G-protein-coupled receptor kinases in inflammation and disease

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G-protein-coupled receptor kinases (GRKs) are serine/threonine protein kinases originally discovered for their role in G-protein-coupled receptor (GPCR) phosphorylation. Recent studies have demonstrated a much broader function for this kinase family including phosphorylation of cytosolic substrates involved in cell signaling pathways stimulated by GPCRs, as well as by non-GPCRs. In addition, GRKs modulate signaling via phosphorylation-independent functions. Because of these various biochemical functions, GRKs have been shown to affect critical physiological and pathophysiological processes, and thus are considered as drug targets in diseases such as heart failure. Role of GRKs in inflammation and inflammatory diseases is an evolving area of research and several studies including work from our lab in the recent years have demonstrated critical role of GRKs in the immune system. In this review, we discuss the classical and the newly emerging functions of GRKs in the immune system and their role in inflammation and disease processes.

INTRODUCTION

Cells are exposed to myriad of extracellular agents including hormones to which the cells have developed sophisticated mechanisms for receiving, processing and transmitting signals. Receptors have a critical role in receiving these signals, and are present both on the plasma membrane and inside the cells. Among these receptors, G-protein-coupled receptors (GPCRs) form the largest family of membrane receptors that are encoded by ~950 genes.1 These GPCRs are characterized by their seven-transmembrane domain and detect a range of extracellular signals including neurotransmitters, chemoattractants, lipids, peptides, hormones, light and odors. Transmission of signals via GPCR activation modulates a variety of physiological processes including sense of vision, olfaction, hormonal signal transduction, cellular proliferation, differentiation and cell survival. Because of the multitude of signals received by these GPCRs, these receptors are now direct drug targets for ~50% of the currently used therapeutics.2 Classical GPCR activation by agonist binding causes conformational change in the receptor, which results in the activation of the heterotrimeric GTP-binding proteins (G proteins).3 G proteins are a complex of subunits composed of α-subunit (Go encoded by 16 genes) and βγ dimers (Gb encoded by 5 genes and Gy encoded by 12 genes).4 Exchange of GTP for GDP in Ga leads to dissociation of Ga from Gβγ. However, there is also evidence that in some cases the heterotrimers may not fully dissociate.5 Instead, they may undergo structural rearrangement following GPCR activation. Subsequent to GPCR activation and exchange of GTP for GDP in Ga, Ga-GTP and Gβγ activate a number of effector proteins, leading to various biological outcomes (Figure 1). The intrinsic GTPase activity in Ga-subunit causes GTP hydrolysis and the formation of Ga-GDP, leading to reassociation of Gαβγ trimer. For a comprehensive review of G-protein activation please see other reviews.6–8

One of the fundamental mechanisms in the regulation of GPCR signaling is the ability of the receptor to ‘shut down’ upon continuous stimulation. This phenomenon called ‘desensitization’ is mediated by two protein families: G-protein-coupled receptor kinases (GRKs) and arrestins. Members of these two protein families have critical roles in desensitization of most GPCRs. GRKs specifically phosphorylate agonist-occupied GPCRs and this results in arrestin translocation and high affinity binding to the phosphorylated receptor. Arrestin binding interdicts GPCR and G-protein binding and this event functionally uncouples GPCRs from their cognate G proteins, thereby terminating G-protein activation.9,10

In addition to the classical desensitization functions, studies within the past decade clearly emphasize functions of GRKs and arrestins that are distinct from this canonical role. It is now clear that GRKs (and arrestins) have GPCR-dependent but G-protein-independent functions in cell signaling and biology. Importantly, GRKs and arrestins have also been shown to modulate GPCR-independent functions in physiological processes (Figure 2). In recent years, this role of GRKs has especially become apparent in the context of inflammation and inflammatory diseases. In this review, we discuss the emerging themes of GRK functions, especially those of non-visual GRKs, in both GPCR-dependent and -independent functions relevant to inflammatory processes.

The GRK family

During 1970s and mid-1980s, agonist-induced dampening of G-protein-mediated signaling was discovered for rhodopsin and β2-adrenergic receptor (β2AR). The enzyme phosphorylating rhodopsin receptor (which controls vision) was identified in the late 1970s and was aptly termed ‘rhodopsin kinase’ (aka GRK1).11 Few years later, a novel kinase was demonstrated to phosphorylate β2AR and dampen G-protein-mediated signaling.12
This kinase was initially named as βAR kinase or βARK (aka βARK1, GRK2). Simultaneously, cloning of the mammalian βAR revealed a sequence similarity with rhodopsin receptor and this led to the recognition of GPCR family, with rhodopsin as its founding member. Another protein (termed as S antigen) discovered initially for its role in allergic uveitis was found to associate with rhodopsin and dampen G-protein-mediated retinal signaling. In 1986, with the discovery of this ‘arresting’ function in retinal signaling, this protein was renamed as ‘arrestin’. Further, functional characterization of GRKs and arrestin established the idea of two-step inactivation process of desensitization of GPCRs. Meanwhile, other members of the GRK family were identified: GRK3 (aka βARK2), GRK4, GRK5, and GRK7.

GRKs are grouped broadly into visual and non-visual GRKs. Expression of the visual GRKs (GRK1 and GRK7) is restricted to the eyes and pineal gland. The non-visual GRKs are further grouped into two subgroups: GRK2-like (GRK2 and GRK3, otherwise known as βARK1 and βARK2) and GRK4-like (GRK4, GRK5 and GRK6) based on their structural similarity. Non-visual GRKs are widely distributed throughout the body with the exception of GRK4, which is found restricted in the testis and proximal tubule of the kidney. Therefore, most GPCRs in the body are regulated by at least one of the four GRKs (GRK2, GRK3, GRK5 and GRK6). Given that there are hundreds of GPCRs in the mammalian system, each GRK must regulate more than one GPCR, thus increasing the complexity of our understanding the role of GRKs.

