Mycobacterium tuberculosis Rv2005c Induces Dendritic Cell Maturation and Th1 Responses and Exhibits Immunotherapeutic Activity by Fusion with the Rv2882c Protein

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Abstract

Immunotherapy represents a promising approach for improving current antibiotic treatments through the engagement of the host's immune system. Latency-associated antigens have been included as components of multistage subunit tuberculosis vaccines. We first identified Rv2005c, a DosR regulon-encoded protein, as a seroreactive protein. In this study, we found that Rv2005c induced dendritic cell (DC) maturation and Th1 responses, and its expression by *Mycobacterium tuberculosis* (Mtb) within macrophages was enhanced by treatment with $CoCl_2$, a hypoxia-mimetic agent. T cells activated by Rv2005c-matured DCs induced antimycobacterial activity in macrophages under hypoxic conditions but not under normoxic conditions. However, Rv2005c alone did not exhibit any significant vaccine efficacy in our mouse model. The fusion of Rv2005c to the macrophage-activating protein Rv2882c resulted in significant activation of DCs and antimycobacterial activity in macrophages, which were enhanced under hypoxic conditions. Furthermore, the Rv2882c-Rv2005c fusion protein showed significant adjunctive immunotherapeutic effects and led to the generation of long-lasting, antigen-specific, multifunctional $CD4^+$ T cells that coproduced TNF-α, IFN-γ and IL-2 in the lungs of our established mouse model over.

Keywords: ***Mycobacterium tuberculosis***; **hypoxia**; **DosR**; **chemo-immunotherapy**; **multifunctional T cell**

Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (Mtb), remains a major global health problem that urgently needs to be controlled because 1.67 million people died of TB in 2016 [1]. The appearance of drug-resistant TB and the imperfect protection provided by the *M. bovis* BCG (Bacillus Calmette–Guérin) vaccine, which is currently the only TB vaccine, have led to the development of a more effective TB vaccine [2]. In particular, vaccines for adjunctive immunotherapy are urgently needed to shorten the duration of chemotherapy and to prevent the reactivation of latent TB.There are still several challenging issues in TB treatment, including long treatment periods, drug resistance, and various side effects [3,4]. In the case of TB, increasing numbers of studies have been conducted to develop immunotherapeutics, particularly with regard to the fight against MDR-TB [5].

Material and Methods

2.1. Mice

Specific pathogen-free (SPF) female WT C57BL/6 mice, TLR2 knockout (KO) mice, TLR4 KO mice, and OT-2 TCR transgenic mice (all at 5–6 weeks of age) were purchased from the Jackson Laboratory All animals were maintained under SPF barrier conditions at the Medical Research Center, Chungnam National University (Daejeon, Korea). All animals were kept under controlled conditions, sterilized food and water was provided ad libitum.

Bone marrow-derived macrophages were differentiated as described [30]. Briefly, flushed from the femurs of mice, then RBC were lysed. After washing the cells, the total cells were suspended in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 50 ng/mL mouse macrophage colony-stimulating factor (M-CSF; Rocky Hill, NJ, USA), and 1% antibiotics .Bone marrow-derived dendritic cells were cultured at 37 °C in the presence of 5% CO_2 using Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% FBS, 1% antibiotics 0.1% 2-mercaptoethanol, 5 mM HEPES buffer, 1% MEM solution, 20 ng/mL granulocyte-macrophage colony-stimulating factor and 2 ng/mL IL-4. The nonadherent cells and loosely adherent proliferating DC aggregates were harvested on day 7 or 8 and were used for further experiments.

Results

3.1. The Recombinant Rv2005c Protein Induces the Maturation and Activation of Dcs

Proteins from Mtb culture filtrates were fractionated by biochemical chromatography as previously described, and the immunoreactivity of each fraction was measured [22]. The protein that strongly reacted with the sera of active TB patients was identified as Rv2005c by liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) as previously described [34] (**Figure S1**). To define the immunological effect of Rv2005c on DCs, first, the protein was expressed in *E. coli* BL21 and purified by His-affinity chromatography. The purified Rv2005c protein showed a major single band at 30 kDa by SDS-PAGE and strongly reacted with anti-His antibody in immunoblotting (**Figure 1**A). Endotoxin content of prepared Rv2005c was below 15 pg/mL (<0.1 UE/mL) according to an LAL assay. Next, we investigated whether Rv2005c could induce DC maturation. BMDCs treated with Rv2005c significantly increased the expression of the costimulatory molecules CD40, CD80 and CD86, as well as MHC class II molecules, in a dose-dependent manner (**Figure**

1B). Rv2005c-stimulated BMDCs also secreted high levels of IL-1β, TNF-α, and IL-12p70 in a dose-dependent manner, whereas untreated BMDCs secreted negligible amounts of these cytokines (Figure 1C).

Discussion

Several TB vaccines are in clinical trials, but only a few antigens, such as Ag85 or ESAT-6, have been used in these vaccines [23]. There is accumulating evidence that the inclusion of latency-associated Ags, which are specifically encoded by the DosR regulon, will be important in the development of a more potent TB vaccine [38]. For example, H56 and ID93, the most advanced multistage subunit vaccines in clinical trials, contain the latency-associated Ags Rv2660c and Rv1813, respectively [18,39]. Therefore, the discovery and selection of target antigens shown immunogenicity and protective efficacy in preclinical animal models are required for the development of an improved TB vaccine. It has been reported that the expression of the Rv2005c gene in hypoxic conditions is regulated by DosR [40], and the Rv2005c protein is upregulated during the dormancy and reactivation of Mtb [35]. However, there is no report about the immunoreactivity of Rv2005c, which is a predicted vaccine candidate. Therefore, we investigated whether Rv2005c had potential as a vaccine, and the Rv2005c protein was first identified as a seroreactive antigen in TB patients. We found that T cells activated by Rv2005c-matured DCs triggered antimycobacterial activity in hypoxic macrophages, but the single Rv2005c protein did not exhibit significant efficacy as a vaccine (prophylactic, pre-exposure, or post-exposure).

Conclusions

Collectively, our study demonstrated that the Rv2882c-Rv2005c fusion protein was enhanced antimycobacterial effect in vitro/in vivo, and induced antigen-specific multifunctional $CD4^+$ T cells in Mtb infected mice lung. Like this, construction of fusion protein and validation of immunotherapeutic efficacy will be used as a basis to effectively improve the therapeutic vaccines against tuberculosis.

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