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Strong PPD responses are associated with poor mycobacterium inhibition in latent tuberculosis

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ABSTRACT

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

INTRODUCTION

The tuberculin skin test (TST), representing delayed type hypersensitivity to purified protein derivative (PPD) of *Mycobacterium tuberculosis* (Mtb), 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], since various *Mycobacterium* species have many genes in common. Thus, using 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 10 (CFP-10) are immunogenic TB proteins encoded by genes absent in all BCG strains and most environmental mycobacteria [4]. Hence, the use of *in vitro* interferon-gamma (IFN-γ) release assays, with ESAT-6/CPF-10 as Mtb-specific antigens, avoids cross-reactivity in BCG-vaccinated subjects, thereby overcoming some limitations of TST. The utility of these assays has been extensively reviewed [5, 6]. Numerous human studies worldwide have demonstrated that ESAT-6/CFP-10 is more specific than PPD in detecting LTBI [7, 8]. Although some studies suggest that sensitivity of ESAT-based assays is lower than 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 noted [6, 11]. However, the immune status of TB contacts with discordant results in PPD-based and ESAT-based tests has yet to be fully resolved.

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 this study, we asked whether the magnitude of PPD responses, whether by IFN-γ assays or 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, because we believe PPD reactivity reflects protective immunity in the former group, but not the latter.

This study was performed in Singapore where there is moderate TB endemicity, routine BCG vaccination in newborns and revaccination of TST-nonresponders in adolescents (between the years 1958 – 2001). Prevalent environmental mycobacteria exposure is likely due to the equatorial climate [3]. Hence, there are 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 of 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. TST, using 1 TU of PPD RT23 (Mantoux method), was read at 48-72 hrs. Contacts were recruited from amongst those offered chemoprophylaxis on clinical suspicion of LTBI, i.e. minimum criterion of TST ≥ 10 mm in those with one BCG scar and ≥ 16 mm for those with two BCG scars. These criteria were based on a prior local study relating TST size in schoolchildren and 4-year risk of reactivation [18]. Chest X-ray cavities and smear positivity in the index case were also considered in offering prophylaxis. Analysis for tuberculin reactivity amongst TB Contacts was adjusted for age, gender and BCG vaccination status, of which none were found to be sources of bias.

As comparator groups for TB Contacts, we also recruited 13 culture-positive pulmonary TB patients and 152 healthy community volunteers. The TB patients were PPD- and ESAT-6/CFP-10-reactive subjects within the first month of treatment. The 'Community' subjects (n=152) were from the local resident community 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 1 past BCG vaccination. Subjects aged above 85 or below 18 were excluded in all groups, and the age distribution was comparable in all healthy groups.

HIV infection was excluded on clinical history alone, as HIV rate amongst TB cases in Singapore is low. Informed consent from 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 institutional review boards.

Cell stimulation and IFN-γ release assay

IFN-γ released by 2 x 10^5 viable PBMCs in response to 20 μg/ml of either 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 enzyme-linked immunosorbent assay (OptEIA human IFN-γ, BD). The 5-day stimulation assay, rather than overnight stimulation, was used because it reflects memory response to the antigens [20], and minimises risk of weak reactors being missed as prolonged incubation increases sensitivity for detecting LTBI [21]. IFN-γ levels four standard deviations 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 [22]. Briefly, 2 × 10^5 viable PBMCs were seeded in RPMI 1640 (Sigma-Aldrich) supplemented with 2 mM L-glutamine (Sigma-Aldrich) and 10% foetal calf serum within 96-well plates. A

sonicated preparation of dead Mtb (H37Rv strain, 1 μg/ml), or phosphate buffered saline (control wells), was included in the cultures to expand mycobacterium-specific lymphocytes. After 2 days, 2×10^5 live *Mycobacterium bovis* BCG (Pasteur strain) were added per well to infect the macrophages. Concurrently, supplementary ferric ammonium citrate (50 μg/ml) was added for supporting optimal mycobacterium growth [23]. Three days later, non-adherent 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), then pulsed with 1 μCi of ^{3}H-uridine (Amersham) for 48 hours [24]. The radiolabelled BCG cultures were harvested onto membranes (Tomtec cell harvester, PerkinElmer) 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 studied in this assay – 13 TB subjects, 8 ESAT-6/CFP-10 responsive Contacts, 8 ESAT-6/CFP-10 responsive Community, 27 ESAT-6/CFP-10 nonresponsive Contacts and 17 ESAT-6/CFP-10 nonresponsive Community (total n = 73).

