Acta Biochim Biophys Sin, 2015, 47(8), 597–603
doi: 10.1093/abbs/gmv058
Advance Access Publication Date: 20 June 2015
Original Article

Original Article

IL-37b suppresses T cell priming by modulating dendritic cell maturation and cytokine production via dampening ERK/NF-κB/S6K signalings

Wantong Wu[1,2], Weiqiang Wang[3], Yun Wang[4], Wenwen Li[3], Gang Yu[1], Zhonglong Li[1], Chunmin Fang[1], Yue Shen[1], Zhina Sun[1], Ling Han[1], Juan Yu[1], Lijun Fang[1], Song Chen[1], Kui Dong[3], Zhongchao Han[1], Hanzhi Liu[1], Yuechen Luo[1,*], and Xiaoming Feng[1,*]

[1]State Key Laboratory of Experimental Hematology, Institute of Hematology and Hospital of Blood Disease, Chinese Academy of Medical Sciences, Tianjin 300020, China, [2]Tianjin Dongli Hospital, Tianjin 300300, China, [3]Department of Gastroenterology and Hepatology, General Hospital, Tianjin Medical University, Tianjin 300052, China, and [4]Center of Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing 100191, China

*Correspondence address. Tel/Fax: +86-22-23909409; E-mail: xfeng1979@hotmail.com (X.F.)/ycluo2013@163.com (Y.L.)

Received 9 March 2015; Accepted 16 April 2015

Abstract

Interleukin 37b (IL-37b) plays a key role in suppressing immune responses, partially by modulating the function of dendritic cells (DCs). However, the precise mechanisms are still largely unknown. Here, we investigated the effects of IL-37b on DC maturation and T cell responses induced by DCs, and explored the involved signaling pathways. It was found that IL-37b down-regulated the expressions of co-stimulatory molecules CD80 and CD86 on DCs *in vitro*. At the same time, the expressions of pro-inflammatory cytokines, such as TNF-α and IL-6, were suppressed, while the expression of the T cell inhibitory cytokine TGF-β was increased in IL-37b-treated DCs. In addition, the activation effect of DCs on T cells was impaired by IL-37b. We further revealed that extracellular single-regulated kinase (ERK), nuclear factor-κB (NF-κB), and mTOR-S6K signaling pathways were involved in the inhibition of DCs induced by IL-37b. This was confirmed by the similarly suppressive effect of chemical inhibitors against NF-κB, ERK, and S6K on the expressions of IL-6 and TNF-α in DCs. In conclusion, these results demonstrated that IL-37b suppressed DC maturation and immunostimulatory capacity in T cell priming by involving in ERK, NF-κB, and S6K-based inhibitory signaling pathways.

Key words: IL-37b, dendritic cells, cytokines

Introduction

Interleukin (IL)-1 is a family of protein molecules that possess a variety of immunoregulatory properties in inflammation and immune diseases [1,2]. In this family, IL-37 which is recently identified and originally defined as IL-1 family member 7 (IL-1F7) is a fundamental inhibitor of innate immunity [3,4]. It has been verified to be expressed in a variety of normal tissues, such as tonsils, skin, esophagus, placenta, and also carcinomas of the breast, prostate, colon, and lung, albeit at low levels [4–7]. IL-37 can also be induced in peripheral blood mononuclear cells (PBMCs) and dendritic cells (DCs) [3]. IL-37 has various splice forms (IL-37a–e), and IL-37b is the most studied one [8]. Recently, McNamee *et al.* [9] and Bulau *et al.* [10] found that the murine IL-37b transgenic model is protected from lipopolysaccharide (LPS)-induced shock and dextran sulfate sodium-induced colitis. In addition, IL-37 shows anti-inflammatory effects on liver cells and can reduce liver injury induced by hepatic ischemia/reperfusion [11].

DCs constitute a rare cell population, accounting for ~0.1%–2% of the total cells in most tissues. DCs are professional antigen-presenting cells, playing a key role in the activation of immune responses by capturing, processing, and presenting antigens, and by activating naive T cells [12]. Under steady-state conditions, DCs maintain an immature status. Once activated by inflammatory signal, the expressions of co-stimulatory molecules, such as CD80, CD86, and CD40, and pro-inflammatory cytokines were increased in DCs to facilitate T cell activation and expansion [13,14].

Although it was reported that the presence of IL-37b conferred a marked reduction in LPS-induced DC activation, and IL-37b also impaired the activation of effector T cells [15], the effects of exogenous IL-37b on DC activation and the related signaling pathways are still unclear. In this study, we investigated the role of exogenous IL-37b in modulating the function of DCs on T cell responses and the underlying molecular mechanisms.

