**Abstract**

**Background:** Effector CD4 T cells represent a key component of the host's anti-tuberculosis immune defense. Successful differentiation and functioning of effector lymphocytes protects the host against severe *M. tuberculosis* (Mtbd) infection. On the other hand, effector T cell differentiation depends on disease severity/activity, as T cell responses are driven by antigenic and inflammatory stimuli released during infection. Thus, tuberculosis (TB) progression and the degree of effector CD4 T cell differentiation are interrelated, but the relationships are complex and not well understood. We have analyzed an association between the degree of Mtbd-specific CD4 T cell differentiation and severity/activity of pulmonary TB infection.

**Methodology/Principal Findings:** The degree of CD4 T cell differentiation was assessed by measuring the percentages of highly differentiated CD27lowIFN-γ+ cells within a population of Mtbd-specific CD4 T lymphocytes ("CD27lowIFN-γ" cells). The percentages of CD27lowIFN-γ+ cells were low in healthy donors (median, 3.1%) and TB contacts (21.8%) but increased in TB patients (47.3%, *p* < 0.0005). Within the group of patients, the percentages of CD27lowIFN-γ+ cells were uniformly high in the lungs (>76%), but varied in blood (12–2%). The major correlate for the accumulation of CD27lowIFN-γ+ cells in blood was lung destruction (*r* = 0.65, *p* = 2.7 × 10^-7). A cutoff of 47% of CD27lowIFN-γ+ cells discriminated patients with high and low degree of lung destruction (sensitivity 89%, specificity 74%); a decline in CD27lowIFN-γ+ cells following TB therapy correlated with repair and/or reduction of lung destruction (*p* < 0.01).

**Conclusions:** Highly differentiated CD27low Mtbd-specific (CD27lowIFN-γ+) CD4 T cells accumulate in the lungs and circulate in the blood of patients with active pulmonary TB. Accumulation of CD27lowIFN-γ+ cells in the blood is associated with lung destruction. The findings indicate that there is no deficiency in CD4 T cell differentiation during TB; evaluation of CD27lowIFN-γ+ cells provides a valuable means to assess TB activity, lung destruction, and tissue repair following TB therapy.

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**Introduction**

Tuberculosis (TB) is one of the most common infectious diseases worldwide. Prevention of TB dissemination in the community critically depends upon efficient TB diagnostics and treatment. Current strategies to diagnose TB disease and monitor TB treatment are based on microbiological, clinical and radiographic examinations, all having limitations. Microbiological diagnosis is limited because patients with less-extensive pulmonary TB often lack identifiable *Mtb* in their sputum, or sputum is unavailable for microbiological analysis; clinical symptoms are often non-specific; chest radiography does not allow to distinguish between active TB, inactive infection, and other lung pathologies. Thus, new TB tests are needed. Ideally, such tests should be based on blood sample analysis and should evaluate TB activity, i.e., they should distinguish TB disease from latent infection and assess disease activity in patients with diagnosed TB [1–3].

Immunological tests based on T cell analysis have a high potential for TB diagnostics and monitoring. Immunological assays based on the evaluation of T-cell mediated IFN-γ responses (i.e., T-SPOT.TB, Quantiferon-TB Gold) have proved to be useful for detecting *Mtb* infection [4–8]. Unfortunately, they have demonstrated poor ability to distinguish between active and latent TB [9–13], two forms of *Mtb* infection that differ in their contagiousness and treatment strategies. To overcome these
limitations, new approaches have been suggested, including phenotypic analysis of IFN-γ producing CD4 T cells [14,15] and quantification of polyfunctional and TNF-α producing CD4 T cells [16,17]. The applicability of these assays for discriminating between active and latent infections is being tested. In contrast, tests to evaluate disease activity in patients with diagnosed TB are unavailable. This is in spite of the fact that TB may have a spectrum of activities characterized by different degrees of lung pathology and disease severity and may require alternative treatment strategies.

Our previous studies performed in a mouse model of Mtb infection, suggested that it is possible to evaluate the infectious process ongoing in the lungs during TB by analyzing the proportion of the CD27<sub>low</sub> effector CD4 T cell subset. CD27, a member of the TNF-receptor superfamily [18], is constitutively expressed by naive T cells and early effector lymphocytes, but is downregulated at late stages of effector cell differentiation; accordingly, late effector lymphocytes exhibit low to no CD27 expression [19–24]. Late CD27<sub>low</sub> effector T cells differentiate from CD27<sub>hi</sub> effector precursors under antigenic and/or inflammatory stimuli [19,21]. Our studies in mice have demonstrated that during Mtb infection, CD27<sub>low</sub> effector CD4 T cells differentiate from CD27<sub>hi</sub> effector precursors directly in the lungs and their differentiation is promoted by lung Mtb infection [24,25]. We also showed that in mice, active Mtb infection leads to the accumulation of CD27<sub>low</sub> effector CD4 T lymphocytes in the lungs, blood, and other organs of infected mice [24,25]. In humans, patients with active pulmonary TB have higher frequency of CD27<sub>low</sub> Mtb-specific CD4 T cells than latently-infected individuals [14]. Based on these observations, we hypothesized that the degree of CD27<sub>hi</sub>→CD27<sub>low</sub> differentiation may serve as an indicator of disease activity within the lungs during TB. Here we addressed our hypothesis by evaluating CD27<sub>low</sub> Mtb-specific CD4 T cells in patients with pulmonary TB. We show an association between Mtb-induced lung tissue destruction and the accumulation of CD27<sub>low</sub> Mtb-specific CD4 T cells in the blood of TB patients and present evidence that evaluation of CD27<sub>low</sub> cells provides a means to assess lung destruction and tissue repair following TB therapy.

### Methods

#### TB Patients

All studies were approved by the IRB #1 of the Central Tuberculosis Research Institute of Russian Academy of Medical Sciences and were conducted in accordance to the principles expressed in the Helsinki Declaration. Seventy two patients under treatment in the CTRI gave written informed consent and were enrolled in the study. Two TB suspects with unconfirmed diagnosis were further excluded from the study. In 70 patients with diagnosed TB, diagnosis was based on clinical and/or radiographic evidence of TB plus either identification of Mtb in the sputum (60 patients) or response to anti-TB therapy (10 patients). Fifty one patients had recently diagnosed TB; 19 patients had chronic (>1 year) TB and had received several courses of therapy.

Of 70 patients included in the study, blood cell analysis was performed in 62 patients; in 8 patients, lung and blood cells were analyzed. Of 62 patients, 50 patients formed the main group (median age, 31 years; range 18–76 years; 30 women), and 12 patients – the validation group (median age, 31 years; range 18–76 years; 5 women, Table 1). In all 62 patients, immunological analysis was performed within 2.0±0.1 weeks of patients’ admittance to the hospital, and the clinical follow-up for these patients was at least 2 months. In 22 patients of the main and validation groups, additional immunological analysis was performed after 2-mo TB therapy (“dynamic” group). The clinical follow-up for these patients was 4 months.

Immunological analysis of lung cells was performed in 8 patients who underwent lung tissue surgery because of extensive lung disease or to distinguish between tumor and tuberculosis lesion (“surgery” group; median age, 34 years; range, 21–51 years; 4 women). In these patients, lung derived cells, blood cells and (where available) pulmonary lymph node cells were analyzed on the day of surgery.

