60 (70)	26 (30)
Community	152	100 (66)	20 (13)		
LTBI community+	20	20 (100)	20 (100)		
ESAT-6/CFP-10 nonresponsive community+	132	80 (61)	0 (0)		

Data are presented as n (%), unless otherwise stated. TST: tuberculin skin test; LTBI: latent tuberculosis infection. #: the criteria for defining positive antigen-induced interferon-γ response are given in the Methods section. ¶: TST was not performed for community subjects. Contacts with TST <10 mm were excluded from the study. The TST response was dichomotised at 16 mm based on a previous local study of schoolchildren which suggested that those with ⩾16 mm TST readings had a higher risk of developing tuberculosis within 4 yrs [18]. +: LTBI subjects had positive responses to ESAT-6/CFP-10 and were regarded as having latent tuberculosis infection, ESAT-6/CFP-10 nonresponding subjects were regarded as having no latent infection.

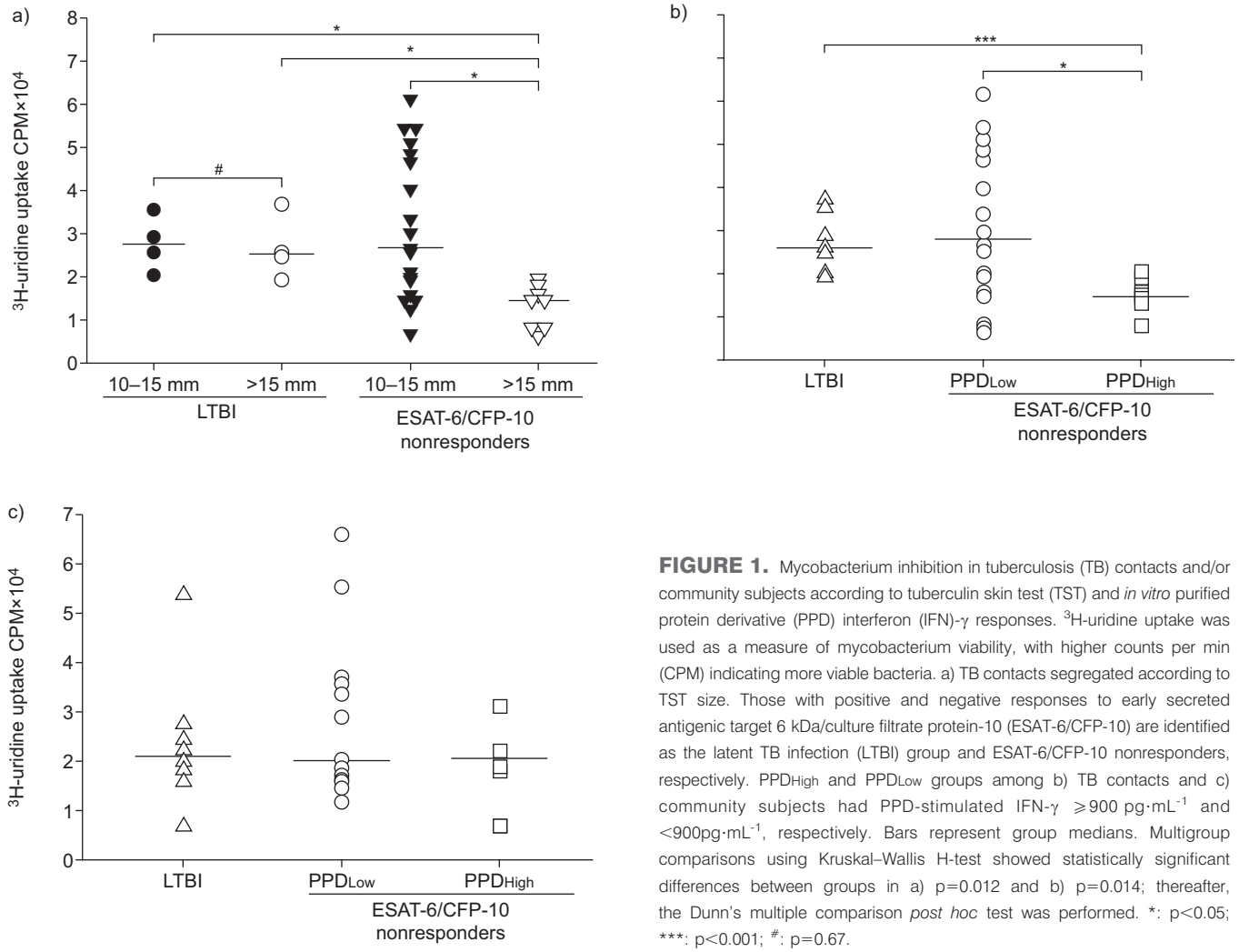

FIGURE 1. Mycobacterium inhibition in tuberculosis (TB) contacts and/or community subjects according to tuberculin skin test (TST) and *in vitro* purified protein derivative (PPD) interferon (IFN)-γ responses. ^{3}H-uridine uptake was used as a measure of mycobacterium viability, with higher counts per min (CPM) indicating more viable bacteria. a) TB contacts segregated according to TST size. Those with positive and negative responses to early secreted antigenic target 6 kDa/culture filtrate protein-10 (ESAT-6/CFP-10) are identified as the latent TB infection (LTBI) group and ESAT-6/CFP-10 nonresponders, respectively. PPD$_{High}$ and PPD$_{Low}$ groups among b) TB contacts and c) community subjects had PPD-stimulated IFN-γ $\geqslant$900 pg·mL^{-1} and $<$900pg·mL^{-1}, respectively. Bars represent group medians. Multigroup comparisons using Kruskal–Wallis H-test showed statistically significant differences between groups in a) p=0.012 and b) p=0.014; thereafter, the Dunn's multiple comparison *post hoc* test was performed. *: p$<$0.05; ***: p$<$0.001; $^{\#}$: p=0.67.

of response. Since IFN-γ activates infected macrophages to kill intracellular *M. tuberculosis* [25], we hypothesised that the strength of the PPD-specific IFN-γ responses might be positively associated with mycobacterium killing activity.