Materials and Methods

Isolation and culture of bone marrow-derived DCs

Six-to-eight-week-old male C57BL/6 mice (18–20 g) were purchased from Institute of Hematology, Chinese Academy of Medical Sciences (Tianjin, China). All mice were maintained in specific pathogen-free barrier facilities at Institute of Hematology, Chinese Academy of Medical Sciences. All animal experiments were conducted according to guidelines made by Animal Care and User Committee at the Institute of Hematology, Chinese Academy of Medical Sciences. Mice were sacrificed by cervical dislocation. Both femurs and tibiae were dissected free from adherent tissue. Bone marrow was eluted by flushing the bones with RPMI 1640 medium (Invitrogen, Carlsbad, USA) supplemented with 1% horse serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were dispersed by vigorous pipetting, washed by centrifugation and cultured in T75 flask in medium at 37°C in a humidified 5% CO_2 atmosphere. Cells were re-suspended at 2×10^7 cells/ml in DC complement medium (RPMI 1640 plus Glutamax (Invitrogen), supplemented with 1% fatal bovine serum, 15 mM HEPES, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin) containing 50 ng/ml IL-4 and 100 ng/ml GM-CSF (BD Biosciences, San Jose, USA), and cultured for 6 days by replacing half of the medium with fresh cytokines every other day. After 6 days, the cells were harvested and $CD11c^+$ DCs were separated with magnetic bead sorting technique (Miltenyi Biotec, San Diego, USA).

Flow cytometric analysis

Unless otherwise specified, antibodies used in this work were purchased from eBioscience (San Diego, USA). To analyze the levels of co-stimulatory molecules CD80, CD86, and CD40 on DCs after treatment with IL-37b, cells were incubated with anti-CD80 (FITC), anti-CD86 (PerCP/Cy5.5), anti-CD40 (PE), or isotype Abs for 30 min on ice. To analyze the intracellular expression levels of phosphorylated ERK, NF-κB, and S6K, cells were fixed for 15 min with 10% methanol at room temperature, treated with 0.1% Triton-100 for 30 min, and then treated with cold 50% methanol on ice for 15 min. After being washed with phosphate-buffered saline containing 1% BSA, cells were stained for 30 min with PE-conjugated antibodies. Detection was conducted with a BD FACS Canto II flow cytometer (BD Biosciences) and analyzed with FlowJo software (Treestar, San Carlos, USA).

T cell proliferation assay *in vitro*

To explore the effect of IL-37b-treated DCs on T cell activation and proliferation, we performed *in vitro* T cell proliferation assay as described previously [16]. Briefly, 5×10^4 DCs treated with or without 200 ng/ml recombinant IL-37b (R&D Systems, Minneapolis, USA) for 24 h were co-cultured with lymph node cells labeled with carboxyfluorescein succinimidyl ester (CFSE; Invitrogen) at a ratio of 1:4 in anti-CD3 antibody-coated 96-well plates. After 72 h, cell size (indicated by forward scatter, FSC), T cell proliferation (indicated by CFSE), and the expression levels of CD25 and CD69 were examined by flow cytometry. Data were analyzed by FlowJo software.

Quantitation of IL-6, IL-10, TNF-α, TGF-β, and MCP-1

Concentrations of pro-inflammatory cytokines IL-6, IL-10, MCP-1, and TNF-α in culture supernatants of DCs were measured by mouse inflammation cytokine cytometric bead array. Concentration of TGF-β was determined with ELISA kit (eBioscience).

Quantitative RT-PCR

Total RNA was isolated from DCs by TRIzol reagent (Invitrogen) and cDNA was obtained using MMLV-RT (Gibco-BRL, Eggenstein, Germany). Quantitative RT-PCR was performed with SYBR green PCR mastermix (QIAGEN, Hilden, Germany) on ABI 7500 (Applied Biosystems, Foster City, USA). The primers used were as follows: *IL-10*, forward: 5′-GCTGCCTGCTCTTACTGACT-3′, and reverse: 5′-CTGGGAAGTGGGTGCAGTTA-3′; *TNF-α*, forward: 5′-AGGG GATTATGGCTCAGGGT-3′, and reverse: 5′-CCACAGTCCAGGT CACTGTC-3′; *TGF-β*, forward: 5′-GCTCCCCTATTTAAGAA CACCC-3′, and reverse: 5′-TTGAGGTTGAGGGAGAAAGC-3′; *IL-6*, forward: 5′-GAAAGCCAGTCCTTCAGAG-3′, and reverse: 5′-GTCCTTAGCCACTCCTTCTG-3′; *IL-18*, forward: 5′-GCCTC AAACCTTCCAAATCAC-3′, and reverse: 5′-GTTGTCTGATTCC AGGTCTCC-3′; *β-actin*, forward: 5′-CGGTTCCGATGCCCT GAGGCTCTT-3′, and reverse: 5′-CGTCACACTTCATGATGG AATTGA-3′.

