Cellular Immunology 311 (2017) 28–35

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

BTLA-expressing CD11c antigen presenting cells in patients with active tuberculosis exhibit low capacity to stimulate T cell proliferation

Wan-Dang Wang [a,d,1], Yu-Chi Gao [a,c,1], Yuan-Bin Lu [a,c,1], Jun-Ai Zhang [a,c], Gan-Bin Liu [b], Bin Kong [a,c], Wen-Yu Xiang [a,c], You-Chao Dai [a,c], Shi-Yan Yu [a,c], Yan Jia [a,c], Xiao-Xia Fu [a], Lai-Long Yi [b], Bin-Ying Zheng [a], Zheng W. Chen [d], Jixin Zhong [e], Jun-Fa Xu [a,c,*]

[a] Department of Clinical Immunology, Institute of Laboratory Medicine, Guangdong Medical University, No. 1 Xincheng Road, Dongguan 523808, China
[b] Department of Respiration, Dongguan 6thHospital, Dongguan 523008, China
[c] Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, No. 1 Xincheng Road, Dongguan 523808, China
[d] Department of Microbiology and Immunology, Center for Primate Biomedical Research, University of Illinois College of Medicine, Chicago, IL, USA
[e] Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

ARTICLE INFO

Article history:
Received 25 March 2016
Revised 11 September 2016
Accepted 30 September 2016
Available online 30 September 2016

Keywords:
B and T lymphocyte attenuator
CD11c APCs
Stimulatory capacity
Antigen uptake
HLA-DR
HLA-I(A,B,C)
CD86
IL-6

ABSTRACT

Despite past extensive studies on B and T lymphocyte attenuator (BTLA)-mediated negative regulation of T cell activation, the role of BTLA in antigen presenting cells (APCs) in patients with active pulmonary tuberculosis (ATB) remains poorly understood. Here, we demonstrate that BTLA expression on CD11c APCs increased in patients with ATB. Particularly, BTLA expression in CD11c APCs was likely associated with the attenuated stimulatory capacity on T cells (especially CD8+ T cell) proliferation. BTLA-expressing CD11c APCs showed lower antigen uptake capacity, lower CD86 expression, higher HLA-DR expression, and enhanced IL-6 secretion, compared to counterpart BTLA negative CD11c APCs in healthy controls (HC). Interestingly, BTLA-expressing CD11c APCs from ATB patients displayed lower expression of HLA-DR and less IL-6 secretion, but higher expression of CD86 than those from HC volunteers. Mixed lymphocyte reaction suggests that BTLA expression is likely associated with positive rather than conventional negative regulation of CD11c APCs stimulatory capacity. This role is impaired in ATB patients manifested by low expression of HLA-DR and low production of IL-6. This previous unappreciated role for BTLA may have implications in the prevention and treatment of patients with ATB.

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 [1]. Despite extensive investigations on the immunopathogenesis of TB, the mechanisms leading to complete clearance, latent infection or chronic disease have not been elucidated fully. Both innate and adaptive immune responses are required for host control of tuberculosis infection [2,3]. *Mycobacterium tuberculosis* is spread by aerosol route and is first encountered by antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs). CD11c is a surface marker expressed in most APCs, including dendritic cells [4,5], macrophages [6], monocytes [7], neutrophils [8] and B cells [9]. It has been reported that depletion of CD11c+ cells in vivo delayed CD4+ T cells response to MTB and exacerbated the outcome of infection [10]. However, the frequencies and function of CD11c+ APCs in the peripheral blood of patients with active pulmonary tuberculosis (ATB) is not well characterized.

B and T lymphocyte attenuator (BTLA), a recently discovered inhibitory receptor belonging to the CD28 family sharing structural and functional similarities with cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death 1 (PD1) [11]. BTLA is expressed by most lymphocytes and other immune cells, such as dendritic cells (DC), monocytes, macrophages, neutrophils, and NK cells. BTLA interacts with the herpesvirus entry mediator (HVEM; TNFRSF14), a TNFR super family member found on T, B, natural killer (NK), dendritic (DC) and myeloid cells [12]. The function of BTLA has been described in several inflammatory disorders and autoimmune diseases [13–15], suggesting that BTLA is crucial for dampening overreactive immune responses. More recent studies further demonstrated emerging evidence that BTLA could also

* Corresponding author at: Department of Clinical Immunology, Institute of Laboratory Medicine, Guangdong Medical University, No. 1 Xincheng Road, Dongguan 523808, China.
E-mail addresses: imxujunfa@163.com, xujunfa@gdmu.edu.cn (J.-F. Xu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cellimm.2016.09.015
0008-8749/

initiate pro-survival signals for activated effector T cells and mediate immune memory [16–20]. We demonstrated previously that BTLA exhibits immune memory for αβT cells in patients with active pulmonary tuberculosis [21], and BTLA associates with increased Foxp3 expression in CD4+ T cells in dextran sulfate sodium-induced colitis [22]. Therefore, BTLA expression on T cells might correlate with T-cell dysfunction and disease pathogenic events.

