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Tuberculosis-sensitized monocytes sustain immune response of interleukin-37

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Abstract

Roles of human IL-37 in infections remain poorly characterized. Although plasma IL-37 is elevated in patients with tuberculosis (TB), IL-37 source and immune correlate in TB have not been investigated. It is also unknown whether and how TB can influence the ability of immune cells to mount innate responses of IL-37 and pre-inflammatory cytokines. Here, we demonstrated that IL-37b-producing monocytes coincided with a source of elevated plasma IL-37b in TB patients. While IL-37b production in TB was associated with prolonged/complicated TB, TB burdens and inflammatory reactions, it negatively correlated with immune responses of pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α or IL-10. Interestingly, mycobacterial re-infection of monocytes from TB patients, but not healthy BCG-vaccinated controls, enhanced or sustained IL-37b production by cultured monocytes. TB-sensitized monocytes from TB patients mounted more robust immune responses of IL-37b than those of pre-inflammatory cytokines during mycobacterial re-infection in culture. Our data represent new findings in terms of IL-37b responses, immune correlates and potential mechanisms in TB patients.

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Competing interests

The authors have no conflict of interests.

Keywords

IL-37b; Monocytes; Cytokines; Innate immunity; Active tuberculosis

1. Introduction

Tuberculosis (TB) remains one of the leading causes of global morbidity and mortality among infectious diseases largely due to HIV pandemics and multidrug-resistance (Yates et al., 2016). Both innate and adaptive immune responses appear to contribute the host control of *M. tuberculosis* (Mtb) infection (Cooper, 2009; Orme et al., 2015). While monocytes/macrophages or dendritic cells are infection targets of Mtb, they function as APC and innate immune cells to initiate adaptive responses and to produce various pre-inflammatory cytokines mediating inflammatory milieu in the lungs (Cooper, 2009; Ginhoux and Jung, 2014). In fact, we recently demonstrated that *in vitro* production of HMGB1, IL-6, IL-10 and TNF- α by mycobacterium-infected monocytes could also be seen *in vivo* both in peripheral blood and sputum of TB patients (Zeng et al., 2016). Importance of monocytes/innate responses in TB is implicated by a recent observation that impaired production of IL-1 β , TNF- α and IL-7 by monocytes could be associated with poor clinical outcomes during the intensive phase of TB treatment (Waitt et al., 2015).

Interleukin-37 (IL-37), a newly discovered member of the IL-1 family, can act as a natural inhibitor of immune responses (Nold et al., 2010). IL-37 has five splice variants including IL-37a-e (Boraschi et al., 2011), and IL-37b is the largest and most studied isoform, with significant sequence similarity to IL-18 (Kumar et al., 2002). IL-37 can be produced by various types of cells including PBMCs, epithelial cells, macrophages, dendritic cells (DCs) and T cells following the stimulation by pro-inflammatory cytokines such as IL-18, IFN- γ , IL-1 β , TNF, and Toll like receptor (TLR) ligand lipopolysaccharide (LPS) (Boraschi et al., 2011; Bouali et al., 2015; Ye et al., 2014, 2015). Increasing evidence indicates that the modulation of immune system by IL-37 involves the suppression of both innate and adaptive immunity (Boraschi et al., 2011; Chen and Fujita, 2015; Ye et al., 2014). Recent studies have shown that IL-37 is highly expressed in the serum and PBMCs of patients with systemic lupus erythematosus (SLE) (Ye et al., 2014) and in the plasma and synovial tissues of patients with rheumatoid arthritis (Ye et al., 2014; Zhao et al., 2014). In this context, recombinant human IL-37 can suppress the *in vitro* expression of pro-inflammatory cytokines IL-1 β and IL-6 in PBMCs from SLE patients (Ye et al., 2014). Mice transgenic for IL-37 exhibited markedly reduced production of IL-17, IL-1b and IL-6, following LPS stimulation (Lopetuso et al., 2013).

