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Tuberculosis-sensitized monocytes sustain immune response of interleukin-37

Jun-Ai Zhang[a,c,1], Gan-Bin Liu[b,1], Bi-Ying Zheng[a,1], Yuan-Bin Lu[a,c], Yu-Chi Gao[a,c], Xiao-Zhen Cai[d], You-Chao Dai[a,c], Shi-Yan Yu[a,c], Yan Jia[a,c], Chen Chen[a,c], Ze-Gang Zhuang[a,c], Xin Wang[a,c], Wan-Dang Wang[a,e], Xiao-Xia Fu[a], Lai-Long Yi[b], Ling Shen[e], Zheng W. Chen[e], Jun-Fa Xu (Dr.)[a,c,*]

[a] Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, No. 1 Xincheng Road, Dongguan, 523808, China
[b] Department of Respiration, Dongguan 6th Hospital, Dongguan, 523008, China
[c] Department of Clinical Immunology, Institute of Laboratory Medicine, Guangdong Medical University, No. 1 Xincheng Road, Dongguan, 523808, China
[d] Department of Respiration, Affiliated Houjie Hospital of Guangdong Medical University, Dongguan, China
[e] Department of Microbiology and Immunology, Center for Primate Biomedical Research, University of Illinois College of Medicine, Chicago, IL, United States

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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-1β, 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.

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-1β, 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-1β, 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

* Corresponding author at: Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, No. 1 Xincheng Road, Dongguan, 523808, China.
E-mail address: xujunfa@gdmu.edu.cn (J.-F. Xu).
[1] These authors contributed equally to this work.

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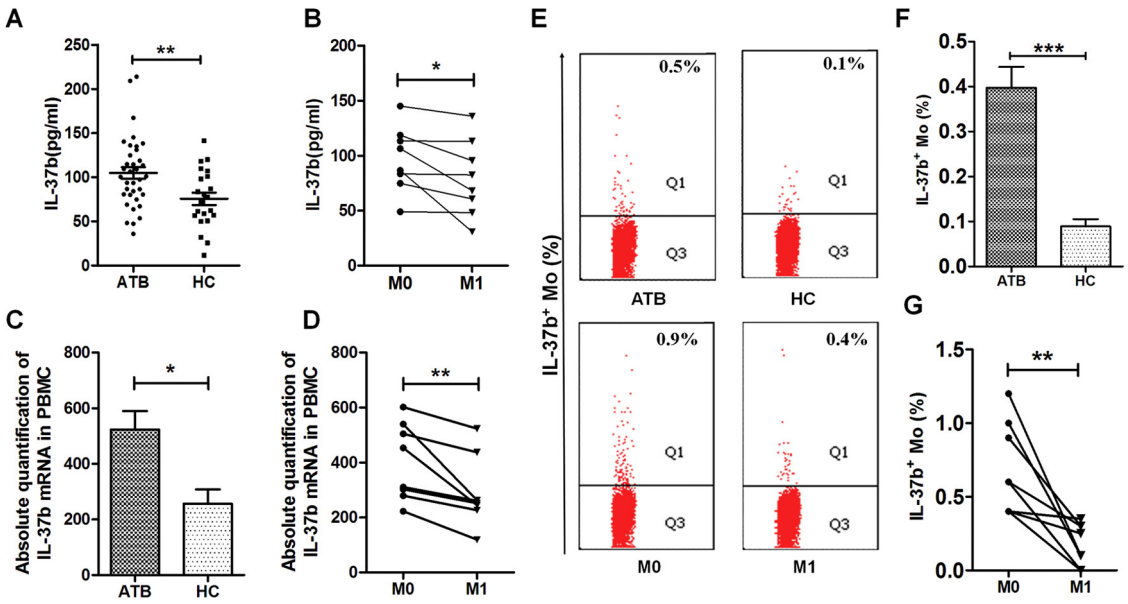

Fig. 1. Consistent increases in plasma IL-37b and IL-37b-producing monocytes in ATB patients.
IL-37 mRNA and protein in ATB patients were measured by qRT-PCR, ELISA and flow cytometry, respectively. (A) Statistic comparison of plasma levels of IL-37b between ATB patients and healthy controls (HCs) detected 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. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

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 L-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-1β 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 influences 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 Dongguang 6st 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 Dongguang 6st People's Hospital, and a written informed consent was obtained from all study subjects prior to the participation in this study.

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	–