All enrolled patients were HIV seronegative. All analyses were performed during years 2008–2011. Detailed clinical characteristics of enrolled patients are presented in Table S1.

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### Table 1. Characterization of groups included in the analysis.

| Characteristic | TB patients<sup>1</sup> | Mtb-unexposed<sup>1</sup> | TB contacts<sup>1</sup> |
|---------------|-----------------|-----------------|-----------------|
| **Gender**    |                 |                 |                 |
| Male          | 30 (60%)        | 5 (42%)         | 4 (50%)         |
| Female        | 20 (40%)        | 7 (58%)         | 8 (63%)         |
| **Age**       |                 |                 |                 |
| Median        | 31              | 31              | 34              |
| Range         | 18–71           | 18–76           | 21–51           |
| **TB duration**|                |                 |                 |
| Recent        | 39 (78%)        | 10 (83%)        | 2 (25%)         |
| Chronic       | 11 (22%)        | 2 (17%)         | 6 (75%)         |
| **Mtb in the sputum** |       |                 |                 |
| Mtb+          | 43 (86%)        | 12 (100%)       | 5 (63%)         |
| Mtb−          | 7 (14%)         | 0               | 3 (37%)         |
| **Form of TB pathology** |       |                 |                 |
| Tuberculosis  | 5 (10%)         | 1 (8%)          | 2 (25%)         |
| TB infiltrate | 30 (60%)        | 9 (76%)         | 0               |
| Cavitary TB   | 10 (20%)        | 1 (8%)          | 6 (75%)         |
| Caseous pn.   | 5 (10%)         | 1 (8%)          | 0               |

<sup>1</sup>Indicated are numbers (%).

<sup>2</sup>NA, not applicable.

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TB Contacts and Healthy Donors
Healthy CTRI employees (n = 21) working in tight contacts with TB patients for at least 1 year (median, 7 years; range 1–51 years) and having no clinical or radiographic evidence of tuberculosis were enrolled in the study as *Mtb*-exposed individuals ("TB contacts"); median age, 43 years; range 25–74 years; 16 women; Table 1). The results of QuantiFERON-TB Gold in-tube test (QFT; Cellestis Ltd, Carnegie, Australia) were positive in 10 persons ("QFT TB contacts") and negative in 11 persons ("QFT TB contacts", Table S2).

A group of healthy individuals included 15 participants with no records of *Mtb* exposure, negative results of QFT test and no clinical signs of TB ("*Mtb*-unexposed"); median age, 20 years; range, 19–71 years; 8 women; Table 1).

Clinical and Radiographic Evaluations of TB Patients
Disease manifestations were evaluated and scored by independent clinicians and radiologists who were unaware of the results of immunological assays. Clinical disease severity was scored from 0 to 3 based on the extent of systemic intoxication symptoms (fatigue, sweating, fever, weight loss); 0 – no symptoms; 1 – one to two symptoms without fever; 2 – several symptoms plus sub-febrile body temperature; 3 – several symptoms plus febrile temperature. Hematologic abnormalities were scored based on changes in erythrocyte sedimentation rate, leukocytosis, lymphopenia, left neutrophilic shift (0 – no abnormalities; 1 – one abnormality; 2 – two abnormalities; 3 – at least three severe abnormalities).

TB extent, the form of lung TB pathology and the degree of lung tissue destruction were evaluated based on radiographic examinations. TB extent (the area affected by TB lesions) was scored as: 1 – one to three segments in different lobes; 2 – four or more segments in different lobes or one-two whole lobe(s); 3 – three lobes in different lungs; 4 – one whole lung or both lungs. The degree of lung tissue destruction was assessed based on the number and size of destructive (lucent) foci: 0 – no foci; 1 – one small (<2 cm diameter) focus; 2 – several small (<2 cm) foci or one large (≥2 cm) transparent focus; 3 – multiple foci of which at least one is large (≥2 cm, Fig. 1). The forms of lung TB pathology included: tuberculoma, TB infiltrate, cavitary TB, caseous pneumonia (Table 1, Table S1).

**CD27**

**CD4 T Cell Analysis**

Percentages of CD27** low** lymphocytes were determined within: i) the total population of CD4 T cells ("CD27** low** cells"); ii) *Mtb*-specific population of CD4 T cells ("CD27**low**IFN-γ**+**" cells). *Mtb*-specific lymphocytes were identified as CD4 T cells producing IFN-γ in response to stimulation with *Mtb*-specific antigens ("IFN-γ**+**" cells, Fig. 2A–C). For CD27**low** cells, 200 ul of freshly isolated blood were stained with PerCP-Cy5.5 anti-CD4 and PE-anti-CD27 mAbs (BD Biosciences, San Diego, USA; 10 min, room temperature). Red blood cells were lysed with BD FACS Lysing solution (BD Biosciences); the cells were washed and analyzed. Following analysis, the percentages of CD27**low** cells within total population of CD4 T lymphocytes were determined (Fig. 2A). For CD27**low**IFN-γ**+** cells, 0.5 ml of whole blood was diluted 1/1 in RPMI and cultured in the presence of *Mtb* sonicate (37°C, 5% CO2, 10 mg/ml protein of *Mtb* sonicate prepared as described earlier, [26]). The stimulation was performed within 5 hours of blood collection (preliminary analysis showed that this storage did not affect the results of the test). As a negative control, an aliquot of blood was always cultured without addition of *Mtb* sonicate. After 4 hours of culture, GolgiPlug (BD Biosciences) was added and cells were incubated for additional 14 hours. Cells were then stained with PerCP-Cy5.5-anti-CD4 and PE-anti-CD27 mAbs, treated with BD FACS Lysing solution and BD FACS Permeabilizing solution II, stained with APC-anti-IFN-γ mAbs (BD Biosciences), fixed and analyzed.

Following analysis, percentages of IFN-γ-producing cells were determined within the CD4**+** population (Fig. 2B); percentages of CD27**low** cells – within IFN-γ**+** lymphocytes (Fig. 2C). The details of the gating strategy are presented in Fig. 2 A–C. The validity of the gates for IFN-γ**+** cells was confirmed by fluorescence minus one control (not shown). In *Mtb* sonicate un-stimulated samples, IFN-γ**+** cells were largely absent, supporting that IFN-γ**+** cells identified in stimulated samples represented *Mtb*-specific lymphocytes.

Absolute numbers of CD27**low**IFN-γ**+** cells per one mL of blood were calculated as follows: *N*CD27**−**–IFN-γ**+** / *N*CD27**−** – percentages of CD27**low** cells within IFN-γ**+** cells; *P*IFN-γ**+** – percentages of IFN-γ**+** cells within CD4 T lymphocytes, *P*CD4**+** – percentages of CD4**+** cells within all lymphocytes, *N*lymph – numbers of lymphocytes in 1 mL of blood (determined in hematology test on the day of analysis).

For lung and lymph node (LN) cell analysis, suspensions were prepared from samples of surgically resected lung tissue or lymph nodes. Cells were stimulated, stained and analyzed as described above. To compare pools of IFN-γ**+** and CD27**low**IFN-γ**+** cells in the lungs, lymph nodes and blood, absolute numbers of these lymphocytes per one million of acquired cells were calculated. All cells were analyzed using a BD Biosciences FACSCalibur with CellQuest (BD Biosciences) and FlowJo (Tree Star) softwares. Several reagents were kindly gifted to us by BD Biosciences.

