Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Short Communication

Resveratrol increases phagocytosis and lipopolysaccharide-induced interleukin-1β production, but decreases surface expression of Toll-like receptor 2 in THP-1 monocytes

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ARTICLE INFO

Keywords:
Monocytes
Resveratrol
Phagocytosis
IL-1β
Toll-like receptors

ABSTRACT

THP-1 monocytes were used to evaluate the effects of physiological levels of resveratrol aglycone, resveratrol-3-O-glucuronide, resveratrol-4′-O-glucuronide, and resveratrol-3-O-sulfate on phagocytosis, IL-1β, IL-1α, and IL-18 production, viability, and TLR2 and TLR4 expression. THP-1 cells were treated with 1, 5, 10, and 15 μM resveratrol or metabolites. Resveratrol-3-O-glucuronide, resveratrol-4′-O-glucuronide, and resveratrol-3-O-sulfate had no effect on the functional parameters tested. Resveratrol aglycone increased phagocytosis at concentrations of 5, 10, and 15 μM and LPS-induced IL-1β production at concentrations of 10 and 15 μM. Expression of TLR4 increased slightly after resveratrol treatment, but surface expression of TLR2 was reduced as resveratrol concentrations increased. Our data suggest that resveratrol may be effective in modulating monocyte function in an environment where there is direct exposure to the aglycone, such as at the gut epithelium.

1. Introduction

The primary roles of monocytes during infection are phagocytosis and destruction of pathogens and the activation of both the innate and adaptive immune system by releasing pro-inflammatory cytokines [1]. Toll-like receptors (TLRs) on monocytes recognize and bind to pathogen-associated molecular patterns (PAMPs) on bacteria or viruses and certain nucleic acids released by apoptotic or necrotic tissues [2]. Interactions of PAMPs with TLRs induce the expression of pro-IL-1β and pro-IL-18 that are processed by caspase-1 leading to the secretion of mature IL-1β and IL-18, as well as pyroptotic cell death [3]. Procaspase-1 is activated by multi-protein inflammasome complexes.

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a phytochemical with reported activities that include inhibition of transcription factors, enzymes involved in cell signaling, and cell proliferation [4]. The majority of circulating resveratrol is transformed into metabolites by the gut epithelium and the liver. A recent study in humans showed that oral doses of 0.5–5 g of resveratrol over 21–28 d resulted in maximum plasma levels of 4.2, 17.1, 10.2, and 18.3 μM for resveratrol and three of its major metabolites, resveratrol-3-O-glucuronide, resveratrol-4′-O-glucuronide, and resveratrol-3-O-sulfate, respectively [5].

THP-1 cells are used widely as a model for analyses of monocyte functions and display a similar morphology to primary monocytes, produce pro-inflammatory cytokines in response to lipopolysaccharide (LPS), express Toll-like receptors, and have phagocytic activity [6]. In this study, we used THP-1 cells to determine if physiological levels of resveratrol and its major glucuronidated and sulfated metabolites (resveratrol-3-O-glucuronide, resveratrol-4′-O-glucuronide, and resveratrol-3-O-sulfate) altered phagocytic activity, the production of LPS-induced cytokines, and/or the expression of TLR2 and TLR4.

2. Materials and methods

2.1. Reagents and cell culture

Resveratrol (≥99%), 2-mercaptoethanol, cell culture-grade dimethylsulfoxide (DMSO), fetal bovine serum (FBS), and unconjugated mouse IgG1 were purchased from Sigma (St. Louis, MO). Phycoerythrin (PE)-conjugated anti-human TLR4 and Alexa Fluor 647-conjugated anti-human TLR2 antibodies, PE- and Alexa Fluor 647-conjugated isotype controls, human Fc block, FVS780 dye, and human IL-1β enzyme linked immunosorbant assay (ELISA) kits were purchased from BD Biosciences (San Jose, CA). The human IL-1α plus IL-18 U-plex kit was purchased from Meso Scale Discovery (MSD; Rockville, MD). Resveratrol 3-O-D-glucuronide (R3G, ≥95% purity), resveratrol 4′-O-D-glucuronide (R4′G, ≥95% purity), and resveratrol-3-O-sulfate (R3S,

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http://dx.doi.org/10.1016/j.cyto.2017.07.023
Received 21 April 2017; Received in revised form 28 July 2017; Accepted 29 July 2017
1043-4666/ Published by Elsevier Ltd.