Blockage of MEK-ERK, S6K, and NF-κB in DCs

Purified DCs were seeded in 96-well plates and treated with inhibitors of MEK-ERK (U0126, 26 mM), S6K (PF-4708671, 1 μM), and NF-κB (BAY11-7082, 48 mM) separately. After 48 h, DCs were collected and RT-PCR was performed as described in the above session.

Statistical analysis

All statistical tests were performed with GraphPad Prism software. Each experiment was repeated at least three times. Relative mRNA expression levels, cytokine levels, and relative fluorescence values were expressed as the mean ± SD. Unpaired Student's *t*-test was used to define differences between the two groups. A *P*-value of <0.05 was considered of significant difference.

Results

IL-37b inhibited DC maturation

After sorting by magnetic bead, flow cytometric analysis showed that the purity of $CD11c^+$ DCs was ~92% (**Fig. 1A**), and the percentage of $CD14^+$ cells was ~2% (**Supplementary Fig. S1**), ruling out the contamination of a significant number of monocytes. The DC maturation markers CD80, CD86, and CD40 were evaluated after being treated with IL-37b for 48 h. CD80 and CD86 were slightly down-regulated, while no significant effect was observed on CD40 expression (**Fig. 1B**). This indicated that IL-37b treatment can mildly inhibit the expressions of co-stimulatory molecules on DCs.