**Statistical Analysis**

In the text, if not indicated otherwise, medians, 25% and 75% percentiles are shown. Differences between unpaired and paired samples were analyzed using non-parametric Mann-Whitney and Wilcoxon tests. Sensitivity and specificity were determined by Receiver Operating Characteristic (ROC) curve analysis (GraphPad Software, Inc., San Diego, CA); cutoffs were selected based on the maximum sensitivity and specificity summary. Correlations were performed using Spearman analysis (GraphPad Software; program R, http://www.r-project.org). For multiple correlation analyses, p-value of 0.007 (7 variables) was considered significant to account for multiple testing. Best models were selected by multiple regression analysis using F-test for nested models and Akaike Information Criterion [27–29]. In bootstrapping analysis a pseudo dataset was created by re-sampling the data; 1000 simulations were performed and in each the best minimal model was selected using Akaike Information Criterion [28].

**Results**

**TB Patients have Higher Percentages of *Mtb*-specific CD27**

**low** CD4 T Cells in their Blood than *Mtb* Un-exposed Individuals and TB Contacts

Our previous studies in mice suggested that the degree of CD27**low**→CD27**low** differentiation may serve as an indicator of disease activity within *Mtb*-infected lungs. To test this hypothesis, in the present study we evaluated the degree of CD27**low**→CD27**low** differentiation of CD4 T cells in patients with pulmonary TB.

The differentiation status of CD4 T cells was evaluated by determining the percentages of CD27**low** cells within the total population of CD4 T cells ("CD27**low** cells") and within *Mtb*-specific subset of CD4 T cells ("CD27**low**IFN-γ**+**") cells. *Mtb*-specific cells were identified as CD4 T cells producing IFN-γ in response to stimulation with mycobacterial antigens ("IFN-γ**+**") cells.

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CD27**low** Cells and Lung Tissue Destruction
In the first part of the study we compared the percentages of CD27\textsuperscript{low}, IFN-\(\gamma\), and CD27\textsuperscript{low}IFN-\(\gamma\) cells in the blood of TB patients (\(n = 50\)), TB contacts (\(n = 21\)) and \(Mtb\)-unexposed individuals (\(n = 15\)).

The percentages of CD27\textsuperscript{low} cells were higher in TB patients, relative to \(Mtb\)-unexposed individuals (12.2 [8.3–23.0] vs 8.2 [5.5–15.8], respectively; \(p = 0.03\)), but did not differ between TB patients and TB contacts (12.2 [8.3–23.0] vs 11.9 [10.1–17.6]; \(p > 0.5\); Fig. 2D). Similarly, the percentages of IFN-\(\gamma\) cells were higher in TB patients as compared to \(Mtb\)-unexposed individuals (0.3 [0.2–0.5] vs 0.1 [0.1–0.3]), but were similar in TB patients and TB contacts (0.3 [0.2–0.5] vs 0.3 [0.1–0.4]; \(p > 0.5\), Fig. 2E). The later finding is consistent with other studies, which revealed that IFN-\(\gamma\) assays were not useful for discriminating active TB disease and latent \(Mtb\) infection [11]. Thus, neither the expression of CD27 on bulk CD4 T cells...
lymphocytes, nor the frequency of *Mtb*-specific IFN-γ producing CD4 T cells, discriminated active TB from latent infection.

In contrast, the percentages of CD27lowIFN-γ+ cells were significantly higher in TB patients (47.3 [33.2–63.6]) compared to either *Mtb*-unexposed participants (33.1 [16.8–39.5], p<0.0005)

Figure 2. TB patients have increased percentages of CD27lowIFN-γ+ CD4 T cells in their blood. A–C, Strategies for determining percentages of CD27low (A), IFN-γ+ (B) and CD27lowIFN-γ+ (C) CD4 T cells. A, CD27low cells were gated within the total population of CD4+ T cells. B, To identify IFN-γ+ CD4 T cells, an aliquot of blood was stimulated with *Mtb* sonicate; another aliquot was left un-stimulated. During the analysis, the gates for IFN-γ+ cells in *Mtb*-stimulated samples were plotted based on *Mtb* un-stimulated samples (Fig. B, dotted line). To identify CD27lowIFN-γ+ cells, the expression of CD27 was first analyzed in IFN-γ+ population. Because this population was always numerous, CD27low and CD27hi cells could be easily separated. The gates for CD27low IFN-γ+ population (C, dotted line). D–F, Percentages of CD27low (D), IFN-γ+ (E), and CD27lowIFN-γ+ (F) cells in TB patients (n = 50), TB contacts (n = 21) and *Mtb*-unexposed individuals (n = 15). G, Lack of correlation between the percentages of IFN-γ+ and CD27lowIFN-γ+ cells in TB patients, TB contacts and *Mtb*-unexposed individuals (n = 86). H, ROC-curve of CD27lowIFN-γ+ cell percentages for discriminating TB patients from healthy individuals (TB contacts and *Mtb*-unexposed). I, Percentages of CD27lowIFN-γ+ cells in TB contacts with positive and negative results of QFT assay *p*<0.0005 compared to TB patients.

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or TB contacts (21.8 [18.0–28.5], p<0.0001, Fig. 2F), which was in line with results published previously by Kern’s group [14]. The percentages of IFN-γ+ and CD27lowIFN-γ+ cells were not statistically correlated (r = −0.03, p = 0.8, Fig. 2G). Thus, TB patients and TB contacts differed, not by the frequency of Mtb-specific CD4 T cells, but rather, by the degree of their differentiation.

ROC curve analysis confirmed that the percentages of CD27lowIFN-γ+ cells (but not CD27low or IFN-γ+ cells) provided a means to distinguish TB patients and TB contacts (82% sensitivity and 91% specificity, Table 2). Moreover, TB patients could be distinguished from all healthy participants (i.e., TB contacts and Mtb-unexposed individuals; 74% sensitivity and 83% specificity; Table 2, Fig. 2H). A cut-off value for the percentages of CD27lowIFN-γ+ cells that separated TB patients from all healthy participants with best specificity and sensitivity was 35.1%; this value was used in our further analyses as an upper limit of the norm.

Because some of TB contacts included in our study had positive results of QFT assay (QFT+, n = 10), while others were negative (QFT−, n = 11), we analyzed whether these two subgroups differed by the percentages of CD27lowIFN-γ+ cells. The percentages were similar in both subgroups (p = 0.6); furthermore, the percentages did not differ from those observed in Mtb-unexposed individuals (p > 0.2, Fig. 2F), but differed significantly from TB patients (p < 0.0005). On ROC curve, similar cut-off values for the percentages of CD27lowIFN-γ+ cells separated TB patients from QFT+ and QFT− contacts (Table 2). Overall, the percentages of CD27lowIFN-γ+ cells were uniformly low in all healthy individuals and were significantly increased in patients with active TB. These results were in line with previous reports [14] and suggested that CD27lowIFN-γ+ cells could serve as a measure of TB activity.

