CD8$^+$ T Cells Provide an Immunologic Signature of Tuberculosis in Young Children

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Rationale: The immunologic events surrounding primary *Mycobacterium tuberculosis* infection and development of tuberculosis remain controversial. Young children who develop tuberculosis do so quickly after first exposure, thus permitting study of immune response to primary infection and disease. We hypothesized that *M. tuberculosis*–specific CD8$^+$ T cells are generated in response to high bacillary loads occurring during tuberculosis.

Objectives: To determine if *M. tuberculosis*–specific T cells are generated among healthy children exposed to *M. tuberculosis* and children with tuberculosis.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Production of IFN-γ by host T cells is known to be essential to the human immune response to *Mycobacterium tuberculosis* (Mtb). However, little is understood regarding the immune response to a primary Mtb exposure that results in disease, an outcome that is common in young children.

What This Study Adds to the Field

Mtb-specific CD8$^+$ T cells were present among young children with tuberculosis disease, but not healthy children recently exposed to Mtb. This indicates that an Mtb-specific CD8$^+$ T cell response is generated in response to high bacillary load and may be able to distinguish children with TB disease from those with Mtb exposure.

Methods: Enzyme-linked immunosorbent spot assays were used to measure IFN-γ production in response to *M. tuberculosis*–specific proteins ESAT-6/CFP-10 by peripheral blood mononuclear cells and CD8$^+$ T cells isolated from Ugandan children hospitalized with tuberculosis (n = 96) or healthy tuberculosis contacts (n = 62).

Measurements and Main Results: The proportion of positive CD8$^+$ T-cell assays and magnitude of CD8$^+$ T-cell responses were significantly greater among young (<5 yr) tuberculosis cases compared with young contacts ($P = 0.02$, Fisher exact test, $P = 0.01$, Wilcoxon rank-sum, respectively). *M. tuberculosis*–specific T-cell responses measured in peripheral blood mononuclear cells were equivalent between groups.

Conclusions: Among young children, *M. tuberculosis*–specific CD8$^+$ T cells develop in response to high bacillary loads, as occurs during tuberculosis, and are unlikely to be found after *M. tuberculosis* exposure. T-cell responses measured in peripheral blood mononuclear cells are generated after *M. tuberculosis* exposure alone, and thus cannot distinguish exposure from disease. In young children, IFN-γ–producing *M. tuberculosis*–specific CD8$^+$ T cells provide an immunologic signature of primary *M. tuberculosis* infection resulting in disease.

Keywords: *Mycobacterium tuberculosis*; infant; child; CD8-positive T lymphocytes; enzyme-linked immunosorbent spot

Mycobacterium tuberculosis (Mtb), the etiology of tuberculosis (TB), causes over 9 million cases of disease and 1.7 million deaths annually (1). The only available vaccine to prevent TB, bacillus Calmette-Guérin, offers little protection against the most

common disease manifestations (2) and efforts to develop an improved vaccine are hampered by poor understanding of immunologic events that occur after Mtb exposure. Scientific studies of immunologic responses to initial Mtb infection are difficult because most individuals living in TB-endemic settings have experienced multiple Mtb exposures. Young children, however, suffer disproportionately after exposure to Mtb, because they are at substantial risk for developing TB after primary infection (3–5). Therefore, young children with TB offer a valuable window into the human immune response to primary Mtb infection.

The fundamental importance of IFN-γ production in response to Mtb has been shown in animal models, and through identification of severe mycobacterial disease in humans with genetically altered IFN-γ signaling pathways (6). Although CD4$^+$ T cells are an important source of IFN-γ, evidence suggests that CD8$^+$ T cells also contribute to Mtb containment (7–13). Because our prior work has shown that Mtb-specific CD8$^+$ T-cell clones preferentially recognize dendritic cells heavily infected with Mtb (9), we hypothesized that IFN-γ producing Mtb-specific CD8$^+$ T cells are generated in response to high bacillary burden. Therefore, Mtb-specific CD8$^+$ T cells would be present in individuals with TB and, unlike Mtb-specific T-cell responses elicited from unfractionated peripheral blood mononuclear cell (PBMC) populations, absent in healthy individuals with recent Mtb exposure.

