International Immunopharmacology 47 (2017) 190–198

Contents lists available at ScienceDirect

International Immunopharmacology

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

Glucocorticoids differentially regulate the innate immune responses of TLR4 and the cytosolic DNA sensing pathway

Jinli Wang, Ruining Wang, Jiahui Yang, Xiaofan Yang, Shengfeng Hu, Hui Wang, Chaoying Zhou, Wenjing Xiong, Qian Wen, Li Ma *

Institute of Molecular Immunology, School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou 510515, China

ARTICLE INFO

Article history:
Received 7 January 2017
Received in revised form 22 March 2017
Accepted 22 March 2017
Available online 14 April 2017

Keywords:
Glucocorticoid
Macrophage
Autophagy
TLR4
DNA sensor

ABSTRACT

Glucocorticoids (GCs) are widely used to treat the chronic inflammatory disorders because of their powerful anti-inflammatory properties; however, their effects on macrophage-mediated immune responses are not completely understood. In the present study, we found that GCs decreased LPS-mediated TBK1 activation and the expression of IFN-β, RANTES and CXCL-10; however, poly(dA:dT)-induced TBK1 activity and cytokine expression were not affected by GCs treatment. Furthermore, GCs decreased the expression of key autophagy-related genes (ATGs), including ATG5, ATG7 and ATG12, and inhibited autophagy in macrophages after LPS stimulation. However, GCs had no effect on poly(dA:dT)-mediated autophagy and ATG expression in macrophages. Collectively, this study demonstrates that GCs inhibit the TLR4-mediated innate immune response, but do not affect the cytosolic DNA sensing pathway. This provides new insights into the immunomodulatory mechanisms of GCs in macrophages, which may provide useful information for the clinical use of GCs in treating chronic inflammatory disorders.

1. Introduction

Macrophages are the predominant type of innate immune cells and play an important role in pathogens recognition and the induction of inflammatory responses, which leads to immediate pathogen control and the initiation of adaptive immunity [1]. Macrophages recognize microbial pathogens via multiple pattern-recognition receptors (PRRs) [2]. Toll-like receptors (TLRs) were the first described class of PRR that recognize pathogen-associated molecular patterns (PAMPs) [3]. Bacterial lipopolysaccharide (LPS) was first defined as the specific ligand for mammalian TLR4. Upon recognition of its ligand, TLR4 activates both myeloid differentiation factor 88 (MyD88)-dependent and MyD88-independent TIR domain-containing adaptor inducing interferon (IFN)-β (TRIF)-dependent pathways, which subsequently leads to the activation of nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPKs), and TANK-binding kinase 1 (TBK1)/IFN-regulatory factor 3 (IRF3) signaling pathways [3]. The activation of the NF-κB and MAPKs is required for the induction of pro-inflammatory cytokines, whereas the induction of IFN-β and IFN-inducible genes requires the activation of TBK1/IRF3 in macrophages [3]. Cytokines produced by macrophages are generally part of the protective innate immune response in the host, but inappropriate activation or inefficient regulation of inflammatory reactions can contribute to chronic inflammatory disorders, such as rheumatoid arthritis, multiple sclerosis, and chronic obstructive pulmonary disease (COPD) [4].

To limit macrophages activation and restore homeostasis, endogenous glucocorticoids (GCs), a type of steroid hormone, function via the GC receptor, to execute an anti-inflammatory response and control a variety of fundamental metabolic and homeostasis functions [5]. As an immunosuppressive agent, synthetic GC, such as dexamethasone and hydrocortisone, is usually prescribed in clinics to treat autoimmune and inflammatory diseases, including rheumatoid arthritis, ulcerative colitis and systemic lupus erythematosus. Despite the desired anti-inflammatory effects, prolonged GC treatment can also have detrimental effects on host adaptive immunity by decreasing lymphocyte activation, proliferation and survival [6,7]. However, the immunoregulatory effects of GCs on macrophages are not clearly understood.

Autophagy is an evolutionarily conserved biological process, which is mediated by a reaction cascade that consists of a range of autophagy-related genes (ATGs), including ATG5, ATG7 and ATG12 [8]. Autophagy is responsible for the degradation of intracellular aggregated

Abbreviations: GC, glucocorticoid; DEX, dexamethasone; HCS, hydrocortisone; TLR, Toll-like receptor; ATG, autophagy-related gene; PRR, pattern-recognition receptor; PAMP, pathogen-associated molecular pattern; LPS, lipopolysaccharide; MyD88, myeloid differentiation factor 88; TRIF, TIR domain-containing adaptor inducing interferon (IFN)-β; NF-κB, nuclear factor κB; MAPK, mitogen-activated protein kinase; TBK1, TANK-binding kinase 1; IRF3, IFN-regulatory factor 3; COPD, chronic obstructive pulmonary disease; STING, stimulator of IFN genes; Baf. A1, bafilomycin A1; MDC, monodansylcadaverine; ISRE, interferon-stimulated response element; mTOR, mammalian target of rapamycin.

* Corresponding author.
E-mail address: maryhmz@126.com (L. Ma).

http://dx.doi.org/10.1016/j.intimp.2017.03.022
1567-5769/

proteins and damaged mitochondria, and functions to maintain cytoplasmic homeostasis [8]. Importantly, the critical role of autophagy in macrophage-mediated antimicrobial defense against intracellular pathogens is newly established [9]. Autophagy can be induced by the activation of TLR4 signaling in a p38 MAPK-dependent manner [10]. GCs have been reported to inhibit TLR4-induced cytokine expression through multiple mechanisms, such as inhibition of TBK1 kinase activity [11] and p38 MAPK activation [12]; however, whether GCs affect TLR4-mediated autophagy in macrophages remains unclear.

