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Institut Pasteur

Microbes and Infection xx (2014) 1−10

www.elsevier.com/locate/micinf

Original article

Increased resistin may suppress reactive oxygen species production and inflammasome activation in type 2 diabetic patients with pulmonary tuberculosis infection

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Received 5 August 2014; accepted 27 November 2014

Abstract

Although it has been known for decades that patients with type 2 diabetes mellitus (DM) are more susceptible to severe tuberculosis (TB) infection, the underlying immunological mechanisms remain unclear. Resistin, a protein produced by immune cells in humans, causes insulin resistance and has been implicated in inhibiting reactive oxygen species (ROS) production in leukocytes. Recent studies suggested that IL-1β production in patients with *Mycobacteria tuberculosis* infection correlates with inflammasome activation which may be regulated by ROS production in the immune cells. By investigating the level of resistin in different patient groups, we found that serum resistin levels were significantly higher in severe TB and DM-only groups when compared with mild TB cases and healthy controls. Moreover, elevation of serum resistin correlated with impairment of ROS production of neutrophils in patients with both DM and TB. In human macrophages, exogenous resistin inhibits the production of ROS which are important in the mycobacterium-induced inflammasome activation. Moreover, macrophages with defective ROS production had poor IL-1β production and ineffective control of mycobacteria growth. Our results suggest that increased resistin in severe TB and DM patients may suppress the mycobacterium-induced inflammasome activation through inhibiting ROS production by leukocytes.

Keywords: Resistin; Tuberculosis; Diabetes mellitus; ROS; Inflammasome

1. Introduction

Mycobacterium tuberculosis (TB) infection remains an important disease in the modern world. In the year 2012 alone, 8.6 million people developed active pulmonary TB and 1.3 million died from this disease [1]. The emergence of the combination of TB and type 2 diabetes mellitus (DM) has become a new global challenge not only because of the rising prevalence of type 2 DM in populations with higher TB infection rates, but also due to the fact that type 2 DM patients tend to have higher TB incidence and increased disease severity [2,3]. The immunological mechanisms underlying this TB susceptibility in diabetic patients, however, still remain unclear [4].

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http://dx.doi.org/10.1016/j.micinf.2014.11.009
1286-4579/

Resistin, a 12-kDa soluble serum protein, was initially considered an adipokine which can cause insulin resistance and mediates the progression from obesity to type 2 DM [5]. Further investigations revealed that activated immune cells, rather than adipocytes, are the main source of resistin in humans [6]. Serum resistin level hence has been used as an inflammatory biomarker in various diseases including sepsis, rheumatoid arthritis, and atherosclerosis [7–9]. Furthermore, resistin was recently reported to impair the chemotaxis and production of reactive oxygen species (ROS) in neutrophils [10].

ROS not only are key weapons used by the phagocytic leukocytes to kill microorganisms but also serve as signaling mediators to coordinate innate and adaptive immune responses [11]. Recent studies have shown that ROS regulate important macrophage functions including apoptosis, autophagy, and chemokine production during mycobacterial infection and may affect the development of T cell immunity against mycobacteria [12]. Since neutrophils, which produce abundant ROS when stimulated, have been shown to be the predominant phagocytic cells in the airways of patients with active pulmonary TB [13], ROS production by leukocytes may be quite abundant and play a central role in defense against tuberculosis in the infected tissues. Inflammasomes are multi-protein complexes responsible for caspase-1 activation and subsequent proteolytic processing and secretion of interleukin-1β (IL-1β), which is a pivotal cytokine for anti-TB immune response [14]. Based on recent studies implicating the role of ROS in activating inflammasome in mycobacterial infection [15], we postulated that resistin may weaken the immune defense against TB infection in type 2 DM through affecting ROS production by immune cells. Here, we analyzed the resistin level in TB patients with or without type 2 DM and investigated its relationship with ROS production and IL-1β secretion in defense against mycobacteria.

2. Materials and methods

2.1. Patients

We prospectively enroll tuberculosis patients at Chest Hospital, a TB referral center in southern Taiwan with 51 negative-pressure isolation beds. We enrolled culture-proven pulmonary TB subjects in this study. DM is defined by glycated hemoglobin (HbA1c) ≧6.5%, fasting blood glucose ≧126 mg/dl, or a random glucose ≧200 mg/dL. Venous blood of 10 ml was sampled from each subject for laboratory analysis. The clinical data including Chest X-ray, sputum culture results, HbAlC, and other important clinical data of the study subjects were recorded. Our previous study and other studies showed that DMTB patients tend to have more severe disease severity when compared with non-DM TB patients [2,3]. We hence used sputum acid-fast stain (AFS) grades to classify the severity of TB, and the severity classification by sputum AFS correlated well with the cavity formation on chest X-ray in this study. This study was approved by the Institutional Review Board of Taichung Veteran General Hospital (C09194).

2.2. Cytokine and prostaglandin E2 measurement

Serum resistin, CRP, IFN-γ, and IL-10 of all cases were measured with ELISA, using Duoset human resistin and Duoset human CRP kits (R&D systems, Minneapolis, MN). IL-1β concentrations in sera were measure by high sensitivity ELISA kits with the detection ranged from 0.16 to 10.0 pg/mL (eBioscience, USA). Prostaglandin E2 (PGE2) concentration of macrophage supernatants was determined by an ELISA kit (Cayman Chemical, USA for PGE2).

2.3. Mycobacterium marinum *preparation*

Given that RD1 locus plays critical role in TB virulence, we used *M. marinum (Mycobacterium marinum)* in this study, which is an RD-1 containing non-tuberculosis mycobacterium. *M. marinum*, obtained from American Type Culture Collection (ATCC), was further confirmed using chip hybridization and 16S rRNA sequencing.

2.4. Monocytic cell culture and mycobacterial stimulation

THP-1 cells were grown in RPMI 1640 (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Phorbol-myristate-acetate (PMA, 100 nM) was added for 24 h to induce differentiation of THP-1 cells into macrophages. Macrophages were then infected with *M. marinum* at an MOI of 1, and cell free supernatants were harvested, double filtered with 0.2 micron filters, and assayed for cytokines by ELISA and Western blot.

2.5. Preparation and mycobacterial stimulation of monocyte-derived macrophages (MDMs)

MDMs were prepared from peripheral venous blood from healthy donors and a chronic granulomatous disease (CGD) patient with a dinucleotide deletion (711-712 AG) in exon 7 [16]. CGD patients have defect in ROS generation from granulocytes and are extremely susceptible to mycobacteria infection [17]. Freshly sampled venous blood was mixed with 6% dextran for 2 h for RBCs sedimentation to get leukocyte-enriched supernatant. The supernatants were then overlaid on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) and centrifuged at 400× g for 20 min to separate PBMC and neutrophils. Monocytes were then isolated from PBMCs by positive selection using $CD14^+$ magnetic beads (Miltenyi Biotech). The purity of monocytes, determined by flowcytometry, was consistently more than 95%. The monocytes were then cultured for 7 days with GM-CSF (2 ng/ml) for differentiation and activated by human IFN-γ (5 ng/ml) for 2 days before infected with *M. marinum* at an MOI of 0.1. After being infected for 3 days, macrophages were lyzed with 0.1% Triton-X and the amount of intracellular bacteria was

Please cite this article in press as: Chao W-C, et al., Increased resistin may suppress reactive oxygen species production and inflammasome activation in type 2 diabetic patients with pulmonary tuberculosis infection, Microbes and Infection (2014), http://dx.doi.org/10.1016/j.micinf.2014.11.009