Roles of IL-37 in infections remain poorly characterized. A recent study has reported that IL-37 expression was up-regulated in patients with TB (Huang et al., 2015). However, source of IL-37 production and immunological and clinical correlates of IL-37 in TB patients have not been investigated. In addition, it is unknown whether and how Mtb infection can influence the ability of immune cells to mount innate responses of IL-37 and pre-inflammatory cytokines. Elucidation of these questions will help to define roles of IL-37

in TB and improve our understanding of TB pathogenesis. In the present study, we have recruited TB patients and control subjects to address these questions.

2. Materials and methods

2.1. Subjects and ethics statement

A total of 48 cases of active pulmonary tuberculosis (ATB) diagnosed as previously described (Zeng et al., 2015) were recruited from Dongguan 6th People's Hospital (Dongguan, China). ATB was confirmed based on typical clinical symptoms, chest X-radiography, positive Ziehl-Neelsen acid fast bacilli staining, positive Lowenstein-Jensen slants bacterial culture for sputum, or apparent anti-TB drugs efficacy. Bronchoscopy was performed to confirm a lack of evidence for respiratory diseases, lesions, or tumors. Subject exclusion criteria included HIV+ test results, diabetes, cancer, autoimmune diseases, immunosuppressive treatment. Twenty one healthy volunteers served as controls (HCs), with the absence of bacteriological and clinical evidence of TB disease (Table 1 and Table S1). All HC subjects had a documented history of BCG vaccination at birth. All subjects were recruited from July 2013 to June 2014 in Dongguan 6th People's Hospital (Dongguan, China). Subjects with HIV infection, diabetes, cancer, autoimmune diseases, immunosuppressive treatment, and history of pulmonary TB as previously described were excluded from the study (Zeng et al., 2014). All subjects underwent sputum smears and Ziehl-Neelsen acid fast staining to record smear positivity, along with cultures on Lowenstein-Jensen slants according to the standard method. The individualized treatment of patients with ATB was described previously (Zeng et al., 2014). Patients with ATB were given initial anti-tubercular drug (ATD) treatments, namely, M0 phase (duration, 0–4 days), to M1 phase treatment (duration, 25–35 days) as manifested by the absence of TB-relapsed symptoms. No significant differences in terms of age and gender were noted between patients and HCs. The study was approved by the Internal Review and the Ethics Boards of Guangdong Medical University and Dongguan 6th People's Hospital, and a written informed consent was obtained from all study subjects prior to the participation in this study.

2.2. Peripheral blood mononuclear cells preparation and plasma conservation

Peripheral blood mononuclear cells (PBMCs) were prepared as previously reported (Zeng et al., 2015). Briefly, approximately 5 mL blood was collected from each subject in an acid citrate dextrose (ACD)-containing blood collection tube. Blood samples were centrifuged at 1500 rpm for 5 min, and plasma samples were collected and stored at –80 °C until analysis. PBMCs were freshly isolated from blood by standard Ficoll (GE health) density gradient centrifugation. Cell viability was determined by trypan blue exclusion (>95% in all experiments). PBMCs were then aliquoted for the following experiments.

2.3. Antibodies and reagents

The following Abs were used for flow cytometry: Mouse anti-human IL-37b (6A6, LifeSpan BioSciences), Goat anti-Mouse IgG/IgM-FITC (BD Biosciences). IL-6, IL-10, IL-17A, IL-1 β and TNF- α ELISA Kits were from eBioscience (product BMS213/2, BMS215/2, BMS2017, BMS224/2, BMS223/4; eBioscience, San Diego, CA). IL-37b ELISA Kit was from AdipoGen (product AG-45A-0041YEK-KI01, AdipoGen, Switzerland). M. bovis

BCG was obtained from Zheng W. Chen laboratories, University of Illinois College of Medicine, Chicago, Illinois, USA. Mycobacterial strains were grown to mid-log phase in MB7H9 medium supplemented with albumin-dextrose-catalase (1×), glycerol (0.5%) and Tween 80 (0.05%) according to the method described by Huang et al. (2015). PBS stocks were prepared and stored at -80°C until use. The CFU of stocks was enumerated by plating appropriate dilutions in duplicates on MB7H11 agar supplemented with oleic acid-albumin-dextrose-catalase (1×) and glycerol (0.5%) (Huang et al., 2007). The Fixation/Permeabilization kit was from BD Biosciences (product 554715, BD Biosciences, San Jose, CA).