All GRKs are multidomain proteins, sharing a 25-residue N-terminal region (unique for GRKs), followed by a regulator of G-protein signaling homology domain (RH) and a catalytic serine/threonine protein kinase domain responsible for phosphorylating substrates (Figure 3). The N-terminal basic region in the GRK4-like family (GRK4–6) aid in membrane translocation. However, the C-terminal domain is the most essential domain for targeting the GRKs to the plasma membrane. GRK1 and GRK7 are membranes associated by virtue of their short farnesylated C termini. The C termini of GRK2-like kinases are longer compared with that of GRK4-like kinases and contain a 125-amino-acid pleckstrin homology domain. Pleckstrin homology domain has binding sites for phosphatidylinositol bisphosphate and Gβγ. GRK4 and GRK6 are thought to be membrane associated via their palmitoylated residues within the last 15–20 amino acids of the C terminus and also by their N-terminal basic region. GRK5 is also predominantly membrane bound, through polybasic regions found in both the C and N termini.

Interestingly, GRK5 also contains a nuclear localization signal and has been shown to accumulate inside the nucleus. Indeed, GRK5 can phosphorylate class II histone deacetylase and therefore mediate gene transcription in...
cardiomyocytes.\textsuperscript{25} Interestingly, GRK6A, one of the three splice variants of GRK6, has also been detected inside the nucleus; however, its physiological role is yet to be ascertained.\textsuperscript{26} In addition to these differences, recent studies have also shown that the GRK2/3 and GRK5/6 family members differ in their ability to phosphorylate inactive GPCRs. Although it was originally assumed that all GRKs phosphorylate only active GPCRs, GRK5 and GRK6 were shown to phosphorylate $\beta_2$-adrenergic and M2 muscarinic receptors even in their inactive state.\textsuperscript{27}

![Domain structure of GRK family of proteins](image)

Figure 3. Domain structure of GRK family of proteins: GRK have a short N-terminal domain, a catalytic domain and a variable C-terminal domains. See text for further information. Numbers above the domains represent the amino-acid residue based on Lodowski et al.\textsuperscript{146}

Regulation and activation of GRKs

Activity of GRKs is regulated by both protein–protein interactions and phosphorylation events. $G_\beta_\gamma$-subunits were one of the earliest known proteins known to interact with and activate GRKs.\textsuperscript{28} In addition, lipids can bind and activate GRKs. Interestingly, GRK2 family members are activated by phospholipids (phosphatidylinositol bisphosphate) binding to the C-terminal PH domain, whereas GRK4-like family members are activated by phosphatidylinositol bisphosphate binding to the N-terminal polybasic regions.\textsuperscript{29} Furthermore, calmodulin, caveolin-1 and actin are also known to affect GRK activity by direct binding.\textsuperscript{30,31}

Phosphorylation can lead to either activation or deactivation of GRKs depending on which kinase phosphorylates GRKs or whether GRKs undergo autophosphorylation. A decrease in kinase activity of the respective GRKs has been observed when visual GRKs are phosphorylated by protein kinase-A and GRK5 by protein kinase C.\textsuperscript{32,33} In contrast, GRK2 phosphorylation by protein kinase C enhances its kinase activity.\textsuperscript{34} In addition to phosphorylation, GRK2 can also be S-nitrosylated by nitric oxide synthase, which leads to inhibition of its activity.\textsuperscript{35} These findings suggest that multiple pathways are involved in regulating the activities of GRKs.

Recent studies looking at the crystallographic structures of GRK1, GRK2 and GRK6 have provided key information on how these GRKs phosphorylate receptors.\textsuperscript{36} These studies revealed GRKs in a closed conformation in which the conserved N-terminal 18–20 residues form the a-(aN) helix and interact with the kinase domain. Formation of aN helix stabilizes the closed (active) conformation of the kinase domain, leading to allosteric activation and favoring catalysis by kinase domain. Furthermore, the aN helix is proposed to act as a docking site for the activated receptors, and removal of aN helix abolishes GPCR phosphorylation, suggesting that aN helix formation is indeed the primary step in the activation of GRKs.

Animal models of GRK deficiency

Phenotypes of GRK knockout mice have enabled researchers to identify the various pathophysiological roles of GRKs. In some cases, phenotypes were less obvious likely because of compensation by other GRKs. The most striking phenotype was that of embryonic lethality in GRK2 homozygous knockout because of defective cardiac development.\textsuperscript{37} Using targeted knockouts and heterozygous mice, GRK2 has been shown to be important in the heart development, lymphocyte chemotaxis, experimental autoimmune encephalomyelitis, sepsis, atherosclerosis and so on. Closer examination of other GRK knockout mice revealed distinct phenotypes—GRK3 in olfaction;\textsuperscript{38} GRK5 in cholinergic responses and inflammation; GRK6 in chemotaxis, behavioral responses, locomotor stimulating effect of cocaine and so on.\textsuperscript{39} A detailed summary of these phenotypes is listed in Table 1.

In addition to the knockout mice, transgenic overexpression of GRKs has also revealed important functions for GRKs.\textsuperscript{40,41} Thus, research from over the past decade demonstrates multiple functions of GRKs in different organ systems. However, the role of GRKs in immune system is only beginning to be understood. In this review, we will focus mainly on our recent understanding of GRKs in the regulation of immune system, in particular their effects on inflammatory signaling pathways and in inflammatory diseases.

REGULATION OF INFLAMMATORY SIGNALING PATHWAYS BY GRKs

GRKs in NF-$\kappa$B signaling

Nuclear factor-$\kappa$B (NF-$\kappa$B) signaling pathway is intricately tied with many inflammatory processes, and thus regulatory molecules in the NF-$\kappa$B signaling pathway are potential therapeutic targets in a number of inflammatory diseases. Under unstimulated conditions, NF-$\kappa$B transcription factors (p65 (RelA), p50, RelB, cRel and p52) are
our group demonstrated that GRK5 is a noncanonical Iκκ

