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Research article

Ginsenoside fractions regulate the action of monocytes and their differentiation into dendritic cells

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ABSTRACT

Background: *Panax ginseng* (i.e., ginseng) root is extensively used in traditional oriental medicine. It is a modern pharmaceutical reagent for preventing various human diseases such as cancer. Ginsenosides—the major active components of ginseng—exhibit immunomodulatory effects. However, the mechanism and function underlying such effects are not fully elucidated, especially in human monocytes and dendritic cells (DCs).
Methods: We investigated the immunomodulatory effect of ginsenosides from *Panax ginseng* root on $CD14^+$ monocytes purified from human adult peripheral blood mononuclear cells (PBMCs) and on their differentiation into DCs that affect $CD4^+$ T cell activity.
Results: After treatment with ginsenoside fractions, monocyte levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 increased through phosphorylation of extracellular signal-regulated kinase (ERK)1/2 and c-Jun N-terminal kinase (JNK), but not p38 mitogen-activated protein kinase (MAPK). After treatment with ginsenoside fractions, TNF-α production and phosphorylation of ERK1/2 and JNK decreased in lipopolysaccharide (LPS)-sensitized monocytes. We confirmed that DCs derived from $CD14^+$ monocytes in the presence of ginsenoside fractions (Gin-DCs) contained decreased levels of the costimulatory molecules CD80 and CD86. The expression of these costimulatory molecules decreased in LPS-treated DCs exposed to ginsenoside fractions, compared to their expression in LPS-treated DCs in the absence of ginsenoside fractions. Furthermore, LPS-treated Gin-DCs could not induce proliferation and interferon gamma (IFN-γ) production by $CD4^+$ T cells with the coculture of Gin-DCs with CD4+ T cells.
Conclusion: These results suggest that ginsenoside fractions from the ginseng root suppress cytokine production and maturation of LPS-treated DCs and downregulate $CD4^+$ T cells.

1. Introduction

Panax ginseng (i.e., ginseng) is a well-known traditional oriental medicine used to prevent various human diseases such as inflammatory diseases and cancer [1,2]. Ginsenosides are a major component of ginseng and more than 25 ginsenosides reportedly exist [3]. Ginsenosides can activate macrophages to produce reactive nitrogen intermediates and induce a tumoricidal effect [4]. However, they may also attenuate cytokine production [5].

Monocytes comprise approximately 5–10% of blood leukocytes in humans [6] and mice [7]. They have an important role in establishing innate immune responses. Monocytes differentiate

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into macrophages or dendritic cells (DCs) in the presence of appropriate mediators such as granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), or interleukin 4 (IL-4) [8]. On stimulation with lipopolysaccharide (LPS), monocytes and macrophages produce proinflammatory cytokines such as tumor necrosis factor (TNF)-α and the chemokines. Dendritic cells have a major role in initiating and inducing innate immunity and, perhaps more importantly, bridging with antigen-specific immune responses elucidated by T cells. Compared to monocytes, immature DCs display higher expression levels of CD80, CD86, CD11c, and major histocompatibility complex (MHC) class II, and have increased antigen uptake [9]. After antigen uptake, immature DCs become mature and sensitize naive T cells, which leads to clonal expansion and differentiation into effector helper T cells and cytotoxic T cells, which produce IFN-γ.

Mouse DCs treated with ginsenosides in a recent study showed a suppressed maturation process [10]. In mouse DCs stimulated with LPS, the ginsenosides inhibit the secretion of IL-12, an important cytokine that induces T cell activation. However, no reports have revealed the effect of ginsenosides on the differentiation of immature DCs from human monocytes. In the present study, we therefore explored the effect of ginsenoside fractions on the differentiation of $CD14^+$ monocytes to DCs, and explored the expression of cell surface markers (e.g., CD80, CD86, CD40, and MHC class II) on the differentiated DCs and interferon gamma (IFN-γ) production in $CD4^+$ T cells when cocultured with DCs that were differentiated in the presence of ginsenoside fractions.

2. Materials and methods

2.1. Reagents and chemicals

Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), and antibiotics (e.g., penicillin and streptomycin) were purchased from Gibco-BRL (Grand Island, NY, USA). *Escherichia coli* LPS (026:B6), the c-Jun N-terminal kinase (JNK) inhibitor SP600125, and polymyxin B (PMB) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The mitogen-activated protein kinase (MAPK) inhibitor U0126 was purchased from EMD Millipore (San Diego, CA, USA). Human recombinant IL-4, GM-CSF, and anti-Annexin-V-FITC antibody were purchased from R&D Systems (Minneapolis, MN, USA). Rabbit antiphospho-extracellular signal-regulated kinase 1/2 (antiphospho-ERK1/2), anti-ERK1/2, antiphospho-JNK, anti-JNK, antiphospho-p38, anti-p38, and anti-inhibitory kappa B (anti-IκB) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Goat antimouse immunoglobulin G-horseradish peroxidase (IgG-HRP), mouse antirabbit IgG-HRP, and mouse monoclonal anti-β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The specific antibodies for flow cytometric analysis, which included human anti-CD80-PE, anti-CD86-antigen-presenting cell (APC), anti-CD40-fluorescein isothiocyanate (FITC), anti-CD14-FITC, anti-CD11c-APC, and anti-human leukocyte antigen DR (HLA-DR)-FITC were purchased from BD Biosciences (San Diego, CA, USA). Unless otherwise noted, all other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Ginsenoside preparation