To test our hypotheses, we studied children because their unique vulnerability to develop TB after initial exposure offers an opportunity to capture the immunologic response to a recently established Mtb infection. In Uganda, we compared Mtb-specific T-cell responses elicited from unfractionated PBMC with those elicited from CD8$^+$ T cells, between young and older healthy children exposed to Mtb. In addition, we compared Mtb-specific T-cell responses elicited from PBMC with those elicited from CD8$^+$ T cells, between healthy children exposed to Mtb and age-matched acutely ill children with probable and confirmed pulmonary TB. We provide evidence that young children with a heavy burden of disease harbor Mtb-specific CD8$^+$ T cells, thus offering insight into the human immune response to primary Mtb infection. Some of these results have been previously reported in poster and platform presentations (14, 15).

METHODS

Participants and Procedures

Healthy children exposed to Mtb (HE) and children acutely ill with confirmed or probable pulmonary TB (CP-TB) were enrolled from separate recruitment sites in Kampala, Uganda (Figure 1). For the HE group, we evaluated child household contacts (age <15 yr) of adults with culture-confirmed pulmonary TB (16). For the CP-TB group, we enrolled acutely ill, HIV-uninfected, hospitalized children (age <10 yr) meeting World Health Organization criteria for confirmed or probable intrathoracic TB (*see* Table E1 in the online supplement) (17). Subject recruitment and study coordination were sponsored by the Tuberculosis Research Unit at Case Western Reserve University, Cleveland, OH.

For all participants, demographic and clinical information were collected and a physical examination and anterior chest radiograph were performed. Blood was drawn at enrollment for enzyme-linked immunosorbent spot (ELISPOT) analysis and HIV testing, before or within 72 hours of tuberculin skin test (TST) placement. Nutritional status was determined by comparing individuals' body mass index (BMI) with World Health Organization child growth standards to generate a Z-score. Hospitalized children with suspected TB had a mycobacterial smear and culture of one induced sputum sample performed.

For the HE cohort, children were followed for 6–24 months and those with symptoms concerning for TB evaluated. Children with a positive TST, and children less than 5 years, were offered 9 months of isoniazid prophylaxis after TB was excluded. Children with a history of prior or current TB, children who developed TB within 6 months of enrollment, and children who were HIV-infected or immunosuppressed were excluded.

Children with suspected TB received a standard four-drug regimen per Ugandan national treatment guidelines. Children received a final designation of confirmed TB, probable TB, or not TB based on results of their diagnostic work-up and response to treatment at 2 months. Children classified as not TB were excluded.

IFN-γ ELISPOT Assay

Overnight IFN-γ ELISPOT assays were performed as described previously (18) using a single synthetic peptide pool consisting of 15-mers overlapping by 11 aa, representing Mtb-specific proteins CFP-10 and ESAT-6. For the CD8 ELISPOT assay, CD8$^+$ T cells were negatively selected from PBMCs using a combination of CD4 and CD56 magnetic beads (Miltenyi Biotec, Auburn, CA). For the PBMC ELISPOT, unfractionated PBMCs were used as the source of responding T cells.

Study Design and Statistical Analysis

We performed a cross-sectional study examining Mtb-specific T-cell responses elicited from PBMC (PBMC ELISPOT]) and isolated CD8$^+$ T cells (CD8 ELISPOT) at baseline from two clinical study groups: children with CP-TB and HE children. All analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). All categorical comparisons between study groups were performed using Student *t* test for continuous variables, and chi-square test (or Fisher exact test where indicated) for categorical variables. The magnitude of spot-forming units (SFU) above background between groups was compared using a nonparametric analysis (Wilcoxon rank-sum test). A positive ELISPOT assay was defined by an antigen-specific response at least two SD above the background control plus 5 SFU. If these criteria were met, the background was subtracted out to determine the antigen-specific response.