Subsequent to the discovery of GRK5 regulation of p105, Sorriento

Later studies however underscored further complexity in the regulation of NF-κB pathway by GRK5. Studies by Valanne et al. demonstrated that the role of GRK5 in NF-κB signaling has an evolutionarily conserved significance and that GRK5 is a critical mediator of NF-κB signaling in different models (and species) including Drosophila, zebrafish and human cell lines. In their studies, GRK5 was shown to be required for normal microbial resistance in vivo in zebrafish and Drosophila. Later studies from our group demonstrated that GRK5 is a noncanonical IκB kinase and that it phosphorylates Ser32/36—same sites phosphorylated by IκB kinaseβ, the canonical IκB kinase. Consistent with these biochemical findings, levels of cytokines and chemokines were largely attenuated in GRK5 knockout mice compared with the wild-type mice in endotoxemia model. In addition, IκB phosphorylation and p65 nuclear translocation were significantly reduced in lipopolysaccharide (LPS)-treated GRK5-deficient peritoneal macrophages. Interestingly, using a different GRK5 knockout mice generated by the Lefkowitz group, Wu et al. found that endothe
tial GRK5 indeed stabilized IκBα similar to earlier studies by Sorriento et al. Also, unlike our studies in macrophages, Wu et al. did not find any role for GRK5 in IκBα phosphorylation and p65 translocation. It is yet unclear if these contrasting results are because of different cell types being studied, different knockouts used (complete deletion of GRK5 gene versus part of the gene) or background of the mice. It should be noted, however, that a recent study by Islam et al. using GRK5 knockout mice from the Lefkowitz group found that GRK5 indeed positively regulates the NF-κB pathway in cardiomyocytes. In earlier studies, Koch’s lab had shown that GRK5 expression itself is regulated by the NF-κB pathway, suggesting a positive feedback loop wherein GRK5 mediates the NF-κB pathway, and NF-κB signaling increases GRK5 expression. In primary peritoneal macrophages, however, Toll-like receptor ligands downregulate GRK5 expression, suggesting that both regulation of GRK5 expression and its role in NF-κB pathway are distinct in different cell types. Given that NF-κB signaling is a double-edged sword, the physiological relevance of GRK5 regulation of the NF-κB pathway can only be deduced from in vivo disease models (discussed later).

Table 1. Mouse phenotypes

| GRKs | Phenotype | Phenotype | References |
|------|-----------|-----------|------------|
| GRK2 | Whole-body knockout | Embryonic lethal because of its role in cardiac development | Jaber et al. |
| Heterozygote GRK2 | Increase in lymphocyte chemotaxis toward the CCR5 ligand CCL4 | Vroon et al. |
| Myeloid-specific knockout | Early-onset experimental autoimmune encephalomyelitis with increased infiltration of the CNS by lymphocytes and macrophages | Vroon et al. |
| Vascular smooth muscle-specific overexpression | Increased inflammation during endotoxemia and polymicrobial sepsis | Parvataneni et al. |
| Cardiac-specific knockout | Decreased atherosclerotic lesions in LDL-mimyeloid GRK2 dual knockout mice | Patial et al. |
| Adrenal-specific knockout | Decreased resting blood accompanied by vascular thickening and cardiac enlargement | Otten et al. |
| Cardiac-specific knockin | Increased inotrope responsiveness to isoproterenol, enhanced cardiac contractile performance and reduced ventricular remodeling postinfarction | Eckhart et al. |
| GRK3 | Whole-body knockout | Loss of olfactory receptor desensitization and neuropathic pain induced opioid tolerance | Terman et al. |
| GRK4 | Complete knockout | Normal fertility and sperm function. No obvious phenotype | Pfeffer et al. |
| GRK5 | Whole-body knockout | Enhanced hypothermia, hypoactivity, tremor and salivation by oxotremorine | Gainetdinov et al. |
| Myocardial overexpression | Decreased NF-κB activation in thioglycollate- induced peritoneal macrophages and cardiomyocytes | Patial et al. |
| Vascular smooth muscle-specific overexpression | Increased NF-κB activation in endothelial cells | Islam et al. |
| | Increased apoptotic response to genotoxic damage | Gainetdinov et al. |
| | Decreased thymocyte apoptosis during sepsis | Sorriento et al. |
| | Attenuation of contractility and heart rate in response to β-agonist | Chen et al. |
| | VSM-specific overexpression of GRK5 increases blood pressure by regulating β1AR and Ang II receptors | Rockman et al. |
| GRK6 | Whole-body knockout | Enhanced locomotor stimulating effects of cocaine and amphetamine | Keys et al. |
| | Impaired T lymphocyte chemotaxis | Gainetdinov et al. |
| | Enhanced neutrophil chemotaxis | Eijkelkamp et al. |
| | Increased in vasoconstriction and vasodilation, increased in inflammation during endotoxemia and polymicrobial sepsis | Vroon et al. |

Abbreviations: β1AR, β1-adrenergic receptor; CCR5, CC chemokine receptor-5; CNS, central nervous system; GRK, G-protein coupled receptor kinase; LDL, low-density lipoprotein; NFKB, nuclear factor-κB; VSM, vascular smooth muscle.
review was in preparation, Ohba et al. showed that GRKs is also able to phosphorylate IkBα at Ser32/36. Similar to the role of GRK5, tumor necrosis factor-α (TNFα)-induced inflammation in peritoneal macrophages was shown to be dependent on GRK6. Importantly, TNFα was shown to induce a conformational change in GRK6.57

Compared with GRK5, GRK2 has also been shown to interact with IkBα and p105. Unlike GRK5 (and GRK6), GRK2 phosphorylates IkBα at very low stoichiometry.52 Similar to GRK5, however, GRK2 negatively regulates p105 signaling in primary peritoneal macrophages, via interaction with p105.53 Interestingly, Toll-like receptor ligands enhance GRK2 expression in primary macrophages.54 In addition, immune cells from septic patients exhibit high GRK2 levels,54 suggesting that GRK2 levels and the associated signaling pathways have potential clinical relevance in inflammatory diseases.

GRKs in MAPK signaling
Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases that mediate fundamental biological processes and cellular responses to extracellular signals. Typically, activation of MAPKs lead to phosphorylation events that culminate in translocation of transcription factors from the cytoplasm to the nucleus, leading to altered gene expression. Three major groups of distinctly regulated MAPK cascades are known to lead to transcription of various genes: extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), Jun N-terminal kinase (JNK) and p38 MAPK. ERK is activated by MAPK kinase1 (MKK1) and MKK2, JNK by MKK4 and MKK7 and p38 MAPK by MKK3, MKK4 and MKK6. Upon activation of the MAPKs, transcription factors present in the cytoplasm or nucleus are phosphorylated and activated, leading to the expression of target genes resulting in biological responses.