Statistics

Kruskal-Wallis H test was used for multi-group comparisons to determine if the difference between groups was statistically significant. If this was significant, it was followed up with Dunn's multiple comparison test (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 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 noted 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 mm and 14.5 mm respectively in LTBI (ESAT-6/CFP-10 responding) Contacts and ESAT-6/CFP-10 nonresponsive Contacts (not shown); this difference was not statistically significant. Amongst 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, not shown).

TABLE 1. *In vitro* responses to PPD- and ESAT-6/CFP-10 based assays in different clinical groups

Group (no. per group)	No. tested positive† (% positive)		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%)	-	-

Footnotes:

*'LTBI' 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.

† Criteria for defining positive antigen-induced IFN-γ response given in Methods. Percentage positive responders is expressed out of each sub-group total.

‡ TST was not performed for Community subjects. Contacts with TST < 10 mm were excluded from study. The reason for dichotomising the TST responses at 16 mm was that a previous local study on schoolchildren suggested that those with ≥ 16 mm TST readings had a higher risk of developing TB within 4 years [18].

Strong TST responders in ESAT-6/CFP-10 nonresponding 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 magnitude of the TST response related to *in vitro* mycobacterium inhibition in Contacts. Higher viable BCG counts per minute 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 strongest TST responses (≥ 16 mm) showed the best *in vitro* mycobacterium inhibition, better than all LTBI groups. Amongst ESAT-6/CFP-10 nonresponsive Contacts, the TST ≥ 16 mm group also showed better mycobacterium killing than the TST 10-15 mm group. However, amongst 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 if 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. Therefore, Contacts and Community were divided into PPD^{Hi} and PPD^{Low} groups based on IFN-γ responses (≥ 900 pg/ml = PPD^{Hi}, < 900 pg/ml = PPD^{Low}),

then the mycobacterium inhibition was compared. The cut-off value was based on 70% of all LTBI subjects having at least this level of response. Since IFN-γ activates infected macrophages to kill intracellular Mtb [25], we hypothesised that strength of PPD-specific IFN-γ responses might be positively associated with mycobacterium killing activity.

By this *in vitro* PPD IFN-γ release assay (fig. 1B), the Contacts showed a similar pattern of responses as for the TST – i.e. PPD^{Hi} 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^{Hi} 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, supplementary fig. 1A). In contrast, the Community subjects showed no differential mycobacterium killing activity either based on PPD IFN-γ reactivity (supplementary fig. 1B) or LTBI status (fig. 1C).

Since 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 nonresponders 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 anti-mycobacterium immunity in such people). Conversely, the magnitude of PPD reactivity in infected persons might not correlate with killing activity. This would explain prior observations that PPD responses are not always linked to protection [13]. Therefore, we separately assessed 'infected' persons, including latent and active TB groups (healthy ESAT-6/CFP-10 responders and TB patients), versus the 'uninfected' group (ESAT-6/CFP-10 nonresponders in both Contacts and Community). All subjects across the entire spectrum of PPD responses for both 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.

There was an interesting dichotomy in the correlation graphs. Amongst ESAT-6/CFP-10 nonresponders, the higher the PPD IFN-γ responses, the better the mycobacterium killing activity (fig. 3A). Conversely, amongst '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 population 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. TB Contacts in our study had an average age of 40 years. 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 Mtb in Singapore, where TB incidence is moderately high.

