

Original Article

CD11c antigen presenting cells with high B7-H4 expression in active tuberculosis patients are associated with low stimulatory capacity to T cell proliferation

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Abstract: B7-H4 is a co-inhibitory molecule that negatively regulates T lymphocytes, playing a role in immune suppression many disorders. However, the role of antigen presenting cells (APCs)-expressing B7-H4 in active pulmonary tuberculosis (ATB) remains poorly understood. Here, we demonstrated that B7-H4 expression on CD11c APCs increased in patients with ATB. B7-H4 expression on CD11c APCs was associated with high expression of HLA-DR and CD86 both in healthy controls (HCs) and in ATB patients. However, CD86 expression in B7-H4-expressing CD11c APCs was significantly lower in ATB patients compared to that in HCs. Importantly, B7-H4 expressing CD11c APCs from ATB patients showed a lower stimulatory capacity to T cell proliferation than those from HCs in mixed leukocyte culture test. Collectively, our data suggest that the evaluation of B7-H4 expression in CD11c APCs in patients with active tuberculosis might be one of the key molecular mechanisms for the dysfunction of APCs in TB patients. B7-H4 may have important implications in the prevention and treatment of patients with ATB.

Keywords: B7-H4, CD11c APCs, stimulatory capacity, T cell proliferation

Introduction

Tuberculosis (TB) remains a serious global public health problem that accounts for upto 2 million deaths each year [1]. TB is caused by the human pathogen, *Mycobacterium tuberculosis* (*Mtb*), which primarily infects innate immune cells patrolling the lung. Innate immune cells serve as barometers of the immune response against *Mtb* infection by determining the inflammatory milieu in the lungs and promoting the initiation of adaptive immune responses [2]. Antigen presenting cells (APCs) such as dendritic cells, macrophages and monocytes are the major components of innate immune cells

[3]. CD11c is a surface marker expressed in most APCs. It has been reported that depletion of CD11c⁺ cells in vivo delayed CD4⁺ T cells response to *Mtb* and exacerbated the infection [4]. We found that the frequencies of CD11c-expressing APCs (CD11c APCs) increased in active pulmonary tuberculosis (ATB) patients and the expression of HLA-DR and CD86 were up-regulated in CD11c APCs isolated from ATB patients (data not published). However, the function of CD11c⁺ APCs in the peripheral blood of patients with ATB is not fully characterized.

B7-H4 (B7x, B7S1, VTCN1), a newly identified co-stimulatory molecule [5, 6], is a transmem-

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Table 1. The clinical data of studied subjects

Groups	PTB (n=39)	HC (n=15)
Age (years)	(18~60)	(19~53)
Mean \pm SEM	36.79 \pm 1.81	34.07 \pm 2.47
Female/Male	14/25	4/11
IT/RT	32/7	-/-
Sputum smear +/-	7/32	0/15
ATD treatment (0~40 days)	22/17	-/-
PRT/POT		

brane protein belonging to the Ig superfamily with one IgV domain and one IgC domain. The mRNA expression of B7-H4 has been found in both lymphoid and non-lymphoid tissues [6, 7]. However, B7-H4 protein expression is more restricted and not expressed on ex vivo human immune cells (T, B, DC, and monocytes), but can be induced after in vitro stimulation [6, 7]. Studies have indicated that B7-H4 is a negative regulator of T cell activation but only plays a minor role in fine-tuning T cell immunity [6, 7]. B7-H4-deficient mice displayed only a mildly enhanced Th1 response and cytotoxic T-lymphocyte reactions against viral infections [8]. Activation of B7-H4 pathway leads to the inhibitions of TCR-mediated T cell proliferation, cell-cycle progression, and IL-2 production [6, 7]. In addition to the direct impact on T and B cell immune response, B7-H4 also acts as a negative regulator for neutrophil response [9]. Although soluble B7-H4-immunoglobulin (B7-H4-Ig) fusion protein has been shown to bind activated T cells and act as an inhibitory signal molecule just as the transmembrane expressed B7-H4 [10-12], the ligand on T cells for B7-H4 binding has not been identified. Studies have reported that B7-H4 is expressed on APCs, such as dendritic cells [13-15], monocytes [16, 17] and macrophages [18-20]. Interestingly, B7-H4 is also expressed in many tumor cells and highly expressed in immune cells in tumor patients [21, 22]. It was confirmed that B7-H4 acts as a biomarker for immune escape of cancer [21, 22]. However, the role of B7-H4-expressing CD11c APCs in ATB patients remains to be characterized. We thus in the present study detected the expression of B7-H4 in human pulmonary tuberculosis granuloma tissues and in the peripheral blood CD11c APCs in ATB patients and investigated its effects on the stimulatory capacity to T cell proliferation.

Materials and methods

Ethics statement

Written informed consent was obtained from each patient before participation in the study. Ethics approval for the present study was obtained from the Ethics Boards of Guangdong Medical University (GDMU) and the Ethics Boards of Dongguan 6th Hospital.