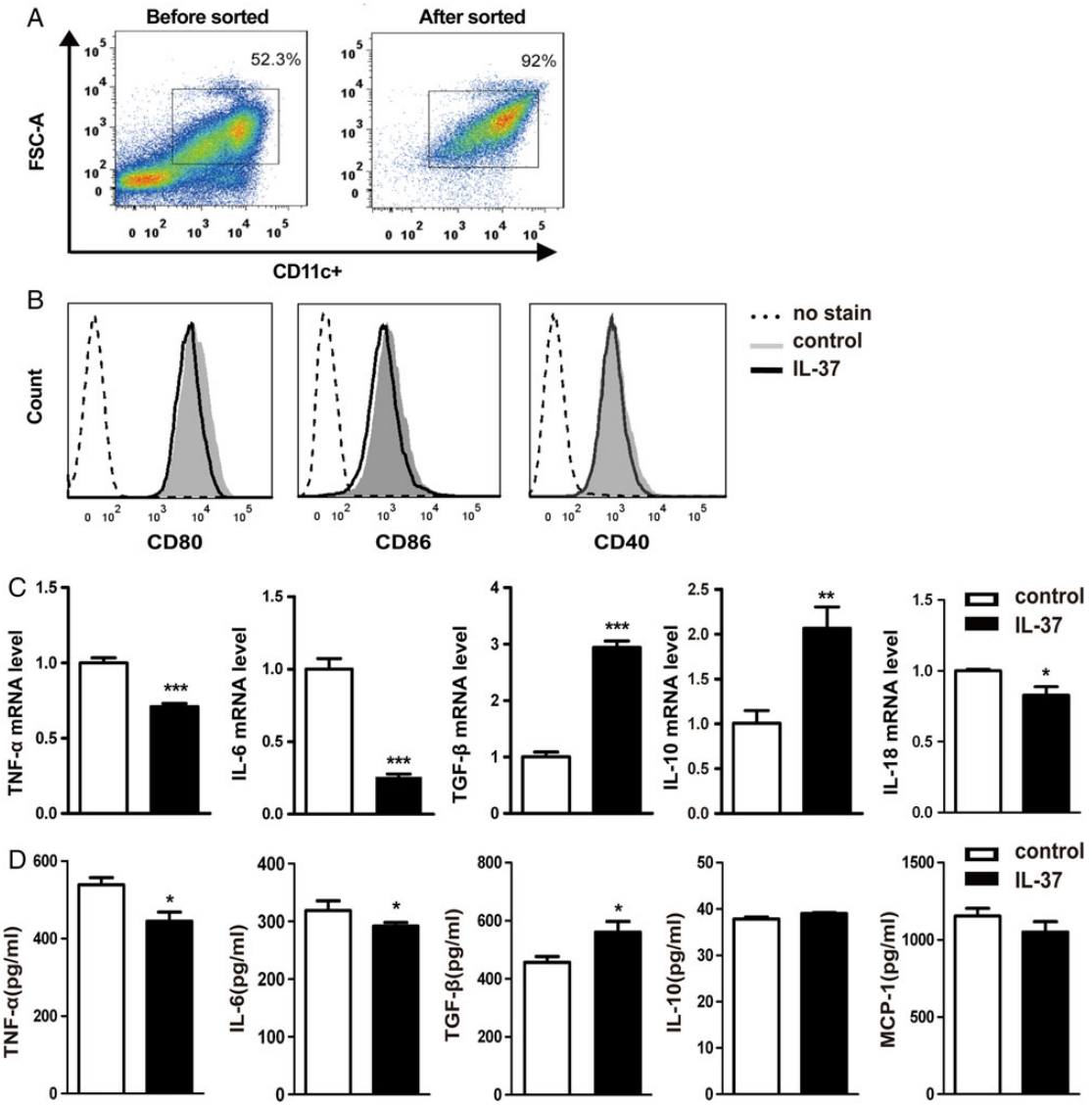

Figure 1. Expression of co-stimulatory molecules on DCs and the cytokine levels in DC culture supernatants after treatment with IL-37b (A) Flow cytometry plots of DC purity before and after being separated with magnetic bead sorting technique. (B) Flow cytometric analysis of the expression levels of CD40, CD80, and CD86 on DCs with/without IL-37b treatment. (C) mRNA expression levels of TNF-α, IL-6, IL-18, IL-10, and TGF-β in DCs with/without IL-37b treatment. (D) The concentrations of TNF-α, IL-6, IL-10, TGF-β, and MCP-1 proteins in DC culture supernatants with/without IL-37b treatment. Values are the mean ± SD of three independent experiments. $*P<0.05$, $**P<0.01$, $***P<0.001$.

We further analyzed the effect of IL-37b on the production of TNF-α, IL-6, IL-10, IL-18, and TGF-β in DCs. IL-37b treatment significantly down-regulated the mRNA levels of pro-inflammatory cytokines TNF-α, IL-18, and IL-6, while up-regulated those of anti-inflammatory cytokines TGF-β and IL-10 (**Fig. 1C**). The impact of IL-37b on the expressions of TNF-α, IL-6, and TGF-β in DCs was confirmed by ELISA, but no changes in the protein expressions of IL-10 and MCP-1 were noted in comparison with the control (**Fig. 1D**).

IL-37b reduced T cell activation capability of DCs

As potent antigen-presenting cells, DCs present processed peptides to naïve T cells and induce T cell activation and differentiation. Since the treatment with IL-37b impaired DC maturation and cytokine production, we further explored whether IL-37b can influence DC-mediated T cell activation and proliferation. DCs pre-treated with IL-37b were co-cultured with lymphocytes stimulated by anti-CD3 antibody. Results showed that the activation and proliferation of $CD4^+$ T cells were inhibited as determined by decreased cell size (FSC), reduced expression of surface markers CD25 and CD69, and suppressed proliferation (CFSE) (**Fig. 2A,B**). Similar effects were observed in $CD8^+$ T cells (**Fig. 2A,B**).

IL-37b inhibited DC maturation via repressing ERK1/2, NF-κB, and S6K signaling

We further explored the signaling pathways that might mediate the effects of IL-37b on DCs. By flowcytometry, we detected the phosphorylation levels of ERK1/2, S6K, and NF-κB that are important signaling effectors of DC maturation. The levels of phosphorylated ERK1/2, S6K, and NF-κB in DCs were all declined after treatment with IL-37b (**Fig. 3A,B**), especially that of NF-κB which had been verified by western blotting (**Supplementary Fig. S2**).

