Engagement of Toll-Like Receptor 2 on CD4+ T Cells Facilitates Local Immune Responses in Patients with Tuberculous Pleurisy

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Background. Although it has been recognized that Mycobacterium tuberculosis contains large amounts of Toll-like receptor 2 (TLR2) ligands, their direct effects on CD4+ T cells and the clinical implications have not been determined.

Methods. With the recent finding that activated CD4+ T cells express TLR2 as a costimulatory receptor, we hypothesized that M. tuberculosis and its components may directly affect CD4+ T cells by engaging TLR2, thus facilitating the expansion and function of these lymphocytes in tuberculous pleura.

Results. Our results indicate that CD4+ T cells from the pleural fluid and peripheral blood of patients with tuberculosis show significantly increased TLR2 expression, compared with those from healthy donors. TLR2 ligand activity was also significantly higher in the tuberculous pleural fluid than in the serum from healthy donors or patients with pulmonary tuberculosis. M. tuberculosis TLR2 ligands, 19-kDa lipoprotein, and live bacillus Calmette-Guérin all modulated cytokine production (interferon-γ and interleukin 17), cellular proliferation, survival, and migration of CD4+ T cells isolated from pleural fluid and activated with anti-CD3 and anti-CD28.

Conclusion. These data indicate that direct interaction between M. tuberculosis TLR2 ligands and CD4+ T cells facilitated local CD4+ T cell immune responses in patients with tuberculous pleurisy.

Toll-like receptors (TLRs) expressed by immune and nonimmune cells recognize pathogen-associated molecular patterns on microorganisms. Signaling induced by TLR engagement provides a rapid protective response against pathogens [1]. The interaction between Mycobacterium tuberculosis or extracellular products of the pathogen and TLRs has been studied in both mice and humans [2–5]. By full-exon sequencing of TLR1, TLR2, TLR4, TLR6, and TLR10, Ma et al [3] revealed that TLR variants contribute to human susceptibility to tuberculosis. In terms of TLR2, Chang et al [2] have reported that M. tuberculosis infection causes up-regulation of TLR2 in the mononuclear leukocytes of patients with active tuberculosis. TLR2 polymorphisms in human are associated with increased susceptibility to tuberculosis [4,6], and TLR2-deficient mice die rapidly after M. tuberculosis infection [7].

M. tuberculosis expresses numerous TLR2 ligands, such as 19-kDa lipoprotein and the lipoprotein LprA. The interactions between TLR2 and M. tuberculosis 19-kDa lipoprotein, LprA, or live M. tuberculosis have multiple effects on the phenotypic and functional regulation of TLR2–expressing macrophages and dendritic cells, for example, the expression of HLA-DR and response to interferon-γ (IFN-γ) [8–12]. However, these studies have focused on the role played by TLR2 in innate immune cells in tuberculosis. Whether M. tuberculosis may directly modulate adaptive immune responses through TLR2 remains unclear. In particular, the effects...
that *M. tuberculosis*–derived TLR2 ligands have on TLR2-expressing CD4+ T cells and their clinical implications require further investigation.

Recent findings have shown that CD4+ T cells express some TLRs that may regulate T cell activity independently of antigen-presenting cells (APCs) [13–17]. TLR2, expressed on activated T cells, may increase cellular proliferation and IFN-γ secretion after T cell receptor (TCR) stimulation [17, 18]. Because CD4+ T lymphocytes play a critical role in controlling *M. tuberculosis* infection and are predominant among the immune cells infiltrating the pleural fluid in patients with tuberculosis [19–24], we sought to determine the direct effects that *M. tuberculosis* TLR2 ligands have on CD4+ T lymphocytes infiltrating into tuberculous pleura. We hypothesized that *M. tuberculosis* and its TLR2 ligands may modulate the function and survival of CD4+ T cells during *M. tuberculosis* infection, which may in turn facilitate local CD4+ T cell responses observed in patients with tuberculous pleurisy.

**METHODS**

**Patients and samples.** Thirty-nine patients with newly diagnosed pulmonary tuberculosis and 22 patients with tuberculous pleurisy were prospectively recruited at Shenzhen Third Hospital, Shenzhen, China. The diagnosis was based on positive cultures for *M. tuberculosis*, clinical and radiological features, and a good response to antituberculosis treatment.

For comparison, 38 healthy donors were recruited in this study. Regular physical examinations, bacillus Calmette-Guérin (BCG) purifie protein derivative skin test, and *M. tuberculosis* antigen-specific IFN-γ enzyme-linked immunospot assay were used to exclude those with latent *M. tuberculosis* infection.

