Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Molecular Immunology 45 (2008) 2312–2322
Molecular Immunology
www.elsevier.com/locate/molimm

Toll-like receptors differentially regulate GPCR kinases and arrestins in primary macrophages

Katie Loniewski [a], Yuhui Shi [b], James Pestka [b,c], Narayanan Parameswaran [a,*]

[a] 3175 Biomedical Physical Sciences Building, Department of Physiology, Division of Pathology, Michigan State University, East Lansing, MI 48824, USA
[b] Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA
[c] Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA

Received 25 October 2007; received in revised form 19 November 2007; accepted 21 November 2007
Available online 4 January 2008

Abstract

G-protein coupled receptor kinases (GRKs) and arrestins (ARRs) are ubiquitously distributed crucial signaling proteins that are critical in the regulation of responsiveness of G-protein coupled receptors (GPCRs). Toll-like receptors (TLRs) (class of pattern recognition receptors) play a vital role in macrophage biology and innate immunity. Because GPCR responsiveness is regulated in part by the expression levels of GRKs/ARRs, the focus of this work was to uncover potential cross-talk mechanisms between TLRs and GPCRs via regulation of GRK/ARR expression in primary mouse macrophages. We demonstrate here that activation of TLR2 and 4 (but not TLR3 and 7) significantly decrease ARR2 but not ARR3 protein levels in macrophages. Compared to this, activation of TLR2, 4, and 7 (but not TLR3) significantly decrease GRK5 and 6 protein levels. Surprisingly, GRK2 protein levels are markedly increased by TLR2, 3, 4 and 7. Mechanistically, expression of ARR2 and GRK5 are regulated at transcriptional as well as post-translational levels. Downregulation of GRK6 by LPS is regulated primarily at the post-translational level. TLR4-induced GRK2 level, however, is both transcriptionally and post-transcriptionally regulated. Our results demonstrate previously unknown crucial regulatory mechanisms that alter ARR/GRK expression levels in macrophages that might modify many, if not all, GPCR-mediated innate immune responses.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Arrestin-2; Arrestin-3; Beta-arrestin; TLR; GPCR; TLR4; GRK2; GRK5; GRK6; GPCR kinase; TLR3; TLR2; TLR7

1. Introduction

G-protein coupled receptors (GPCRs) constitute one of the major therapeutic target areas in the US and worldwide. More than 50% of the drug targets in the pharmaceutical industry are targeted against GPCRs, thus underscoring the important role GPCRs play in human diseases (Howard et al., 2001). In addition, GPCR agonists (host- and pathogen-derived) regulate inflammatory responses by modulating a number of macrophage functions including chemotaxis, cell survival and inflammatory mediator production (Lattin et al., 2007; Vroon et al., 2006). Therefore, GPCRs play a vital role in regulating macrophage biology and innate immunity.

A fundamental mechanism by which GPCR responsiveness to ligands is regulated, is via desensitization, where by, upon continuous stimulation, the G-protein-dependent GPCR signaling is shut down (Lefkowitz et al., 1992; Lefkowitz and Shenoy, 2005). GPCR kinases (GRKs) and arrestins (ARRs) are two major protein families that primarily regulate receptor desensitization of almost all GPCRs. GRKs phosphorylate the agonist occupied receptor and arrestins bind to the phosphorylated receptor and inhibit G-protein-dependent signaling. GRKs are serine/threonine kinases with a central catalytic domain, flanked by an amino terminal “regulator of G-protein like homology domain” (RH-domain) and a carboxy terminal lipid-binding domain. The seven known GRKs can also be divided into three subfamilies based on overall structural organization and homology: (1) GRK1 (rhodopsin kinase) and GRK7; (2) GRK2 (βARK1) and GRK3 (βARK2); and (3) GRK4, GRK5 and GRK6. Arrestins (visual arrestin-1 and -4, nonvisual arrestin-2 and -3), however, are scaffolding proteins with no known

* Corresponding author. Tel.: +1 517 355 6475x1134.
E-mail address: paramesw@msu.edu (N. Parameswaran).

0161-5890/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2007.11.012

enzymatic activity (Lefkowitz and Shenoy, 2005; Reiter and Lefkowitz, 2006).

Responsiveness to agonist-induced GPCR signaling by GRKs and ARRs is regulated in part by the expression levels of these two protein families. Modulation of cellular levels of GRKs and ARRs by over-expression, knockdown or knockout have profound effects on GPCR signaling *in vivo* and *in vitro* (Ahn et al., 2003; Bohn et al., 2003; Krupnick and Benovic, 1998; Lefkowitz and Shenoy, 2005; Pierce et al., 2002). In addition, several studies have reported dynamic regulation of expression of ARRs and GRKs in various diseases (Iaccarino et al., 2005; Lombardi et al., 2001, 1999; Mak et al., 2002; Penela et al., 2006; Vroon et al., 2005, 2003). It is, however, not known whether the altered expression of ARRs/GRKs represent a mechanism by which cells are responding to a disease state, or whether it is a maladaptive state that contributes to the disease progression. Thus, understanding the biochemical mechanisms by which the expression levels of ARRs/GRKs are regulated in macrophages is of great significance.