Subjects

39 active pulmonary tuberculosis (ATB) patients aged from 18 to 60 years and 15 healthy individuals from 19 to 53 years were recruited in this study (Table 1 and Table S1). All the ATB subjects were diagnosed based on clinical symptoms, chest X radiography, Acid fast bacilli (AFB) staining of sputum smears, positive bacterial culture, Bronchoalveolar lavage (BAL) or biopsy direct examination and culture, which were done in Dongguan 6th Hospital (Dongguan, China). Exclusion criteria were HIV+, >65 years, <16 years, pregnancy, alcoholism, diabetes, cancer, autoimmune diseases, or on immunosuppressive treatment. ATB patients received individualized anti-tuberculosis drugs (ATDs) treatment with isoniazid, rifampicin, pyrazinamide and ethambutol. All blood samples were collected before or within ~1 week after the patients received ATDs. 15 healthy control (HC) volunteers had no bacteriological and clinical evidence of TB disease.

Peripheral blood mononuclear cells preparation

Peripheral blood mononuclear cells (PBMCs) were prepared as previously reported [23]. Approximately 5 ml blood was collected from each subject in acid citrate dextrose (ACD)-containing blood collection tubes. PBMCs were freshly isolated from blood by standard Ficoll (GE health) density gradient centrifugation. Cell viability were determined by trypan blue exclusion (>95% in all experiments). PBMCs were then aliquoted for the following experiments.

Antibodies and reagents

The following mouse anti-human Abs were used for flow cytometry: CD11c-APC-eFluor780 (BU15, eBioscience), HLA-DR-PE-Cy7 (LN3, eBioscience), CD86-PerCP-eFluor710 (IT2.2, eBio-

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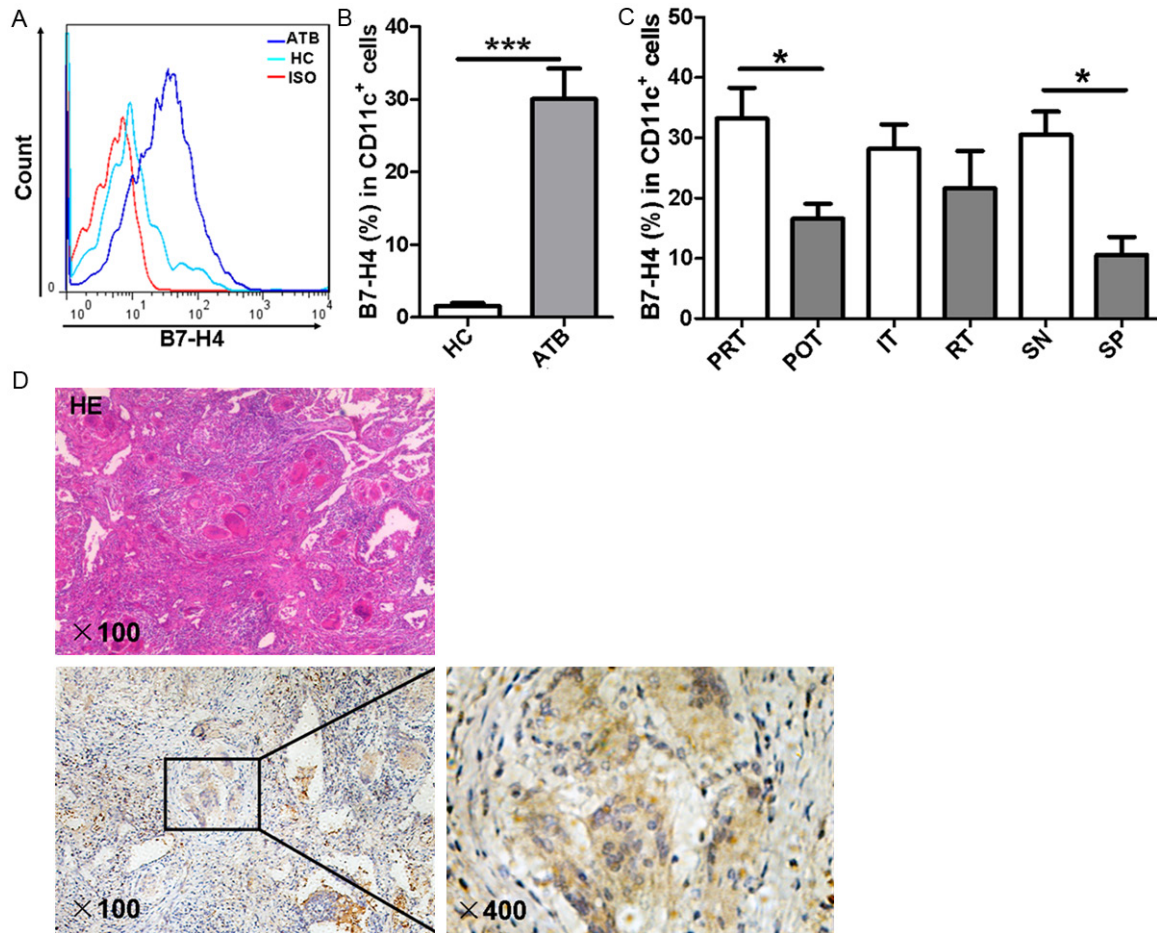