2.4. Flow cytometry analysis

The expression of IL-37b in monocytes was determined with intracellular flow cytometry staining (Zeng et al., 2015). PBMCs (10×10^6) were fixed and permeabilized using a Fixation/Permeabilization kit, followed by staining with a mouse anti-human IL-37b antibody for 30 min and goat anti-mouse IgG/IgM-FITC for 20 min. After washing, the stained cells were resuspended in 200 μL PBS containing 2% paraformaldehyde and 2% FBS. The samples were then acquired on a BD FACS-Canto II flow cytometer and analyzed using FlowJo software (Tree Star).

2.5. ELISA detection

The IL-6, IL-10, IL-17A, IL-1 β , TNF- and IL-37b in plasma or culture medium were tested with commercial ELISA kits following the protocol from the manufacturer.

2.6. Real time PCR

Absolute quantitative Real Time PCR was performed to detect the expression of IL-37 mRNA in PBMCs from ATB patients and HCs (Forlenza et al., 2012). PBMCs (10×10^6) were resuspended in RNAiso Plus (TaKaRa) and stored at -80°C until RNA extraction. Total RNA was isolated by Trizol (TaKaRa, Japan) according to the manufacturer's instruction. The amount of RNA was determined using the Eppendorf Biophotometer (Eppendorf AG, Hamburg, Germany). An aliquot of RNA (450 ng) was reverse transcribed into cDNA using PrimeScript RT Master Mix (TaKaRa, Japan), following the manufacturer's protocol. Real-time PCR assay was carried out in duplicate in a 20 μL volume using SYBR Green (Applied Biosystems) and the following primers: human IL-37b forward 5'-GTGCTGCTTAGAAGACCCG-3' and reverse 5'-ATGAGATTTCCAGAGTCCAG-3' and human β -actin forward 5'-GGTCGGAGTCAACGGATTTG-3' and reverse 5'-ATGAGCCCCAGCCTTCTCCAT-3'. The plasmid expressing IL-37b mRNA (pcDNA3.1 + IL-37 + GFP, presented by Professor Chen in our lab) was used as the standard; Normalized to 1 $\mu\text{g}/\text{mL}$ for each subsequent real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) process and detected in ABI7500. The copies of IL-37b mRNA in sample were analyzed.

2.7. 7 In vitro stimulation experiment

PBMCs were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin at 37°C in a humidified

incubator with 5% CO₂. The cells were seeded in 24-well microplates at a concentration of 1×10^6 cells/well, followed by adding living BCG and lipopolysaccharide (LPS) respectively for 6, 12 and 24 h. BCG was washed and suspended with PBS (0.05% Tween 80). The concentration of BCG is MOI = 1:20. 1 µg/mL lipopolysaccharide (LPS) was used as a positive control and PBS (0.05% Tween 80) was used as a negative control. After culture for an indicated period, cell culture supernatants were collected, filtered through a 0.22 µm filter, and stored at -80°C until analysis.

2.8. Statistical analysis

Normality test was first performed to decide whether the data were in normal distribution. Data were shown as Mean ± SEM (standard error of the mean, SEM). Student's *t*-test or ANOVA (analysis of variance) were employed to compare the differences of measured data, and *Pearson* correlation was used to measure the degree of dependency between variables by the GraphPad Prism version 5.0 software (GraphPad Software Inc., San Diego, CA, USA). A *P* value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Consistent increases in plasma IL-37b and IL-37b-producing monocytes in ATB patients

