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Diarylheptanoid from rhizomes of *Curcuma kwangsiensis* (DCK) inhibited imiquimod-induced dendritic cells activation and Th1/Th17 differentiation

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ABSTRACT

Background and object: Dendritic cells (DCs) are critical for initiating the activation and differentiation of T cells in inflammatory diseases including psoriasis. *Curcuma kwangsiensis S.G. Lee & C.F. Liang* is a herb for treating psoriasis and we previously found Diarylheptanoid from rhizomes of *Curcuma kwangsiensis* (DCK) inhibited keratinocytes proliferation. However, it is unknown whether DCK influences DC functions. Thus we aimed to explore whether DCK affect the major immunological functions of DCs.

Materials and methods: Primary DCs derived from mouse bone marrow cells and spleen were used for examining their general immunological functions, and OVA-specific T cells from OT-II mice were used for examining the DC-mediated T-helper (Th) 1 and Th17 cells differentiation and effect.

Results: We demonstrated DCK suppressed DC uptake of FITC-labeled ovalbumin (OVA) and DC maturation characterized by decreased MHCII, CD80 and CD86 following imiquimod (IMQ) stimulation. DCK also reduced DC expression of the lymphoid-homing chemokine receptor CCR7, and DC migration towards CCL21, the ligand for CCR7. Importantly, DCK significantly reduced the production of proinflammatory cytokines including IL-12, IL-6 and IL-1β by IMQ-stimulated DCs. Moreover, in the coculture of $OVA_{323–339}$ peptide-pulsed DCs and OVA-specific T cells from OT-II mice, DCK significantly inhibited T cell proliferation and the differentiation of Th1 and Th17 cells. Furthermore, DCK treatment greatly reduced phosphorylation of p65-associated cell signaling pathway in IMQ-stimulated DCs.

Conclusion: These data together demonstrate a potential role of DCK in suppressing the biological function of DCs, and provide a possible mechanism for understanding the effects of herb *Curcuma kwangsiensis* in treating psoriasis.

1. Introduction

Dendritic cells (DCs), which bridge the innate and adaptive immunity, are recognized as professional antigen presenting cells (APCs) crucial for the presentation of various antigens to T cells and induce T-helper (Th) cells differentiation. Th1 cells are the host immunity effectors against intracellular bacteria by activating macrophages and $CD8^+$ T cells, following interleukin (IL)-12 priming and the production of effector cytokine interferon (IFN)-γ. Th2 helper cells protect against extracellular parasites by mainly activating eosinophils, basophils, and mast cells. They are primed by IL-4 and their effector cytokines are IL-4, IL-5 and IL-13. Th17 cells are a subset of pro-inflammatory T helper cells defined by their production of IL-17, and play an important role in maintaining mucosal barriers and contributing to pathogen clearance at mucosal surfaces. Besides the physiological functions of Th cells, these cells are also implicated in inflammatory disorders, and the interplay between DCs and Th cells accounts for the main process of the development and chronicity of these inflammatory diseases, including skin

Abbreviations: APCs, antigen presenting cells; CCL, chemokine (C−C motif) ligand; CCR, C−C chemokine receptor type; DAPI, 40,6-diamidino-2-phenylindole; DCK, diarylheptanoid from *Curcuma kwangsiensis*; DCs, dendritic cells; ERK, extracellular signal-regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IMQ, imiquimod; MFI, mean fluorescence intensity; OVA, ovalbumin; PAMP, pathogen-associated molecular patterns; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; TGF, transforming growth factor; Th, T-helper; TLR, toll-like receptors

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diseases such as psoriasis [1–4].

Psoriasis is most broadly defined as a Th17 and Th1 cells-induced inflammatory skin disease. IL-17/IL-22 synthesized from Th17 cell stimulate keratinocytes proliferation, and activate innate immune defense of keratinocytes and neutrophil-recruiting chemokines which work together to initiate the typical psoriatic phenotype [5]. Activated DCs produce cytokines to prime Th cells differentiation and activation. For example, IL-6 and transforming growth factor (TGF)-β, contribute to the priming and skewing, and the independent factor IL-23 promotes the terminal differentiation and sustaining the self-renewing of Th17 cells [6]; while IL-12 and IFN-γ are required for Th1 differentiation and development [7,8]. Moreover, given their distribution that DCs are involved in both epidermis and dermis, together with their irreplaceable function to initiate a cascade of immune responses, DCs are thought to display a pivotal pathogenic role in psoriasis [1,9].

Clinically, the markedly elevated levels of IL-17, IL-23, and IFN-γ, tumor necrosis factor (TNF)-α, were observed in psoriatic skin lesions of patients, accompanying the hyper-proliferation and altered differentiation of keratinocytes [10–12]. Correspondingly, the elevated expressions of IL-17 and IL-23 and increased Th17 cells were also observed in a mouse model of psoriasis, which was induced by imiquimod (IMQ) [13]. IMQ is a toll-like receptors (TLR) 7/9 agonist to mimic the pathological process of psoriasiform in mouse. IMQ induced accumulation of DCs populations and increased IL-23, as well as enhanced IL-6, IL-1β and TNF-α production in mouse skin and isolated human skin resident DCs [5,14]. Psoriasis can be exacerbated by IMQ-promoted DCs migration, e.g. from skin to draining lymph nodes, to initiate naïve $CD4^+$ T cells activation and Th subsets differentiation [15,16]. Therefore, IMQ-induced mouse model of psoriasiform is well used in investigating the immunological functions of the interplay of DCs and Th subsets in psoriasis.