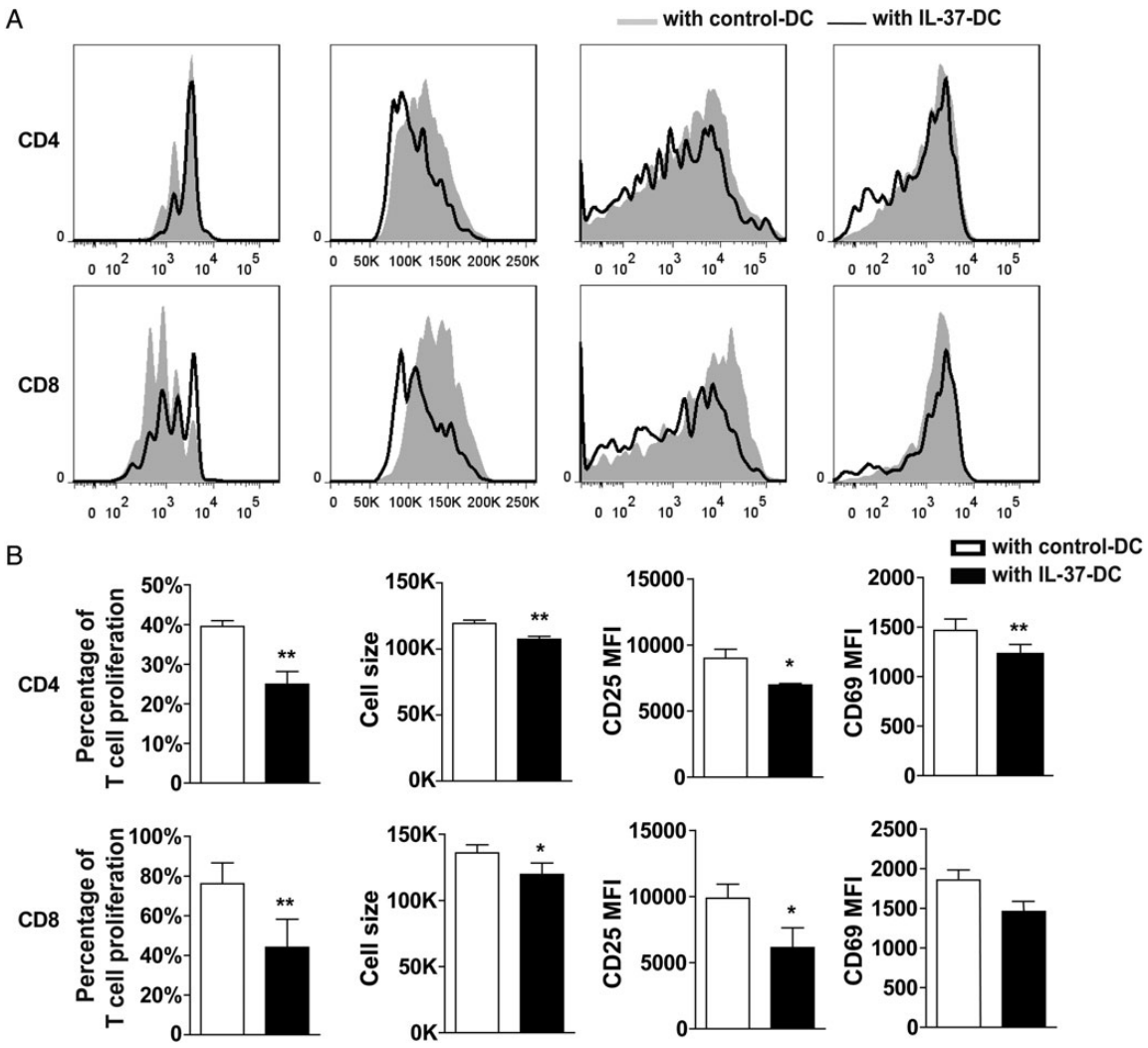

Figure 2. Treatment of IL-37b dampens the stimulatory function of DCs on T cell activation and proliferation (A) Cell proliferation (CFSE), cell size (FSC), and expression of activation markers CD25 and CD69 were analyzed by flow cytometry for $CD4^+$ and $CD8^+$ lymphocytes stimulated with anti-CD3 antibody for 72 h, in the presence of control DCs or IL-37b-treated DCs. (B) Percentage of T cell proliferation, cell size, and mean fluorescence intensity (MFI) of CD25 and CD69 were determined based on Phospho flowcytometric analysis. Data are the mean ± SD of three independent experiments. $*P < 0.05$, $**P < 0.01$.

ERK, S6K, and NF-κB inhibitors reduced mRNA levels of IL-6 and TNF-α in DCs

In order to confirm the involvement of ERK, S6K, and NF-κB in IL-37b-mediated suppression of pro-inflammatory cytokines, chemical inhibitors against these three molecules were used to detect the expression changes of TNF-α and IL-6. All the three inhibitors declined the expression levels of TNF-α and IL-6 (**Fig. 4**). These results support the notion that IL-37b suppresses the expression of TNF-α and IL-6 via inhibition of mTOR, ERK1/2, and NF-κB signaling pathways.

Discussion

IL-37, one member of the IL-1 family, has been recognized as a potent inhibitor of innate and adaptive immunity [17]. Transgenic expression of IL-37b suppresses the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β in RAW macrophages, THP-1 cells, etc. [17,18]. IL-37b transgenic mice were protected from LPS-induced shock, and DC activation was suppressed in peripheral blood of these LPS-treated mice [19]. Moreover, DCs from IL-37b transgenic mice showed strong capability in inducing regulatory T cells which is an important cell type to exert immuno-suppressive function [15].

Up to now, most anti-inflammatory properties of IL-37b rely on results obtained using transgenic expression or induced expression of IL-37 *in vivo* or *in vitro*, and have demonstrated that IL-37b acts as an intracellular signaling effector to affect gene transcription through binding Smad3 [17]. However, few studies are engaged in investigating the immuno-suppressive function of exogenous recombinant IL-37b protein, which is more practical for potential clinical application.