All participants were from the Chinese Han ethnic population. Mean ages were 38.3 years (range, 21–58 years) for patients with pulmonary tuberculosis, 33.2 years (range, 20–59 years) for patients with tuberculous pleurisy, and 30.2 years (range, 22–59 years) for healthy donors. The male-to-female ratios for the 3 groups were 26:13, 15:7, and 20:18, respectively. For both patient groups, the white blood cell counts in blood ranged from 4.53 × 10^9 to 10.57 × 10^9 cells/L, and lymphocyte counts ranged from 1.14 × 10^9 to 2.79 × 10^9 cells/L. For patients with tuberculous pleurisy, the cell numbers in pleural fluid ranged from 0.35 × 10^9 to 1.21 × 10^9 cells/L. The white blood cell and lymphocyte counts for healthy donors were within normal ranges for healthy individuals (4.0 × 10^9 to 10.0 × 10^9 cells/L and 1.5 × 10^9 to 4.0 × 10^9 cells/L, respectively).

**Flow cytometry analysis.** Monoclonal antibodies against CD3 (clone SK7), CD4 (clone SK3), CD8 (clone SK1), TCR-αβ (clone WT31), CD14 (clone MΦP9), CD27 (clone L128), CD38 (clone HB7), CD44 (clone L178), HLA-DR (clone 243), CD69 (clone UCHL1), CD45RO (clone 78), IFN-γ (clone 25723.11), and isotype-matched control immunoglobulin were obtained from BD Biosciences. Anti–human TLR2–APC (clone TL2.1) and immunoglobulin G isotype control antibodies (eBioscience) were used to evaluate the specific role of TLR2. Apoptosis of cultured CD4+ T cells was identified by flow cytometry after cells were stained with fluorescein isothiocyanate V (Bender MedSystems), in accordance with the manufacturer’s instructions, and by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) technology with the In Situ Cell Death Detection kit (Roche Diagnostics). To detect intracellular IFN-γ, PFMCs from patients with tuberculous pleurisy were stimulated with the indicated TLR2 ligands for 72 h. Cells were exposed to brefeldin A (10 mg/mL; Sigma-Aldrich) for 6 h before collection, stained with anti-CD3 and anti-CD8 antibodies, fixed and permeabilized with Cytofix/Cytope m Plus (BD Biosciences), and then stained with anti–IFN-γ antibody before being analyzed by flow cytometry. A minimum of 100,000 events were acquired with a BD FACSCanto flow cytometer and analyzed using BD FACSDiva software (version 5.0.2; BD Biosciences).
**Carboxyfluorescein succinimidyl ester staining and cell proliferation assay.** To measure cell proliferation in response to TLR2 ligands, purified CD4+ T cell suspensions were labeled with carboxyfluorescein succinimidyl ester (CFSE) at a final concentration of 1.25 μmol/L. Cells were incubated at 37°C for 10 min and washed twice. Cells were then resuspended in complete medium, and 2 x 10^5 cells/well were cultured for 3 days in the presence or absence of the M. tuberculosis-derived 19-kDa lipoprotein, Pam3Cys-SK4, or live BCG, with or without anti-CD3 and anti-CD28 mAbs. At the end of culturing, cells were harvested and analyzed by flow cytometry. The proliferation of CD3+CD4+ T cells was determined in relation to the decrease in CFSE fluorescence.

**RNA isolation and reverse transcription.** Total RNA was extracted from purified CD4+ T cells isolated from fresh PBMCs and PFMCs by means of an RNeasy Mini kit (Qiagen). RNA was reverse transcribed using a SensiScript reverse transcriptase kit (Qiagen) and oligo(dT)15 (Takara) with 20 ng of RNA as the starting material (×10 reverse-transcriptase buffer, 0.5 mmol/L deoxynucleotidetriphosphate, 10 U of RNase inhibitor, 1 μmol/L oligo(dT)15, 10 U of reverse transcriptase, and RNase-free water).

**Quantitative real-time polymerase chain reaction.** The following primers and probe for TLR2 were used: forward, 5′-AGAGTGATGGTGCAAGATGAA-3′; reverse, 5′-AAATGCAGCATCATTCTTCTC-3′; and probe, 5′-TGGACCTTCTCC CATTTCGT-3′. Primers and probes were synthesized by Shanghai GeneCore BioTechnologies. The housekeeping gene β-actin was used as a reference, as described elsewhere [21]. Complementary DNA was amplified by quantitative real-time polymerase chain reaction (PCR) using the ABI 7500 real-time PCR detection system (ABI). Relative messenger RNA (mRNA) expression compared with β-actin mRNA molecules was calculated using a method that has been described elsewhere [26].