With this *in vitro* PPD IFN-γ release assay (fig. 1b), the contacts showed a similar pattern of responses as with the TST; *i.e.* PPD$_{High}$ ESAT-6/CFP-10 nonresponders showed better mycobacterium inhibition than both the LTBI contacts and the PPD$_{Low}$ ESAT-6/CFP-10 nonresponders. The difference in mycobacterium killing between PPD$_{High}$ ESAT-6/CFP-10 nonresponders and LTBI subjects was not due to differential PPD IFN-γ responses as these two groups had similar median IFN-γ responses (p=0.67; see fig. 1a of the online supplementary material). In contrast, the community subjects showed no differential mycobacterium killing activity based on PPD IFN-γ reactivity (see fig. 1b of the online supplementary material) or LTBI status (fig. 1c).

As an association was found between TST and killing activity in the contacts (fig. 1a), we studied the correlation between these parameters. In the ESAT-6/CFP-10-nonresponsive group the two parameters were significantly correlated: the larger the TST size, the better the mycobacterium inhibition (fig. 2a). There was no correlation in the LTBI contacts group (fig. 2b).

Stronger PPD-specific IFN-γ responses correlated with better mycobacterium inhibition activity in ESAT-6/CFP-10 nonresponders but opposite correlation found in LTBI and TB subjects

We hypothesised that, in uninfected persons, mycobactericidal activity should increase progressively with increases in PPD reactivity (if the latter is a marker of protective antimycobacterium immunity). Conversely, the magnitude of PPD reactivity in infected persons might not correlate with killing activity. This would explain previous observations that PPD responses are not always linked to protection [13]. We therefore separately assessed infected persons, including latent and active TB groups (healthy ESAT-6/CFP-10 responders and TB patients) *versus* uninfected persons (ESAT-6/CFP-10 nonresponders in both contacts and community groups). All subjects across the entire spectrum of PPD responses for both the infected and uninfected groups were included as we did not wish to make any assumptions regarding the impact of any specific range of PPD values on the correlation trend.