Figure 1. Expression of B7-H4 in peripheral blood CD11c APCs from ATB patients and healthy controls and in human pulmonary tuberculosis granuloma tissues. Expression of B7-H4 in circulating CD11c APCs and in human pulmonary tuberculosis granuloma tissues was determined by flow cytometry and by Immunohistochemistry. For detection of B7-H4-expressing CD11c APCs, 100 μ L heparin sodium anticoagulated whole blood was stained with anti-human B7-H4 and CD11c antibodies. After the lysis of red blood cells, the expression of B7-H4 on CD11c APCs was analyzed with flow cytometry. A is a representative flow cytometric histogram showing the frequencies B7-H4 expressing CD11c APCs; B is Bar graph data showing the percentage (%) of B7-H4-expressing CD11c APCs from ATB patients and healthy controls. C shows the percentage (%) of B7-H4-expressing CD11c APC in PRT/POT, IT/RT and SN/SP patients. D is showing the representative HE stain picture and the representative Immunohistochemistry stain picture under different magnification. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

science), B7-H4-PE (H74, eBioscience), B7-H4 (H-108, SANTA CRUZ BIOTECHNOLOGY, INC.), and CD3-PerCp-Cy5.5 (BD Pharmingen). Appropriate isotype controls were used. CFSE was purchased from Beyotime (Beyotime, Shanghai, China).

Flow cytometry analysis

The expression of B7-H4, HLA-DR and CD86 on CD11c APCs were determined by flow cytometry [24]. 100 μ L heparin sodium anticoagulated whole blood was stained with antibodies for 30 min. Then 2 ml red blood cell lysis buffer were

used to lyse red blood cells. After washing, the stained cells were resuspended in 200 μ L 2% FBS-PBS containing 2% paraformaldehyde and the samples were then acquired on a BD FACS-Canto II flow cytometer and analyzed using FlowJo software (Tree Star).

T cell proliferation assay

Mixed leukocyte culture (MLC) was used to test the capacity of B7-H4⁺ CD11c APCs to stimulate T cell proliferation [25]. B7-H4⁺ CD11c APCs were sorted by flow cytometry from PBMCs in 6 ATB patients with sputum smear

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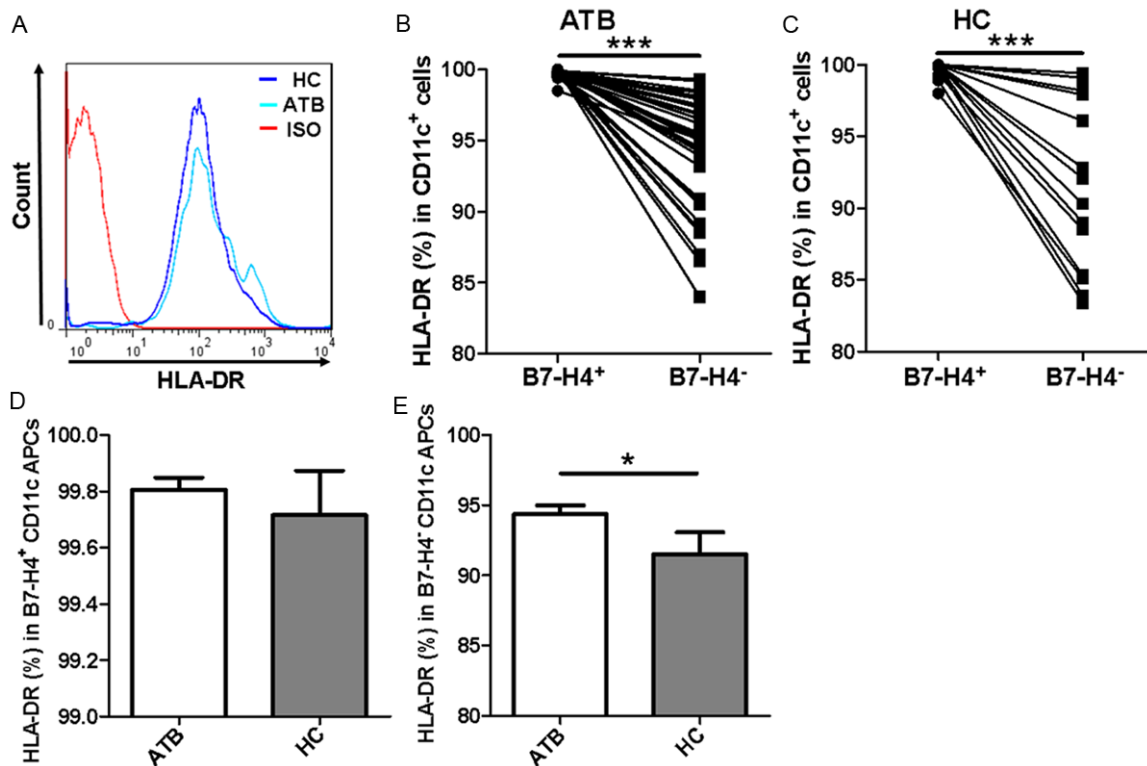