Ginsenoside fractions were extracted from *Panax ginseng*, as previously described [11]. In brief, the dried root of *Panax ginseng* was refluxed twice with 80% methanol and concentrated with a vacuum-evaporator. The concentrate was diluted with water and the solution was extracted with 1 L of diethyl ether. The aqueous phase was briefly evaporated under vacuum to remove the remaining ether. The solution was then extracted with *n*-butanol. The organic phase was finally collected and evaporated. Endotoxin levels in the ginsenoside preparations were determined using a *Limulus* amebocyte lysate test kit (Cambrex Bio Science, Walkersville, MD, USA) in accordance with the manufacturer's instructions. For each experiment, 100 mg of the ginsenoside fractions were dissolved in 5 mL sterile double-distilled water and diluted 1:1 with phosphate-buffered saline (PBS, Gibco-BRL) for a final concentration of 10 mg/mL.

2.3. Thin-layer chromatography and high-performance liquid chromatography analyses

For TLC, 8 μL of ginseng extract solution in butanol was spotted onto a TLC plate (silica gel 60) with standard samples and developed to 5.5 cm distance in a chamber containing a mobile phase chloroform-methanol-water mixture (65:35:10, v/v/v; lower phase). The bands on the TLC plates were detected by spraying with 10% sulfuric acid, followed by heating at 110°C for 10 min. High-performance liquid chromatography was performed by using the NS 3000i system (Futecs Co., Ltd, Jinju, Korea), which is equipped with a UV detector and a gradient pump. A 20-μL sample was injected into a C18 column (250 mm × 4.6 mm, 5μm), and the eluent was withdrawn at a flow rate of 1.6 mL/min using a solvent gradient consisting of acetonitrile (A) and water (W). The solvent A/solvent W ratios were 15:85, 21:79, 58:42, 65:35, 90:10, 90:10, and 15:85 with runtimes of 0–5 min, 5–25 min, 25–70 min, 70–85 min, 85–87 min, 87–97 min, and 97–110 min, respectively. Each ginsenoside fraction peak was monitored and compared with the peak corresponding to the standards (i.e., Rb1, Rc, Rd, Rh2, Rg1, Rg3, and compound K) prepared from steamed and dried *Panex ginseng* root (KT&G, Daejeon, Korea).

2.4. Preparation of $CD14^+$ monocytes and differentiation into DCs

The Institutional Review Board (IRB Number 0705/001-002) of the Seoul National University (Seoul, South Korea) approved all experiments using human blood. Peripheral blood mononuclear cells (PBMCs) were prepared by density gradient centrifugation of blood obtained from healthy donors by using the Ficoll-Paque Plus centrifuge (Amersham Bioscience, Buckinghamshire, UK). Mononuclear cells in the buffy coat were collected and washed three times with PBS. The $CD14^+$ monocytes were isolated from the PBMCs by using an IMag anti-human CD14 antibody kit (BD Biosciences). The $CD14^+$ monocytes were suspended in a complete medium composed of RPMI-1640 glutamax supplemented with 10% FBS and 1% antibiotics/antimycotics. To generate DCs, 1×10^6 $CD14^+$ monocytes were cultured for 3 d or 5 d at 37°C under 5% carbon dioxide in RPMI complete medium containing 500 U/mL IL-4 and 800 U/mL GM-CSF in a 24-well culture plate (Nalgene Nunc International, Rochester, NY, USA). The medium was changed every 3 d.

2.5. Enzyme-linked immunosorbent assay

For 24 h, $CD14^+$ monocytes (1×10^6 cells) were treated with ginsenoside fractions at a concentration of 0 μg/mL, 1 μg/mL, or 10 μg/mL in the presence or absence of LPS (50 ng/mL). The supernatants were then harvested. In some experiments, $CD14^+$ monocytes were pretreated for 1 h with U0126, SP600125, or PMB. The levels of IL-1β, IL-6, IL-10, and TNF-α in the supernatants were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) in accordance with the manufacturer's instructions.

2.6. Western blot analysis

The $CD14^+$ monocytes (1×10^6 cells) were stimulated with ginsenoside fractions at a concentration of 0 μg/mL, 1 μg/mL, and 10 μg/mL in the presence or absence of LPS (50 ng/mL). The cells were