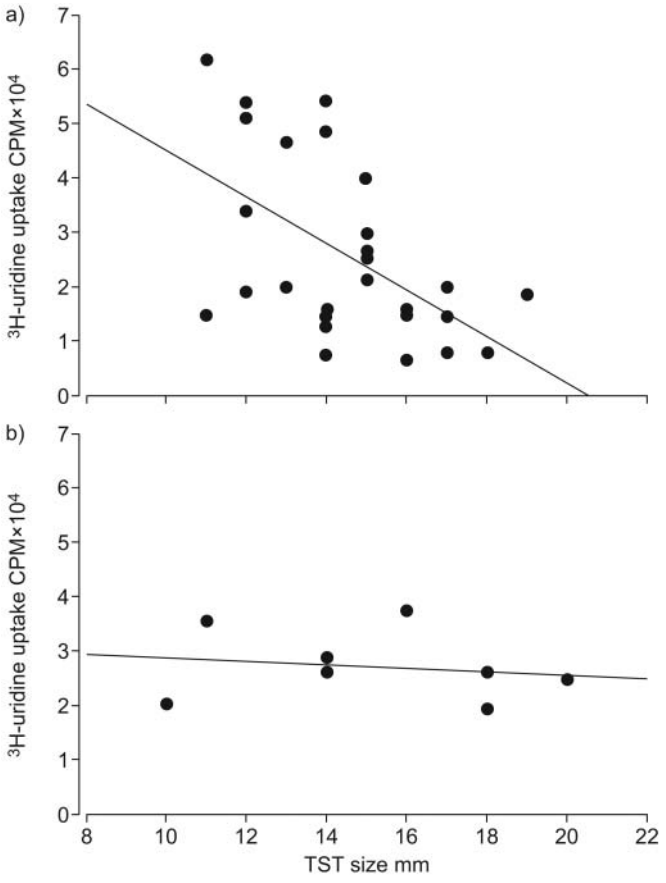

FIGURE 2. Correlation of tuberculin skin test (TST) outcomes in relation to mycobacterium inhibition in tuberculosis (TB) contacts. Regression lines and coefficients for correlations between TST sizes and counts per minute (CPM) ^{3}H-uridine uptake for a) early secreted antigenic target 6 kDa/culture filtrate protein 10 (ESAT-6/CFP-10) nonresponder contacts (n=27) and b) latent TB infection contacts (n=8). a) r=-0.5, p=0.01; b) r=-0.27, p=0.53 (Spearman rank sum correlation test).

There was an interesting dichotomy in the correlation graphs. Among ESAT-6/CFP-10 nonresponders, the higher the PPD IFN-γ responses, the better the mycobacterium killing activity (fig. 3a). Conversely, among infected subjects, the higher the PPD IFN-γ levels, the poorer the mycobacterium inhibition (fig. 3b). Both correlations were significant but the trends were opposite. Therefore, stronger PPD-specific IFN-γ responses were associated with better mycobacterium killing only in subjects without latent or active infection.

DISCUSSION

In our study, of the subjects with moderate TB endemicity, only 18% of the healthy TST-reactive TB contacts responded to ESAT-6/CFP-10 (table 1). This discordance is probably due to prior BCG vaccination and/or environmental mycobacteria exposure priming TST responses in those without TB exposure. The TB contacts in our study had an average age of 40 yrs. The extent of sensitisation with environmental mycobacteria is known to increase with age [26], and TST readings are strongly influenced by environmental mycobacteria exposure [27]. Moreover, BCG priming in childhood may potentially be boosted by repeated exposure to *M. tuberculosis* in Singapore where the incidence of TB is moderately high.

Two main points were shown by our data. First, strongly PPD-reactive ESAT-6/CFP-10 nonresponders in our population had good antimycobacterium immunity. They not only showed better mycobacterium killing than the ESAT-6/CFP-10 nonresponders with lower PPD reactivity (fig. 1a and b), but the magnitude of PPD reactivity was also correlated with killing (fig. 2). These findings support the probability that, in those without latent infection in our study population, PPD reactivity reflects the strength of antimycobacterium immunity in each person, consistent with a previous report that tuberculin-positive subjects show better inhibition of mycobacterial growth than tuberculin-negative individuals [17]. Secondly, our strongly PPD-reactive ESAT-6/CFP-10-nonresponsive contacts had distinct antimycobacterium immunity from LTBI contacts as the former had better mycobacterium inhibition activity