In TB Patients, Accumulation of Mtb-specific CD4 CD27low T Cells in Peripheral Blood is Associated with Lung Tissue Destruction

In comparison with Mtb-unexposed participants and TB contacts, TB patients had higher percentages of CD27lowIFN-γ+ cells. Yet, these percentages varied substantially among individual patients (i.e., from 12 to 92%, Fig. 2F). Previously, several authors had also observed variability among TB patients in the percentages of CD27low Mtb-specific CD4 T cells [14,30]. However, the mechanisms underlying this variability and its relevance to TB monitoring remained unaddressed.

To start addressing these mechanisms, we analyzed whether the percentages of CD27lowIFN-γ+ cells were associated with different TB manifestations. The following characteristics of TB disease were taken into account: duration of TB disease, the presence of Mtb in sputum smear/culture, Mtb drug resistance, the form of lung pathology, the extent of pulmonary TB, the degree of lung tissue destruction, disease severity (assessed separately based on clinical symptoms and hematologic abnormalities). All characteristics were evaluated blindly by physicians, radiologists and microbiologists who were unaware of the results of immunological assays.

As a first step, we divided TB patients into groups with different levels of a given factor and compared the percentages of CD27lowIFN-γ+ cells in these groups. We found that the percentages of CD27lowIFN-γ+ cells did not differ between groups of patients with different TB extent, with different disease durations, and with high and low content of Mtb in the sputum (p = 0.01, p = 0.04, and p = 0.01, respectively, insignificant for multiple parameter testing; Fig. 3A–C). In contrast, the percentages of CD27lowIFN-γ+ cells differed significantly between groups of patients having different degrees of lung destruction, clinical disease severity, and different forms of lung TB pathology (p < 0.007, Fig. 3D–F).

Next, we looked at correlations between the percentages of CD27lowIFN-γ+ cells and magnitude of a given factor. In this analysis, factors that correlated most significantly with the percentages of CD27lowIFN-γ+ cells were: lung tissue destruction (r = 0.65, p = 2.7 × 10−7), clinical disease severity (r = 0.63, p = 7.7 × 10−7), hematologic abnormalities (r = 0.49, p = 5 × 10−3). The presence of Mtb in the sputum and TB extent correlated less strongly (r = 0.41, p = 0.003, and r = 0.42, p = 0.003, respectively; Table 3). The correlations between the percentages of CD27low “IFN-γ+ cells and Mtb drug resistance or TB duration were insignificant (p = 0.01 and p = 0.04, respectively, insignificant when adjusted for multiple comparisons). The correlation between the percentages of CD27lowIFN-γ+ cells and the form of lung pathology was highly significant (r = 0.63, p = 8.7 × 10−7), but it was likely secondary to lung destruction: it appeared only when higher scores were assigned to more destructive TB forms (i.e., tuberculum – score 1; TB infiltrate – score 2; cavity TB – score 3; caseus pneumonia – score 4); within a group of patients with the same form of lung TB pathology (e.g., TB infiltrate) the percentages of CD27lowIFN-γ+ cells correlated with lung destruction (r = 0.45, p < 0.01).

While the percentages of CD27lowIFN-γ+ cells correlated strongly with several TB characteristics (e.g., TB severity, lung destruction), the numbers of CD27lowIFN-γ+ cells (per ml of blood) did not correlate significantly with either of the factors analyzed (Table S3). This result can be explained by the fact that percentages of CD27lowIFN-γ+ cells represent a simple parameter that mirrors only the degree of CD27low→CD27− CD4 T cell differentiation. In contrast, the number of CD27lowIFN-γ+ cells

| Group of comparison | AUC | 95% CI | p-value | Best cutoff, % | Sensitivity, % | Specificity, % | LR | OR |
|---------------------|-----|--------|---------|---------------|---------------|--------------|----|----|
| All healthy participants (Mtb-unexposed and TB contacts) | 0.86 | 0.78–0.93 | <0.0001 | 35.1 | 74 | 83 | 3.2 | 14.2 |
| All TB contacts | 0.89 | 0.81–0.97 | <0.0001 | 31.2 | 82 | 91 | 5.0 | 41.5 |
| QFT+ TB contacts | 0.87 | 0.75–0.99 | 0.0003 | 31.2 | 82 | 90 | 5.0 | 41.5 |
| QFT− TB contacts | 0.92 | 0.83–1.00 | <0.0001 | 28.2 | 90 | 91 | 9.1 | 90 |
| Mtb-unexposed | 0.80 | 0.69–0.92 | 0.0004 | 35.1 | 74 | 73 | 2.8 | 7.9 |

The percentages of CD27lowIFN-γ+ cells were compared in TB patients and indicated groups of healthy participants. AUC, area under curve; CI, confidence interval; LR, likelihood ratio; OR, odds ratio.

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represents a complex parameter that, besides the percentages of CD27lowIFN-\(\gamma\)^+ cells in TB patients \((n = 50)\) grouped based on different characteristics of TB disease. For multiple (seven) parameter testing, \(p\) value \(< 0.007\) was considered significant. G, ROC curve for discriminating TB patients with high (score 3) and low (scores 0–2) degrees of lung tissue destruction \((n = 50)\). H, The degree of lung tissue destruction in patients with "low" (<47%) and high (>47%) percentages of CD27lowIFN-\(\gamma\)^+ CD4 T cells \((n = 12,\) validation analysis). I, J, Lack of correlation between the percentages (I) and the numbers (J) of CD27lowIFN-\(\gamma\)^+ cells in the lungs and in the blood of TB patients \((n = 8)\). Indicated are numbers of CD27lowIFN-\(\gamma\)^+ cells per 1 million of acquired cells.

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Figure 3. Association between blood CD27lowIFN-\(\gamma\)^+ cells and different manifestations of TB disease. A–F, Percentages of CD27lowIFN-\(\gamma\)^+ cells in TB patients \((n = 50)\) grouped based on different characteristics of TB disease. For multiple (seven) parameter testing, \(p\) value \(< 0.007\) was considered significant. G, ROC curve for discriminating TB patients with high (score 3) and low (scores 0–2) degrees of lung tissue destruction \((n = 50)\). H, The degree of lung tissue destruction in patients with "low" (<47%) and high (>47%) percentages of CD27lowIFN-\(\gamma\)^+ CD4 T cells \((n = 12,\) validation analysis). I, J, Lack of correlation between the percentages (I) and the numbers (J) of CD27lowIFN-\(\gamma\)^+ cells in the lungs and in the blood of TB patients \((n = 8)\). Indicated are numbers of CD27lowIFN-\(\gamma\)^+ cells per 1 million of acquired cells.

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Because the percentages of CD27lowIFN-\(\gamma\)^+ cells correlated significantly with several different manifestations of TB disease, we next addressed, which of the identified correlates were most important for determining the percentages of CD27lowIFN-\(\gamma\)^+ cells in the blood of TB patients. In multiple regression analysis, high percentages of CD27lowIFN-\(\gamma\)^+ cells correlated significantly with lung destruction and clinical TB severity (Table 3). In several additional analyses (F-test for nested models and model selection using Akaike Information Criterion), lung destruction, clinical disease severity and hematologic abnormalities, or lung destruction and clinical disease severity alone predicted the percentages of CD27lowIFN-\(\gamma\)^+ cells (Table 4). Any of these factors taken separately did not predict the percentages of CD27lowIFN-\(\gamma\)^+ cells well, suggesting that lung tissue destruction and TB severity were important and both were needed to
explain increased percentages of CD27lowIFN-γ+ cells in TB patients. When a method for random data resampling was used [26], lung destruction and clinical TB severity also appeared most often as factors determining high percentages of CD27lowIFN-γ+ cells in TB patients (13% and 15% of cases, respectively).