Figure 2. HLA-DR expression in B7-H4⁺ or B7-H4⁻ CD11c APCs. HLA-DR expression on CD11c APCs was determined by flow cytometry. (A) is the representative flow cytometric histogram showing the frequency of HLA-DR⁺ CD11c APCs; (B and C) show the expression of HLA-DR on B7-H4⁺ or B7-H4⁻ CD11c APCs from ATB (B) patients and HC volunteers (C), respectively. (D and E) show the expression of HLA-DR on B7-H4⁺ (D) or B7-H4⁻ (E) CD11c APCs in ATB patients and HC volunteers. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

positive and in 6 HC volunteers, respectively. PBMCs were stained with mouse anti-human CD11c-APC-eFluor780 (BU15, eBioscience) and B7-H4-PE (H74, eBioscience) monoclonal antibodies, and then sorted on a FACS Aria II cytometer to isolate B7-H4⁺CD11c APCs. Meanwhile, CD3⁺ T cells were isolated from an allogeneic healthy donor using a human T cell isolation kit following the instruction from the manufacturer (Stem Cell Biotech, Vancouver, Canada). Isolated cells with purity higher than 97% were used for experiments. The purified CD3⁺ T cells were labeled with 20 μ M CFSE (Beyotime, Shanghai, China). Thereafter, 5×10^4 /well purified T cells were co-cultured with sorted B7-H4⁺ CD11c⁺ APCs (5×10^3 cells/well) in 96-well round-bottom plates for 7 days. T cells alone were used as a negative control. After the co-culture, T cells were harvested and stained with CD3-PerCp-Cy5.5 (BD Pharmingen) for 30 min at 4°C, and their proliferation were detected by CFSE dilution on a BD FACS-Canto II flow cytometer and analyzed with FlowJo soft-

ware. The CFSE-low cells were quantified as a percentage of proliferating cells in the culture.

Immunohistochemistry and histopathology

The expression of B7-H4 in human pulmonary tuberculosis granuloma tissue was determined by immunohistochemistry as previously reported [26, 27]. Paraffin embedded human pulmonary tuberculosis granuloma tissues were collected from the department of pathology of Dongguan 6th Hospital. Following clearing in xylene and rehydration, antigen retrieval was performed by heating in Tris/EDTA antigen retrieval solution. Sections were then blocked using a ScyTek Biotin Blocking kit followed by blocking in PBS-tween solution with 2% bovine serum albumin/5% donkey serum (Jackson ImmunoResearch). Following blocking, sections were incubated with biotinylated anti-B7-H4 Ab (1:200 dilution) or isotype IgG control (R&D Systems) overnight at 4°C and then blocked for endogenous peroxidase for 10 min in 1% H₂O₂.

B7-H4-expressing CD11c APCs display low stimulatory capacity

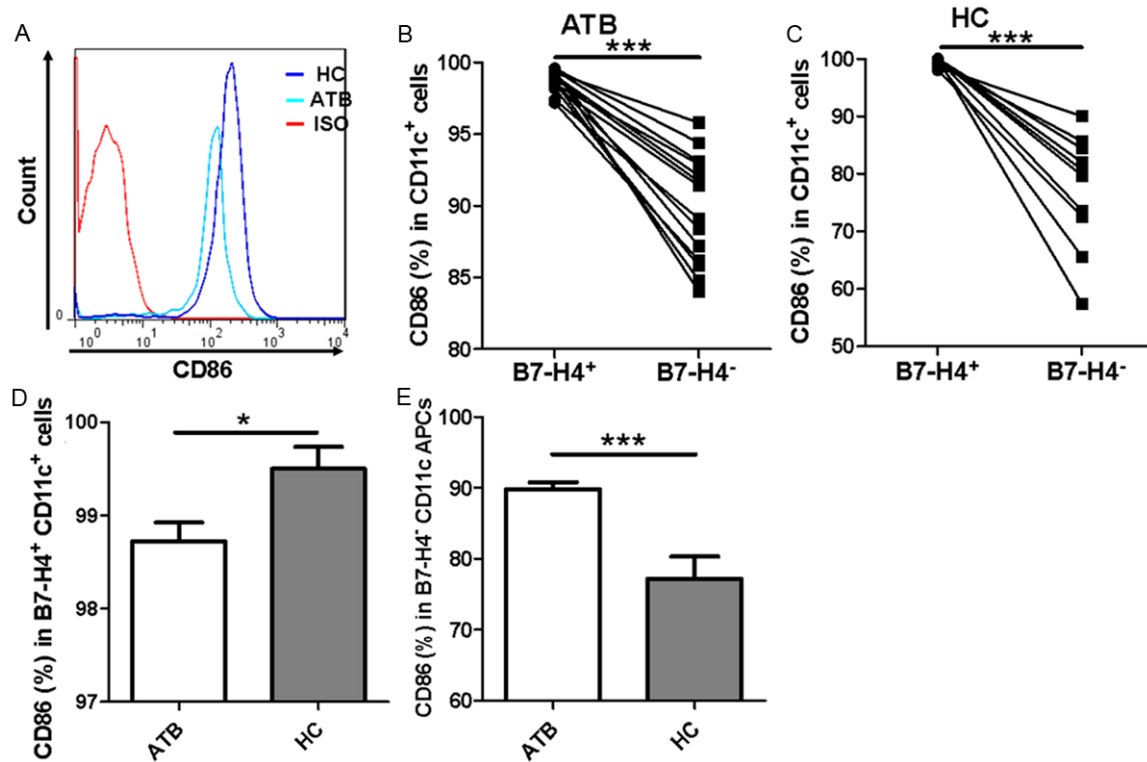


Figure 3. CD86 expression on B7-H4⁺ or B7-H4⁻ CD11c APCs. CD86 expression on CD11c APCs was determined by flow cytometry. (A) is a representative flow cytometric histogram showing the frequency (%) of CD86 expressing CD11c APCs; (B and C) show the expression of CD86 on B7-H4⁺ or B7-H4⁻ CD11c APCs from ATB (B) patients and HC volunteers (C), respectively. (D and E) show the expression of CD86 on B7-H4⁺ (D) or B7-H4⁻ (E) CD11c APCs in ATB patients and HC volunteers. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