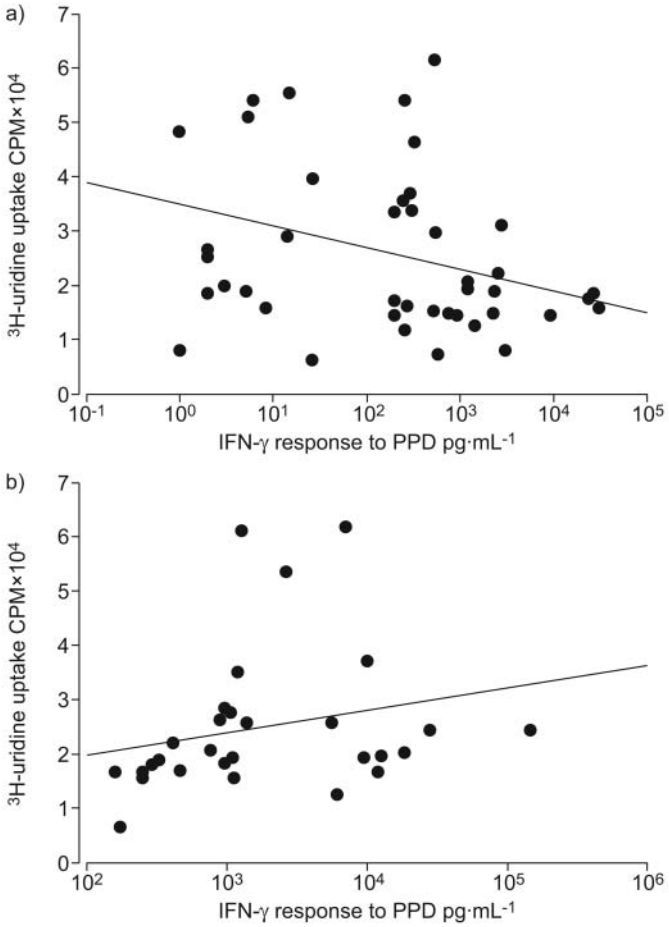

FIGURE 3. Opposite correlation trends between mycobacterium inhibition and purified protein derivative (PPD) interferon (IFN)-γ response in a) the uninfected group (early secreted antigenic target 6 kDa/culture filtrate protein 10 nonresponders, n=44) and b) the infected group (tuberculosis (TB) and latent TB infection subjects, n=29). Both groups included contacts and community subjects. Regression lines and coefficients are shown. a) r=-0.32, p=0.03; b) r=0.4, p=0.03 (Spearman rank sum correlation test). CPM: counts per minute.

(fig. 1a and b). There were also opposite trends in the association between PPD reactivity and mycobacterium killing in infected and uninfected groups (fig. 3). We acknowledge, however, that the sample of LTBI contacts studied in figure 3 was small (n=8). Nonetheless, taken together, we believe that in communities with other known sources of mycobacterium antigen priming besides LTBI, strongly PPD-reactive ESAT-6/CFP-10 nonresponders should not generally be regarded as having LTBI and could even have some protective immunity [2]. In stating this, we have assumed that inhibition of mycobacterium survival is a marker of protective immunity. We acknowledge that there is no direct prospective epidemiological evidence that this assay predicts long-term protection.

However, a number of published studies support this assumption. Using a mycobacterium inhibition assay, SILVER *et al.* [28] showed an association between *in vitro* lymphocyte responses and inhibition of intracellular *M. tuberculosis* growth; the assay therefore reflected the contribution of mycobacteria-specific T-cells to protection. HOFT and co-workers [15, 16] found that inhibition of intracellular *M. tuberculosis* replication significantly increased after BCG vaccination of initially PPD-nonreactive persons, thus reflecting BCG-induced protective immunity. Taken together with evidence that tuberculin-positive persons can restrict growth of BCG better than those who are tuberculin-negative [17], these studies demonstrate that assays of BCG killing by blood immune cells reflect the host's mycobacterium-specific immunity, not just innate immune mechanisms. Moreover, we and others [22] have shown that the assay is specific for mycobacterium-reactive T-cells (see fig. 2 of the online supplementary material) by demonstrating that unstimulated T-cells or cells specific for non-mycobacterium antigens have weaker killing activity than mycobacterium antigen-specific cells. The utility of the mycobacterium growth inhibition assay in reflecting protective immunity has led to its recent use as a surrogate marker for vaccine-induced protection in a recombinant BCG vaccine study [14].

