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Mouse β-defensin-14 for inducing the maturation of dendritic cells

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ABSTRACT

Background: β-defensins are an excellent antimicrobial peptide against microbial infection in which dendritic cells (DCs) play a crucial role by improving the innate and adaptive immune defense. However, it is unclear whether BDs affect DC maturation. This work aimed to study the effects of mouse β-defensin-14 (MBD-14) on DC maturation.

Methods: Via in vitro using mouse bone marrow DCs, the maturation of DCs was evaluated by cell morphological staining, flow cytometry, endocytosis assay, and allogeneic mixed lymphocyte reaction, respectively. And it was also assessed by in vivo establishing a mouse air-pouch model for flow cytometric determination, cytokine analysis, and histological staining.

Additionally, CLI-095, an inhibitor of Toll-like receptor-4 (TLR-4), was used to determine whether TLR-4 is possibly involved in DC maturation.

Results: It was found MBD-14 promoted DCs to form more filopodia and lamellipodia, increased the expression of DC maturation markers (CD40 and MHC-II), decreased their endocytic capacity, and enhanced T-cell proliferation. The analyses of the air-pouch exudates were consistent with the in vitro results of MBD-14 activating DCs. And when CLI-095 was applied, DC maturation was inhibited partly.

Conclusions: This work demonstrates that MBD-14 can promote the maturation of DCs in which TLR-4 is possibly involved.

1. Introduction

β-defensins (BDs) are an important subfamily of antimicrobial peptides comprising α-, β-, and θ-defensins, which are small molecular cationic peptides of 18–45 amino acid residues that exhibit a strong broad-spectrum antimicrobial activity at micromolar to nanomolar concentrations [1,2]. As one member of the β-defensin family, human β-defensin-3 (HBD-3) has a broader antimicrobial spectrum, more stable physicochemical properties, and a stronger sterilization effect on Gram-positive and Gram-negative bacteria as well as fungi compared with those of other antimicrobial peptides. Furthermore, it also plays important roles in linking the innate and adaptive immune responses through different Toll-like receptors (TLRs) from different immune cells which play a vital role in preventing infection and killing bacteria. Through a mechanism similar to that by which TLR-1 and -2 are expressed, HBD-3 may induce the maturation of monocytes [3]. Furthermore, during the response to viral infection and the development of autoimmunity in humans, HBD-3 suppresses TLR-3 responses [4]. HBD-3 also acts on TLR-4 to affect the pro-inflammatory activity of macrophages and glioma cells [5–7]. However, it is unclear what roles HBD-3 plays in the maturation of dendritic cells (DCs) which are regarded as the most powerful antigen presenting cells (APCs).

In this aspect, APCs play a vital role in linking the innate and adaptive immune systems and are indispensable for recognizing, taking up, processing, and eventually presenting various foreign antigens to T cells to initiate the adaptive immune response to cope with infection [8]. Among the three mainly professional APCs (DCs, macrophages, and B cells), DCs include immature DCs (imDCs) and mature DCs (mDCs). Compared to imDCs, the latter mDCs could express higher levels of major histocompatibility complex class II (MHC-II) and co-stimulatory molecules such as CD40 on their surface [9,10], and are considered to be the most efficient APCs for activating helper-T and cytotoxic cells, as well as inducing a protective immune response [10–15]. As described in previous reports, TLRs recognize and bind to ligands and then trigger relevant signaling pathways with components distributed on the surface of different immune cells [16–18]. And TLR-4, as one of important markers of DC maturation, is involved in the maturation of DCs through endogenous and exogenous adjuvants, induces T-cell activation, and enhances the innate and adaptive immune responses against bacterial and fungal infections [17–20].

Biragyn et al. suggested that, as an endogenous ligand for TLR-4, murine β-defensin 2 acts directly on imDCs to induce DC maturation

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and the upregulation of co-stimulatory molecules [21]. Moreover, HBD-3 activates APCs through TLR-1 or -2, which is similar to the action of the TLR-1/2 agonist, Pam(3) CSK(4) [22]. However, few studies have focused on whether HBD-3 induces the maturation of DCs and whether TLR-4 is involved or not. On the other hand, according to a BLAST search, the mouse β-defensin-14 (MBD-14) sequence is highly homologous to that of HBD-3. In vitro antibacterial and immunological experiments have shown that recombinant MBD-14 has a broad antibacterial spectrum and cytokine activity similar to those of HBD-3 [23,24]. Taken together, the structural and functional similarities between MBD-14 and HBD-3 suggest that MBD-14 could be considered to a functional substance substituting for HBD-3 in some applications.

In this work, we tested the HBD-3 homologue MBD-14 to determine whether it effectively promotes the phenotypic and functional maturation of DCs, and whether TLR-4 is involved in the process of DC maturation induced by MBD-14 or not. We hope our work could provide a theoretic basis for β-defensins possibly as an excellent immunotherapeutic candidate for infection.

2. Materials and methods

2.1. Materials

Pathogen-free male C57BL/6 mice (aged 6–8 weeks) used for in vitro and in vivo experiments were kept under specific pathogen-free conditions at our animal care facility. All animal experiments described in this work were approved by the Animal Care and Experiment Committee of Shanghai Sixth People's Hospital affiliated with Shanghai Jiao Tong University (Approval No. 2017-0253). In addition, animal maintenance and experiments were conducted in accordance with the policy of the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University, the National Institutes of Health Guide for the Care and Use of Laboratory Animals (GB14925-2010), and the regulations for the Administration of Affairs Concerning Experimental Animals (China, 2014).