Chinese herbal medicine is one of the therapies in wide range of application for treating psoriasis in Asia. *Curcuma Kwangsiensis S.G. Lee & C.F. Liang* (named E'zhu in Chinese) is a Chinese herb reported in modulating various biological functions [17–20], and our previous findings showed that Diarylheptanoid from *Curcuma kwangsiensis* (DCK) could inhibit the proliferation of keratinocytes [21], however, it is unknown whether DCK influences the biological functions of DCs. In the present study, we hypothesized that DCK might regulate the interplay of DCs and Th subsets in the pathological process of psoriasis, and we aimed to explore whether DCK could affect the major immunological functions of DCs upon IMQ stimulation. Our results demonstrate that DCK could suppress the multiple functions of DCs, including antigen uptake, maturation, migration, and the production of proinflammatory cytokines including IL-12, IL-6 and IL-1β. More importantly, DCK could impair the ability of IMQ-stimulated DCs to induce the antigen-specific T cell response, as evidenced by decreased OVA-specific T cell proliferation and Th1 and Th17 cell differentiation. Furthermore, we demonstrate DCK could inhibit the phosphorylation of p65-associated cell signaling pathway in IMQ-stimulated DCs. Thus, this study provides a possible mechanism for understanding the effects of Chinese herbal medicine in treating psoriasis.

2. Methods and materials

2.1. Animals and cell culture

For the DCs generation, C57BL/6 male mice aged 4–6 weeks were purchased from the Chinese Academy of Sciences (Shanghai, China), and OT-II mice were a gift from Prof. Leng's laboratory in Chinese Academy of Sciences (Shanghai, China). The animals were kept in a specific pathogen-free (SPF) environment. DC 2.4 cell line (between 3 and 10 passages) used here was a gift from Prof. Chu's Lab in Fudan University, and both of the DC 2.4 cell line and primary DCs were cultured in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C and 5% CO_2. DC 2.4 cells were passaged when they achieved 80–90% confluent. All animal experiments were approved by the Animal Care and Use Committee at Fudan University (Shanghai, China), and all methods were performed in accordance with the relevant guidelines and regulations.

2.2. Generation of primary bone marrow-derived dendritic cells (BMDC)

A widely used method for generating DCs was applied here to culture bone marrow cells in granulocyte-macrophage colony-stimulating factor (GM-CSF)-containing medium for 8 days. Bone marrow cells were obtained from C57BL/6 male mice and seeded at 1×10^6 cells/well in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 ng/ml GM-CSF (R&D System, Wiesbaden-Nordenstadt, Germany) for 8 days. Fresh 1640 medium with GM-CSF was changed every 3 days. On Day 8, the non-adherent cells and loosely adherent cells were harvested by pipetting gently before being sorted with FACS.

2.3. Isolation and purification of Diarylheptanoid from Curcuma kwangsiensis (DCK)

The isolation, purification and basic bioactivity of the Diarylheptanoid from *Curcuma kwangsiensis* used in this study was previously described [21]. Briefly, for the plant material, rhizomes of *C. kwangsiensis* originating from Guangxi Province were supplied by Kangmei Pharmaceutical Co. Ltd. (Puning, China). The rhizomes of *C. kwangsiensis* were refluxed twice with 95% (v/v) aqueous ethanol (2×80 L) for 2 h each time. The filtrate was concentrated under reduced pressure. The concentrated solution was suspended in water, centrifuged, and passed through a macroporous resin HP-20 column (10×120 cm), successively eluted with 0%, 30% and 95% EtOH-H_2O, to afford extracts. After the isolation and the determination of chemical structure, DCK was chemically synthesized (purity > 98%) for the following functional examination.

2.4. Lactic acid dehydrogenase (LDH) assay

LDH is normally retained in the cytosol until the cell membrane is ruptured, after which it is free to diffuse into the surrounding media. To determine the amount of cell injury induced by herb-derived compounds, both the culture medium and cell lysate were collected and immediately assayed for LDH activity. LDH Cytotoxicity Detection Kit was used according to the manufacturer's instruction (Clontech, Mountain View, CA), and result was determined with an Infinite M200 pro Microplate Reader (Tecan, Männedorf, Switzerland) at Optical density (OD) 490 nm. Cell viability was calculated as the ratio of LDH amount in the cell lysate to the total LDH amount from both the medium and cell lysate.

2.5. Antigen uptake assay

FACS sorted BMDCs in 10% FBS 1640 medium were added into 24-well plates for 12 h incubation. Medium for BMDCs culture was added with or without FITC-labeled OVA (2 μg/ml) (AnaSpec, Fremont, CA). Then BMDCs were stimulated with or without IMQ (10 μg/ml) (InvivoGen, San Diego, CA) in the absence or presence of DCK. After 12 h, cells were collected and the mean fluorescence intensity (MFI) of FITC-OVA was detected by flow cytometry.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Cells were cultured in 24-well plates in 1 ml of complete RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin and 10% heat inactivated FBS (all from Gibco). After experimental treatments, concentrations of cells-derived proteins in the