Cytosolic DNA sensors are a type of newly defined PRR, and include cyclic GMP-AMP synthase [13], absent in melanoma 2 [14], DNA-dependent activator of IRFs [15] and IFN-γ-inducible protein 16 (also called p204 in mice) [16]. Cytosolic DNA sensors detect intracytoplasmic double-stranded DNA and signal through the central adaptor protein, stimulator of IFN genes (STING), which recruits and activates downstream TBK1/IRF3 signaling axis to induce the production of type-I IFN [17,18], which is critical for innate immune defense against viral infections [19,20]. Furthermore, cytosolic DNA also activates STING-dependent and STING-independent autophagy to promote host defense against bacterial infection [21,22]. However, whether GCs modulate intracellular DNA sensor-mediated immune responses remains unknown.

In the present study, we investigated the role of GCs in the modulation of the PRR-driven innate immune response. LPS-induced TBK1 activation and the expression of cytokines, including IFN-β, RANTES and CXCL-10 were decreased in macrophages after GC treatment; however, poly(dA:dT)-induced TBK1 activation and cytokines expression were not affected after GC treatment. Furthermore, we found that GCs inhibited ATGs expression and autophagy in macrophages upon LPS treatment, whereas GCs had no effect on poly(dA:dT)-induced autophagy and ATG expression. Our findings provide new insight into the modulation of PRR-mediated innate immune responses in macrophages by GCs, which enhances our understanding of the immunomodulatory mechanisms of GCs, specifically regarding the macrophage-mediated innate immune response. Our study may provide useful information for the clinical use of GCs in treating chronic inflammatory disorders.

2. Materials and methods

2.1. Ethics statement

All animal experiments in this study were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. For all experiments of human primary macrophages, the study was approved by the Ethics Committee of Southern Medical University and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before the commencement of the study. All experimental protocols were reviewed and approved by the Medical Ethics Board and the Biosafety Management Committee of Southern Medical University.

2.2. Reagents

The following reagents were used in this study: dexamethasone (Sigma-Aldrich, D4902), hydrocortisone (Sangon Biotech, A610506), monodansylcadaverine (MDC, Sigma-Aldrich, D4008), DMSO (Sigma-Aldrich, D2650), bafilomycin A1 (Santa Cruz, sc-201550), p38 MAPK inhibitor SB203580 (Selleck Chemicals, S1076), Trizol reagent (Invitrogen, 15596-018). The following antibodies were used in this study: LC3 (Novus Biologicals, NB100-2220), GAPDH (ZSGB-BIO, TA-08), ATG5 (Cell Signaling, 12994), ATG7 (Cell Signaling, 8558), ATG12 (Cell Signaling, 4180), Tubulin (Cell Signaling, 2128), TBK1/NAK (Cell Signaling, 3013), Phospho-TBK1/NAK (Ser172) (Cell Signaling, 5483).

2.3. Cell culture and stimulation

RAW264.7 cells (ATCC; TIB-71) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin, 100 μg/ml streptomycin. For bone-marrow derived macrophages (BMDMs) preparation, firstly, eight-week-old female C57BL/6 mice were purchased from Southern Medical University Animal Supply Center. Secondly, single-cell suspensions of bone marrow cells from the femurs and tibiae were cultured in DMEM containing 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, supplemented with 100 ng/ml GM-CSF. Fresh medium was provided on days 3 and 5 of culture. At day 7 of culture, the isolated macrophages were used for further in vitro studies. For human monocyte-derived macrophages (MDMs) preparation, human peripheral blood samples from healthy donors were obtained from Guangzhou Blood Center. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll (TBDsciences, Tianjin) density gradient centrifugation. Human monocytes were purified from PBMCs by anti-CD14 microbeads (BD Biosciences) according to the manufacturer's directions. Monocytes were cultured in RPMI-1640 supplemented with 10% FBS and 100 ng/ml GM-CSF for a week to generate MDMs. Cells were treated with LPS (μg/ml), or transfected with 1 μg/ml poly(dA:dT) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

2.4. RAW264.7 GFP-LC3 stable cell line

RAW264.7 cells were transfected with pEX-GFP-LC3 using Lipofectamine 2000 according to manufacturer's instructions. Stable transfectants were selected for 3 weeks with 1 mg/ml G418 and maintained in 0.2 mg/ml G418. The transfectants were confirmed by Western blotting, and the formation of LC3 puncta in response to rapamycin was assessed by fluorescence microscopy (data not shown).

2.5. Western blotting

Western blotting was performed as described previously [23]. Briefly, the whole-cell extract was resolved by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked in 5% bovine serum albumin and then incubated with diluted primary antibodies at 4 °C overnight. Western blotting was developed using horseradish peroxidase-conjugated secondary antibody, followed by detection with enhanced chemiluminescence.

2.6. Confocal microscopy

Confocal microscopy was performed as described previously [24]. In fluorescence experiments, RAW264.7 GFP-LC3 cells were treated as indicated, followed by fixation, permeabilization. Nuclei were labeled with 4,6-diamidino-2-phenylindole (DAPI) staining. MDC staining was performed by adding MDC (50 μM) to cells and incubating at 37 °C for 30 min before fixation. Coverslips were mounted with ProLong Gold antifade reagent (Invitrogen) and visualized by using Olympus confocal microscope (FV10-ASW).

2.7. RNA isolation and real time-PCR

Total RNA was isolated with Trizol reagent as described before [25] and cDNA was synthesized with TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, AT311-03). TransStart Top Green qPCR SuperMix and an Eppendorf Mastercycler ep realplex^4 Real-Time PCR system (Eppendorf) were used for quantitative RT-PCR analysis. β-actin was used as housekeeping gene for data normalization. The primer sequences used for PCR were listed in Table 1.