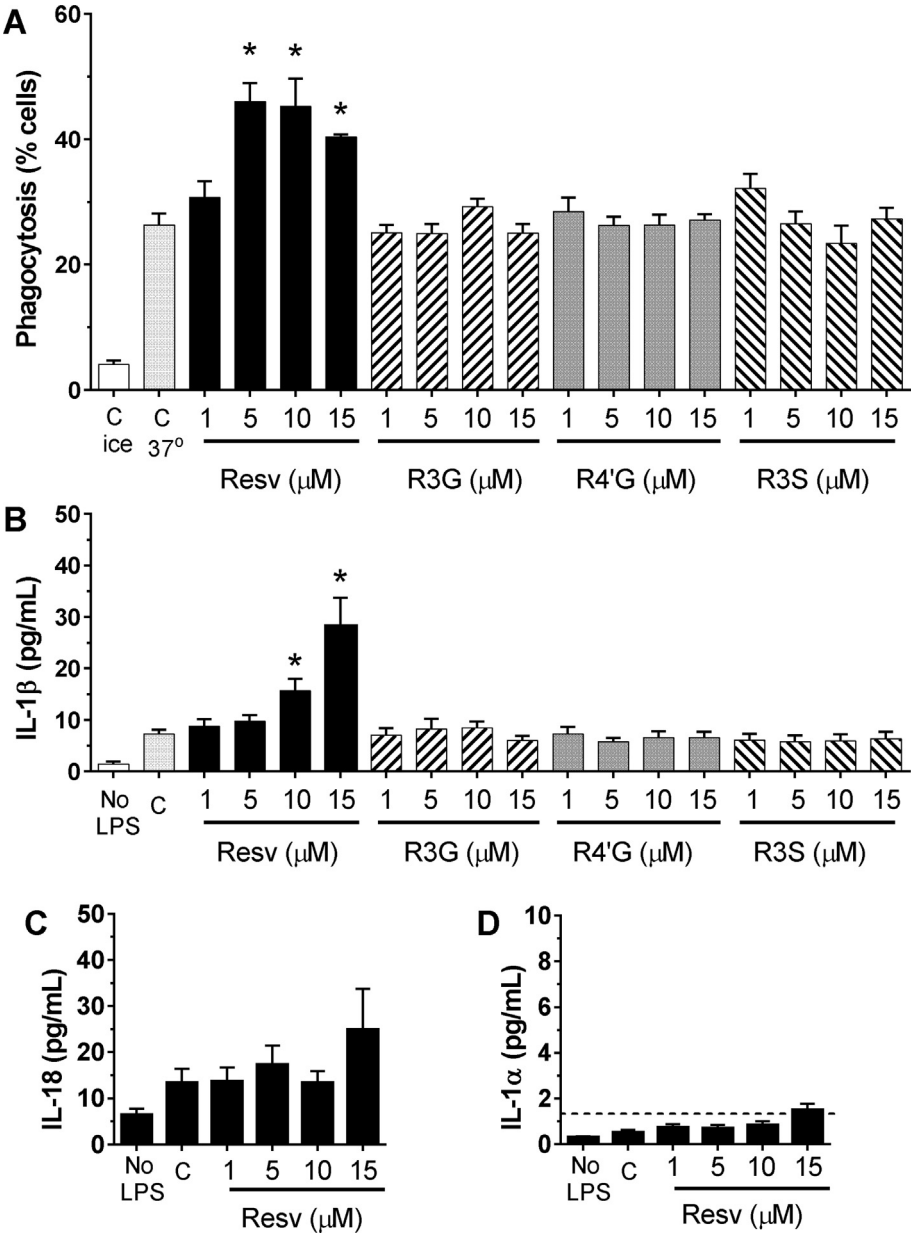

Fig. 1. Phagocytosis and LPS-induced IL-1β production increases in THP-1 cells after pretreatment with resveratrol aglycone. (A) THP-1 cells were pretreated for 24 h with the indicated concentrations of resveratrol (Resv), resveratrol 3-O-D-glucuronide (R3G), resveratrol 4′-O-D-glucuronide (R4′G), or resveratrol-3-O-sulfate (R3S). Phagocytosis was measured using opsonized pHrodo™ Green *E. coli* Bioparticles® and flow cytometry. The vehicle (0.1% DMSO)-treated control cells (C) were incubated on ice to inhibit phagocytosis or incubated at 37 °C. The asterisks represent a significant difference in percent of phagocytic cells compared to the vehicle control ($P < 0.05$). The data presented represent the mean of the phagocytic cells ± SEM from 3 separate experiments. (B) THP-1 cells were pretreated for 24 h with Resv, R3G, R4′G, or R3S and treated with 10 ng/mL lipopolysaccharide (LPS) for another 24 h. The control (C) consisted of pretreatment with vehicle (0.1% DMSO) for 24 h and LPS for another 24 h. Supernatants were collected and IL-1β was measured using ELISA. The asterisks represent a significant difference in IL-1β levels compared to the vehicle control ($P < 0.05$). The data presented represent the mean IL-1β concentrations ± SEM from 5 separate experiments. (C) THP-1 cells were treated with resveratrol and LPS as described above. IL-18 was measured in supernatants from 5 experiments using a Meso Scale U-plex kit. No differences were observed between treatment groups. (D) THP-1 cells were treated with resveratrol and LPS as described above. IL-1α was measured in supernatants from 5 experiments using a Meso Scale U-plex kit. No differences were observed between treatment groups. The dotted line indicates the limit of detection (lowest standard) of the kit for IL-1α. Data below the limit of detection and above the Y-intercept of the standard curve for IL-1α were extrapolated from the standard curve.

