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Overlapping and distinct roles of GRK5 in TLR2-, and TLR3-induced inflammatory response in vivo

Nandakumar Packiriswamy^{1,2}, Sitaram Parvataneni¹, and Narayanan Parameswaran^{1,2,*}

¹Department of Physiology, Michigan State University, East Lansing, MI

²Graduate program in Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI

Abstract

G-protein coupled receptor kinase-5 (GRK5) is a recently described NFκB regulator in TLR4 signaling pathway. To determine whether the role of GRK5 is MyD88- or TRIF-dependent, we injected wild type and GRK5 knockout mice with Pam3CSK4 (MyD88-dependent TLR1/2 ligand) and Poly(I:C) (TRIF-dependent TLR3 ligand) and examined the in vivo systemic inflammatory response. Our results demonstrate that GRK5 regulates IL-12p40 and G-CSF via a mechanism that is common to both MyD88 and TRIF. However, GRK5 regulates IL-5 and MCP-1 in a MyD88-dependent but TNFα in a TRIF-dependent manner. Together, our results demonstrate multiple roles of GRK5 in TLR signaling.

Keywords

GRK5; TLR4; TLR2; TLR3; inflammatory cytokines; Poly(I:C); Pam3CSK4

INTRODUCTION

Toll-like receptors (TLRs) are a well-characterized family of receptors involved in the recognition of pathogen-associated molecular patterns (PAMPs) derived from microbes. Eleven TLRs in the humans and 13 in the mice have been identified [1]. Activation of TLRs by PAMPs leads to transcriptional regulation of many genes and the pattern of gene regulation and the outcome is crucially dependent on the TLR that is activated and the specific cell type. In general, TLR signaling can be broadly classified into MyD88-dependent and TRIF-dependent pathways [1]. MyD88 signaling has generally been linked to NFκB and MAPK signaling, whereas TRIF-dependent signaling has been linked to activation of interferon response factors. Studies have also shown crosstalk and overlap between these two pathways depending on the cell type involved [2]. All TLRs except TLR3 utilize MyD88-dependent signaling, whereas TLR4 and TLR3 utilize TRIF-dependent signaling. TLR4 is unique in that it utilizes both MyD88- and TRIF-dependent pathways.

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*Corresponding author: Narayanan Parameswaran, BVSc., PhD, Associate Professor, Department of Physiology, Michigan State University, East Lansing, MI 48824, Ph: 517-884-5115, paramesw@msu.edu.

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TLRs are involved in a number of inflammatory diseases including non-infectious diseases [3]. Therefore, understanding the mechanistic basis of TLR signaling is a rapidly expanding field. In this regard, we recently showed that TLR4 signaling is regulated by G-protein coupled receptor kinases (GRKs) in vitro in macrophages and in vivo in mice [4]. GRKs are a family of serine/threonine kinases discovered for their role in G-protein coupled receptor desensitization [5]. Of the seven GRKs, recent studies have suggested that GRK5 has a unique role in regulating TLR4-stimulated NF κ B signaling in different cell types and in vivo in mice [4, 6–8]. In addition, studies have also shown important roles for GRK5 in signaling from microbes in *Drosophila* and Zebra Fish models [8]. Even though initial studies suggested a critical role for GRK5 in TLR4 signaling, it is not known whether the role of GRK5 is specific for TLR4 or whether it regulates other TLRs. In addition, since TLR4 activates both MyD88- and TRIF-dependent pathways, an important question still remains as to whether GRK5 selectively regulates one of these pathways. To address these issues we utilized our previously described GRK5 knockout mice as model and administered a TLR1/2 ligand (Pam3CSK4) that activates only the MyD88 pathway, and a TLR3 ligand (Poly(I:C)) that activates only the TRIF pathway. Surprisingly, our results suggest that GRK5 has unique and overlapping roles in inflammation induced by TLR2, and TLR3.

MATERIALS AND METHODS

Animals and protocol

All animal procedures were approved by the Michigan State University Institutional Animal Care and Use Committee and conformed to NIH guidelines. Generation and characterization of GRK5 null mice has been described before [4]. Male mice, 6–8 weeks of age were used for the experiments with appropriate littermate controls. Animals were housed 4–5 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*. Wild type GRK5^{+/+} and Knockout GRK5^{-/-} were segregated into two different groups for intra-peritoneal injection of Pam3CSK4 (100 μ g/mouse) and Poly(I:C) (200 μ g/mouse) ligands [9–11]. Both ligands were obtained from Invivogen (San Diego, CA). The animals were euthanized and samples collected 9 hours post-injection.

Cytokine analysis

A mouse 23-plex multiplex-based assay (from Biorad, Hercules, CA) was used to determine the cytokine/chemokine concentrations according to manufacturer's instructions via Luminex 100 technology as described previously [12, 13]. Plasma from mice treated with Pam3CSK4 and Poly(I:C) were used to assess the cytokine/chemokine levels.