Sections were incubated in ABC reagent (Vector Labs) for 20 min. Then the tyramide amplification kit (Invitrogen) was used, followed by incubating with ABC reagent again for 20 min. Sections were developed with the DAB kit (Vector Labs), counterstained with hematoxylin. For histopathological examination, lungs were inflated with 10% NBF through the trachea using an 18G needle until fully expanded and incubated in 10% NBF overnight. After fixation, lungs were transferred to 70% ethanol for at least one hour prior to paraffin embedding. Sectioned tissues were stained with hematoxylin and eosin (H&E) in the Pathology Core facility and images were captured with an AxioCam Mrc Zeiss microscope. A board certified veterinary pathologist performed histopathological examination.

Statistical analysis

A normality test was first performed to determine whether our dataset was well-modeled by

a normal distribution. Student's *t*-test of two-sample and two-tailed comparisons was employed using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA) as previously described [24]. In all cases, $P < 0.05$ was considered as statistically significant.

Results

BTLA expression increased in tuberculosis granuloma from TB patients and in CD11c APCs in the patients with ATB

CD11c APCs, including dendritic cells, monocytes and B lymphocytes, in peripheral blood are the most comprehensive antigen presenting cells in human being. It has been reported that antigen presenting function was decreased in TB patients. We found that the frequency of CD11c APCs in peripheral blood was increased in ATB patients (unpublished data). However, the exact mechanism of APC dysfunction in TB

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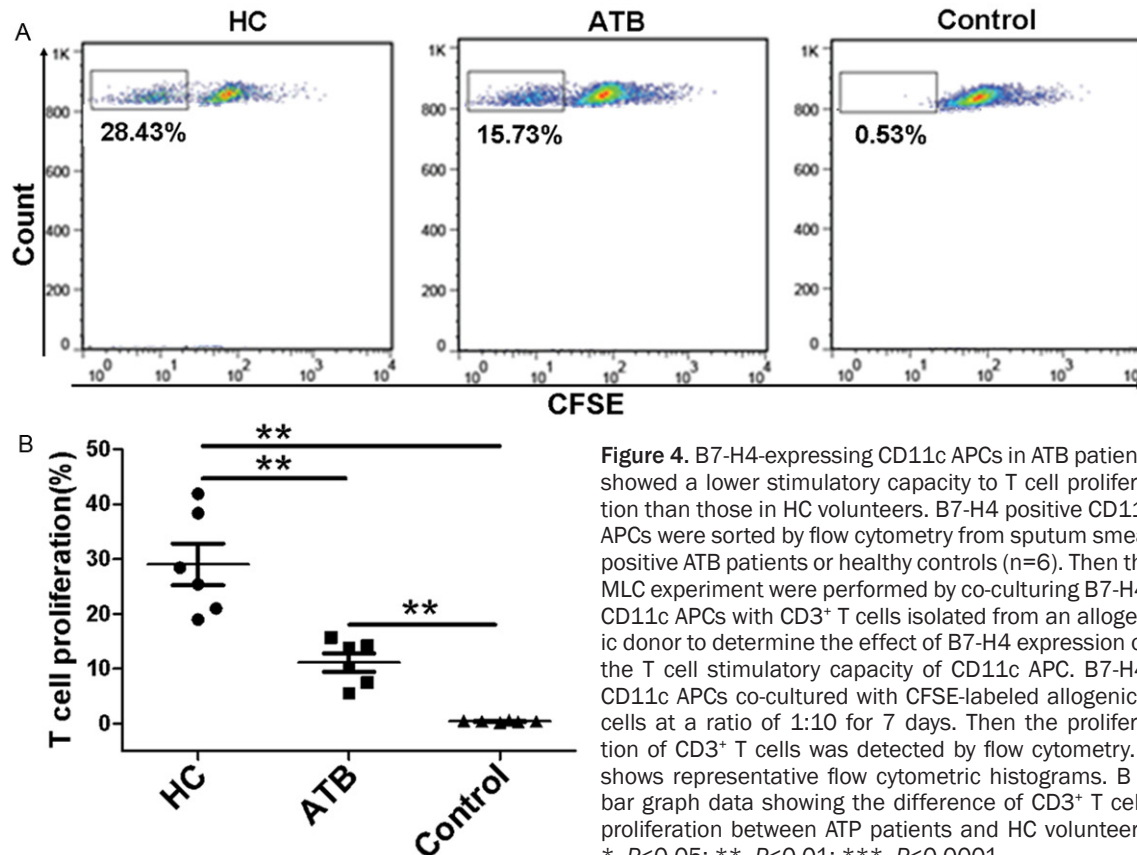