**Chemotaxis assay.** CD4+ T cells isolated from PFMCs were plated (1 x 10^6 cells/mL) in 96-well plates. Cells were treated with Pam3Cys-SK4, 19-kDa lipoprotein, or BCG with anti-CD3 and anti-CD28 mAbs and incubated for 48 h at 37°C. Chemotaxis assays were then performed as described elsewhere [27]. Briefly, cell migration was assessed with a modified transwell chamber assay; CD4+ T cells (1 x 10^6 cells/mL in 0.1 mL of medium) were seeded in the upper compartment of a transwell chamber (5-μm-pore size; Corning Costar). Interleukin 8 (IL-8) (10 ng/mL) was introduced in the bottom well of the chamber, with untreated wells used as a control. After incubation for 3 h at 37°C, cells were recovered from the bottom chambers and counted. A chemotaxis index was determined for each treatment, defined as the number of migrating cells in response to each treatment divided by the number of migrating cells with no treatment.

**Detection of cytokine by ELISA.** IFN-γ, interleukin 17 (IL-17), interleukin 4 (IL-4), IL-8, and interleukin 2 (IL-2) concentrations in culture supernatants were determined using sandwich DuoSet ELISA kits, in accordance with the manufacturer’s instructions (R&D Systems).

**TLR2 ligand activity assay.** Human embryonic kidney (HEK) 293 cells transfected with human TLR2 gene (HEK293-hTLR2) were obtained from InvivoGen. In response to stimulation with TLR2, HEK293-hTLR2 cells secrete IL-8 [11]. The activity of TLR2 ligands was thus assessed by measuring IL-8 production in the supernatant of stimulated HEK293-hTLR2 cells [11]. Serum and pleural fluid were incubated with HEK293-hTLR2 cells, and the production of IL-8 was quantified by ELISA.

**Statistical analyses.** One-way analysis of variance with the Newman-Keuls multiple-comparison test was used to compare the differences between multiple groups (GraphPad Prism software; version 3.0). The Wilcoxon matched-pair t test was used to analyze the effect of TLR2 ligand treatment on CD4+ T cells. Differences were considered significant at P < .05.

**RESULTS**

**Increased TLR2 expression by CD4+ T lymphocytes in patients with active tuberculosis.** Previous reports have indicated that M. tuberculosis infection is associated with an increase in TLR2 mRNA expression by PBMCs [2]. To determine more specifically the expression profile of TLR2 by CD4+ T cells, we evaluated TLR2 mRNA levels in CD4+ T cells isolated from fresh PBMCs and PFMCs. Our results indicated that TLR2 mRNA levels were significantly increased in CD4+ T cells from patients with tuberculosis, compared with levels for healthy donors. In addition, TLR2 mRNA expression was significantly higher in CD4+ T cells isolated from PFMCs than in CD4+ T lymphocytes from the blood of patients with tuberculosis (figure 1A).

The percentage of activated CD4+ T lymphocytes (cells expressing CD27, CD45RO, HLA-DR) that expressed TLR2 was significantly higher than the percentage in resting CD4+ T cells, as determined by flow cytometry (figure 1B and data not shown). TLR2 expression by both activated and resting CD4+ T cells was significantly increased in patients with tuberculosis, compared with that in healthy donors (figure 1B–1D). TLR2 expression by CD3+CD4+ T cells was significantly higher than that in CD3+CD4- T cells among PFMCs and PBMCs (figure 1B and data not shown).