A wide range of extracellular signals including LPS and TNFα can induce the ERK1/2 pathway. Activation of the ERK1/2 pathway leads to the induction of various inflammatory mediators (e.g., TNFα, interleukin-1 (IL-1), IL-8 and prostaglandin E2). Both GRK2 and GRK5 can negatively regulate LPS-induced ERK pathway in macrophages.42,55 Also, overexpression of GRK5 and/or GRK6 was found to enhance β-arrestin2-mediated ERK1/2 activation, whereas overexpression of GRK2 and/or GRK3 abolished β-arrestin2-mediated ERK1/2 activation.56 These effects were observed with the activation of β2-adrenergic receptor, cannabionoid receptor-2,57 angiotensin 1A receptor,43,58 and insulin-like growth factor-1 receptor.59

The p38 MAPK pathway shares many similarities with the other MAPK cascades, and is also associated with inflammation, cell growth, cell differentiation and cell death. A number of pathogenic stimuli, including LPS, staphylococcal peptidoglycan and enterotoxin B, echovirus 1 and herpes simplex virus 1 activate p38 through different toll-like receptors.60-62 The main biological response of p38 activation in the immune system involves the expression and production of inflammatory mediators to initiate leukocyte recruitment and activation. P38 MAPK mediates the expression of many genes involved in inflammation, such as TNFα, IL-1β, IL-6, IL-8, cyclooxygenase-2 and collagenase-1 and -3.63 Inhibition of p38 MAPK with SB203580 reduces proinflammatory cytokine production in monocyte/macrophages, neutrophils and T lymphocytes.64 Interestingly, GRK2 and p38 have bidirectional functional roles. Whereas GRK2 inhibits p38 function by directly phosphorylating it, p38 blocks GRK2-mediated GPCR desensitization.65,66 GRK2 phosphorylates p38 MAPK at Thr123 and interferes with the ability of p38 to bind to MKK6 and therefore prevents p38 activation.67 In addition, modulating GRK2 levels alters the activation of p38 and its dependent processes such as differentiation of adipocytes and cytokine production. Consistent with this role, GRK2/−/− macrophages55 and microglial cells57 have increased p38 activation and produce increased amounts of TNFα in response to LPS and this results in accelerated brain damage during hypoxic ischemic injury in neonatal mice.67 In contrast, GRK2 silencing decreases cytokine production (IL-6 and IL-13) in a p38-dependent manner during antigen-induced mast cell degranulation.68 Interestingly, GRK2 and GRK5 can phosphorylate a constitutively active, virally encoded GPCR (US28) and inhibit its activation, but simultaneously mediate p38 MAPK activation.69 Both GRK2 and GRK6 also regulate cytokine-induced pain in a p38 MAPK-dependent manner. These kinases reduce neuronal responsiveness to cytokines such as IL-1β and TNFα, by downregulating p38 activation, thereby reducing cytokine-induced hyperalgesia.70,71

Compared with GRK2 regulation of p38 activity, p38 directly phosphorylates GRK2 at Ser670 and inhibits GRK2 translocation to the membrane, thereby preventing GRK2-initiated internalization and desensitization of CC chemokine receptor-2 (CCR2) in response to MCP-1.72 In addition, p38 inhibits GRK2-mediated desensitization by acting as a noncanonical GRK for the FPR1 (formyl peptide receptor1) and facilitating neutrophil chemotaxis.66

JNKs are activated by mitogens, as well as by a variety of environmental stresses (heat shock, ionizing radiation, and oxidants), genotoxins (topoisomerase inhibitors and alkylating agents), ischemic–reperfusion injury, mechanical shear stress, vasoactive peptides, proinflammatory cytokines and pathogen-associated molecular pattern molecules/danger-associated molecular patterns.73-77 JNK induces transcription of AP-1, c-Jun, ATF-2 and ELK-1, all of which are important mediators of inflammatory gene transcription.75 JNK activation of AP-1 is important for the synthesis of TNFα as well as for the proliferation and differentiation of lymphocytes, and hence have a vital role in the immune system.77,78 Role of GRKs in JNK signaling, particularly related to the immune system, is not well characterized. However, studies with transgenic mice overexpressing cardiac-specific GRK5 and constitutively active mutant of α1B AR showed attenuation of JNK activation compared with controls. GRK5 also had variable effects on α1B AR signaling, and the complexity of GRK5 regulation in vivo α1B AR signaling remains to be fully elucidated.79

Taken together, these studies suggest that while some aspects of GRK regulation of the MAPK pathways have been explored in the immune system, most of these studies are focused more on GRK2 and some on GRK5. Role of GRK3 and GRK6 in these pathways remain less understood. Moreover, it remains to be seen if regulation of MAPK pathways by GRKs is therapeutically targetable in inflammatory diseases.

REGULATION OF IMMUNE PROCESSES BY GRKs
GRKs in immune cell migration
Chemotaxis is an important function, which enables immune cells to arrive at the site of inflammation. Cells producing chemokines act on chemokine receptors (mostly GPCRs), and initiate chemotactic response.80 The chemotactic response depends on the amount of chemokines produced and their gradient, and also on the expression levels of their receptors. Chemotaxis also depends on the integrated modulation of different steps of the chemotactic processes (receptor sensing, cell polarization, membrane protrusion, adhesion/de-adhesion cycles) in a given cell type and in response to a specific stimuli.81 Chemokine receptors being GPCRs also undergo desensitization upon continuous presence of the stimuli. Therefore, it is not surprising that GRKs are critical regulators of chemotaxis. Intriguingly, GRK2, GRK3, GRK5 and GRK6 are expressed at high levels in immune cells, suggesting that modulation of these GRKs during the disease might change the outcome or progression of the disease via modulation of immune cell chemotaxis.
Of the various GRKs, GRK2 is widely studied and better characterized in terms of chemotaxis. Studies have shown that GRK2 regulation of cell migration is complex—it is both cell type- and stimulus-dependent. In most cell types, GRK2 negatively regulates chemotactic responses consistent with its canonical negative regulatory role in GPCR signaling.