TB-driven immune responses of IL-37 and source of IL-37 production have not been demonstrated (Huang et al., 2015). To address these questions, we first detected the level of IL-37b in plasma from ATB patients and HCs using a sandwich ELISA kit (product AG-45A-0041YEK-KI01, AdipoGen, Switzerland). We found that the level of plasma IL-37b in ATB patients was much higher than that in HCs (Fig. 1A). Interestingly, the level of plasma IL-37b in eight initially-treated (IT) ATB patients decreased significantly one month after anti-tuberculosis drug treatment (ADT) (Fig. 1B). Since IL-37 could be produced in PBMCs of humans who are not infected with *Mtb* (Bouali et al., 2015), we sought to determine if IL-37 mRNA expression was increased in PBMCs of ATB patients using real time quantitative PCR (Forlenza et al., 2012) and if such an increase could be reversed one month after ADT. We found that the expression of IL-37 mRNA in PBMCs from ATB patients was much higher than that from HCs (Fig. 1C), and it decreased significantly one month treatment (Fig. 1D). We then used an intracellular cytokines staining method (Zeng et al., 2015) to detect IL-37 in PBMCs using an IL-37b monoclonal antibody. Surprisingly, we found that IL-37b was predominantly produced in monocytes, but not lymphocytes in ATB patients and HCs, and the frequency of IL-37b-producing monocytes in ATB patients was higher than that in HCs (Fig. 1E, F, Fig. S2). Consistently, the frequency of IL-37b-producing monocytes decreased significantly one month after ADT (Fig. 1E and G). These results suggested that IL-37b-producing monocytes, not lymphocytes, could serve as a source of elevated plasma IL-37b in ATB patients.

3.2. IL-37b-producing monocytes were associated with prolonged/complicated TB, TB burden and inflammatory reactions

Since IL-37b expression has not been shown to correlate with clinical severity of TB, we sought to determine if increased production of IL-37b in ATB patients was linked to

inflammatory responses and clinical status of *Mtb* infection. Interestingly, IL-37b expression appeared to be associated with prolonged/complicated TB as TB patients re-treated (RT) with advanced regimens due to TB relapse, severe TB or drug resistance could exhibit higher frequencies of IL-37b-producing monocytes than those early TB patients who just initiated (IT) their TB therapy after diagnosis (Fig. 2A and B). In addition, TB patients with positive sputum smears had higher frequencies of IL-37b-producing monocytes than those with negative sputum smears (Fig. 2A and D). Of note, TB patients with detectable *Mtb*-specific antibodies showed higher frequencies of IL-37b-producing monocytes than those with negative status (Fig. 2A and C). Furthermore, IL-37b-producing monocytes seemed to be associated with inflammatory reactions as frequencies of IL-37b-producing monocytes in ATB patients coincided with TB-driven changes in numbers of lymphocytes and monocytes (Fig. 3A–C), and detectable C-reactive protein (CRP) (Fig. 3D). It is noteworthy that negative correlation between IL-37b production by monocytes and numbers of monocytes/lymphocytes implicates that TB sensitizes the ability of monocytes to produce IL-37b. Nevertheless, these results suggest that IL-37b expression associates with prolonged/complicated TB course, TB bacilli burden and inflammatory reactions.

3.3. Plasma IL-37b and IL-37b-producing monocytes in ATB coincided with immune responses of pro-inflammatory cytokines, with negative correlation between them

We then measured inflammatory/pro-inflammatory cytokines that are also involved in innate immunity using ELISA, and examined whether these pro-inflammatory cytokines' responses correlated with IL-37b production. The results showed that pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α , and anti-inflammatory cytokine IL-10 were elevated in the peripheral blood from ATB patients compared to HCs (Fig. 4A–D), despite no difference in IL-17 between ATB patients and HCs (Fig. 4E). Interestingly, plasma level of IL-37b negatively correlated with IL-1 β (Fig. 4F) and IL-10 (Fig. 4I) in ATB patients. Virtually, frequencies of IL-37b-producing monocytes correlated negatively with all the evaluated pro-inflammatory cytokines IL-1 β , IL-6, TNF- α and IL-10 except for IL-17 (Fig. 4K–O). Of note, high-level of IL-37b production coincided with low levels of the pro-inflammatory cytokines in plasma of ATB patients. Thus, plasma IL-37b and IL-37b-producing monocytes coincide with immune responses of pro-inflammatory cytokines in ATB, with negative correlation between them.