**Increased TLR2 ligand activity at the site of infection in patients with tuberculosis.** TLR2 ligand activity in the serum and pleural fluid samples was determined using HEK293-hTLR2 cells, which produce IL-8 in response to TLR2 agonists. We first established that HEK293-hTLR2 cells produced significant amounts of IL-8 in response to stimulation with Pam3Cys-SK4. M. tuberculosis–derived 19-kDa lipoprotein, and live BCG in a dose-dependent manner, but not in response to...
Figure 1. Enhanced Toll-like receptor 2 (TLR2) expression by CD4+ T cells in patients with active tuberculosis. Real-time polymerase chain reaction (PCR) and flow cytometry were used to analyze TLR2 expression by CD4+ T cells in the blood of healthy donors (HD; n = 38) and patients with pulmonary tuberculosis (TB; n = 39) and in pleural fluid mononuclear cells (PFMCs) from patients with tuberculous pleurisy (TP/PF; n = 22). A, TLR2 messenger RNA (mRNA) expression. Real-time PCR was used to quantify relative TLR2 mRNA expression by purified CD4+ T cells isolated from peripheral blood mononuclear cells (PBMCs) and PFMCs, with actin expression used as the standard. B, Results of flow cytometry. PFMCs were stained with anti-CD3, anti-CD4, and anti-TLR2 antibodies. TLR2 expression was analyzed in differentially gated (resting and activated) CD3+CD4+ and CD3+CD4− T cells. Representative dot plots are shown, and percentages of TLR2-positive cells are indicated in the quadrants. FSC, forward scatter; SSC, side scatter. C and D, TLR2 protein expression. TLR2 protein expression was determined by flow cytometry in resting (C) and activated (D) CD4+ T cells from PBMCs or PFMCs in different groups. Data are expressed as means ± standard errors of the mean; differences between groups were analyzed by 1-way analysis of variance with the Newman-Keuls multiple-comparison test.

hepatitis B surface antigen (HBsAg) (used to rule out non-TLR2 stimulation) (figure e 2A and 2B). We then directly quantify the levels of IL-8 in blood samples obtained from healthy donors and patients with tuberculosis and in pleural fluid from patients with tuberculosis (figure e 2C). Consistent with previous reports [28, 29], we found that IL-8 was significantly increased in pleural fluid samples from patients with tuberculosis. HEK293-hTLR2 cells were then treated with serum or pleural fluid samples, and IL-8 production was evaluated by ELISA. For each sample, the IL-8 concentration was corrected by subtracting the values shown in figure e 2C (IL-8 background in the patient serum or pleural fluid sample). The data depicted in figure e 2D indicate that TLR2 ligand activity was significantly higher in tuberculous pleural fluid than in serum samples from healthy donors or from patients with pulmonary tuberculosis.

Modulation of cytokine production of CD4+ T cells from PFMCs by M. tuberculosis TLR2 ligands. Although M. tuberculosis TLR2 ligands did not directly affect TLR2 expression by CD4+ T cells, they may be capable of modulating the activity of these lymphocytes [18]. Our results indicate that the TLR2
agonist Pam3Cys-SK4 (used as positive control), 19-kDa lipoprotein, and live BCG significantly enhanced IFN-γ production by CD3+CD4+ T cells (CD3+CD8− T cells) in PFMC culture, with a less marked effect for CD3+CD8+ T lymphocytes (figure 3A). Furthermore, this effect was abrogated or significantly impaired by anti-TLR2 blocking antibodies, which evidenced the involvement of the TLR2 pathway (figure 3A). It was possible, however, that the TLR2 ligands had indirectly promoted IFN-γ production by T lymphocytes by stimulating TLR2-expressing APCs among PFMCs.

To investigate further whether \textit{M. tuberculosis}–derived TLR2 ligands may directly affect CD4+ T cell cytokine production, CD4+ T lymphocytes purified from the pleural fluid of patients with tuberculosis were cultured with plate-bound anti-CD3 and anti-CD28 mAbs in the presence or absence of \textit{M. tuberculosis} 19-kDa lipoprotein, Pam3Cys-SK4, live BCG, or HBsAg. Production of type 1 T-helper (Th1)–related (IFN-γ and IL-2), type 2 T-helper (Th2)–related (IL-4), or type 17 T-helper (Th17)–related (IL-17) cytokines was then analyzed in the culture supernatants (figure 3B). The results depicted in figure 3B indicate that Pam3Cys-SK4 significantly enhanced CD4+ T cell production of IFN-γ and IL-17 (figure 3B). Similar effects were observed when cells were cultured with live BCG or 19-kDa lipoprotein but not with HBsAg (figure 3B). The production of IFN-γ and IL-17 induced by Pam3Cys-SK4, BCG, or 19-kDa lipoprotein was significantly impaired by the presence of anti-TLR2 blocking antibody but not by control immunoglobulin G, demonstrating the involvement of the TLR2 pathway (figure 3 and data not shown). In contrast, IL-2 or IL-4 production by CD4+ T cells was not modified by stimulation of the cells with 19-kDa lipoprotein, BCG, or Pam3Cys-SK4 (figure 3B).