Cell lines transfected with GRK2 and chemokine receptors show increased agonist-induced phosphorylation and/or desensitization of chemokine receptors such as CCR2β, CCR5 and CXCR1. Consistent with its negative regulatory role, reduced GRK2 levels in T lymphocytes increase chemotactic response to CCL3, CCL4 and CCL5, which act through chemokine receptors CCR1 and CCR5. In addition, myeloid-specific GRK2 knockout and low-density lipoprotein receptor knockout chimeric mice exhibit increased mobilization of macrophages to inflammatory sites and have increased circulating neutrophils, conforming to the desensitizing effect of GRK2 on chemokine receptors. Moreover, neutrophils obtained from patients with different disease conditions such as malaria and sepsis revealed increased GRK2 levels associated with decreased CXCR2 expression and reduced response to IL-8. This increased GRK2 expression might be deleterious from the host perspective as this could reduce appropriate chemotactic response and eventually the ability of the host to contain the pathogen. On that note, IL-33 reverses sepsis-induced expression of GRK2, resulting in increased CXCR2 expression in neutrophils, leading to increased chemotaxis of neutrophils, increased bacterial clearance and enhanced survival in mice. In contrast to these studies, GRK2 positively mediates chemotactic responses in few other cell types. These roles of GRK2 might depend on the cell type and its polarization state. In polarized cells such as epithelial cells, GRK2 mediates chemotaxis, whereas in the less polarized cells such as immune cells, GRK2 does the opposite. Interestingly, positive regulation of chemotactic responses by GRK2 does not require its catalytic activity, thus suggesting protein–protein interaction being a potential molecular mechanism. In keeping with this notion, membrane-targeted kinase mutant strongly enhances cell motility, and GRK2 interacts with GIT1 (GRK2-interacting factor), and is present at the leading edge of polarized/migrating epithelial cells in wound-healing assays. Also, this transient association of GRK2 with GIT1 is critical for proper ERM/ERK1/2 activation and efficient cell migration. GRK2 has also been shown to directly phosphorylate histone deacetylase 6, a cytoplasmic histone deacetylase responsible for deacetylation of tubulin and other substrates involved in cell migration. Furthermore, GRK2 also phosphorylates ERM proteins ezrin and radixin, which contribute to the F-actin polymerization-dependent motility. These novel roles of GRK2 in cell migration might shed light in comprehending the noncanonical role of GRK2 in cell motility.

Similar to GRK2, GRK5 has also been shown to regulate GPCRs that are critical in chemotaxis in a canonical as well as a noncanonical manner. However, unlike GRK2, GRK5 has been shown to regulate cell migration in very few cell types. In monocytes, GRK5 regulates cell migration by modulating CCR2, a GPCR for monocyte chemoattractant protein-1. GRK5 also modulates monocyte chemotaxis in a non-GPCR-dependent manner by regulating the colony-stimulating factor-1 receptor, a receptor tyrosine kinase. Furthermore, GRK5 was reported to attenuate atherosclerosis by desensitizing CCR2 and inhibiting migration of monocytes. GRK5 was also shown to regulate CXCR4 (CXC chemokine receptor-4) desensitization via phosphorylation of HIP (HSP70-interacting protein) in an in vitro system. However, in vivo evidence of GRK5-mediated desensitization of CXCR4 is currently lacking. In our studies using a clinically relevant model of polymicrobial septic peritonitis, there was no evidence for a role for GRK5 in immune chemotaxis. Similar findings have been reported for arthritis. GRK5 has been shown to regulate prostate cancer cell migration and invasion by forming a complex with moesin (ERM (ezrin–radixin–moesin) proteins). In this study, the authors found that GRK5 phosphorylates moesin principally on Thr-66 residue, thereby regulating cellular distribution of moesin. Interestingly, a recent study demonstrated that GRK5 can function as an actin-bundling scaffold and promote neuronal filopodial formation and neurite outgrowth.

GRK6 has been shown to regulate chemokine receptors CXCR2, CXCR4 and LTβR. By desensitizing these receptors, GRK6 modulates neutrophil and lymphocyte recruitment in vivo in different disease models. In epithelial cells, a functional screening identified GRK6 as a critical mediator in integrin-mediated cell adhesion and migration of tumor cells, and GRK6 deficiency also appears to promote CXCR2-mediated tumor progression and metastasis in a lung carcinoma model. These roles of GRK6 are mostly related to its canonical role in GPCR desensitization. Evidence for GRK6 in the noncanonical role of immune cell chemotaxis is currently lacking.

Taken together, these studies reveal critical functions of GRKs in immune cell chemotaxis, and therefore suggest that if specific GRKs could be selectively modulated by small-molecule compounds, then it could have a potential therapeutic effect in disease processes.

GRKs in cell apoptosis

Aptosis or programmed cell death regulates development, selection and maturation of immune cells at various stages of their life cycle. Hence, normal rate of apoptosis is critical in immune system development. Inappropriate apoptosis (either too little or too much) is a factor in many human conditions including sepsis, neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. In addition to the role of apoptosis in the development of immune system, apoptotic bodies/cells can alter the course of the inflammation. Apoptotic cells are usually taken up by macrophages and dendritic cells. In response to the uptake, macrophages induce production and release of immunosuppressive cytokines such as IL-10, tumor growth factor-β, prostaglandin E2 and platelet-activating factor and suppress the production of proinflammatory cytokines IL-1β, IL-6, IL-12 and TNFα. Similarly, ingestion of apoptotic cells by dendritic cells also results in the suppression of IL-12 and IFN-γ expression, upregulation of cohibitory molecules and production of anti-inflammatory cytokines. Thus, apoptotic cells have significant impact on the function of these phagocytes and that in turn modulates inflammatory disease pathogenesis.

Consistent with their broad biological functions, GRKs have also been reported to have a critical role in apoptosis; however, there have been only a few studies looking at the role of GRKs in immune cell apoptosis, especially in vivo disease models. GRK2 overexpression was shown to increase caspase-3 levels and induce increased cardiomyocyte apoptosis following ischemia–reperfusion injury in the myocardium. Conversely, GRK2 inhibition reduced apoptosis via increased NO production and AKT levels in cardiomyocytes. These data suggest positive regulatory role of GRK2 in apoptosis. Interestingly, GRK2 levels were increased in apoptotic lymphocytes obtained from heart failure patients. Even though the implications of this is not yet clear, it is possible that increased GRK2 levels may drive more lymphocyte apoptosis.