To study the factors associated with CP-TB, logistic regression models were constructed to examine the independent influence of the CD8$^+$ T-cell response (CD8 ELISPOT, categorical), the PBMC response (PBMC ELISPOT, categorical), age group (<5 and >5), nutritional status (BMI Z score, continuous), and TST (categorical) on the odds of having TB compared with the reference group (HE). Backward logistic regression was performed with significance level of 0.05. The online supplement provides additional details.

Ethics Approval

This study received Institutional Review Board approval at all sponsoring institutions. Written, informed consent was obtained from participants' parents or guardians before enrollment.

Role of the Funding Source

The funders of the study had no role in study design; in the collection, analysis, and interpretation of data; in writing of the report; or in the decision to submit the paper for publication.

RESULTS

Patient Enrollment and Clinical Characteristics

To study the effect of age on PBMC and CD8$^+$ T-cell responses within the HE group, we evaluated 105 child household contacts age less than 15 years (Figure 1A). Exclusions included 19 children who developed TB within 6 months of enrollment and four children infected with HIV. Thus, we performed ELISPOT assays on 82 HE children age less than 15 years; 76 PBMC ELISPOT assays and 59 CD8 ELISPOT assays were included in the final analysis. To compare PBMC and CD8$^+$ T-cell responses between the HE and CP-TB group (age <10 yr), we included ELISPOT assay data from 62 eligible HE children who were age less than 10 years (Figure 1B).
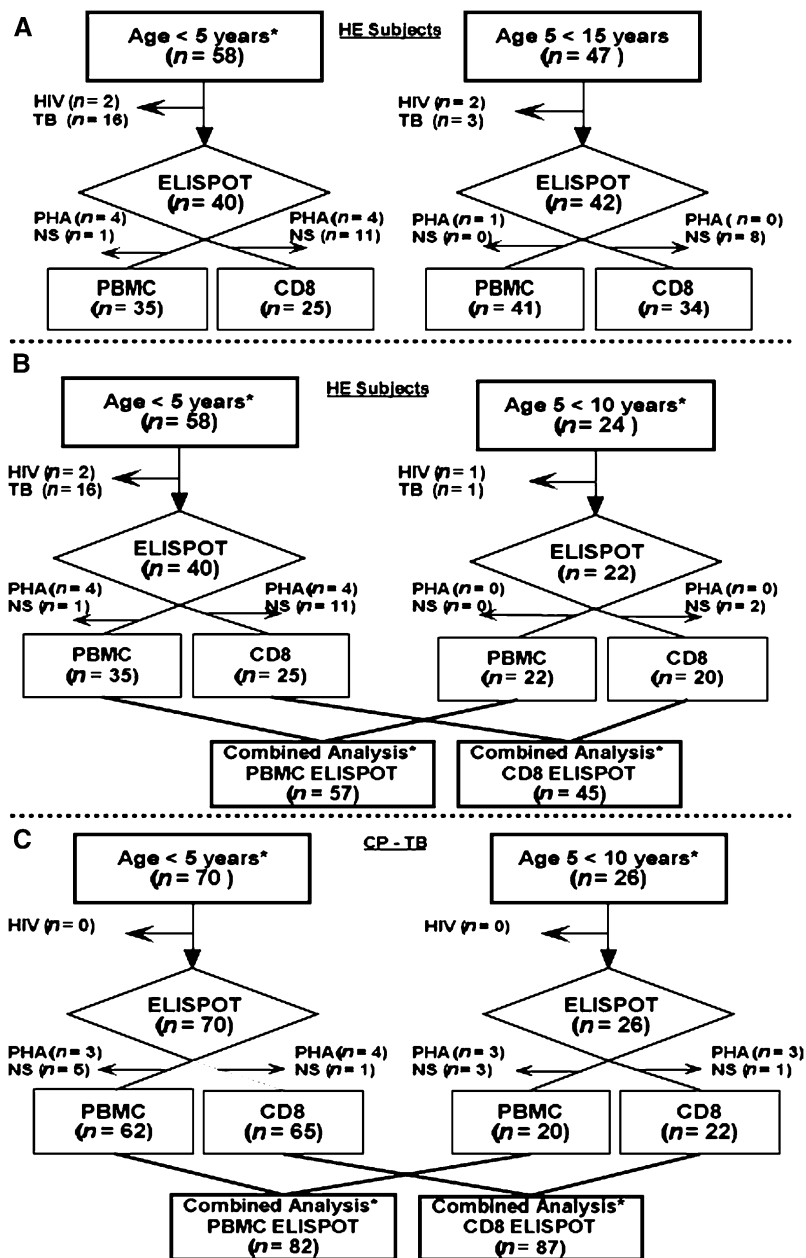
Figure 1. Flow diagram depicting enrollment into the study. Flow diagram of enrollment of (*A*) healthy exposed (HE) children stratified for ages 0–5 and 5–15 years, (*B*) HE children stratified for ages 0–5 and 5–10 years, and (*C*) children with confirmed or probable tuberculosis (CP-TB) stratified for ages 0–5 and 5–10 years. Children excluded because of HIV infection or TB (HE group only), and children excluded because the positive control response was low (phytohemagglutinin [PHA] response ≤20 spot-forming units) or peripheral blood mononuclear cell (PBMC) sample was insufficient (NS) for analysis, are shown. *Age groups of HE children used for comparison with the CP-TB group.