In the present study, we determined the effects of recombinant IL-37b protein on DC activation and further investigated the involved signaling pathways. Pretreatment with recombinant IL-37b protein reduced the ability of DCs to activate T cells, indicating that exogenous IL-37b might exert immuno-suppressive function via DCs. Then, how does recombinant IL-37b protein suppress DCs? On one hand, upon encountering foreign antigen, DCs rapidly convert from immature stage to mature stage and express more co-stimulatory molecules

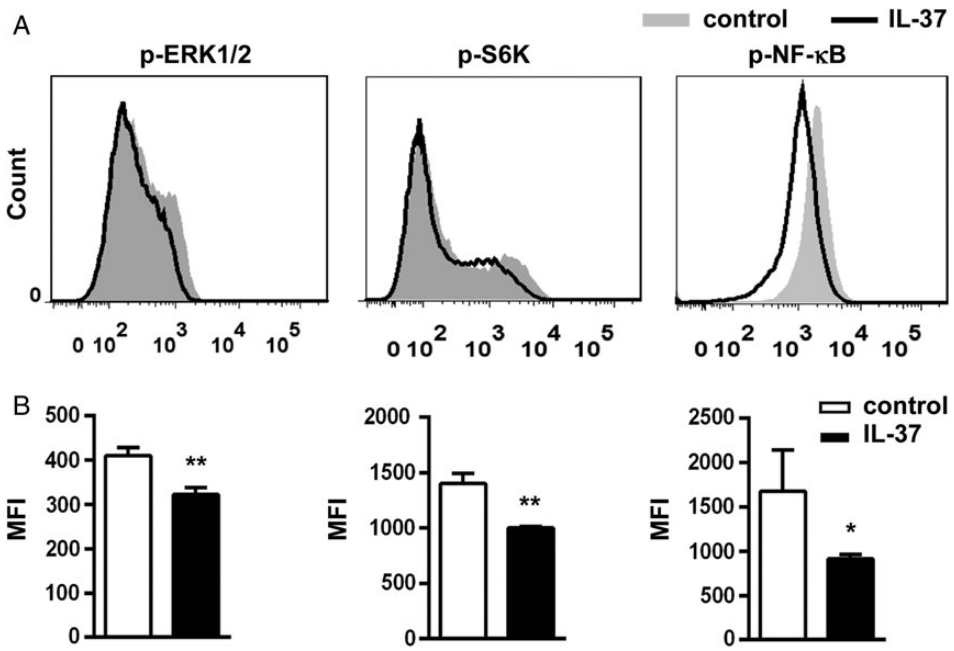

Figure 3. The phosphorylation levels of ERK1/2, S6K, and NF-κB in DCs are reduced by IL-37b (A) Purified DCs were treated with or without IL-37b for 48 h, and the phosphorylation levels of ERK1/2, S6K, and NF-κB were detected by Phospho flowcytometric analysis. Data were representative of three independent experiments. (B) Mean fluorescence intensity (MFI) was determined based on Phospho flowcytometric analysis. Data are the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

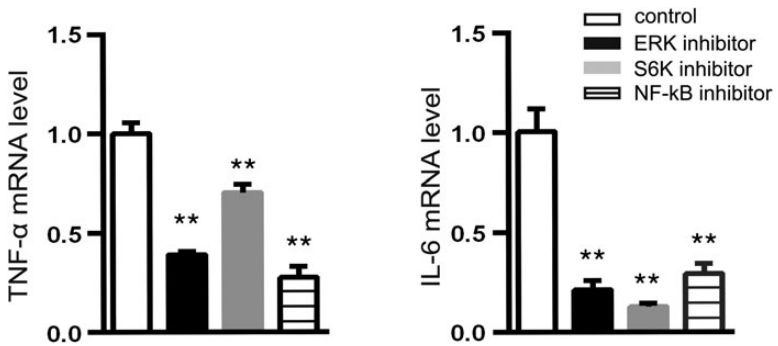

Figure 4. mRNA levels of TNF-α and IL-6 are decreased by inhibitors of ERK1/2, S6K, and NF-κB After DCs were treated for 48 h with inhibitor of ERK (U1026), S6K (PF-4708671), or NF-κB (BAY11–7082), RT-PCR was performed to determine the mRNA levels of TNF-α and IL-6. Data were represented as the mean ± SD of three independent experiments. $^{**}P < 0.01$.