Complete RPMI medium was prepared by adding 25 mM HEPES, 2 mM glutamine, 100 kU penicillin/L, 100 mg streptomycin/L, and 10% heat-inactivated fetal bovine serum (FBS) into RPMI 1640 medium (Gibco, Grand Island, NY, USA). Maturation medium comprised complete RPMI medium containing 20 ng/mL granulocyte-macrophage colony-stimulating and 20 ng/mL interleukin-4 (R&D Systems, Minneapolis, MN, USA). Antibodies for flow cytometry included phycoerythrin (PE)-labeled anti-mouse CD11c, fluorescein isothiocyanate (FITC)-labeled anti-mouse MHC Class II (I-A/I-E) (eBioscience, San Diego, CA, USA), and FITC-labeled anti-mouse CD40 (BD Biosciences, San Jose, CA, USA). Appropriate isotype controls were used for the corresponding antibodies. MBD-14 was customized and purchased from China Peptides Co., Ltd. (Shanghai, China). Other main reagents used were CLI-095 (TLR-4 inhibitor, InvivoGen, San Diego, CA, USA), lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, MO, USA), FITC-dextran (average molecular weight = 40,000, Sigma-Aldrich), and enzyme-linked immunosorbent assay (ELISA) kits (TNF-α and IL-12p70, Invitrogen, Carlsbad, CA, USA). Endotoxin contamination was detected using Tachypleus Amebocyte Lysate (TAL, Zhanjiang A & C Biological Ltd., China) with a sensitivity of 10–0.01 EU/mL, and only endotoxin-free materials were used in this work.

2.2. Bone marrow-derived DCs

Bone marrow-derived DCs (BMDCs) were generated in accordance with methods described previously [25,26], as follows. BM cells in tibiae and femurs of C57BL/6 mice were washed repeatedly with complete RPMI medium using a 5-mL syringe and a 0.45-mm-diameter needle under aseptic conditions. Then, 1–2 × 107 cells/mL were seeded onto 60-mm-diameter Petri dishes containing maturation medium. All cells were cultured at 37 °C in 5% CO_2 at 95% humidity. Half of the medium was replaced with fresh maturation medium after 2 and 4 days of culture. Non-adherent cells were collected on day 6, seeded into 24-well plates with fresh maturation medium, and randomly divided into four groups. The control group (Control) was maintained in RPMI alone, the LPS group (LPS) was supplemented with a final concentration of 1 μg/mL LPS, the MBD group (MBD) was supplemented with a final concentration of 10 μg/mL MBD-14, and the MBD + CLI group (MBD + CLI) was supplemented with a final concentration of 1 μg/mL CLI-095 plus 10 μg/mL MBD-14. The second day after these s were added, DCs were collected for further experiments.

2.2.1. DC morphological characteristics

DCs were stained with FITC-phalloidin (Sigma-Aldrich) and 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) to evaluate the early morphological characteristics of DCs during the exposure to the experimental s including LPS, MBD-14, CLI-095 and MBD-14. At 8 h and 24 h after the experimental s added, the DCs in each group were gently washed twice with phosphate-buffered saline (PBS), and next fixed in 4% paraformaldehyde for 15 min at room temperature. Then the DCs were incubated and stained by using FITC-phalloidin and DAPI at 37 °C in the dark for 1 h and 10 min, respectively. Finally, the cells were observed using fluorescence microscopy (Olympus, Japan) and counted based on five randomly selected microscopic fields.

2.2.2. Flow cytometry for DCs

DCs were centrifuged at 1200 rpm for 5 min, re-suspended into a single-cell suspension with 1 mL of PBS containing Fc-receptor-blocking antibodies (Miltenyi Biotec, Cambridge, MA, USA), and incubated for 30 min at room temperature. Then, the DCs were incubated with FITC-labeled anti-mouse CD40, FITC-labeled anti-mouse MHC-II, and PE-labeled anti-mouse CD11c for 30 min at room temperature in the dark. Next, the DCs were centrifuged, re-suspended, and 5000 cells were analyzed for each test using the Guava EasyCyte™ HT Instrument (Millipore, Billerica, MA, USA). Finally, the data were processed using the GuavaSoft 3.1.1 software.

2.2.3. Endocytosis assay

To evaluate the antigen uptake activity of DCs, the endocytosis assay was performed. DCs were incubated at 37 °C for 1 h after FITC-dextran (final concentration, 1 mg/mL) had been added, and then washed twice gently using the cold PBS containing 1% FBS. Uptake of FITC-dextran by DCs was determined by flow cytometry. In addition, a control experiment was carried out at 4 °C. Results were obtained by subtracting the fluorescence of the cells held at 4 °C from the fluorescence of the cells held at 37 °C.

2.2.4. Allogeneic mixed lymphocyte reaction

DC suspension was supplemented with mitomycin C (final concentration, 30 μg/mL), and washed twice gently after being incubated at 37 °C for 30 min. Then, the DCs were re-suspended in complete medium were co-cultured with interleukin (IL)-2-dependent cytotoxic-T-cell line-2 (CTLL-2) in new 96-well plates at a ratio of 1:10. Triplicate experiments were employed for the results of each group. Two controls were expressed by adding CTLL-2 alone and complete medium alone. All groups were cultured at 37 °C for 3 days, and then CCK-8 reagent (20 μL/sample) was added, followed by continuous culture for 4 h. Absorbance at 450 nm was determined with an ELISA reader, and the stimulation index (SI) of each group was determined as follows: SI = (experimental sample A value − complete medium A value) / (CTLL-2 A value − complete medium A value).

2.3. Mouse air pouch model

The mouse air pouch model was prepared in accordance with the methods adapted and described in previous studies [27,28]. Briefly, 20 mice (five mice for each group) were anesthetized by the