Statistical methods

All values are represented as mean $\pm$ SEM. Data were analyzed and statistics performed using GRAPHPAD PRISM software (La Jolla, California). The Student's t-test was used to compare mean values between two experimental groups. P value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Although originally discovered as a regulator of G-protein coupled receptor desensitization, GRK5 has now been shown to broadly regulate other aspects of cell signaling, independent of GPCRs [4, 6–8, 14]. In this regard, recently we showed that GRK5 is an important regulator of NF κ B signaling in mice and this was further confirmed in *Drosophila*, and Zebra fish, suggesting an evolutionarily conserved role for GRK5 in NF κ B signaling [4, 8]. Using cells from mice that are deficient in GRK5, we showed that lipopolysaccharide

(TLR4 ligand)-induced activation of NF κ B signaling and the consequent cytokine production are critically regulated by GRK5. In addition, in the endotoxemia model, several cytokines/chemokines were significantly attenuated in the GRK5 knockout mice.

To begin to understand the specificity of GRK5 in various TLR signaling pathways, we used ligands for TLR2, and TLR3 to separately activate MyD88 and TRIF respectively. We administered these ligands in wild type and GRK5 knockout mice. Nine hours after administration, we examined the levels of various cytokine/chemokines using a 23-plex ELISA. In the Pam3CSK4 injected group, IL12p40, IL-5, G-CSF and MCP1 were significantly inhibited in the GRK5 knockout mice (Fig 1 and 2). IL-6 and MIP1 β levels showed similar results but were not statistically significant (Fig 1). Interestingly, three of these cytokines/chemokines (IL12p40, IL5, and MCP1) overlapped with our previously reported findings using LPS. When Poly(I:C) was administered, only IL-12p40, TNF α , and G-CSF were significantly inhibited in the GRK5 knockout mice compared to the wild types (Fig 2 and 3). Poly(I:C)-induced RANTES was also inhibited in the GRK5 knockout, but did not reach statistical significance. All these except G-CSF were also inhibited in the LPS group in our previous report. Unlike Pam3CSK4, IL1- α , and Eotaxin levels were not at detectable levels in the plasma after Poly(I:C) administration. There was no significant difference in the levels of IL1 β , IL-13, IFN γ , IL-2, IL-3, IL-4, IL-9, IL12p70 and GM-CSF when compared between the wild type and the GRK5 knockout mice in either of the ligand groups (data not shown).

These results clearly indicate several outcomes as follows: 1. GRK5 regulation of IL-12p40 and G-CSF production occurs at a level that is common to MyD88 and TRIF pathways and possibly all TLRs. 2. Regulation of IL-5, and MCP-1 production by GRK5 occurs at a level that is specific to MyD88-dependent pathway. It is important to note that IL-5 and MCP-1 were also inhibited in the LPS-induced response, in our previous study. 3. Regulation of TNF α and possibly RANTES production by GRK5 occurs at a level that is likely to be specific for the TRIF-dependent pathway because these were inhibited in both the LPS group [4] and the Poly(I:C) but not Pam3CSK4 group. 4. In previous study, we reported that LPS-induced production of IFN γ , MCP-1, TNF α , IL-1 β , IL-12(P40), IL-12(P70), IL-5, IL-17, IL-10, EOTAXIN, IL-2, IL-3, IL-4, IL-9, IL-13, and RANTES are significantly inhibited in the GRK5 knockout mice [4]. Of these cytokines/chemokines IFN γ , IL-1 β , IL-12p70, IL-17, IL-10, Eotaxin, IL-2, IL-4, IL-9 and IL-13 are not inhibited in either TLR2 or TLR3 ligand groups suggesting that regulation of these factors by GRK5 is likely to be unique to TLR4.

Previous studies have shown that GRK5 regulates NF κ B signaling and consistent with that many cytokines/chemokines induced by LPS were inhibited in the GRK5 knockout mice. Similarly, in this study many cytokines/chemokines induced by Pam3CSK4- and Poly(I:C)- were inhibited in the knockout mice. While this regulation at least in part is likely due to GRK5's role in NF κ B pathway, it is possible that other signaling pathways are also being regulated by GRK5, given its roles in many cellular processes [4–7, 14–22]. The observation that GRK5 appears to regulate various cytokines/chemokines at levels that are common to MyD88 and TRIF, as well as unique to MyD88 or TRIF or TLR2/TLR4 pathways suggests that GRK5 mediates the production of these inflammatory factors via multiple regulatory pathways. Identifying these novel roles of GRK5 will be the focus of future studies.

Highlights

TLR-induced IL-12p40 and G-CSF are mediated by GRK5 via both MyD88- and TRIF-dependent pathways.

TLR-induced increase in IL-5 and MCP-1 are regulated by GRK5 in a MyD88-dependent fashion.

TLR-induced TNF α and RANTES are regulated by GRK5 in a TRIF-dependent manner.

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Figure 1. Plasma IL-12p40 and G-CSF are regulated by GRK5 in both Pam3CSK4 and Poly(I:C) treatment groups: WT and GRK5 knockout mice were injected with Pam3CSK4 and Poly(I:C) and sacrificed 9 hours after administration. Plasma samples were collected and cytokines measured as described in the methods. N=6 per group. *p<0.05

Figure 2.

Plasma IL-5, MCP-1, MIP-1 β and IL-6 production are regulated by GRK5 only in the Pam3CSK4 treatment group: Mice were injected with respective ligands and samples collected and analyzed as described in Fig 1. N=6 per group. *p<0.05.

Figure 3.

Plasma TNF α and RANTES are regulated by GRK5 only in the Poly(I:C) treatment group: Mice were injected with ligands and samples collected and analyzed as described in Fig 1. N=6 per group. *p<0.05.