3.4. Mycobacterial infection of monocytes from TB patients, but not BCG-vaccinated HC, sustained IL-37b production

Since ATB sensitized monocytes to constitutionally produce IL-37b without antigen stimulation *in vitro* (Fig. 1E–F, Fig. 3A–B), we sought to determine whether re-exposure of monocytes to TB antigens by mycobacterial re-infection in culture could alter the ability of monocytes to mount IL-37b immune responses. Thus, monocytes/PBMCs from ATB patients and healthy controls (HC) with documented BCG vaccination were infected with *M. bovis* BCG, and assessed for production of IL-37b using ICS methods. As control, LPS stimulation of TLR4 in monocytes/PBMCs culture could induce low-level production of IL-37b by monocytes similarly from both ATB patients and HCs at 6, 12 and 24 h (Fig. 5A and B). In contrast, mycobacterial infection/stimulation steadily enhanced and sustained IL-37b production by monocytes from ATB patients at 6 through 24 h after BCG infection

of PBMCs, whereas HC with documented BCG vaccination exhibited transient production of IL-37b by monocytes at 6 and 12 h, but not 24 h after BCG infection (Fig. 5A, C). Thus, these results suggest that mycobacterial infection in PBMC culture could enhance or sustain IL-37b production by monocytes from ATB patients but not HC.

3.5. TB-sensitized monocytes from ATB patients mounted more robust immune responses of IL-37b than those of pre-inflammatory cytokines during mycobacterial re-infection in culture

The BCG-enhanced production of IL-37b by monocytes from ATB patients raised an interesting question as to whether TB-sensitized monocytes mounted more favorable response of IL-37b than pre-inflammatory cytokines (Fig. 4) in response to mycobacterial infection *in vitro*. To address this question, we detected IL-37b in monocytes through ICS in BCG-infected PBMC cultures using ELISA while simultaneously measuring those pre-inflammatory cytokines. Surprisingly, while BCG infection enhanced the ability of TB-sensitized monocytes to produce IL-37b at 24 h (Fig. 5), monocytes/PBMC from ATB patients produced and secreted significantly lower levels of IL-1 β , IL-6, TNF- α , IL-10 and IL-17 (Fig. 6A–E) in the culture when compared with those from HCs. Consistent with the negative correlation between IL-37b and plasma inflammatory cytokines (Fig. 4), the percentage numbers of IL-37b-producing monocytes negatively correlated with IL-1 β , IL-6, TNF- α and IL-10 (Fig. 6F–I) in the PBMC culture, but not IL-17 (Fig. 6J). Thus, these results support the hypothesis that TB-sensitized monocytes mounted more favorable IL-37b response than the above pre-inflammatory cytokines in response to mycobacterial re-infection in culture.

4. Discussion

The current study presents the following previously undescribed findings: (i) IL-37b-producing monocytes coincide with a source of elevated plasma IL-37b in ATB patients; (ii) IL-37b production in ATB appear to associate with prolonged/complicated TB, TB burdens and inflammatory reactions, and negatively correlate with immune responses of pro-inflammatory cytokines; (iii) While mycobacterial re-infection of monocytes from TB patients, but not BCG-vaccinated HC, enhances or sustains IL-37b production, TB-sensitized monocytes from TB patients mount more robust immune responses of IL-37b than pre-inflammatory cytokines, with negative correlation, during mycobacterial re-infection in culture. Compared to the recent report (Huang et al., 2015), our data represent new and useful findings in terms of IL-37b responses, immune correlates and potential mechanisms in TB patients.