Regarding the CP-TB group, 101 children uninfected with HIV with suspected pulmonary TB (*see* Table E1) were assessed for eligibility. Of these, 96 children were enrolled into the CP-TB group with 28 (29%) classified as confirmed TB (C-TB) and 68 (71%) as probable TB (Prob-TB) at 2 months (*see* Table E1; 5 of 101 children with suspected TB were classified as not TB). There were 82 interpretable PBMC ELISPOT assays and 87 interpretable CD8 ELISPOT assays included in the final analysis (Figure 1C). There was no significant difference between the number of interpretable ELISPOTs between children with C-TB and Prob-TB.

Characteristics of children age less than 10 years enrolled in the HE and CP-TB groups, after exclusions for HIV infection (both groups) and TB (HE children only), are compared in Table 1. Children with CP-TB were more malnourished ($P = 0.006$) than the HE group and were slightly younger ($P = 0.01$). The frequency of a positive TST was equivalent between groups, as was the presence of bacillus Calmette-Guérin scar (data not shown). Baseline clinical characteristics (age, sex,

BMI Z-score, and TST status) of children included in the final analysis did not differ from those of children excluded because of failed ELISPOT assays (data not shown).

Mtb-specific PBMC and CD8^{+} T-cell Responses in Healthy Children Exposed to Mtb

We sought to determine if Mtb-specific PBMC and CD8^{+} T-cell responses differed between healthy individuals who had experienced their primary exposure to Mtb, and healthy individuals who had experienced multiple, lifetime exposures to Mtb and would be at increased risk for persistent infection. Therefore, we analyzed the magnitude of PBMC and CD8^{+} T-cell responses in the HE cohort by age group, because younger (<5 yr) children were less likely to have been exposed to Mtb previously compared with their older (5–15 yr), school-aged counterparts (4). We observed robust PBMC (PBMC ELISPOT) responses in both age groups, whereas CD8^{+} T-cell (CD8 ELISPOT) responses were decreased in children age less than 5 years compared with older children ($P = 0.055$) (Figure 2).

TABLE 1. BASELINE CHARACTERISTICS OF HEALTHY EXPOSED CHILDREN COMPARED WITH CHILDREN WITH CONFIRMED AND PROBABLE TB, AGE <10 YEARS

Category	Healthy Exposed (*n* = 62)	CP-TB (*n* = 96)	*P* Value
Female, n (%)	29 (47)	51 (53)	0.4*
Mean age, yr	4.7	3.6	0.01[†]
Age IQR	2.5 to 6	1 to 6	
Mean BMI Z score	0.09	−0.8	0.006[†]
BMI Z score IQR	−0.6 to 0.6	−2.1 to 0.6	
TST+, n (%)	28 (45)	40 (42)	0.6*

Definition of abbreviations: BMI = body mass index; CP = confirmed and probable; IQR = inter-quartile range; TB = tuberculosis; TST = tuberculin skin test.
* Chi-square test.
[†] Student *t* test Satterthwaite/unequal variances.