Chen et al. demonstrated that GRK5 is able to phosphorylate p53 and regulate irradiation-induced apoptosis. P53 is crucial in determining the cellular response to stress not only by inducing apoptosis but also by having a role in growth arrest. Induction of p53-dependent apoptosis occurs via extrinsic or intrinsic pathways depending on apoptotic signals and converges on activation of caspases. GRK5 phosphorylates p53 in vivo in Thr-55, which results in reduced p53 levels. Treatment with the proteasome inhibitor, MG132, prevents the reduction in p53, thus
confirming that its degradation is via the proteasomes. GRK5 knockout mice display tissue-wide upregulation of p53, implying that GRK5 negatively regulates p53 in vivo. GRK5-mediated p53 degradation directly affects apoptosis, as knockdown of GRK5 in the osteosarcoma cell line, U2OS, increases apoptosis by 40% following cisplatin treatment. Apoptosis of Saos-2 cells, which are p53 null, were unaffected following GRK5 knockdown, suggesting that GRK5 induces apoptosis via p53.110 In contrast, our studies suggest that GRK5 positively regulates thymocyte apoptosis in vivo in polymicrobial sepsis.10 Speciﬁcally, lack of GRK5 causes decreased apoptosis of CD4+CD8+ cells in the thymus. Additionally, GRK5 has been shown to negatively regulate Bcl-2 transcription (antiapoptotic protein) in SHSY5Y cells and this is predicted to increase cell death.111 Taken together, these results suggest that the role of GRK5 in apoptosis may be related to multiple factors including the type of cell and context (in vivo versus in vitro).

Recently, Nakaya et al.112 reported that GRK6 is able to regulate clearance of apoptotic cells. GRK6 was shown to cooperate with GIT1 to activate Rac1, which promotes apoptotic engulfment. GRK6 was critical in removing apoptotic B cells by splenic white pulp macrophages and removing senescent red blood cells by splenic red pulp macrophages. GRK6-deﬁcient mice were also shown to have increased iron stores in splenic red pulp in which F4/80+ macrophages are responsible for senescent red blood cell clearance. As a consequence, GRK6-deﬁcient mice were shown to develop autoimmune disease.

In summary, it is clear that GRKs can regulate chemotaxis, inﬂammatory signaling and apoptotic pathways. Because dysregulation of any one of these functions have greater impact in altering the course of many diseases, a number of studies have examined the role of speciﬁc GRKs in various disease processes, especially inﬂammatory diseases using genetically modiﬁed mouse models.

GRKs in Inﬂammatory Diseases

Role of GRKs in neurodegenerative and autoimmune diseases

GRKs are implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (AD),113 multiple sclerosis (MS)114 and Parkinson’s disease.115 GRK2 was shown to serve as a marker for early hypoperfusion-induced brain damage, which is associated with mitochondrial damage found in AD patients.116 GRK2 levels in the brain are increased during early stages of damage in aged human and in AD patients (observed postmortem).116 GRK2 via p38-dependent TNFα production exacerbates brain damage during hypoxic ischemic injury.67 GRK2 was also shown to regulate metabotropic glutamate receptor function and expression, which has been implicated in AD and MS pathogenesis.117,118 Also, studies have shown GRK2 downregulation following prolonged inﬂammation sensitizes human and rodent neurons to excitotoxic neurodegeneration via overactivation of group I mGluRs.118

GRKs are also known to have a role in inﬂammatory hyperalgesia. GRK2-deﬁcient heterozygous mice suffer from chronic hyperalgesia owing to continued microglial activation via p38-dependent TNFα production,70,119 as well as via prolongation of prostaglandin E2-mediated pathways. The latter involves interaction with EPAC1 (exchange protein directly activated by cAMP), and activation of protein kinase Ca- and ERK-dependent signaling pathways.120–122 Interestingly, even a transient decrease in GRK2 levels (by intrathecal injection of GRK2 antisense oligodeoxynucleotides) is sufﬁcient to produce a long-lasting neuroplastic change in nociceptor function leading to chronic pain.123

GRK5 has been shown to regulate desensitization of muscarinic receptors selectively with a preference for M2 and M4 receptors.124 These receptors are present in the presynaptic cells and negatively regulate acetylcholine release in the hippocampal memory circuits. Increased presynaptic cholinergic activity decreases acetylcholine release and decreases postsynaptic muscarinic M1 activity. Interestingly, M1 signaling has been shown to inhibit β-amyloidogenic APP processing and decreased β-amyloid accumulation.125 This might explain the increased incidence of AD-like pathology observed in GRK5-deﬁcient mice.

Recent studies have demonstrated a physiological role for GRK6 in regulating apoptotic cell clearance in splenic red pulp and mice deﬁcient in GRK6 develop autoimmune disease.112 As discussed in the previous section, GRK6 mediates macrophage-dependent apoptotic cell clearance by phosphorylation of radixin and moesin, which are essential in membrane skeleton reorganization. Additionally, GRK6 has also been shown to modulate arthritis and colitis by regulating inﬁltration of immune cells.96,102 In DSS-induced colitis model, GRK6-deﬁcient mice produced more keratinocyte chemokine, causing increased inﬁltration of immune cells and enhanced severity. Similar to colitis model, GRK6-deﬁcient mice also suffer from increased weight loss and severity in the arthritis model.

Taken together, these studies demonstrate critical roles of GRKs in neurodegenerative as well as in autoimmune disease processes, and thus provide rationale for targeting these GRKs and their associated pathways for therapeutic development.

