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Mycobacterium tuberculosis HtdY, a Novel Immunostimulatory Antigen, Drives Th1-type T Cell Immunity via TLR4-mediated Activation of Dendritic Cells

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Abstract

Mycobacterium tuberculosis (MTB), the etiological factor of tuberculosis (TB), is among the most successful of intracellular pathogen that regulates the host immune response. Cellular immune responses perform a pivotal function in host defence against MTB. Thus, it is vital to understand the antigens which drive immune protection, especially Th1-type cellular immunity. The role of 3-hydroxyacyl-l-thioester dehydratase (HtdY, Rv3389c) of MTB in immunological protection was observed in dendritic cell (DC) activation and T cell immunity herein. Recombinant HtdY was applied for inducing maturation and activation of DCs obtained from murine bone marrow. TLR4 and TLR2 knockout mice and pharmacological inhibitors were used to investigate the mechanism by which HtdY activates DCs. MLR assay was performed to characterize HtdY activity with respect to DC activation and T cell polarization. The alteration of cytokines secretion by human PBMCs elicited by HtdY was observed. We found that HtdY prompted DC maturation and activation through augmenting the expression of surface molecule CD80, CD86 and MHC II, and the release of pro-inþammatory cytokines including IL-1ƀ, IL-6, IL-12 and TNF-Ŭ from DCs. HtdY-drived DC activation involved TLR4 activation and mitogen-activated protein kinases (MAPKs) signaling pathway. DCs treated with HtdY induced naïve CD4+ T cells to produce IFN-ɔ. Human PBMCs had higher TNF-Ŭ and lower IL-10 response to HtdY in active TB group than that in control group ($p<0.01$). Our results indicated that HtdY possesses potential to drive Th1-type cellular immunity by TLR4-mediated activation of DCs.

Introduction

(MTB), the pathogen of tuberculosis (TB), is estimated to infect nearly one-third of the world's population and accounted for about 1.5 million deaths globally in 2018 [1]. MTB is among the most successful intracellular bacterium which adapt to the immune system of human and cellular immunity have crucial function in host defense against MTB [2]. Dendritic cells (DCs) are the most e ective antigen-presenting cells (APCs) to activate naïve T cells [3-5]. As DCs capture and process MTB antigens, they present antigens to naïve CD4+ T cells in an MHC-II dependent manner, which bridges the innate and adaptive immunity [5-8].

Polarization of naïve CD4+ T cells drived by mature DCs relies on the ligation of pattern recognition Receptors (PRRs) during MTB antigens recognition, among which TLR2 and TLR4 play a crucial role [9-12]. Activation of NF- B and MAPK signal pathways results in the higher expression of the surface molecules including MHCII and CD80, CD86, and various immune-regulatory cytokines, which could polarize the naïve CD4+ T cells for adaptive immunity [13-15]. e polarized 1, 2, 17, Treg and other immune responses regulate the anti-MTB immunity cooperatively, among which 1-biased T cell responses are essential by producing critical cytokines like IFN- and TNF- [16]. In previous work, 3-hydroxyacyl-1-thioester dehydratase Y (HtdY) screened from the cultured filtrate proteins (CFPs) of MTB clinical isolate by dynamic immune-proteomics was identified as a unique immune regulatory protein [17]. HtdY is encoded by Rv3389c and related to the biosynthesis of major and requisite lipids such as cell

expression in mouse macrophages [17]. ese results highlighted the potential of HtdY for TB

mToScience, School of Medicine, Shanghai Jiao Tong University (China). All experimental procedures were approved by the Ethics Committee at School of Medicine, Shanghai Jiao Tong University.

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or positive molecular detections by real-time PCR (MeltPro , Xiamen Zeesan Biotech Co., Ltd., Fujian, China). Bacteriological negative TB patients had positive IGRA responses but negative sputum smear examination or cultures for 3 times or negative molecular detections. All had the history of receiving BCG vaccine in the past. Blood was obtained before or no later than 1 week of chemotherapy of TB. All subjects were free of diabetes, HIV, HBV or HCV infection, hepatic or renal dysfunction. Healthy controls were from Physical Examination Center, Henan Provincial Chest Hospital with normal chest radiography and no TB clinical symptoms or close contact with TB patients. Written informed consents were acquired from all subjects, and the investigation received approval from the Ethics Committee at School of Medicine, Shanghai Jiao Tong University.

Expression and puri cation of recombinant HtdY

e recombinant HtdY (accession no. NP_217906) was amplified by PCR from MTB H37Rv genomic DNA using the following primers: forward, 5′-TTATCCATGGCGATTGATCCGAACTCC-3′ (NcoI) and reverse, 5′-TATTAAGCTTCTAACCCGCCACGTACTCCAC-3′ (Hind-III). e product was ligated into the pET32a vector as described [17]. e recombinant clone was identified by DNA sequencing and transformed into . . BL21. e recombinant HtdY was purified using Ni-NTA resin after cell disruption by sonication. e endotoxin was removed by phase separation in Triton X-114. LAL assay (Chinese

maturation and activate DCs by reinforcing the expression of MHC II, CD 80 and CD 86 surface markers. DCs undergoing maturation can di erentiate into exceptionally antigen presenting cells with the ability to activate naïve T cells. Moreover, HtdY augmented the release of the pro-inflammatory cytokines including IL-1β, IL-6, IL-12 and TNF- by DCs.

Further, we found that HtdY treated DCs were activated through TLR4 by experiment on TLR4 knock-out mice. e presence of numerous PRRs, such as Toll like receptors on DCs generally promotes host immunity and allow specialized APCs to promptly recognize encroaching pathogens and increasing the expression of surface co-stimulatory molecules and inflammatory and regulatory cytokines as well [24], both of which influence significantly on the consecutive progress of T cell immune responses.

We crudely investigated that the MAPK signaling pathways are critical for HtdY-mediated DC activation, arousing the expression of phenotypic markers for DC maturation as well as production of pro-inflammatory cytokines. Further work needs to be done to observe the phosphorylation of MAPKs induced by HtdY. Defensive immunity against intracellular pathogens such as MTB relies particularly on cellular immunity implemented by powerful anti-infectious performance of 1 subset of CD4+ T cells [25-27]. 1 cell are featured by the secretion of their.1 (p)7 (lem)4 (-1.2 Td[)]TJ0.108 Tw 0 -1.2 T0kin(m)3 ()4 (es)]TJ0.101 Tw6 (y, IF1.2 T0 0 -1.2(ce o)12 (f ,7 (t)-5 pic5.(l)y J05 (33 (a -22)--(r exp-,9 (t)6iw 0 e4 (l)s3(v)8 (e)2[31]]14 ()-6 ss)]T5.(l))4 (d)1 (u)-5 (l)-8(et)-5 (si)m(K p)f sivdlæ

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