Comparison of Mtb-specific PBMC and CD8+ T-cell Responses Between Healthy Children Exposed to Mtb and Children with TB

To determine if Mtb-specific CD8+ T-cell responses are associated with a high bacillary load, we compared Mtb-specific PBMC and CD8+ T-cell responses between HE children and children with TB age less than 10 years. We determined if the magnitude of Mtb-specific PBMC and CD8+ T-cell responses differed among HE, C-TB, and CP-TB cohorts (Figure 3). For children age less than 5 years, the magnitude of the CD8+ T-cell response (CD8 ELISPOT) was greater in children with TB (CP-TB, *P* = 0.0156; C-TB, *P* = 0.009) than HE children, whereas PBMC (PBMC ELISPOT) responses were equivalent between groups (Figures 3A and 3B). For children age 5–10 years, the magnitude of CD8+ T-cell (CD8 ELISPOT) and PBMC (PBMC ELISPOT) responses were equivalent between the HE and CP-TB cohorts; however, because of the small number in the C-TB cohort age 5–10 yr statistical comparison was not performed (*see* Figure E1). These data demonstrate that among children age less than 5 years, Mtb-specific CD8+ T cells productive of IFN-γ are generated in response to TB but not Mtb exposure. This is in contrast to the Mtb-specific T-cell response elicited from PBMC that seems to be generated equally by young children with TB and those exposed to Mtb.

We next examined the proportion of positive CD8 and PBMC ELISPOT assays between young children with TB and HE children (Figure 4). The proportion of positive CD8 ELISPOT assays was significantly greater in children with C-TB (47%; n = 9/19; confidence interval [CI], 0.24–0.71; *P* = 0.02) and children with Prob-TB (35%; n = 16/46; CI, 0.21–0.50; *P* = 0.05) compared with HE children (12%; n = 3/25; CI, 0.03–0.31). The proportion of positive CD8 ELISPOTs was also significantly greater in young children with CP-TB compared with young HE children (data not shown). However, when we compared children age less than 5 years between cohorts, the proportion of positive PBMC

ELISPOT assays was equivalent between HE children (37%; n = 13/35; CI, 0.21–0.55) and children with C-TB (56%; n = 10/18; CI, 0.31–0.78; *P* = 0.2) (Figure 4), and between HE children and children with Prob-TB (32%; n = 14/44; CI, 0.18–0.47; *P* = 0.6). The proportion of positive PBMC ELISPOT assays was also similar between children with CP-TB and the HE children age less than 5 years (data not shown). Therefore, our analysis suggests that in young children less than 5 years, who have likely experienced their primary exposure to Mtb, a detectable CD8+ T-cell response to Mtb distinguishes TB from a history of Mtb exposure. The small number of children enrolled in the 5- to 10-year cohort precluded formal statistical analysis.

Statistical Associations with PBMC and CD8+ T-cell Responses to Mtb Antigens

Logistic regression analysis was performed to identify variables associated with CP-TB and included CD8 and PBMC ELISPOT assay results as a binary variable (positive or negative); age group (age <5 yr as reference); nutritional status (BMI Z score); and TST results (positive or negative) (*see* Table E2). Children with a positive CD8 ELISPOT assay result had a 3.7 times greater odds of having CP-TB compared with that of children who are healthy and exposed (*P* = 0.005) adjusted for age, nutritional status, and TST result (Hosmer-Lemeshow goodness-of-fit test, *P* = 0.2). By comparison, children with a positive PBMC ELISPOT assay did not have increased odds of having CP-TB compared with HE children (Hosmer-Lemeshow goodness-of-fit test, *P* = 0.15). In a model including both CD8 and PBMC ELISPOT assay results as covariates, a positive CD8 ELISPOT assay was significantly associated with CP-TB, adjusted for PBMC ELISPOT assay result, age, nutritional status, and TST result (odds ratio, 5.3; 95% CI, 1.7–15.8; *P* = 0.003; Hosmer-Lemeshow goodness-of-fit test, *P* = 0.13). To increase model fit, backward logistic regression was used. Here, the odds of having TB (CP-TB cohort) in someone with a positive CD8 ELISPOT assay was 3.9 times (CI, 1.5–9.8) that of someone in the HE group adjusted for age group only (*P* = 0.003; data not shown). The PBMC ELISPOT assay result did not add to the overall model fit and was eliminated in the backward iterative selection process along with nutritional status and TST result (Hosmer-Lemeshow goodness-of-fit test, *P* = 0.9; data not shown).