While the source of IL-37b production has not been illustrated in TB patients, our results show that increased plasma IL-37b in TB patients can be linked to the ability of TB-sensitized monocytes, but not lymphocytes, to produce IL-37b. Of note, we recently demonstrated the specificity and utility for direct ICS assay detecting intracellular cytokines without a need for antigen stimulation (Zeng et al., 2015). We therefore employed this useful direct ICS approach and demonstrated that monocytes not lymphocytes served as one of sources of IL-37b-production in ATB patients. Our data are some-how consistent with the

early report that IL-37 is expressed at a very low level in steady-state target cells and tissues of humans without Mtb infection, although published studies showed that IL-37b could be produced under circumstances by various types of cells, including PBMCs, epithelial cells, macrophages, dendritic cells (DCs) and T cells (Boraschi et al., 2011; Ye et al., 2014, 2015).

Whereas precise roles of IL-37 in human infections remain largely unknown (Boraschi et al., 2011; Chen and Fujita, 2015; Tete et al., 2012; Ye and Huang, 2015), the current study demonstrates that IL-37b production appears to be associated with prolonged/complicated TB, TB burdens and inflammatory reactions, and negatively correlate with immune responses of pro-inflammatory cytokines. IL-37b expression is higher in TB patients re-treated for relapse TB, severe TB and/or drug resistance. It is also higher in patients with sputum smear Mtb, and a short-term anti-TB chemotherapy significantly reduce IL-37. Our findings appear to consist with the recent reports indicating that increases in IL-37 expression in infections including HIV-1 and HBV infections are associated with viral loads and inflammation (Anuradha et al., 2015; Hojen et al., 2015; Huang et al., 2015; Li et al., 2013; van de Veerdonk et al., 2014).

Our data suggest that TB-sensitized monocytes can sustain immune response of IL-37. Impressive levels of plasma IL-37 are consistently detected in active TB patients, particularly in those subpopulations with prolonged and complicated TB course. Such sustained IL-37 response correlates with decreasing trends of pro-inflammatory cytokines IL-1 β , IL-6, TNF- α in plasma. Furthermore, interesting data from our mechanistic experiments suggest that TB can sensitize and sustain the ability of monocytes to produce IL-37b in response to mycobacterial re-infection in culture. In contrast, a lack of sustained IL-37 response can only be seen during BCG re-infection of monocytes from HC subjects with documented BCG vaccination. Furthermore, the BCG infection of monocytes/PBMC from ATB patients uncovers a surprising observation that TB-sensitized monocytes from ATB patients favor predominant immune responses of IL-37b, with negative correlation with pro-inflammatory cytokines IL-1 β , IL-6, TNF- α , and anti-inflammatory cytokine IL-10. Such negative correlation in mycobacterial re-infection culture is consistent with what is seen between plasma levels of these cytokines and IL-37b. The consistent data in TB patients provide a rationale or opportunity to undertake further in-depth studies to eventually define roles of IL-37b in TB.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molimm.2016.09.018>.

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Fig. 1.

Consistent increases in plasma IL-37b and IL-37b-producing monocytes in ATB patients. IL-37 mRNA and protein in ATB patients were measured by qRT-PCR, ELISA and flow cytometry, respectively. (A) Statistic comparison of plasma levels of IL-37b between ATB patients and healthy controls (HCs) detected by a sandwich ELISA ($n = 36$ in ATB, $n = 22$ in HCs). (B) Plasma IL-37b level in ATB patients before and after one month of treatment ($n = 8$). (C) Graph data showing mRNA expression of IL-37 in PBMC from ATB patients and HCs ($n = 23$ in ATB, $n = 10$ in HCs). (D) Changes in IL37b mRNA expression in PBMC from ATB patients before and after one month of treatment ($n = 8$). (E) Representative flow cytometry histograms showing percentages of IL-37b-producing cells within monocytes. Control isotype Ig ICS staining yielded very low levels of IL-37b ($<0.1\%$, Fig. S1), (F) Graph data showing percentage of IL-37b-producing cells within monocytes ($n = 43$ in ATB, $n = 11$ in HCs). (G) IL-37b-producing monocytes in ATB patients before and after one month treatment ($n = 8$). M0 and M1 indicate pretreatment and one month after treatment, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 2.