**Promotion of CD4+ T cell proliferation by \textit{M. tuberculosis} TLR2 ligands.** The accumulation of CD4+ T cells in the pleural fluid may result from increased proliferation and recruitment as well as from prolonged survival of these cells. We thus evaluated whether \textit{M. tuberculosis} ligands directly affect the proliferation of CD4+ T cells purified from the pleural fluid of patients with tuberculosis. Isolated CD4+ T lymphocytes were labeled with CFSE before being cultured with plate-bound anti-CD3 and anti-CD28 mAbs. The proliferation of CD4+ T cells activated with anti-CD3 and anti-CD28 mAbs was significantly...
enhanced by 19-kDa lipoprotein, Pam3Cys-SK4, and live BCG. This effect was significantly reversed with anti-TLR2 blocking antibody (figure 4).

Promotion of CD4^+ T cell survival by M. tuberculosis TLR2 ligands. To investigate whether M. tuberculosis affects the survival of CD4^+ T lymphocytes, CD4^+ cells purified from PFMCs were incubated with Pam3Cys-SK4, 19-kDa, or BCG and stained with propidium iodide (PI) and annexin V. The number of apoptotic (PI^+ and annexin V^+) or necrotic (PI^+ and annexin V^+) cells was then determined by flow cytometry. The data depicted in figure 5 indicate that incubation of CD4^+ T cells with Pam3Cys-SK4, 19-kDa lipoprotein, or BCG significantly reduced the number of apoptotic and necrotic cells. This protective effect was abrogated by anti-TLR2 blocking antibody, demonstrating the role played by TLR2 engagement in the promotion of CD4^+ T cell survival by Pam3Cys-SK4, 19-kDa lipoprotein, and BCG (figure 5). These results were verified with TUNEL staining to confirm the apoptotic commitment of the cells (data not shown).

Enhancement of the migration of CD4^+ T cells in response to IL-8 by M. tuberculosis TLR2 ligands. Recruitment of blood CD4^+ T lymphocytes to pleura is a critical factor that
Figure 4. Direct enhancement of the proliferation of CD4+ T cells by Mycobacterium tuberculosis Toll-like receptor 2 (TLR2) ligands. Carboxyfluorescein succinimidyl ester (CFSE)–labeled CD4+ T cells purified from pleural fluid mononuclear cells of patients with tuberculous pleurisy were activated with plate-bound anti-CD3 and anti-CD28 antibodies and cultured for 3 days in the presence or absence of Pam3Cys-SK4 (PAM3), 19-kDa lipoprotein, and live bacillus Calmette-Guérin (BCG), with or without anti-TLR2 blocking antibody. The percentage of CD4+ T cells undergoing proliferation (as evidenced by a decrease in CFSE fluorescence intensity) is indicated for each treatment group. Representative data from 6 patients are shown.

Figure 5. Promotion of activated CD4+ T cell survival by Mycobacterium tuberculosis Toll-like receptor 2 (TLR2) ligands. CD4+ T cells purified from pleural fluid mononuclear cells of patients with tuberculous pleurisy were cultured for 3 days, in accordance with the protocol described for figure 4. Cells were stained with propidium iodide (PI) and annexin V. Percentages of apoptotic (PI+ and annexin V+) or necrotic (PI+ and annexin V−) cells were determined by flow cytometry. Representative dot plots for 6 experiments are shown. 19 kDa, 19-kDa lipoprotein; BCG, bacillus Calmette-Guérin; Pam3Cys-SK4, or BCG significantly increased lymphocyte migration toward an IL-8 source (figue 6A), with a peak at 48 h (figue 6B-6D). This effect was significantl inhibited in the presence of anti-TLR2 antibody, further underlining the importance of the TLR2 signaling pathway in CD4+ T cell activation by M. tuberculosis (figue 6A). Notably, the differences in T cell chemotaxis with 19-kDa lipoprotein at 6–24 h were