DISCUSSION

The evaluation of Mtb-specific immune responses in young children offers a unique window of opportunity to characterize the immunologic signatures of Mtb exposure, primary infection, and disease. Young children who are recently infected often progress to TB disease, providing a more accurate assignment of the timing of their primary infection (3, 5). In addition, young children by virtue of their age are less likely to have had repeated
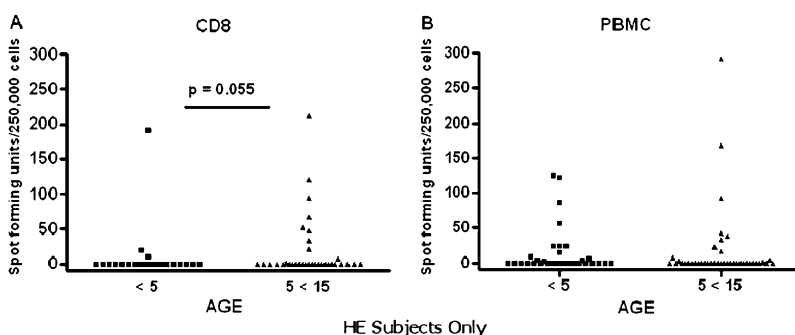
Figure 2. Comparison of *Mycobacterium tuberculosis*–specific T-cell responses in healthy exposed (HE) children stratified by age. (*A*) CD8 enzyme-linked immunosorbent spot (ELISPOT) assay results for HE children age less than 5 years (n = 25) compared with those for HE children age 5–15 years (n = 34) are shown. (*B*) Peripheral blood mononuclear cell (PBMC) ELISPOT assay results for HE children less than 5 years (n = 35) compared with those for HE children age 5–15 years (n = 41) are shown. Spot-forming units per 250,000 cells above background. Wilcoxon rank-sum test (one-sided) was performed comparing HE children less than 5 years with those age 5–15 years for the CD8 ELISPOT assay (*P* = 0.055) and for the PBMC ELISPOT assay (*P* = 0.2).
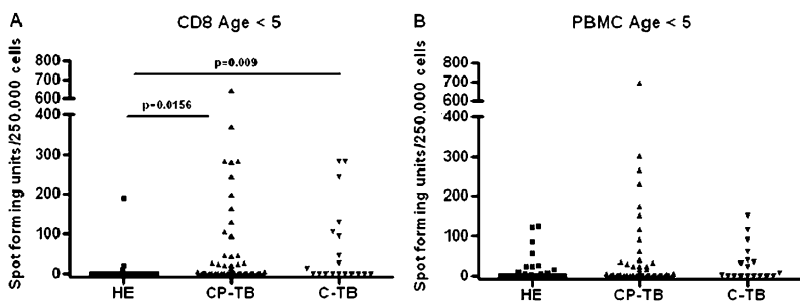
Figure 3. Magnitude of *Mycobacterium tuberculosis* (Mtb)–specific T-cell responses in young children with tuberculosis (TB) or health exposed (HE) children. The magnitude of *M. tuberculosis*–specific T-cell responses is shown. Results of CD8 enzyme-linked immunosorbent spot (ELISPOT) (*A*) and peripheral blood mononuclear cell (PBMC) ELISPOT (*B*) assays among children age less than 5 years are shown. The sample size of the subgroups is (*A*) HE (n = 25), CP-TB (n = 65), C-TB (n = 19); (*B*) HE (n = 35), CP-TB (n = 62), C-TB (n = 18). Spot-forming units per 250,000 cells above background. Two-sided Wilcoxon rank-sum test was performed comparing the HE with CP-TB, and the HE with C-TB groups. C-TB = confirmed tuberculosis; CP-TB = confirmed and probable tuberculosis.

exposures and reinfection that confounds similar studies of adults performed in TB-endemic settings (4).