IL-37b-producing monocytes were associated with prolonged/complicated TB, and TB burden.

Percentage of IL-37b-producing monocytes in ATB patients at different clinical status was analyzed by flow cytometry. (A) Representative flow cytometry histograms showing percentage of IL-37b-producing monocytes in IT/RT, AP/AN and SP/SN patients. (B) Graph data showing percentage of IL-37b-producing monocytes in IT and RT ATB patients ($n = 32$ in IT, $n = 13$ in RT). (C) Graph data showing percentage of IL-37b-producing monocytes in AP and AN ATB patients ($n = 26$ in AN, $n = 15$ in AP). (D) Graph data showing percentage of IL-37b-producing monocytes in SP and SN patients ($n = 10$ in SP, $n = 38$ in SN). IT: initial-treatment, RT: re-treatment, AP: tuberculosis antibody positive, AN: tuberculosis antibody negative, SP: mycobacterium smear positive, SN: mycobacterium smear negative.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 3.

IL-37b-producing monocytes correlated with inflammatory reactions.

The percentage numbers of IL-37b-producing monocytes correlate with the absolute number of monocyte (A), percentages of monocytes (B) in blood white cells, the percentage of lymphocyte in white blood cells (C), and the level of CRP (D) in ATB patients as analyzed by Pearson correlation statistically using GraphPad Prism Version 5.0.

Fig. 4.

Plasma IL-37b and IL-37b-producing monocytes in ATB correlated negatively with immune responses of pro-inflammatory cytokines.

Innate immune cytokines were detected by ELISA and their correlations with IL-37b-producing monocytes or plasma IL-37b were analyzed. (A), (B), (C), (D) and (E) showing the levels of IL-1β, IL-6, TNF-α, IL-10 and IL-17 in ATB patients and HCs. (F), (G), (H), (I), (J) showing that plasma IL-37b correlated negatively with innate immune cytokines IL-1β, IL-6, TNF-α, IL-10 and IL-17, respectively. (K), (L), (M), (N), (O) showing that IL-37b-producing monocytes correlated negatively with IL-1β, IL-6, TNF-α, IL-10 and IL-17, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 5.

Mycobacterial infection of monocytes from TB patients, but not HC, sustained IL-37b production.

PBMCs were isolated from initial treatment ATB patients ($n = 5$) with sputum positive and HCs ($n = 10$), and then stimulated with BCG *in vitro*. IL-37b-producing monocytes were detected by flow cytometry. (A) Representative flow cytometry histograms showing the percentage of IL-37b-producing monocytes stimulated with BCG or LPS for 24 h. (B) and (C) Graph data showing percentage of IL-37b-producing monocytes from ATB patients or HCs stimulated with LPS (B) or BCG (C). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 6.

TB-sensitized monocytes from ATB patients mounted more robust immune responses of IL-37b than those of pre-inflammatory cytokines during mycobacterial re-infection in culture.

PBMCs were isolated from initial treatment sputum smear positive ATB patients ($n = 5$) and HCs ($n = 5$), and then stimulated with BCG for 24hr *in vitro*. The productions of inflammatory cytokines in PBMCs were determined by ELISA. Then the correlations of IL-37-producing monocytes with cytokines were analyzed. (A), (B), (C), (D) and (E) compare the levels of IL-1 β , IL-6, TNF- α , IL-10 and IL-17 in cell culture supernatants, respectively, between active TB patients and healthy controls. (F), (G), (H), (I) and (J) showing that IL-37b-producing monocytes correlated negatively with cytokines IL-1 β (F), IL-6 (G), TNF- α (H), IL-17 (I) and IL-10 (J). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 1

Demographics of the study population.

	ATB	HV	P
Age (Y)	39.90 ± 11.44	36.05 ± 9.40	>0.05
Gender (F/M)	25/27	10/11	>0.05
T (M0/M1)	48/19	21/0	–