CD80 and CD86, developing an improved ability to activate T cells. Since the expression of CD80 and CD86 on DCs was inhibited by pre-treatment with recombinant IL-37b protein, it suggests that exogenous IL-37b might affect DCs at least partly via co-stimulatory molecules. On the other hand, our results implied that down-regulation of pro-inflammatory cytokines TNF-α and IL-6 could contribute to the inhibition of DC activation by IL-37b. In addition to T cells, TNF-α and IL-6 can stimulate some other immune cell types, such as macrophages and DCs [20–22]. Our result is in consistent with a report that increased production of IL-6 and TNF-α by LPS or Pam_3CSK_4 in PBMCs was suppressed, while the anti-inflammatory cytokine IL-10 was unaffected after the treatment with siRNA against IL-37b [17]. In addition, recombinant IL-37b suppressed the production of inflammatory cytokines in PBMCs from systemic lupus erythematosus patients and health controls [23].

Moreover, up-regulation of anti-inflammatory cytokines IL-10 and TGF-β by recombinant IL-37b protein was unveiled in the present study, which might further contribute to the inhibition of DC activation. IL-10 and TGF-β potently inhibit T cell responses by involvement of regulatory T cells and M2 macrophages [24]. Furthermore, TGF-β is well established as an inhibitor of DC itself [25]. Smad proteins are the main intracellular signaling mediators of TGF-β and can be phosphorylated by TGF-β receptors upon binding with the ligand, and subsequently Smad3 can translocate to nucleus and affect gene transcription [26,27].

NF-κB pathway is critical for TLR-induced DC maturation. In addition to NF-κB activation, MAPK family signaling also plays important roles in DC maturation [28]. MAPK activity was determined by assessing ERK1/2 phosphorylation. The signaling mechanistic target of rapamycin (mTOR) has been reported as a major regulator of

DCs in various biological features, including differentiation and maturation [29]. S6K is a major and well-characterized downstream target of mTOR. Treatment with recombinant IL-37b protein decreased the phosphorylation of ERK, S6K, and NF-κB as well as the production of pro-inflammatory cytokines such as TNF-α and IL-6 in DCs. Application of the inhibitors against the three molecules in DCs exerted similar effects as IL-37b did, supporting the notion that IL-37b might function through multiple signaling pathways. However, phosphorylated ERK, S6K, and NF-κB are downstream effectors of different signaling pathways. The upstream effectors, especially the receptors through which IL-37b affects DCs remain to be explored. Recombinant IL-37b binds with the IL-18R chain, thus being the second ligand for this receptor after IL-18. But whether IL-37b acts on DCs through binding with the IL-18R chain or other alternative receptors is still unknown.

In summary, we demonstrated that exogenous recombinant IL-37b protein suppresses T cell responses through modulation of the expression of co-stimulatory molecules and inflammatory cytokines by DCs. We further revealed that the IL-37b-mediated inhibition might involve the ERK/S6K/NF-κB signaling pathways. Thus, exogenous recombinant IL-37b protein shows promise in regulating the function of DCs and adaptive T cell immunity.

Supplementary Data

Supplementary data is available at *ABBS* online.

Funding

This work was supported by the grants from the National Basic Research Program of China (Nos. 2013CB966904 and 2015CB964402), the National Natural Science Foundation of China (Nos. 81273217, 81322007, and 81401295), the Tianjin Research Program of Application Foundation and Advanced Technology (No. 12JCYBJC32800), the Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20121106120037), and the Technology Foundation for Selected Overseas Chinese Scholar, and the Recruitment Program of Global Youth Experts.