Role of GRKs in Cardiovascular Diseases

GRK2, GRK3 and GRK5 are well-known for their role in cardiovascular disease.126,127 Catecholamine signaling via β-adrenergic receptor signaling predominates during heart failure to improve myocardial contractility and cardiac output. However, increased expression/activity of GRK2 and GRK5 during heart failure induces β-adrenergic receptor desensitization. This reduces myocardial contractility and cardiac output and therefore worsens heart failure.128 This further triggers a surge of catecholamines leading to a vicious cycle of persistent β-adrenergic receptor desensitization. Overexpression of GRK2 and GRK5 in vivo has been shown to decrease myocardial contractility and cardiac output in response to adrenergic stimulation, suggesting an impaired adrenergic receptor signaling.128,129 Conversely, when GRK2, GRK3 and GRK5 were inhibited, an increase in cardiac contractility and enhanced survival were observed in heart failure models.126,130 In addition, competitive inhibition of GRK2 or GRK5 using βARKct (C-terminal peptide of GRK2) or adGRK5-NT (N-terminal peptide of GRK5), respectively, prevented cardiomyopathy and improved heart failure.131–134 Furthermore, overexpression of GRK2 and GRK5 in vascular smooth muscle cells led to the development of hypertension.135,136 In addition to these GPCR-dependent roles, GRK5 by virtue of its nuclear localization signal has also been shown to accumulate in the nucleus of cardiomyocytes and function as a histone deacetylase kinase and promote cardiac hypertrophy.29 GRK2 and GRK5 have also been implicated in the development of atherosclerosis. Low-density lipoprotein receptor knockout mice with partial GRK2 deﬁciency in hematopoietic cells develop fewer atherosclerotic plaques.89 Partial GRK2 deﬁciency leads to increased mobilization of macrophages to inﬂammatory sites leading to plaques with smaller necrotic core. In contrast to GRK2, GRK5 deﬁciency in apolipoprotein E knockout mice develop more aortic atherosclerosis.47

Overall, studies in the past decade clearly demonstrate that GRK inhibition has direct beneﬁcial effects in cardiovascular disease conditions by promoting survival signals. Thus, several research groups have embarked on identifying GRK2 and GRK5 inhibitors for therapeutics in heart failure.
GRKs in sepsis

Sepsis and systemic inflammatory response syndrome are leading causes of mortality in intensive care units. GRKs have a major role in the pathophysiological events in sepsis, by regulating cardiovascular, immune and coagulatory responses. GRK2 and GRK5 have a significant role in the pathogenesis of human sepsis by regulating neutrophil chemotaxis. In addition, mouse models of polymicrobial sepsis implicate roles for GRK2 as well as for GRK5 in regulating outcomes of septic shock. Studies from our lab demonstrated that myeloid-specific deficiency of GRK2 leads to enhanced cytokine production in endotoxemic mice. This was shown to be via negative regulation of the NF-κB1p105-TPL2-MEK-ERK pathway by GRK2. Consistent with these results, myeloid-specific GRK2 knockout mice also exhibit exaggerated cytokine response in polymicrobial sepsis model. However, GRK2 deficiency in myeloid cells did not affect immune cell infiltration or bacterial presence. Additionally, enhanced cytokine levels in GRK5 deficiency in GRK2 knockout mice did not significantly affect survival.

Compared with GRK2, our studies demonstrated that GRK5 deficiency led to diminished cytokine levels in both endotoxemia and polymicrobial sepsis models. As discussed previously, GRK5 deficiency led to decreased NF-κB activation in tissues as well as in macrophages. In addition to reduced cytokine responses, GRK5-deficient mice also showed decreased thymocyte apoptosis and immune suppression. Furthermore, decreased immune suppression was attributed to reduced plasma corticosterone levels in GRK5-deficient mice. Overall, these effects protected GRK5-deficient mice from sepsis-induced mortality, especially in the presence of antibiotics. Role of other GRKs in sepsis is not well known and is an area of investigation in our laboratory. Given these effects of GRKs in various cellular processes that impact sepsis pathogenesis, GRKs are also potential therapeutic targets in sepsis.