Because the degree of lung tissue destruction appeared as a good correlate of the percentages of CD27lowIFN-γ+ cells and because destruction of pulmonary matrix is an important pathogenic factor that causes TB progression, morbidity, and bacillary spread, we next asked whether evaluation of CD27lowIFN-γ+ cells could be used as a means to estimate pulmonary destruction.

Table 3. Correlation between TB manifestations and percentages of CD27lowIFN-γ+ CD4 T cells in the blood of TB patients.

| Factors                        | Simple correlation (Spearman) | Multiple linear regression |
|--------------------------------|-------------------------------|---------------------------|
|                                | n    | rho | p-value | Estimate | Std. error | t-value | p-value |
| Lung tissue destruction        | 50   | 0.65| 2.7 x 10^-7 | 4.439   | 2.127     | 2.087   | 0.043   |
|                                | 62   | 0.66| 5 x 10^-9   | 5.8296  | 2.0309    | 2.870   | 0.00578 |
| Clinical TB severity           | 50   | 0.63| 7.7 x 10^-7 | 8.579   | 2.458     | 3.490   | 0.001   |
|                                | 62   | 0.55| 3.2 x 10^-6 | 6.0474  | 2.5473    | 2.374   | 0.02105 |
| Hematologic abnormalities      | 50   | 0.49| 3.0 x 10^-4 | 1.258   | 2.200     | 0.572   | 0.570   |
|                                | 62   | 0.5 | 3.4 x 10^-5 | 1.7958  | 2.2448    | 0.800   | 0.4270  |
| Sputum Mtb positivity          | 50   | 0.41| 0.003      | 1.358   | 2.505     | 0.542   | 0.590   |
|                                | 62   | 0.36| 0.004      | -0.2399 | 2.5495    | -0.094  | 0.92538 |
| TB extent                      | 50   | 0.42| 0.003      | 0.447   | 2.188     | 0.204   | 0.839   |
|                                | 62   | 0.38| 0.002      | 1.1142  | 2.0340    | 0.548   | 0.5860  |
| Mtb multi-drug resistance      | 50   | 0.37| 0.01       | NA      | NA        | NA      | NA      |
|                                | 62   | 0.28| 0.04       | NA      | NA        | NA      | NA      |
| TB duration                    | 50   | 0.29| 0.04       | NA      | NA        | NA      | NA      |
|                                | 62   | 0.28| 0.02       | NA      | NA        | NA      | NA      |

1 Analysis was initially performed in 50 patients. Subsequently, 12 patients from validation cohort were added (n = 62), mainly to check the consistency of the results. The results obtained in both cohorts are shown.

2 Simple correlation analysis selects five major predictors for the accumulation of IFN-γ+CD27low cells in the blood of TB patients (highlighted in bold). For TB duration and Mtb multi-drug resistance p-values were >0.007 (insignificant for multiple (seven) parameter testing); these factors were not included in multiple linear regression analysis. rho, Spearman coefficient, p, significance value of the test.

3 Multiple linear regression identified lung tissue destruction and clinical TB severity as the main correlates for the accumulation of CD27lowIFN-γ+ cells in the blood of TB patients (highlighted in bold).

4 NA, not included in multiple linear regression analysis.

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Table 4. Selection of minimal model to explain variability in the percentages of CD27lowIFN-γ+ cells between TB patients.

| Factor(s)                                           | F test (p), n = 50^2 | F test (p), n = 62^2 |
|-----------------------------------------------------|----------------------|----------------------|
| Minimal model with single factor^1                   |                      |                      |
| Lung tissue destruction                              | 0.01618              | 0.02315              |
| Clinical TB severity                                 | 0.1017               | 0.01493              |
| Hematologic abnormalities                            | 4.6 x 10^-5          | 2.8 x 10^-4          |
| Sputum Mtb positivity                                | 2.7 x 10^-6          | 2.0 x 10^-6          |
| TB extent                                            | 1.7 x 10^-5          | 1.6 x 10^-5          |
| Minimal models with several factors^3                |                      |                      |
| Lung destruction + clinical TB severity + hematologic abnormalities | 0.7972               | 0.8571               |
| Lung destruction + clinical TB severity^2            | 0.7701               | 0.7804               |
| Lung destruction + hematologic abnormalities         | 0.0062               | 0.0890               |
| TB severity + hematologic abnormalities              | 0.1178               | 0.0248               |

1 Best minimal models are those that differ insignificantly from the full model (highlighted in bold).

2 Analysis was initially performed in 50 patients. Subsequently, 12 patients from validation cohort were added (n = 62), mainly to check the consistency of the results. In both cohorts, lung destruction and clinical disease severity predicted best the accumulation of CD27lowIFN-γ+ cells.

3 In Akaike Information Criterion, this combination was the best minimal model to predict the accumulation of CD27lowIFN-γ+ cells in the blood (Δn=50 = 4.7; Δn=62 = 4.8).

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On the Receiver Operating Characteristic (ROC) curve, a cutoff of 47% of CD27lowIFN-γ+ cells appeared as a good separator of TB patients with severe lung destruction (score 3) and other patients (scores 0–2) (AUC = 0.90, p<0.0001, odds ratio = 20.7, likelihood ratio = 7.1, sensitivity, 89%, specificity, 74%; Fig. 3G). Thus, in our study a cutoff of 35.1% of CD27lowIFN-γ+ cells discriminated TB patients and healthy participants; a cutoff of 47% predicted the degree of lung destruction in TB patients.

To validate these results, a group of additional 12 patients was enrolled. In these patients, we determined the percentages of CD27lowIFN-γ+ cells and compared them to 35.1 and 47% thresholds. Disease threshold (35.1%) was exceeded in 9 of 12 patients (data not shown); therefore, the test identified TB patients with 75% sensitivity, which is close to the results obtained in the main group (74%). Severe lung destruction threshold (47%) was exceeded in 7 patients. As judged by the lung radiograms, 5 of these 7 patients had severe destructive lesions. In 5 of 12 patients, the percentages of CD27lowIFN-γ+ cells were below the 47% threshold. As judged by the lung radiograms, none of these 5 patients had severe destructive lesions (Fig. 3H), which is again consistent with our findings in the main group. Overall, these results support our hypothesis that analysis of CD27lowIFN-γ+ cells circulating in peripheral blood can be used to evaluate severity of destructive processes ongoing in the lungs during TB.

At the Site of Lung Mtb Infection, the Majority of Mtb-specific CD4 T Lymphocytes are CD27low

Our previous studies in mice demonstrated that during Mtb infection, CD27low CD4 T cells accumulate preferentially in the lungs and can be generated from CD27high precursors in the lungs [25]. We proposed, therefore, that variability in the percentages of CD27lowIFN-γ+ cells in blood of TB patients was due to differences in the generation of CD27low CD4 T cells in the lungs. To address this question, we analyzed the frequencies and numbers of CD27lowIFN-γ+ and IFN-γ+ cells in the lungs and blood of eight patients who had undergone lung surgery.