Two main points were shown by our data. First, strongly PPD-reactive ESAT-6/CFP-10 nonresponders in our population have good anti-mycobacterium immunity. They not only showed better mycobacterium killing than the ESAT-6/CFP-10 nonresponders with lower PPD reactivity (fig. 1A, 1B), but magnitude of PPD-reactivity was also correlated with killing (fig. 2A, 3A). These support the probability that in those without latent infection in our study population, PPD reactivity reflects the strength of anti-mycobacterium immunity in each person, consistent with a report that tuberculin-positive subjects show better inhibition of mycobacteria growth than tuberculin-negative individuals [17]. Second, our strongly PPD-reactive ESAT-6/CFP-10 nonresponsive Contacts have distinct anti-mycobacterium immunity from LTBI Contacts, as the former had better mycobacterium inhibition activity (fig. 1A, 1B). 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 fig. 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, there are various publications which support this assumption. Using a mycobacterium inhibition assay, Silver showed an association between *in vitro* lymphocyte responses and inhibition of intracellular Mtb growth [28]; thus the assay reflects contribution of mycobacteria-specific T cells to protection. Hoft found that inhibition of intracellular Mtb replication significantly increased after BCG vaccination of initially PPD-nonreactive persons [15, 16], thus reflecting BCG-induced protective immunity. Taken together with evidence that tuberculin-positive persons can restrict growth of BCG better than tuberculin-negative persons [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, that the assay is specific for mycobacterium-reactive T cells has been shown by ourselves (supplementary fig. 2) and others [22], 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 Mtb sonicate stimulation of lymphocytes from LTBI cases may result in expansion of ESAT-specific lymphocytes which 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 nonresponder subjects. However, with our lymphocyte cultures exposed to both whole Mtb sonicate and live BCG, there is likely to be considerable expansion of T cells specific for the many immunodominant antigens shared between BCG and Mtb, 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 persons 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 Mtb-responsive T cells, thus favouring Mtb persistence [31]. Perhaps 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 T

regulatory cells) has been associated with suppressed Mtb-specific immunity [32-34]. In late stage murine TB, transforming growth factor-beta, a suppressive cytokine associated with regulatory T cells, impairs the host's ability to limit Mtb 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 form 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 constitute > 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 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 accounting for strong PPD reactivity other than LTBI. Our findings also suggest it is relevant to assess the latent infection rate in trial populations prior to TB vaccine

studies. This is because we find 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.

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FIGURE LEGENDS

FIGURE 1. Mycobacterium inhibition in TB Contacts and/or Community subjects according to TST and *in vitro* PPD IFN-γ responses. ^{3}H-uridine uptake was used as a measure of mycobacterium viability, higher CPM indicate more viable bacteria.

(A): TB Contacts segregated according to TST size. Those with positive and negative responses to ESAT-6/CFP-10 are called 'LTBI' and 'ESAT-6/CFP-10 nonresponders' respectively. (B) and (C): PPD^{Hi} and PPD^{Low} groups amongst TB Contacts (B) and Community subjects (C) have PPD-stimulated IFN-γ ≥ 900pg/ml and < 900pg/ml respectively. Bars represent group medians. Multi-group comparisons by 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. Each symbol represents one subject.