Previous work has shown that CD8$^+$ T cells preferentially recognize dendritic cells harboring intracellular bacteria, especially cells heavily infected with Mtb (9). Although the *in vivo* relevance of such observations remains difficult to assess, the magnitude of the CD8$^+$ T-cell response has been shown to correlate with mycobacterial burden in TB animal models (19, 20). Moreover, these findings mirror work shown in HIV where there is a robust association between CD8$^+$ T-cell responses and viral set point (21), viral load dynamics (22), and viral control (23). Therefore, we postulated that an Mtb-specific CD8$^+$ T-cell response indicates true intracellular infection and that the magnitude of this response reflects bacillary burden. This is biologically plausible because CD8$^+$ T cells sample the intracellular environment by recognizing antigens presented by major histocompatibility complex class I; thus presence of such a response is both necessary and sufficient to discern an intracellular Mtb infection.

Our data show that Mtb-specific CD8$^+$ T-cell responses immunologically distinguish young children with TB from HE children. We postulate that HE children who demonstrate detectable T-cell responses in PBMC, but absent CD8$^+$ T-cell responses, may not have persistent intracellular infection. Rather, we suggest this PBMC response is dominated by CD4$^+$ T cells and reflects prior exposure or priming to Mtb antigens. Children with TB, however, who are expected to have a high intracellular bacillary burden, demonstrate robust CD8$^+$ and PBMC responses. Therefore, detection of an Mtb-specific CD8$^+$ T-cell response is not reflective of protective immunity, but rather increased mycobacterial load.

Although controversial, the possibility that Mtb exposure does not result in persistent infection is supported by animal models. Transient TST conversions have been observed in healthy guinea pigs with no evidence of underlying Mtb infection that had been exposed to patients with TB (24). More recent work has shown that roughly 30% of guinea pigs exposed to subjects with drug-resistant TB have transient TST conversions but no pathologic or microbiologic evidence of Mtb infection (25). In addition, high rates of TST reversions have been noted in employees with documented Mtb-exposure who undergo repeat TST testing (26). We propose that after Mtb exposure three distinct outcomes are possible: (*1*) an individual remains uninfected, (*2*) Mtb infection takes place but is resolved through a sterilizing immune response, or (*3*) a persistent Mtb infection is established. Therefore, HE children who demonstrated a positive TST or detectable Mtb-specific PBMC response but a negative CD8$^+$ T-cell response may have experienced previous antigen exposure to Mtb, but no longer harbor viable bacilli (the second outcome). Although an alternative interpretation of our data is that CD8$^+$ T cells are insensitive in detecting the low bacillary load associated with clinically asymptomatic Mtb infection, we propose that Mtb-specific CD8$^+$ T-cell responses

do not develop unless a persistent intracellular infection has occurred (the third outcome) as is expected during TB disease or after multiple exposures to Mtb. We speculate that HE children who demonstrated CD8$^+$ T-cell responses (*see* Figure E1), most of whom were school aged, had experienced multiple lifetime exposures to Mtb resulting in persistent infection.

It is unclear why young children are highly susceptible to develop TB. Evidence suggesting that a reduced Th1 cell response predisposes young children to fail to contain Mtb is controversial (27–30), and there are little data regarding the role of CD8$^+$ T cells in the immune response to Mtb in young children. In adults, CD8$^+$ T cells are observed in high frequency in individuals with latent TB infection and TB disease (12, 31–33) and CD8$^+$ T cells are present at extrapulmonary sites of infection (13). Although bacillus Calmette-Guérin vaccination induces IFN-γ–producing CD8$^+$ T cells in infants (34) and clonal expansion of Mtb-reactive CD8$^+$ T cells has been demonstrated in children with TB (35), this is the first study comparing antigen-specific CD8$^+$ T-cell responses between HE children and children with TB. Our finding that young children with TB disease have Mtb-specific IFN-γ–producing T cells suggests that failure to generate a Th1-type response is not responsible for the increased susceptibility of young children to TB.