≥98% purity) were purchased from Cayman Chemical (Ann Arbor, MI). Endotoxin-free, ultrapure lipopolysaccharide (LPS) was purchased from List Biologicals (Campbell, CA). The THP-1 monocytic cell line was obtained from American Type Culture Collection (ATCC TIB202, Manassas, VA). The pHrodo™ Green *E. coli* Bioparticles® Conjugate, the Bioparticles® opsonizing reagent, and Live Cell Imaging solution were purchased from ThermoFisher (Pittsburgh, PA). THP-1 cells were maintained at 37 °C and 5% CO_2 in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin, 0.25 μg/ml amphotericin B, 1 mM sodium pyruvate, 2 mM L-glutamine (Invitrogen), 10% fetal bovine serum (Sigma), and 50 μM 2-mercaptoethanol.

2.2. Phagocytosis assay

The pHrodo™ Green *E. coli* BioParticles® were opsonized and prepared according to the manufacturer's recommendation. THP-1 cells were plated at a final concentration of 0.2×10^6 cells/mL and pretreated for 24 h with vehicle (0.1% DMSO), or with a final concentration of 1, 5, 10, or 15 μM resveratrol, R3G, R4′G, or R3S in 48 well plates. After 24 h, the cells were washed and the phagocytosis assay was initiated by resuspending the pretreated THP-1 cells in 100 μL of the Bioparticles® at a multiplicity of infection (MOI) of 100:1. The cells were incubated in a water bath at 37 °C for 30 min and the assay was halted by placing the cells on ice and washing with 3 mL PBS containing 0.1% bovine serum albumin (PBA buffer). The controls included vehicle-treated THP-1 cells that were kept on ice or at 37 °C, and cells exposed to Bioparticles® but kept on ice. The cells were collected on a BD LSRFortessa flow cytometer using DIVA 8.01 software (BD Biosciences). Twenty thousand events were collected and gated to remove cellular debris and aggregates.

2.3. LPS stimulated IL-1β production and viability analysis

THP-1 cells were pretreated with resveratrol and its metabolites as described above. After 24 h pretreatment, endotoxin-free LPS was added to a final concentration of 10 ng/mL and the cells were incubated another 24 h for measurement of IL-1β, IL-18 and IL-1α production. The cell supernatants were collected and assayed in duplicate using the BD OptEIA Human IL-1β ELISA Set II following the manufacturer's