In all patients, the percentages and numbers of IFN-γ+ and CD27lowIFN-γ+ cells in the lungs were significantly higher than in the blood (p<0.002; Table S4), supporting that Mtb-specific (IFN-γ+) and highly differentiated Mtb-specific (CD27lowIFN-γ+) CD4 T cells accumulated preferentially in the lungs.

Among the patients, the percentages and numbers of IFN-γ+ cells, and the numbers of CD27lowIFN-γ+ cells in the lungs varied (5–31-fold). In contrast, the percentages of CD27lowIFN-γ+ cells in the lungs were uniformly high (>76% of all IFN-γ+ cells), indicating that the majority of Mtb-specific lung CD4 T cells were highly differentiated (Table S4). The percentages of blood CD27lowIFN-γ+ cells differed among the patients (30–74%). No significant correlation was observed between the percentages of CD27lowIFN-γ+ cells residing in the lungs and circulating in the blood (Table S4, Fig. 3, I, J), indicating that variability in the percentages of CD27lowIFN-γ+ cells in blood of TB patients was not due to differences in the generation (accumulation) of these cells in the lungs. A similar lack of correlation was found for IFN-γ+ cells (Table S4). Furthermore, the percentages of CD27lowIFN-γ+ or IFN-γ+ cells in the lungs did not correlate significantly with any of the criteria used to categorize TB disease (i.e., disease severity, lung destruction et al., Table S4). In contrast, the percentages of CD27highIFN-γ+ cells in the blood were well correlated with lung destruction (r = 0.83, p = 0.01, Table S4), which was consistent with our results obtained in the main group.

In two patients, regional lymph nodes were resected during the surgery. The percentages and numbers of CD27lowIFN-γ+ cells were lower in the LNs as compared to the lungs (per 1 million of acquired cells, Table S4). This is in line with our previous findings in mice where we found low percentages of CD27low and IFN-γ+ CD4 T cells in the lymph nodes and their preferential accumulation in the lung tissue [24,25].

Thus, we observed (i) a preferential accumulation of CD27low Mtb-specific CD4 T cells in the lungs; (ii) a poor correlation between the magnitude of CD27low Mtb-specific CD4 T cell response in the lungs and in the blood; (iii) that lung tissue destruction was correlated with the percentages of CD27lowIFN-γ+ cells present in the blood, but not in the lungs. These observations suggested that evaluation of CD27low Mtb-specific CD4 T cells circulating in the blood may provide a means to assess pulmonary destruction during TB.

Following TB Treatment, the Percentages of Mtb-specific CD27low CD4 T Cells Decline Parallel to Reduction/cessation of Lung Tissue Destruction

Next we investigated whether and how the percentages of circulating CD27lowIFN-γ+ CD4 T cells change following TB treatment. Analyses were performed at the start of treatment and 2 months later. Totally, 22 patients with initially elevated percentages of CD27lowIFN-γ+ cells (above 35.1%) were included in the analysis. Based on changes in the percentages of CD27lowIFN-γ+ cells, the patients were divided into three groups (Fig. 4A). For each patient, responsiveness to treatment in terms of repair/reduction of pulmonary destruction, reduction of disease severity and conversion of sputum assay were evaluated blindly and compared to the results of “IFN-γ/CD27” assay.

In 13 of 22 patients, the percentages of CD27lowIFN-γ+ cells did not decline by the end of 2-mo therapy or declined only slightly to remain above 47% threshold (threshold that in our study discriminated TB patients with severe and mild lung tissue destruction; “CD27-high” group). Radiologically, the majority of patients (10 patients, 77%) retained severe lung destruction (score 3) till the end of 2-mo therapy (Fig. 4B). In four of these patients foci of severe destruction persisted during at least 4 months of treatment (follow-up period).

In 6 patients, CD27lowIFN-γ+ cell percentages reduced significantly (by >30% of the initial value) and reached less than 47% threshold level, although they did not normalize completely (i.e., remained >35.1% threshold, “CD27-reduced” group). Radiologically, reduction of lung tissue destructions by the end of 2-mo therapy was registered in 3 patients (50%), and by the end of 4-mo therapy - in all followed-up patients (n = 6).

In 3 patients, the percentages of CD27lowIFN-γ+ cells decreased to achieve normal values by the end of 2-mo therapy (<35.1%, “CD27-normalized” group). Radiologically, in all three patients lung destructive foci repaired by the end of 2-mo therapy.

Thus, high percentages of CD27lowIFN-γ+ cells during therapy were associated with persistence of tissue destruction, while reduction or normalization of CD27lowIFN-γ+ cell percentages was associated with the ongoing or completed repair of the damaged tissue. Overall, there was a significant correlation between a decline in the percentages of blood CD27lowIFN-γ+ cells and repair of lung destruction (r = 0.54, p<0.01).

In contrast to lung destruction, no significant correlation was revealed between changes in the percentages of CD27lowIFN-γ+ cells and conversion of sputum assay or clinical TB improvement (r = 0.23, p = 0.31 and r = 0.22, p = 0.31, respectively). These factors improved prior to lung tissue repair; their improvement was observed even in patients with persistently high percentages of CD27lowIFN-γ+ cells (Fig. 4C–D).

Overall, in our study, blood CD27lowIFN-γ+ cells appeared as a measure of Mtb infection activity in the lungs increased.
percentages of these cells distinguished TB patients from latently infected individuals; in TB patients, extremely high percentages of CD27lowIFN-γ+ cells were indicative of severe lung tissue destruction; following TB treatment, a decrease in the percentages of CD27lowIFN-γ+ cells was predictive of lung tissue repair.

**Discussion**

In this study we have demonstrated that: (i) active TB induces a large increase in the highly differentiated CD27low subset of Mtb-specific CD4 T cells in the lungs and in peripheral blood; (ii) an increase in the percentages of these cells in the blood during TB is associated with lung tissue destruction and disease severity; (iii) evaluation CD27low Mtb-specific CD4 T cells provides a valuable means to assess TB activity, the degree of Mtb-induced lung destruction and lung tissue repair following TB treatment.

CD27 is a member of the TNF-receptor superfamily. Lack of CD27 expression on T lymphocytes marks functionally mature highly differentiated effector T cells [20,21,23,24]. Our data on high percentages of CD27low cells within a population of Mtb-
specific CD4 T lymphocytes in TB patients, indicate that there is no deficiency in the generation of CD4 effectors during severe TB. Instead, active TB is accompanied by a high degree of CD4 T cell differentiation, which is in line with our previous data in mice showing that Mtb infection drives CD27low→CD27high differentiation of effector CD4 T cells [25]. Our results correspond well to the results by Kern’s group [14] who first described an increase in the frequency of CD27low Mtb-specific CD4 T cells in TB patients and suggested that evaluation of these cells can be used to discriminate TB patients from latently infected individuals.

It should be noted that a cutoff discriminating TB patients and healthy individuals in our study (35.1%) was lower than that reported by Kern’s group (49%). The most likely explanation relies on methodological differences (stimulation of whole blood in our study versus stimulation of PBMC in the study by Kern’s group) and/or the differences in patients’ selection. Indeed, in the study by Kern’s group, patients with several lesions and at least one lesion larger than 3 cm diameter were enrolled; in our study, patients with single lung lesion and no signs of tissue destruction (i.e., patients in whom TB diagnostics is most problematic) were also included. As shown in our study, the latter patients have relatively low percentages of CD27lowIFN-γ+ cells, and their inclusion in the analysis could lower the threshold discriminating TB patients from healthy participants. The major focus of the current study is the analysis of CD27low response in TB patients.