In young children, infection with Mtb is associated with severe manifestations of TB, highlighting the importance of accurate and timely diagnosis. However, current diagnostic methods have limited use, because only 5–16% of children with suspected TB have positive acid fast bacillis smears and positive cultures are reported in only 20–50% of suspected cases (36–44). Although commercial IFN-γ release assays are more specific than the TST in adults (45) and children (46), IFN-γ release assays have
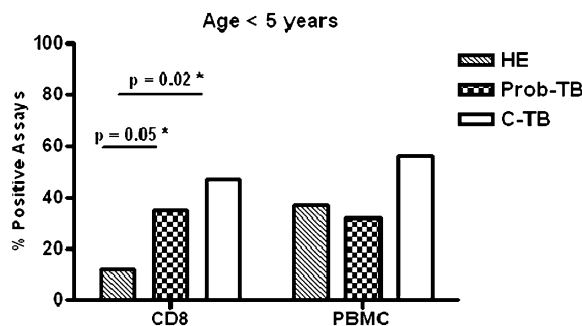
Figure 4. Proportion of positive *Mycobacterium tuberculosis*–specific T-cell responses in young children with tuberculosis (TB) versus healthy exposed (HE) children. The proportion of positive CD8 and peripheral blood mononuclear cell (PBMC) enzyme-linked immunosorbent spot (ELISPOT) assay results for HE children and children in the probable TB (Prob-TB) and confirmed TB (C-TB) groups age less than 5 years are shown. *The differences in proportions were tested using Fisher exact test.

limited use in TB-endemic settings because they do not reliably differentiate TB from latent TB (47–49). In this regard, we also found that measurement of ESAT-6/CFP-10–specific PBMC responses did not distinguish healthy children exposed to Mtb from children with TB. In contrast, we found that detection of an ESAT-6/CFP-10–specific CD8$^+$ T-cell response was specifically associated with TB rather than Mtb exposure. Our results suggest that measurement of CD8$^+$ T-cell responses could be developed as a more accurate diagnostic tool for childhood TB.

Our study has several limitations. First, the cross-sectional design of our study does not permit any insight into the kinetics of T-cell responses in children with Mtb exposure or after treatment for TB. Second, because our only measure of T-cell responses to Mtb was IFN-γ production, we may have failed to detect Mtb-specific T-cell responses characterized by the production of alternative cytokines or cytolytic markers. Third, the small number of children greater than 5 years in our CP-TB cohort limited statistical analysis of the results stratified by age within this cohort. Fourth, although unlikely because all children were recruited from the same geographic area, it is possible that our different recruitment strategies for children in the HE and suspected TB groups introduced biases because of differences in socioeconomic factors or duration of Mtb exposure. Lastly, this current study was not designed to evaluate the diagnostic accuracy of Mtb-specific PBMC or CD8$^+$ T-cell responses for childhood TB. Therefore the potential use of measurement of CD8$^+$ T-cell responses to diagnose childhood TB requires further study.

This is the first study to demonstrate that measurement of a CD8$^+$ T-cell response to ESAT-6 and CFP-10 by ELISPOT assay distinguishes acutely ill, young children with TB from healthy, exposed household contacts in a TB-endemic region. We believe our results are supportive of the hypothesis that Mtb-specific CD8$^+$ T cells develop in response to an established intracellular infection, and that the magnitude of the CD8$^+$ T-cell response is reflective of bacillary burden. This is distinctive from Mtb-specific responses found in PBMC that are generated in response to current or prior antigen exposure and therefore cannot discern exposure from true infection. Finally, these findings may have practical applications for development of a CD8$^+$ T-cell immunodiagnostic for TB in young children, as well as immune-based assays with the capacity to identify latently infected individuals at high risk for reactivation disease and assays that measure response to TB treatment.

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