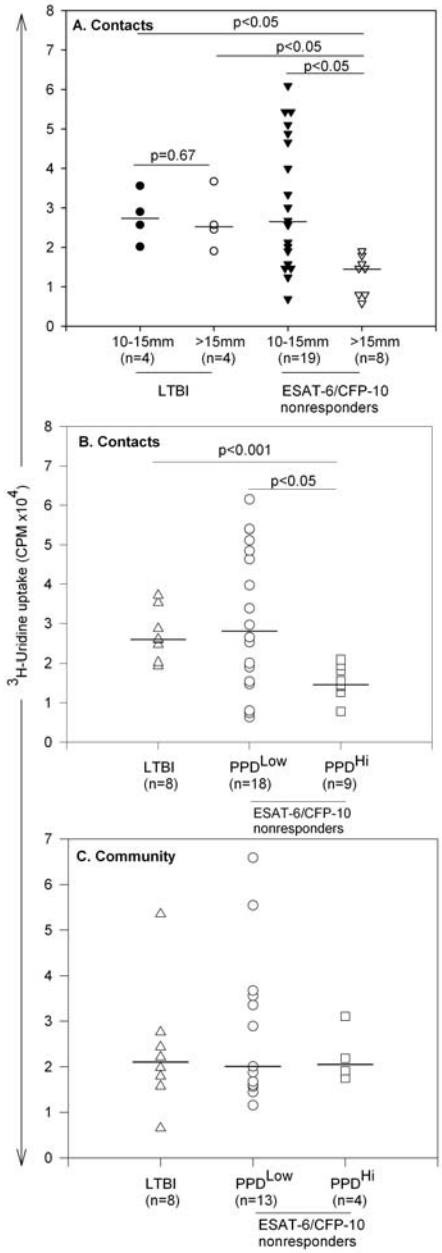


FIGURE 2. Correlation of TST outcomes in relation to mycobacterium inhibition in TB Contacts. Regression line and coefficient shown for correlations between TST sizes and

^{3}H-uridine uptake for ESAT-6/CFP-10 nonresponder Contacts (A) and LTBI Contacts (B). Statistics by Spearman rank sum correlation test.

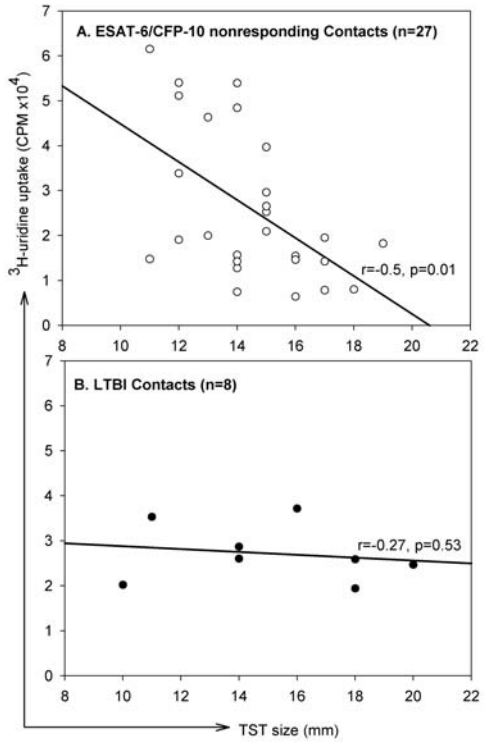
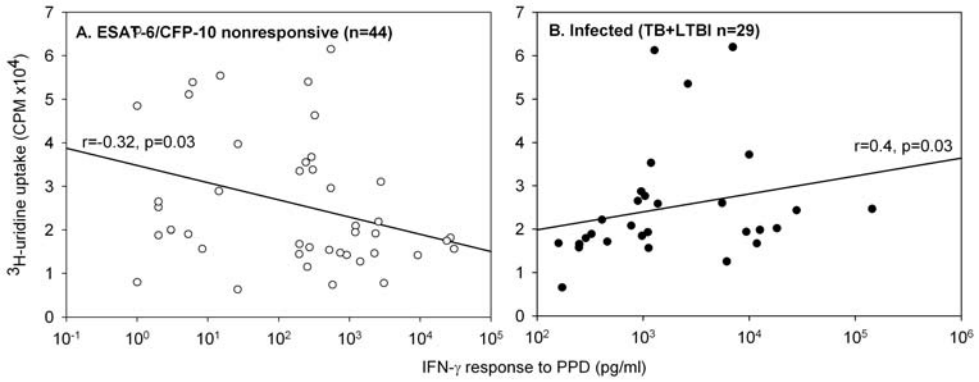

A. ESAT-6/CFP-10 nonresponding Contacts (n=27)
r=-0.5, p=0.01
B. LTBI Contacts (n=8)
r=-0.27, p=0.53
^{3}H-uridine uptake (CPM x10^4)
TST size (mm)

FIGURE 3. Opposite correlation trends between mycobacterium inhibition and PPD IFN-γ response in 'uninfected' group (A) ESAT-6/CFP-10 nonresponders, and in 'infected' group (B) TB and LTBI subjects. Both groups include Contacts and Community subjects. Regression line and coefficient shown, statistics by Spearman rank sum correlation test.


A. ESAT-6/CFP-10 nonresponsive (n=44)
B. Infected (TB+LTBI n=29)
r=-0.32, p=0.03
r=0.4, p=0.03
^{3}H-uridine uptake (CPM x10^4)
IFN-γ response to PPD (pg/ml)