Recent studies have shown that BTLA also plays a role in immune responses against infectious pathogens. BTLA−/− mice exhibited significantly enhanced bacterial clearance in the early phase of bacterial infection compared with WT mice [23]. Furthermore, BTLA−/− mice displayed a higher survival rate in a septic shock model of cecal ligation and puncture [24]. BTLA−/− mice also showed an enhanced parasite clearance before the onset of acquired immune responses [25,26]. These findings suggest that BTLA is involved in the clearance of pathogens in the early phase of immune responses and BTLA on innate cells might be involved in this process. However, the role of CD11c APC-expressing BTLA in ATB patients remains to be characterized. We thus in the present study detected the expression of BTLA in peripheral CD11c APCs in ATB patients and investigated its effects on the stimulatory capacity.

2. Material and methods

2.1. Subjects and ethics statement

52 active pulmonary tuberculosis (ATB) patients aged from 18 to 60 years old and 15 healthy individuals from 19 to 53 years old were recruited in this study (Table 1). 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 Dongguang 6th Hospital (Dongguan, China). Exclusion criteria were HIV+, >65 years, <16 years, pregnancy, alcoholism, diabetes, cancer, autoimmune diseases, 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.

All samples were collected with written informed consent according to protocols approved by the Internal Review and the Ethics Boards of Guangdong Medical University and the Ethics Boards of Dongguan 6th Hospital.

2.2. Peripheral blood mononuclear cells preparation

Peripheral blood mononuclear cells (PBMCs) were prepared as previously reported [21]. 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 following experiments.

2.3. 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), HLA-I(A,B,C)-PE (W6/32, BioLegend), CD86-PerCP-eFluor710 (IT2.2, eBioscience), BTLA-APC (MIH26, Biolegend), BTLA-PE (J168-540, BD Pharmingen), CD3-PerCp-Cy5.5 (BD Pharmingen), CD4-APC (BD Biosciences). Appropriate isotype controls were used. FITC-dextran and CFSE were purchased from Beyotime (Beyotime, Shanghai, China). IL-6 ELISA Kit was from eBioscience (product BMS213HS; eBioscience, San Diego, CA).

Table 1
The clinical data of studied subjects.

Groups	PTB(n = 52)	HC(n = 15)
Age (years)	(18–60)	(19–53)
Age (Mean ± SEM)	36.02 ± 1.58	35.07 ± 2.34
Gender (Female/Male)	18/34	4/11
IT/RT	42/10	−/−
Sputum smear (+/−)	9/43	0/15
ATDs treatment (PRT/POT)	32/15	−/−

Note: IT: Initial treatment; RT: Retreatment; PRT (Prior treatment): ATDs treatment during 0–4 days; POT (Post-treatment): ATDs treatment during 20–40 days. There were no signification differences among age and gender groups ($P > 0.05$).

2.4. Flow cytometry analysis

The expression of BTLA, HLA-DR, HLA-I(A,B,C) and CD86 on CD11c APCs were determined by flow cytometry [27]. 100 μL heparin sodium anticoagulated whole blood was stained with antibodies for 30 min. Then 2 ml red blood cell lysis buffer were used to lyze red blood cells. After washing, the stained cells were resuspended in 200 μL 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).

2.5. FITC-dextran uptake assay

The FITC-dextran uptake assay was setup by incubating cells with FITC-dextran in duplicate plates at 4 °C or 37 °C as previously described [28]. Briefly, 180 μL of whole blood were incubated with 1 mg/ml FITC-dextran in 5 ml Falcon™ Polystyrene Round-Bottom Tubes (BD Biosciences). The FITC-dextran solution was vortexed for 30 s before use. One plate was incubated at 37 °C and the second was incubated at 4 °C (to determine baseline FITC-dextran uptake level) for 1 h. Then, 4 ml red blood cells lysis buffer were added to and put in 4 °C for 10 min. After centrifuged, the cells were washed twice with PBS and stained with mouse anti-human CD11c-APC-eFluor780 (BU15; eBioscience) and mouse anti-human BTLA-APC (MIH26; biolegend) at 4 °C for 30 min. After washing, the stained cells were resuspended in 200 μL 2% FBS-PBS containing 2% paraformaldehyde then analyzed with BD FACS-Canto II flow cytometer. The percentage of phagocytosis was determined as following: Percentage of phagocytosis of CD11c APCs (%) = Percentage of phagocytosis of CD11c APCs at 37 °C(%) − Percentage of phagocytosis of CD11c APCs at 4 °C(%).

2.6. T cell proliferation assay

Mixed leukocyte culture (MLC) was used to test the capacity of CD11c APCs to stimulate T cell proliferation [29]. Firstly, BTLA +CD11c APCs and BTLA−CD11c APCs were sorted by flow cytometry. PBMC were isolated from 5 ATB patients with sputum smear positive and 5 HC volunteers and stained with mouse anti-human CD11c-APC-eFluor780 (BU15, eBioscience) and BTLA-PE (J168-540, BD Pharmingen) monoclonal antibodies, then sorted on a FACS Aria II for BTLA+CD11c APCs and BTLA−CD11c APCs. Meanwhile, CD3+ T cells were isolated from an allogenic donor using a human T cell isolation kit following the instruction from the manufacturer (StemCell Biotech, Vancouver, Canada). Isolated cells with purity higher than 97% were used for experiments. To evaluate T cells proliferation, some of the purified CD3+ T cells