Figure 4. B7-H4-expressing CD11c APCs in ATB patients showed a lower stimulatory capacity to T cell proliferation than those in HC volunteers. B7-H4 positive CD11c APCs were sorted by flow cytometry from sputum smear positive ATB patients or healthy controls (n=6). Then the MLC experiment were performed by co-culturing B7-H4⁺ CD11c APCs with CD3⁺ T cells isolated from an allogenic donor to determine the effect of B7-H4 expression on the T cell stimulatory capacity of CD11c APC. B7-H4⁺ CD11c APCs co-cultured with CFSE-labeled allogenic T cells at a ratio of 1:10 for 7 days. Then the proliferation of CD3⁺ T cells was detected by flow cytometry. A shows representative flow cytometric histograms. B is bar graph data showing the difference of CD3⁺ T cells proliferation between ATP patients and HC volunteers. *, P<0.05; **, P<0.01; ***, P<0.0001.

patients is still to be illustrated. B7-H4, a negative immune-regulatory molecule, expresses in most APCs, but the role of B7-H4 in TB has not been reported. To investigate the potential involvement of B7-H4 in TB, we detected the expression of B7-H4 in CD11c APCs (Figure S1). As expected, the frequency of B7-H4 positive CD11c APCs in ATB patients was higher than that in healthy controls (HC) (Figure 1A, 1B). Interestingly, the frequency of B7-H4-expressing CD11c APCs was higher in prior-treatment (PRT) patients than that in post-treatment patients (POT), and it was higher in sputum smear negative patients than that in sputum smear positive patients (Figure 1C). Interestingly, immunohistochemistry detection showed B7-H4 expressed in human lung tuberculosis granuloma tissues, especially highly expressed in multinucleated giant cells, macrophages and lymphocytes detected (Figure 1D). Thus, these results demonstrated that patients with ATB exhibited high frequency of B7-H4-expressing CD11c APCs, which may be associated with disease progression and bacterium burden.

B7-H4 expression was associated with decreased CD86 expression in CD11c APCs in patients with ATB

HLA-DR, an antigen presenting molecule, provides the first signal for T cell proliferation, while CD86, a costimulatory molecule, provides the secondary signal. Both of them participate in stimulating T cell proliferation. To illustrate the effects of B7-H4 on HLA-DR and CD86 expression in CD11c APC, we further analyzed HLA-DR and CD86 expression in B7-H4⁺/B7-H4⁻ CD11c APCs by flow cytometry (Figure S1). Interestingly, over 95% of B7-H4⁺ CD11c APCs expressed HLA-DR and CD86 both in ATB patients and HC volunteers, which were much higher than those in its counterpart B7-H4⁻ CD11c APCs (Figures 2B, 2C and 3B, 3C). However, there was no difference in the frequencies of HLA-DR⁺ B7-H4⁺ CD11c APCs between ATB patients and Healthy controls (Figure 2D). Surprisingly, the expression of CD86 in B7-H4⁺ CD11c APCs was much lower in ATB patients compared to that in HC volunteers (Figure 3D), while the expressions of

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HLA-DR and CD86 in B7-H4⁻ CD11c APCs were higher in ATB patients compared to those in HC volunteers (**Figures 2E** and **3E**).

B7-H4-expressing CD11c APCs in ATB patients exhibited low stimulatory capacity to allogenic T cells

Antigen presentation is the most important function of APC in anti-tuberculosis immunity. Then, we sought to examine whether the effect of B7-H4 in CD11c APCs on its stimulatory capacity to T cells. B7-H4⁺ CD11c APCs were sorted from the peripheral blood of HC (n=6) and sputum smear positive ATB patients (n=6) by flow cytometry. CD3⁺ T cells were isolated from an allogenic healthy donor and labeled with CFSE. Mixed leukocyte culture (MLC) was performed by mixing B7-H4⁺ CD11c APCs with allogenic CD3⁺ T cells and T cell proliferation was determined using flow cytometry. Interestingly, B7-H4⁺ CD11c APCs from HC volunteers possessed much stronger stimulatory capacity to allogenic T cells proliferation than those from ATB patients (**Figure 4A, 4B**). These results demonstrated that B7-H4-expressing CD11c APC exhibited low stimulatory capacities to allogenic T cells in ATB patients.

Discussion

In the current study we provided previously undescribed findings that B7-H4 highly expressed in APCs in human lung tuberculosis granuloma tissue and the frequency of B7-H4⁺ CD11c APCs increased in ATB patients. Furthermore, HLA-DR and CD86 highly expressed in B7-H4⁺ CD11c APCs both in ATB patients and HC volunteers, with a much higher level compared to that in B7-H4⁻ counterparts. However, there was no difference of HLA-DR expression in B7-H4⁺ CD11c APCs between ATB patients and healthy controls. Interestingly, the expression of CD86 in B7-H4⁺ CD11c APCs was much lower in ATB patients. Notably, B7-H4-expressing CD11c APCs from ATB patients displayed a much lower stimulatory capacity to allogenic T cells proliferation than those from HC volunteers.