may contribute to increased numbers of CD4+ T cells in the pleural flui of patients with tuberculosis. A previous study has indicated that IL-8 detected in the pleural flui may act as a chemoattractant for lymphocytes [28]. These data prompted us to explore further the effect of M. tuberculosis–derived TLR2 ligands on CD4+ T cell migration toward an IL-8 source. Incubation of purifie CD4+ T cells with 19-kDa lipoprotein,
Figure 6. Promotion of the migration of CD4+ T cells toward an interleukin 8 source by Mycobacterium tuberculosis Toll-like receptor 2 (TLR2) ligands. A, CD4+ T cells. CD4+ T cells purified from pleural fluid mononuclear cells of patients with tuberculous pleurisy (n = 10) were cultured for 48 h, in accordance with the protocol described for figure 4. Cells were harvested for chemotaxis assays as described in Methods. Data are chemotaxis indexes of differentially treated CD4+ T cells purified from 10 patients with tuberculous pleurisy and are expressed as means ± standard errors of the mean. Differences between groups and untreated samples (medium) were analyzed by the Wilcoxon matched-paired t test; asterisks indicate statistically significant differences. BCG, bacillus Calmette-Guérin. B–D, Kinetics of migration enhancement by incubation of different TLR2 ligands: Pam3Cys-SK4 (PAM3) (B), 19-kDa lipoprotein (C), and live bacillus Calmette-Guérin (BCG) (D).

not significant. It is possible that 19-kDa lipoprotein differs kinetically from other TLR2 ligands or that the concentration used was less than the optimal concentration required to induce strong migration of CD4+ T cells.

DISCUSSION

We have demonstrated in this study that TLR2 expression by CD4+ T cells was significantly increased in patients with tuberculosis, compared with that for healthy donors. In addition, M. tuberculosis–derived 19-kDa lipoprotein and live BCG directly promoted the function of activated CD4+ T cells. These results extend previous finding indicating that PBMCs from patients with tuberculosis expressed higher levels of TLR2 mRNA. We have also provided evidence that CD4+ T lymphocytes infiltrating the pleural fluid expressed higher levels of TLR2 than did circulating CD4+ T cells. These findings are consistent with previous reports indicating that PBMCs from patients with tuberculosis expressed higher levels of TLR2 mRNA. We have also provided evidence that CD4+ T lymphocytes infiltrating the pleural fluid expressed higher levels of TLR2 than did circulating CD4+ T cells. These findings are consistent with previous reports indicating that PBMCs from patients with tuberculosis expressed higher levels of TLR2 mRNA.

The predominance and expansion of the CD4+ T lymphocyte population in the pleura of patients with tuberculous pleurisy could be due to enhanced proliferation and survival of CD4+ T cells. We confirmed that M. tuberculosis TLR2 ligands promoted the proliferation and survival of CD4+ T cells isolated from pleural fluid mononuclear cells of patients with tuberculous pleurisy.
from PFMCs. Interestingly, these effects of TLR2 ligands on the proliferation and survival of CD4+ T cells were observed only when purified CD4+ T cells were concomitantly activated with anti-CD3 and anti-CD28. One possible explanation was that M. tuberculosis and its associated TLR2 ligands indirectly affect CD4+ T cells by activating macrophages or other APCs contained among PFMCs. This possibility was ruled out, however, because TLR2 agonists were able to modulate highly purified CD4+ T cells from PFMCs.

Recruitment of TLR2-expressing CD4+ T cells may account for the observed increased frequency of these cells in the pleura. Our results indicated that M. tuberculosis TLR2 ligands enhanced the migration of activated CD4+ T cells toward an IL-8 source. This is significant because IL-8 levels are elevated in the pleural fluid of patients with tuberculosis and because chemotaxis toward IL-8 has been identified as an important mechanism for lymphocytic infiltration of the pleura during tuberculosis [28, 29].

CD4+ T lymphocytes represent a heterogeneous population of cells that include Th1 and Th2 cells, regulatory T cells, and Th17 cells [32]. All of these cells have been shown to participate in the immune response against tuberculosis. Although we were not able to evaluate the effect of TLR2 ligands on each individually purified subset of CD4+ T cells, our observation that 19-kDa lipoprotein and live BCG modulated IFN-γ but not IL-4 production suggests that M. tuberculosis–derived TLR2 ligands may have different effects on Th1 and Th2 CD4+ T cells. This result is consistent with a previous report indicating that TLR2 directly promotes the effector function of Th1 but not Th2 cells [18]. In addition, by demonstrating that TLR2 agonists triggered IL-17 production by CD4+ T cells, our study provides further information on the modulatory effects of these ligands on Th17 cells. Because Th1 and Th17 cells have been shown to be critical mediators of the protective immunity against tuberculosis, additional studies are warranted to clarify the in vitro and in vivo function of TLR2 for these cells [33–35].

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References

25. Chen X, Zhou B, Li M, et al. CD4+CD25+FoxP3+ regulatory T cells...


34. Khader SA, Cooper AM. IL-23 and IL-17 in tuberculosis. Cytokine 2008;41:79–83.