We found that at the site of Mtb infection in the lung, most Mtb-specific CD4 T lymphocytes (>76%) were CD27low. These results were obtained in all analyzed patients and supported a propensity of CD27low cells for lung tissue location documented previously in different pathological conditions in mice and humans [25,31–34].

In contrast to the lungs, the percentages of CD27lowIFN-γ+ cells in the blood varied among the patients. There was no significant correlation between the percentages of these cells in the blood and in the lungs, but a significant correlation was found between the percentages of CD27lowIFN-γ+ cells in the blood and lung tissue destruction and TB severity. The percentages of CD27lowIFN-γ+ cells in the lung did not correlate with lung destruction or TB severity. The reasons for discordance between lung and blood CD27lowIFN-γ+ cells and mechanisms associating blood CD27low-IFN-γ- cells, lung destruction and TB severity are not clear.

Effector T cells are thought to be generated in the LNs and migrate to peripheral sites via the circulation. We have shown previously that CD27low CD4 effectors can be generated directly in the lungs from their CD27high effector precursors [25]. Therefore, high percentages of CD27lowIFN-γ+ cells in the blood of patients with severe TB can result from their highly efficient generation in the LNs (e.g., due to extensive antigenic/inflammatory stimulation) and/or preferential retention in the circulation (e.g., due to highly inflammatory milieu). Uniformly high percentages of CD27lowIFN-γ+ cells at the site of lung Mtb infection may be due to their efficient local formation. These scenarios explain an association between CD27lowIFN-γ+ cells and lung tissue destruction and TB severity, but do not explain why the percentages of blood CD27lowIFN-γ+ cells did not depend on TB extent, or why they were tightly associated with lung destruction. There are several possible explanations for the later association.

First, lung destruction and accumulation of CD27lowIFN-γ+ cells in the circulation may represent independent manifestations of severe TB. Second, CD27lowIFN-γ+ cells generated in high quantities during severe TB may directly contribute to tissue destruction, e.g., by producing proinflammatory factors or stimulating inflammatory reactions in phagocytic cells. Indeed, in mice CD27low CD4 T cells mediate these functions more efficiently than CD27high effectors (our unpublished observations); a role for metalloproteinases and proinflammatory cytokines in lung matrix destruction has been directly demonstrated [35,36]; in other pathological conditions, CD27low T cells have been associated with poor disease outcome [34]. However, these scenarios do not answer a question why blood but not lung CD27lowIFN-γ+ cells were associated with lung tissue damage. They also do not explain why the percentages, but not the numbers of cells were associated with TB severity and lung destruction.

In a third hypothesis, accumulation of CD27lowIFN-γ+ cells in blood is a result of lung destruction. In this hypothesis, CD27low CD4 T cells represent a lung tissue resident population and normally have low capacity for tissue exit resembling recently described lung-retentive memory cells [37]. Once the architecture of the lung tissue and/or lung vessels is disrupted, CD27low lymphocytes acquire the capacity to enter the circulation. This may become possible due to mechanical reasons or to a high local production of factors (e.g., proteases) altering cell adhesion to lung parenchyma. Undoubtedly, all described mechanisms may cooperate to promote the accumulation of CD27lowIFN-γ+ cells in the circulation. Exact mechanisms associating high degree of blood CD4 T cell differentiation and lung tissue destruction are yet to be determined.

In conclusion, this study documents that there is no deficiency in the differentiation of effector CD4 T cells during severe pulmonary TB. The study for the first time reveals an association between CD27low Mtb-specific CD4 T cells and lung tissue destruction and suggests an immunological assay to evaluate lung destruction and its repair following TB therapy (processes that until now could have been monitored only by X-ray examination).

Finally, the study raises fundamental questions on mechanisms associating peripheral tissue destruction and differentiation status of circulating effector lymphocytes.

Supporting Information

Table S1 Baseline characteristics of patients included in this study. In all patients, immunological analysis of blood cells was performed at the beginning of treatment. Initial analysis was performed in 50 patients (“main” group). The results were validated in 12 patients (“validation” group). In some patients, additional analysis was performed 2 months following the treatment (“dynamic” group). In patients undergoing lung surgery, blood and lung cells were analyzed on the day of surgery (“Surgery” group). 4Indicated are areas (lobes, segments) of lungs affected by Mtb infection. Lobes: UR, upper right; MR, middle right; LR, lower right; UL, upper left; LL, lower left; S1, S2, etc., segments of lobes. 5Indicated are numbers of destructive foci. Small, the size (diameter) of the focus is <2 cm. For multiple foci the size of the largest is shown in parentheses (if >2 cm); system, system of communicating destructive foci. 6Responsiveness to TB treatment was assessed 2 months following the therapy based on the results of X-ray examination (reduction of lung tissue infiltration; reduction/repair of lung destruction), hematology test and clinical follow-up. 0, no positive dynamics; +, reduction of lung tissue infiltration/repair, normalization of hematologic abnormalities and clinical TB severity; ++, consolidation of pulmonary infiltration, repair of lung destruction, normalization of hematologic abnormalities and
clinical severity; N, parameters were initially normal. + (surgery), positive dynamics was observed in response to lung surgery. (PDF)

Table S2 Characterization of TB contacts and Mtb-unexposed participants. 1 Time of work in TB hospital (years). 2 Not applicable. (PDF)

Table S3 Lack of correlation between TB manifestations and numbers of CD27low IFN-γ+ cells in the blood of TB patients. None of the analyzed factors correlated significantly with the numbers of CD27low IFN-γ+ cells in the blood of TB patients. Initial analysis was performed in 50 patients. Subsequently, 12 patients from validation cohort were added (n = 62). In both cohorts, similar results were obtained (shown are results obtained in 50 patients). ρ, Spearman coefficient, p, significance value of the test. (PDF)

Table S4 Percentages and numbers of IFN-γ+ and CD27low IFN-γ+ cells in the lungs and in blood of surgery operated patients. Blood and lung cells were analyzed on the day of lung surgery. Indicated are percentages and numbers (per 1 million of acquired cells) of IFN-γ+ and CD27lowIFN-γ+ cells. 1 Fold differences between maximal and minimal values in the group. 2 Correlations were analyzed between: the indicated cells in the lungs and blood, lung destruction and indicated cells; TB severity and indicated cells. (PDF)

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Author Contributions
Conceived and designed the experiments: IVL. Performed the experiments: JYN GAK IVL. Analyzed the data: JYN NAK IAV RBA VVG IVL. Wrote the paper: IVL VVG JYN GAK.