Like GPCRs, Toll-like receptors (TLRs) play a crucial role in the regulation of macrophage biology and innate immunity. TLRs are pattern recognition receptors that sense the pathogen-associated molecular patterns in microbes. Thirteen TLRs have been identified to date with TLR1, 2, 4, 5, 6, and 11 displayed on the cell surface, and TLR3, 7, 8 and 9 localized intracellularly. Activation of TLR signaling constitutes one of the earliest responses of an organism to microbe invasion (Akira, 2006; Kawai and Akira, 2007; Lasker and Nair, 2006; Miggin and O'Neill, 2006). Thus, understanding the signaling pathways and biological responses stimulated by these TLRs is an area of intense interest.

Given the important physiological roles for both TLRs and GPCRs in macrophage biology and the regulatory role of ARRs/GRKs in GPCR signaling, we tested the hypothesis that TLR activation modulates the protein levels of ARRs/GRKs in macrophages. We provide evidence here that activation of TLRs selectively decreased arrestin-2 and GRK5/6 protein levels but significantly increased GRK2 expression in primary macrophages. We also demonstrate that the mechanisms involved are highly complex and include regulation at the transcriptional, post-transcriptional and post-translational levels. Our studies uncover novel mechanisms by which TLRs might regulate GPCR signaling in macrophages.

2. Materials and methods

2.1. Materials

Ultra pure LPS, PolyI:C, Pam3CSK4 and R837 were from Invivogen. Protease inhibitor cocktail tablet was from Roche. Monoclonal anti-α-tubulin antibody (mouse IgG1 isotype) was from Sigma. Monoclonal anti-ARR2 antibody (mouse IgG1 isotype) was from Transduction laboratories. Monoclonal anti-GRK2 (mouse monoclonal IgG2aκ) and monoclonal antiGRK-5/6 (mouse monoclonal IgG1κ) antibodies were from Upstate. Polyclonal anti-actin antibody (Actin C-11; Goat polyclonal IgG) was from Santa Cruz. Rabbit anti-arrestin-3 antiserum has been described previously (Kim et al., 2002) and was kindly provided by Dr. Jeffrey Benovic (Thomas Jefferson University).

2.2. Mice

C57BL/six mice (6–8 weeks old) were purchased from Charles River Laboratory and were housed four to five mice per cage at 22–24 °C in rooms with 50% humidity and a 12 h light–dark cycle. All animals were given mouse chow and water ad libitum. Mice were housed for at least 1 week before experimental use, and age-matched animals were employed as described. All animal studies conformed with NIH guidelines.

2.3. Peritoneal macrophage cultures

Murine peritoneal macrophages were elicited by injection of 1 ml of sterile Brewer's thioglycollate medium (4.05 g/100 ml; Sigma) into the peritoneal cavity of C57BL/6 mice. After 4 days, mice were euthanized. PBS (10 ml) was injected into the peritoneum, and lavage fluid was removed (Jia et al., 2006). Peritoneal cells were washed twice by centrifugation, resuspended in 10% fetal bovine serum (US origin, endotoxin tested (less than 0.3 EU/ml), heat inactivated, mycoplasma, virus and bacteriophage tested from Gibco/Invitrogen) supplemented RPMI-1640 medium (Gibco/Invitrogen) and then plated in 12-well plates and allowed to adhere to the wells for 2 h in 5% CO_2 at 37 °C. Non-adherent cells were removed by three washes with fresh culture medium, and the remaining adherent peritoneal macrophages were used for the experiments. By this method more than 90% of the adherent cells were estimated to be macrophages. Elicitation with thioglycollate results in an inflammatory macrophage population. For treatments, cells were serum starved for 4 h and then treated with various TLR ligands for different time points as indicated in the figures. Specific ligands used were Pam3CSK4 for TLR1/2, PolyI:C for TLR3, lipopolysaccharide (LPS) for TLR4, and R837 for TLR7.

2.4. Western blotting

For Western blotting, cells after treatment were quickly washed with cold PBS before lysis with buffer containing 1% Triton X-100, protease and phosphatase inhibitors. The lysates were clarified and then protein concentration determined. Equivalent amounts of protein were loaded on the gels for Western blot analysis. Immunoblotting was performed as described previously (Loniewski et al., 2007; Parameswaran et al., 2006). All the antibodies used here have been extensively used and referenced (please see manufacturer's websites for additional references). For immunoblotting, the secondary antibodies were HRP or fluorescent dye conjugated and analyzed by chemiluminescence or Licor's Odyssey, respectively. The bands were quantified using densitometry (for chemiluminescence) or Licor's Odyssey program (for fluorescence).

Download English Version:

https://daneshyari.com/en/article/2832054

Download Persian Version:

https://daneshyari.com/article/2832054

Daneshyari.com