It is possible that *M. tuberculosis* sonicate stimulation of lymphocytes from LTBI cases may result in expansion of ESAT-specific lymphocytes that do not recognise BCG-infected cells. This may theoretically be one contributory factor to the poorer killing activity of LTBI relative to ESAT-6/CFP-10 nonresponders. However, with our lymphocyte cultures exposed to both whole *M. tuberculosis* sonicate and live BCG, there is likely to be considerable expansion of T-cells specific for the many immunodominant antigens shared between BCG and *M. tuberculosis*, and such cells are known to contribute to mycobacterium killing [29].

When different types of mycobacterium inhibition assays were conducted on PPD-negative persons given BCG immunisation, subsequent BCG-specific IFN-γ production was not correlated with any of the assays [15]. If PPD-positive subjects in our study were taken together, there would also be an apparent lack of correlation of PPD-specific IFN-γ responses with mycobacterium killing because there are opposite correlation trends in ESAT-6/CFP-10 nonresponders and those with LTBI (fig. 3). Some possible explanations for this paradox follow.

TB-specific IFN-γ production activates macrophages to inhibit intracellular mycobacterium growth [30] but, under some circumstances, IFN-γ promotes intracellular mycobacterium replication [28] and contributes to apoptosis of *M. tuberculosis*-responsive T-cells, thus favouring *M. tuberculosis* persistence [31]. This may happen in infected persons. For those with LTBI, it is also possible that stronger PPD reactivity reflects subjects with higher bacterial loads. Alternatively, regulatory T-cells in infected persons may modulate the ability of antigen-specific T-cells to kill mycobacteria. In active TB and LTBI, increased frequency of peripheral blood CD4+CD25+ FoxP3+ T-cells (putative regulatory T-cells) has been associated with suppressed *M. tuberculosis*-specific immunity [32–34]. In late-stage murine TB, transforming growth factor-β, a suppressive cytokine associated with regulatory T-cells, impairs the host's ability to limit *M. tuberculosis* survival [35]. These are some potential reasons why PPD-specific IFN-γ responses were dissociated from mycobacterium inhibition in infected subjects.

In vitro ESAT-6/CFP-10-based tests for diagnosing LTBI are gaining clinical acceptance [36] and discordance with traditional TST readings needs to be resolved for decisions on prophylaxis. In the Singapore population, the discordance in TST and ESAT-6/CFP-10 responses is largely unidirectional; cases of TST <10 mm comprised only 4% of ESAT-6/CFP-10-responsive TB contacts completing prophylactic treatment in a previous local study [37], whereas ESAT-6/CFP-10-nonresponsive cases constituted >80% of our TST-positive contacts (table 1). With regard to stratifying risk in TB contacts, in our epidemiological setting we have shown that the magnitude of the TST responses may not consistently reflect protective outcomes. The good correlation between mycobacterium killing activity and PPD responses in the ESAT-6/CFP-10-nonresponsive contacts supports the probability that strong PPD reactors in this group are unlikely to be at high risk. Conversely, strong PPD reactors in ESAT-6/CFP-10-positive contacts are likely to warrant closer follow-up as they have relatively weaker mycobacterium killing activity. Such a distinction between PPD-reactive contacts could help to channel healthcare resources based on risk. However, we emphasise that this strategy may only be applicable in a setting of moderate to high TB endemicity where there are obvious reasons for strong PPD reactivity other than LTBI. Our results also suggest that it is relevant to assess the latent infection rate in trial populations prior to TB vaccine studies as we found an association between strong PPD reactivity and poorer mycobacterium inhibition in LTBI subjects. Therefore, if LTBI is highly prevalent in the trial population and vaccines strongly inducing PPD reactivity are favoured, this may paradoxically lead researchers to choose vaccines that give rise to relatively poorer mycobacterium inhibition.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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