The expression of B7-H4 has intriguing characteristics. B7-H4 mRNA level was much higher in peripheral non-lymphoid tissues than that in lymphoid tissues [7, 27]. Although its protein is not expressed in immune cells, B7-H4 can be

induced in some types of immune cells including T cells, B cells and APCs in humans [6, 7]. Studies have described that B7-H4 can express in dendritic cells [13-15], monocytes [16, 17], macrophages [18-20] and B cells [9, 28] in different disease conditions. In the current study we found very rare peripheral CD11c APCs expressed B7-H4 in healthy controls, while nearly 30% of CD11c APCs expressed B7-H4 in ATB patients. Notably, the percentage of B7-H4-expressing CD11c APCs decreased to ~14% after 20-40 days of anti-TB treatment. Interestingly, its frequency was lower in sputum smear positive patients than that in sputum smear negative patients. Previous studies have shown that B7-H4 expressed in human lung tissue [27, 29], and abundantly expressed by non-small cell lung cancer. Patients with B7-H4 positive non-small-cell lung tumor have fewer infiltrating lymphocytes and increased lymph node metastasis [30]. B7-H4 highly expressed in tumor associated macrophages and positively correlated to the disease progress [18-20]. We found that B7-H4 expressed in human lung tuberculosis granuloma tissues, especially highly expressed in multinucleated giant cells, macrophages and lymphocytes detected by immunohistochemistry. These results suggest B7-H4 expressed in APCs locating in Mtb infectious site or in peripheral CD11c APCs. B7-H4 expression in APCs might be associated with immune dysfunction of APCs, bacterium burden, and treatment progress.

We found that almost 100% of B7-H4⁺ CD11c APCs expressed HLA-DR and CD86 in both ATB patients and HC, with a much higher level compared to its B7-H4⁻ counterparts. Interestingly, studies have also shown that there was a significant correlation between B7-H4 expression and HLA-DR expression on both circulating monocytes and tumor-associated macrophages in gastric cancer [18]. Our data suggest although B7-H4 is a co-inhibitory molecule, B7-H4⁺ CD11c APCs expressed high levels of antigen presenting molecule and co-stimulatory molecule in ATB patients. Interestingly, we found that CD86 expression was lower in B7-H4-expressing CD11c APCs from ATB patients than that from HCs, but there was no difference in HLA-DR expression. These data suggest that B7-H4-expressing CD11c APCs from ATB patients may exert less stimulatory function to T cell proliferation and activation than that from HCs.

B7-H4-expressing CD11c APCs display low stimulatory capacity

CD11c APCs represent most potent antigen presenting cells within peripheral blood mononuclear cells (PBMC) [31-34], including DC, monocytes and B cells. They mediate innate immunity in early infectious stage and initiate adaptive immunity by stimulating T cell proliferation. However, it has been reported that the function of APCs were decreased in TB patients. Depletion of CD11c⁺ cells in vivo delayed CD4⁺ T cells response to MTB and exacerbated the outcome of infection [4]. The current study suggests that B7-H4-expressing CD11c APCs from ATB patients displayed a lower stimulatory capacity to allogenic T cell proliferation compared to healthy controls. The underlying mechanism might be that B7-H4 expression inhibits T-cell proliferation and decreases cytokine secretion [6-8]. Thus, B7-H4 expression in CD11c APCs may partially contribute to the decreased immune function of APCs in ATB patients.

In summary, our study revealed a previously unappreciated role of B7-H4 in CD11c APCs in ATB patients. B7-H4-expressing CD11c APCs from both healthy controls and ATB patients exhibited activated phenotype with increased expression of HLA-DR and CD86. However, the activation of CD11c APCs was decreased in ATB patients in terms of lower CD86 expression. More importantly, mixed cell culture test directly showed that B7-H4-expressing CD11c APCs in ATB patients displayed a much lower stimulatory capacity. Our data suggest that B7-H4 expressed on CD11c APCs may be associated with impaired immune function in TB although the underlying mechanisms remain to be further investigated.

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Disclosure of conflict of interest

None.

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Table S1. Clinical characteristics of the enrolled subjects

ATB patients						Healthy Control (HC)		
Number	Age	Gender	ATDs treatment (day)	Sputum smear	IT/RT	Number	Age	Gender
1	25	Male	25	SN	IT	1	29	Male
2	41	Female	24	SP	RT	2	34	Male
3	38	Male	23	SN	IT	3	35	Male
4	34	Male	25	SN	IT	4	21	Male
5	19	Male	11	SN	IT	5	19	Male
6	50	Male	20	SN	RT	6	25	Male
7	56	Male	25	SN	IT	7	53	Male
8	46	Female	25	SN	IT	8	24	Femal
9	39	Male	10	SP	IT	9	48	Femal
10	47	Male	7	SP	IT	10	35	Femal
11	18	Male	22	SN	IT	11	42	Male
12	25	Female	8	SN	IT	12	36	Femal
13	30	Female	4	SN	IT	13	34	Male
14	31	Female	8	SN	IT	14	35	Male
15	31	Male	0	SN	IT	15	41	Male
16	32	Male	0	SN	IT			
17	23	Male	0	SN	RT			
18	30	Male	0	SN	IT			
19	42	Female	0	SN	IT			
20	29	Male	0	SN	IT			
21	52	Female	0	SN	RT			
22	18	Female	0	SN	IT			
23	40	Male	1	SN	IT			
24	45	Male	1	SN	IT			
25	50	Female	1	SN	IT			
26	25	Female	1	SN	IT			
27	60	Male	2	SN	IT			
28	32	Female	1	SN	IT			
29	36	Male	1	SN	IT			
30	47	Female	1	SN	IT			
31	35	Female	1	SN	RT			
32	47	Male	0	SP	IT			
33	30	Male	0	SP	IT			
34	58	Male	1	SP	IT			
35	34	Female	1	SN	RT			
36	30	Male	30	SN	IT			
37	53	Male	30	SP	RT			
38	29	Male	30	SN	IT			
39	28	Male	30	SN	IT			