References
1. Jasmer RM, Nahid P, Hopewell PC (2002) Clinical practice. Latent tuberculosis infection. N Engl J Med 347: 1800–1806.
2. Wallis RS, Pai M, Menzies D, Doherty TM, Wald G, et al. (2010) Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet 375: 1920–1937.
3. den Hertog AL, Mayboroda OA, Klatter PR, Anthony RM (2011) Simple Rapid Near-Patient Diagnostics for Tuberculosis Remain Elusive–Is a ‘Treat-to-Test’ Strategy More Realistic? PLoS Pathog 7. Available: http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1002027.htm via the Internet. Accessed 2011 Nov 3.
4. Ever K, Deeks J, Alvarez L, Bryant G, Waller S, et al. (2005) Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet 361: 1168–1173.
5. Mori T, Sakatanii M, Yamagishi F, Takashima T, Kawai E, et al. (2004) Specific detection of tuberculous infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med 170: 59–64.
6. Meier T, Eidenschep Jr, HP, Wrighton-Smith P, Enders G, Regnag T (2005) Sensitivity of a new commercial enzyme-linked immunosorbent assay (T SPOT-TB) for diagnosis of tuberculosis in clinical practice. Eur J Clin Microbiol Infect Dis 24: 529–536.
7. Salvato A (2007) Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest 131: 1898–1906.
8. Pai M, Zuerfing A, Menzies D (2008) Systematic Review: T-Cell-based Assays for the Diagnosis of Latent Tuberculosis Infection: An Update. Ann Intern Med 149: 172–184.
9. Menzies D (2008) Using tests for latent tuberculosis infection to diagnose active tuberculosis: can we eat our cake and have it too? [Editorial]. Ann Intern Med 149: 390–399.
10. Dieb R, Loddenkemper R, Meyaward-K, Niemann S, Nienhaus A (2008) Predictive value of a whole blood IFN-gamma assay for the development of active tuberculosis disease after recent infection with Mycobacterium tuberculosis. Am J Respir Crit Care Med 177: 1164–1170.
11. Sargenti V, Marotti S, Carrara S, Gagardi MC, Teloni R, et al. (2009) Cytometric detection of antigen-specific IFN-γ/IL-2-secreting cells in the diagnosis of tuberculosis. BMC Infectious Diseases 9: 99.
12. Pai M, Minion J, Steinberg K, Ramsay A (2010) New and improved tuberculosis diagnostics: evidence, policy, practice, and impact. Crit Rev Immunol 30: 271–284.
13. Pinto LM, Grenier J, Schumacher SG, Denker CM, Steinberg KR, et al. (2012) Immunodiagnosis of tuberculosis: state of the art. Med Princ Pract 21: 4–16.
14. Streit M, Trosa L, Vyliderin V, Yahayazarah A, Ulrich T, et al. (2007) Loss of Receptor on Tuberculin-Reactive T-Cells Marks Active Pulmonary Tuberculosis. PLoS One 2. Available: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.000735.htm via the Internet. Accessed 2007 Aug 15.
15. Schuetz A, Hasa A, Reiber K, Neugemeny N, Rachou A, et al. (2011) Monitoring CD27 expression to evaluate Mycobacterium tuberculosis activity in HIV-1 infected individuals in vivo. PLoS One 6. Available: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.002204.htm via the Internet. Accessed 2011 Nov 7.
16. Streit M, Fuhrmann S, Powell F, Quassem A, Norouzi L, et al. (2010) Tuberculin-Specific T Cells Are Reduced in Active Pulmonary Tuberculosis Compared to LTBI or Status Post BCG Vaccination. J Infect Dis 203: 378–382.
17. Harari A, Rorot V, Enders FB, Perreaux M, Stalder JM, et al. (2011) Dominant TNF-α Mycobacterium tuberculosis-specific CD4+ T cell responses discriminate between latent infection and active disease. Nat Med 17: 372–376.
18. Watts TH (2005) THF/TNF family members in costimulation of T cell responses. Annu Rev Immunol 23: 23–68.
19. Hamann D, Kostense S, Wohlers KG, Otto SA, Baars PA, et al. (1999) Evidence that human CD4+CD45RA+CD27- cells are induced by antigen and evolve through extensive rounds of division. Int Immunol 11: 1027–1033.
20. Appay V (2002) Characterization of CD4+ CTLs ex vivo. J Immunol 168: 5954–5961.
21. Frisch RD, Shen X, Sims GP, Hulstekt KS, Hodes RJ, et al. (2003) Seropositive differentiation of CD4 memory T cells defined by expression of CD27 and CD227. J Immunol 175: 6489–6497.
22. Nolte MA, van Olffen RW, van Gijsenberg KJP, van Lier RAW (2009) Timing and tuning of CD27-CD72 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. Immunological Reviews 229: 216–231.
23. Kavel DA, Bachy VS, Hewinson RG, Hogarth PJ (2011) Systemic BCG Immunization Induces Persistent Lung Mucosal Multifunctional CD4 TEM Cells which Expand Following Virulent Mycobacterial Challenge. PLoS One 6. Available: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0021566.htm via the Internet. Accessed 2011 Jun 24.
24. Lyadova IV, Oberdorfer S, Kapina MA, Apt AS, Swain SL, et al. (2004) CD4 T cells producing IFN-γ in the lungs of mice challenged with mycobacteria express a CD27-negative phenotype. Clin Exp Immunol 138: 172–182.
25. Kapina MA, Shelpelka GS, Bogacheva PV, Mischenko VV, Sayales P, et al. (2007) CD27+/− CD4 T lymphocytes that accumulate in the mouse lung during mycobacterial infection differentiate from CD27−/− precursors in situ, produce IFN-γ and protect the host against tuberculosis infection. J Immunol 178: 976–985.
26. Apt AS, Avdierko VG, Nikonenko BV, Kramnik IB, Moroz AM, et al. (1993) Distinct H-2 complex control of mortality, and immune responses to tuberculosis infection in virgin and BCG-vaccinated mice. Clin Exp Immunol 94: 322–329.
27. Bates DM (1988) Nonlinear regression analysis and its applications. New York: Second edition. Springer.
28. Efron B (1993) An introduction to the bootstrap. New York: Chapman & Hall.
29. Burnham KP (2002) Model selection and multimodel inference: a practical information – theoretic approach. New York: Second edition. Springer.
30. Jiang J, Wang X, Wang X, Cao Z, Liu Y, et al. (2010) Reduced CD27 expression on antigen-specific CD4+ T cells correlates with persistent active tuberculosis. J Clin Immunol 30: 566–573.
31. Baars PA, Sierro S, Arens R, Tesselaar K, Hooibrink B, et al. (2005) Properties of Memory CD8 T Cells. J Immunol 179: 36–40.
32. de Bree GJ, Daniels H, Schaffgade M, Jansen HM, Out TA, et al. (2007) Characterization of CD4+ memory T cell responses directed against common respiratory pathogens in peripheral blood and lung. J Infect Dis 195: 1718–1725.
33. Zikos TA, Donnenberg AD, Landreneau RJ, Luketich JD, Donnenberg VS (2011) Lung T-cell subset composition at the time of surgical resection is a prognostic indicator in non-small cell lung cancer. Cancer Immunol Immunother 60: 819–827.
35. Elkington P, Shiomi T, Beven R, Nuttall RK, Ugarte-Gil CA, et al. (2011) MMP-1 drives immunopathology in human tuberculosis and transgenic mice. J Clin Invest 121: 1827–1833.

36. O’Kane CM, Elkington PT, Jones MD, Caviedes L, Toscar M, et al. (2010) STAT3, p38 MAPK, and NF-κB Drive Unopposed Monocyte-Dependent Fibroblast MMP-1 Secretion in Tuberculosis. Am J Respir Cell Mol Biol 43: 465–474.

37. Teijaro JR, Turner D, Pham Q, Wherry EJ, Lefrançois L, et al. (2011) Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. J Immunol 187: 5510–5514.