References

1. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996, 87: 2095–2147.
2. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Ann Rev Immunol* 2001, 19: 423–474.
3. Pan GH, Risser P, Mao WG, Baldwin DT, Zhong AW, Filvaroff E, Yansura D, *et al*. IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. *Cytokine* 2001, 13: 1–7.
4. Kumar S, McDonnell PC, Lehr R, Tierney L, Tzimas MN, Griswold DE, Capper EA, *et al*. Identification and initial characterization of four novel members of the interleukin-1 family. *J Biol Chem* 2000, 275: 10308–10314.
5. Busfield SJ, Comrack CA, Yu G, Chickering TW, Smutko JS, Zhou H, Leiby KR, *et al*. Identification and gene organization of three novel members of the IL-1 family on human chromosome 2. *Genomics* 2000, 66: 213–216.
6. Bufler P, Gamboni-Robertson F, Azam T, Kim SH, Dinarello CA. Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide. *Biochem J* 2004, 381: 503–510.
7. Kumar S, Hanning CR, Brigham-Burke MR, Rieman DJ, Lehr R, Khandekar S, Kirkpatrick RB, *et al*. Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production. *Cytokine* 2002, 18: 61–71.
8. Ji Q, Zeng Q, Huang Y, Shi Y, Lin Y, Lu Z, Meng K, *et al*. Elevated plasma IL-37, IL-18, and IL-18BP concentrations in patients with acute coronary syndrome. *Mediators Inflamm* 2014, 2014: 165742.
9. McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, Nold M, *et al*. Interleukin 37 expression protects mice from colitis. *Proc Natl Acad Sci U S A* 2011, 108: 16711–16716.
10. Bulau AM, Fink M, Maucksch C, Kappler R, Mayr D, Wagner K, *et al*. In vivo expression of interleukin-37 reduces local and systemic inflammation in concanavalin A-induced hepatitis. *Sci World J* 2011, 11: 2480–2490.
11. Sakai N, Van Sweringen HL, Belizaire RM, Quillin RC, Schuster R, Blanchard J, Bufler P. Interleukin-37 reduces liver inflammatory injury via effects on hepatocytes and non-parenchymal cells. *J Gastroenterol Hepatol* 2012, 27: 1609–1616.
12. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. *Cell* 2001, 106: 263–266.
13. Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol* 2007, 7: 610–621.
14. Hubert P, Jacobs N, Caberg JH, Boniver J, Delvenne P. The cross-talk between dendritic and regulatory T cells: good or evil? *J Leukoc Biol* 2007, 82: 781–794.
15. Luo Y, Cai X, Liu S, Wang S, Nold-Petry CA, Nold MF, Bufler P, *et al*. Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells. *Proc Natl Acad Sci U S A* 2014, 111: 15178–15183.
16. Hu X, Zhou Y, Dong K, Sun Z, Zhao D, Wang W, Yu G, *et al*. Programming of the development of tumor-promoting neutrophils by mesenchymal stromal cells. *Cell Physiol Biochem* 2014, 33: 1802–1814.
17. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol* 2010, 11: 1014–1022.
18. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, Klunker S, *et al*. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J Allergy Clin Immunol* 2011, 127: 701–721.
19. Arechiga H, de la Fuente JR, de Leon C, Salinas V, Alarcon Segovia D. Medical research in Mexico. Its present status and an outlook. *Gac Med Mex* 1990, 126: 265–282.
20. Pirtskhalaishvili G, Shurin GV, Esche C, Trump DL, Shurin MR. TNF-alpha protects dendritic cells from prostate cancer-induced apoptosis. *Prostate Cancer Prostatic Dis* 2001, 4: 221–227.
21. Chomarat P, Dantin C, Bennett L, Banchereau J, Palucka AK. TNF skews monocyte differentiation from macrophages to dendritic cells. *J Immunol* 2003, 171: 2262–2269.
22. Heusinkveld M, de Vos van Steenwijk PJ, Goedemans R, Ramwadhdoebe TH, Gorter A, Welters MJ, van Hall T, *et al*. M2 macrophages induced by prostaglandin E2 and IL-6 from cervical carcinoma are switched to activated M1 macrophages by CD4+ Th1 cells. *J Immunol* 2011, 187: 1157–1165.
23. Li Y, Wang Z, Yu T, Chen B, Zhang J, Huang K, Huang Z. Increased expression of IL-37 in patients with Graves' disease and its contribution to suppression of proinflammatory cytokines production in peripheral blood mononuclear cells. *PLoS ONE* 2014, 9: 107183.
24. Terabe F, Fujimoto M, Serada S, Shinzaki S, Iijima H, Tsujii M, Hayashi N, *et al*. Comparative analysis of the effects of anti-IL-6 receptor mAb and anti-TNF mAb treatment on CD4(+) T-cell responses in murine colitis. *Inflamm Bowel Dis* 2011, 17: 491–502.
25. Larmonier N, Marron M, Zeng Y, Cantrell J, Romanoski A, Sepassi M, Thompson S, *et al*. Tumor-derived CD4(+)CD25(+) regulatory T cell suppression of dendritic cell function involves TGF-beta and IL-10. *Cancer Immunol Immunother* 2007, 56: 48–59.
26. Andre S, Tough DF, Lacroix-Desmazes S, Kaveri SV, Bayry J. Surveillance of antigen-presenting cells by CD4+ CD25+ regulatory T cells in

autoimmunity: immunopathogenesis and therapeutic implications. *Am J Pathol* 2009, 174: 1575–1587.

27. Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, Chin MT, *et al*. Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. *J Biol Chem* 2000, 275: 36653–36658.
28. Hazzalin CA, Mahadevan LC. MAPK-regulated transcription: a continuously variable gene switch? *Nat Rev Mol Cell Biol* 2002, 3: 30–40.
29. Yanyan Wang HC. mTOR Signaling and Dendritic Cell Biology. *J Immunol Clin Res* 2014, 2: 1015.