B7-H4-expressing CD11c APCs display low stimulatory capacity

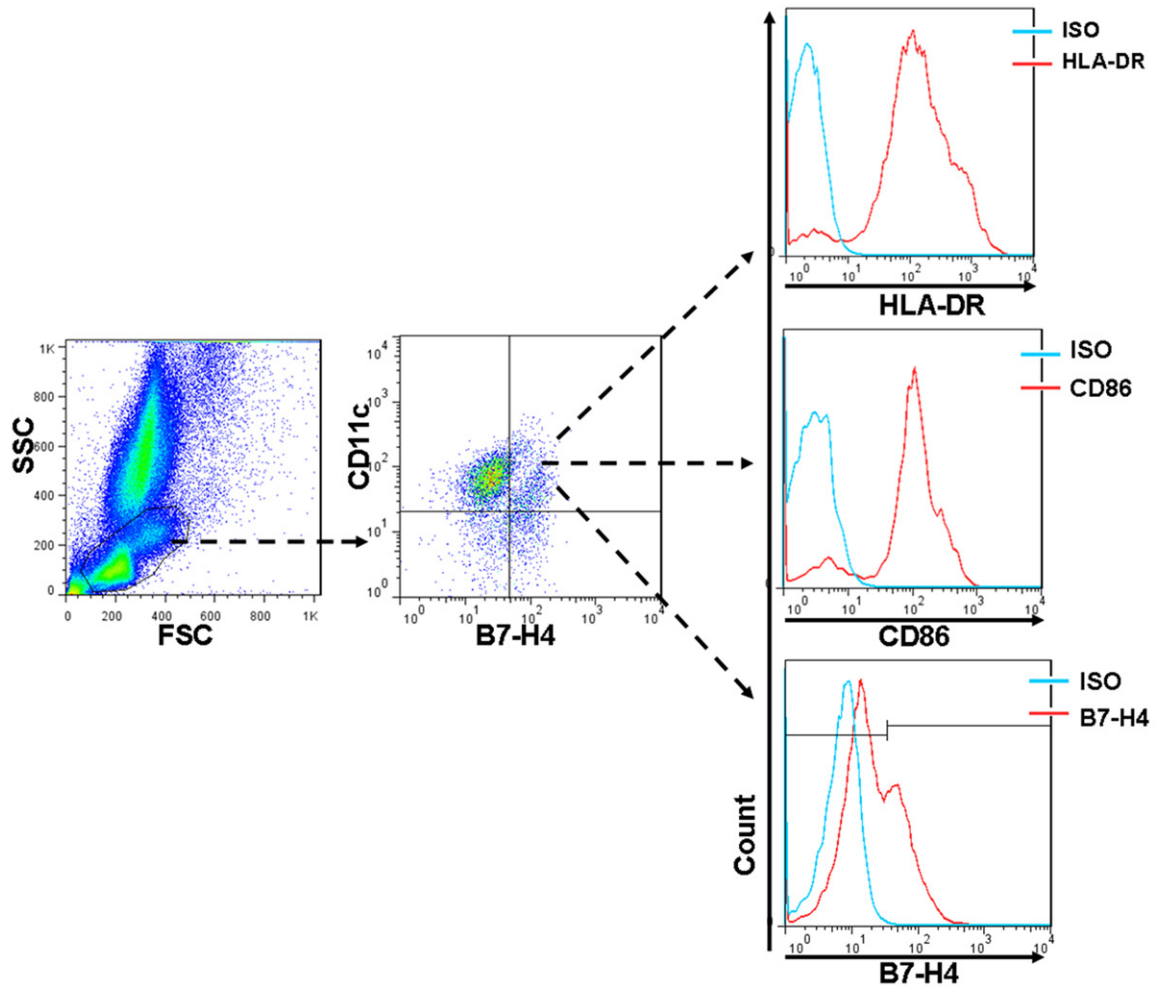


Figure S1. Flow cytometry gating strategy. B7-H4, HLA-DR and CD86 expression in PBMC were detected by flow cytometry. Gating strategies were as following: PBMCs were gated on FSC and SSC dot plots. Then, CD11c⁺ APCs were gated from PBMCs based on CD11c expression, and B7-H4 expression was analyzed in CD11c APCs. Finally, HLA-DR and CD86 expression were analyzed in B7-H4⁺/B7-H4⁻ CD11c APCs.