CONCLUSION

GRKs have numerous physiological roles to maintain homeostasis. Derangement in the processes involving GRKs often leads to pathology. Pathophysiological role of GRKs are attributed both to their canonical GPCR-dependent functions as well as to their noncanonical functions. As more and more novel interactions of GRKs with non-GPCR substrates are uncovered, GRKs are becoming diverse targets for many disease conditions. Thus, it is critical to take into consideration GRKs’ substrate/interaction diversity, as these kinases are targeted for therapeutic purposes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Takeda S, Kadowski S, Haga T, Takaesu H, Mitaka S. Identification of G protein-coupled receptor genes from the human genome sequence. FEBS Lett 2002; 520: 97–101.
2 Howard AD, McAllister G, Feighner SD, Liu Q, Nargund RP, Van der Ploeg LH et al. Orphan G-protein-coupled receptors and natural ligand discovery. Trends Pharmacol Sci 2001; 22: 132–140.
3 Cabrera-Vera TM, Vanhuwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR et al. Insights into G protein structure, function, and regulation. Endocr Rev 2003; 24: 765–781.
4 Downes GB, Gautam N. The G protein subunit gene families. Genomics 1999; 62: 544–552.
5 Lambert NA. Dissociation of heterotrimetric G proteins in cells. Sci Signal 2008; 1: re5.
6 Hewavitharana T, Wedegaertner PB. Non-canonical signaling and localizations of heterotrimeric G proteins. Cell Signal 2012; 24: 25–34.
7 Preininger AM, Hamme HE. G protein signaling: insights from new structures. Sci STKE 2004; 2004: re3.
8 Olah-Sam WM, Hamme HE. Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 2008; 9: 60–71.
9 Barker BL, Benovic JL. G protein-coupled receptor kinase 5 phosphorylation of hip regulates internalization of the chemokine receptor CXCR4. Biochemistry 2011; 50: 6933–6941.
10 Packiriswamy N, Lee T, Raghavendra PV, Durairaj H, Wang H, Parameswaran N. G-protein-coupled receptor kinase-5 mediates inflammation but does not regulate cellular infiltration or bacterial load in a polymicrobial sepsis model in mice. J Innate Immun 2013; 5: 401–413.
11 Parameswaran AC, Ott DA, Hadidi F, Cheong BY. Giant left atrial myxoma everything is bigger in Texas. Tex Heart Inst J 2012; 39: 286–287.
12 Benovic JL, Strasser RH, Caron MG, Lefkowitz R. Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. Proc Natl Acad Sci USA 1986; 83: 2797–2801.
13 Frielle T, Collins S, Daniel KW, Caron MG, Lefkowitz RJ, Kabakia BK. Cloning of the cDNA for the human beta 1-adrenergic receptor. Proc Natl Acad Sci USA 1987; 84: 7920–7924.
14 Wacker WB, Donoso LA, Kalsow CM, Yankeelov JA Jr, Organisciak DT. Experimental allergic uveitis. Isolation, characterization, and localization of a soluble uveotopathogenic antigen from bovine retina. J Immuno 1977; 119: 1949–1958.
15 Wilden U, Hall SW, Kuhn H. Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. Proc Natl Acad Sci USA 1986; 83: 1174–1178.
16 Zuckerman R, Chuey JT, E A 48 kDa protein arrests cGMP phosphodiesterase activation in retinal rod disk membranes. FEBS Lett 1986; 207: 35–41.
17 Parameswaran N, Spielman WS. RAMPs: the past, present and future. Trends Biochem Sci 2006; 31: 631–638.
18 Rittirsch D, Huber-Lang MS, Fielie MS, Ward PA. Innate characterization of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009; 4: 31–36.
19 Kunapuli P, Benovic JL. Cloning and expression of GRK5: a member of the G protein-coupled receptor kinase family. Proc Natl Acad Sci USA 1993; 90: 5588–5592.
20 Benovic JL, Gomez J. Molecular cloning and expression of GRK5. A new member of the G protein-coupled receptor kinase family. J Biol Chem 1993; 268: 19521–19527.
21 Hisatomi O, Matsuda S, Satoh T, Kotaka S, Imanishi Y, Tokunaga F. A novel subtype of G-protein-coupled receptor kinase, GRK7, in teleost cone photoreceptors. FEBS Lett 1999; 424: 159–164.
22 Weiss ER, Raman D, Shirakawa S, Ducceschi MH, Bertram PT, Wong F et al. The cloning of GRK7, a candidate cone opsin kinase, from cone- and rod-dominant mammalian retinas. Mol Vis 1998; 4: 27.
23 Premont RT, Macae A, Aparicio SA, Kendall HE, Welch JH, Lefkowitz R. The GRK4 subfamily of G protein-coupled receptor kinases. Alternative splicing, gene organization, and sequence conservation. J Biol Chem 1999; 274: 29381–29389.
24 Gurevich EV, Tesmer JI, Mushegian A, Gurevich WV. G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. Pharmacol Ther 2012; 133: 40–69.
25 Martinez IS, Baake P, Vinge LE, DeGeorge BR Jr, Chuprun JK, Harris DM et al. Uncovering G protein-coupled receptor kinase 5 as a histone deacetylase kinase in the nucleus of cardiomyocytes. Proc Natl Acad Sci USA 2008; 105: 12457–12462.
26 Jiang X, Benovic JL, Wedegaertner PB. Plasma membrane and nuclear localization of G protein coupled receptor kinase 6A. Mol Biol Cell 2007; 18: 2960–2969.
27 Li L, Homan KT, Vishnivetskiy SA, Manglik A, Tesmer JI, Gurevich WV et al. G Protein-coupled receptor kinases of the GRK4 protein subfamily phosphorylate inactive g protein-coupled receptors (GPCRs). J Biol Chem 2015; 290: 10775–10790.
28 DeBburman SK, Ptasienski J, Benovic JL, Hosey MM. G protein-coupled receptor kinase GRK2 is a phospholipid-dependent enzyme that can be conditionally activated by G protein beta-gamma subunits. J Biol Chem 1996; 271: 22554–22562.
29 Pitcher JA, Fredericks ZL, Stone WC, Premont RT, Stoffel RH, Koch WJ et al. Phosphatidylinositol 4,5-bisphosphate (PI(2P)-enhanced G protein-coupled receptor kinase (GRK) activity. Location, structure, and regulation of the PI(2P) binding site distinguishes the GRK subfamilies. J Biol Chem 1996; 271: 24907–24913.
30 Chuang TT, Paolucci L, De Blasi A. Inhibition of G protein-coupled receptor kinase substrates by Ca2+/-calmodulin. J Biol Chem 1996; 271: 28691–28696.

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50 Islam KN, Koch WJ. Involvement of nuclear factor kappaB (NF-kappaB) signaling by G protein-coupled receptor kinases by cAMP-dependent protein kinase attenuates their enzymatic activities. J Biol Chem 2005; 280: 28241–28250.

Proin AN, Benovic JL. Regulation of the G protein-coupled receptor kinase GRKs by protein kinase C. J Biol Chem 1997; 272: 3806–3812.

Chuang TT, LeVine H II, De Blasi A. Phosphorylation and activation of beta-adrenergic receptor kinase by protein kinase C. J Biol Chem 1995; 270: 18660–18665.

Whalen EJ, Foster MW, Matsumoto A, Ozawa K, Violin JD, Que LG et al. Regulation of beta-adrenergic receptor signaling by S-nitrosylation of G-protein-coupled receptor kinase 2. Cell 2007; 129: 511–522.

Boguth CA, Singh P, Huang CC, Tesmer JJ. Molecular basis for activation of G protein-coupled receptor kinases. EMBO J 2010; 29: 3249–3259.

Jaber M, Koch WJ, Rockman H, Smith B, Bond RA, Sulik KK et al. Essential role of beta-adrenergic receptor kinase 1 in cardiac development and function. Proc Natl Acad Sci USA 1996; 93: 12974–12979.

Peppel K, Boekhoff I, McDonald P, Breer H, Caron MG, Lefkowitz RJ. G protein-coupled receptor kinase 3 (GRK3) gene disruption leads to loss of odorant receptor desensitization. J Biol Chem 1997; 272: 25425–25428.

Gainetdinov RR, Bohn LM, Sotnikova TD, Cyr M, Laakso A, Macrae AD et al. Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. Neuron 2003; 38: 291–303.

Rockman HA, Choi DJ, Rahman NU, Akhter SA, Lefkowitz RJ, Koch WJ. Receptor-specific in vivo desensitization by the G protein-coupled receptor kinase-5 in transgenic mice. Proc Natl Acad Sci USA 1996; 93: 9954–9959.

Iaccarino G, Rockman HA, Shotwell KF, Tomhave ED, Koch WJ. Essential role of beta-adrenergic receptor kinase 1 in cardiac development and function. Proc Natl Acad Sci USA 1996; 93: 12974–12979.

Nick JA, Avdi NJ, Genewins P, Johnson GL, Worthen GS. Activation of a p38 mitogen-activated protein kinase in human neutrophils by lipopolysaccharide. J Immunol 1996; 156: 4867–4875.

Dziarski R, Jin YP, Gupta D. Differential activation of extracellular signal-regulated kinase (ERK) 1, ERK2, p38, and c-Jun NH2-terminal kinase mitogen-activated protein kinases by bacterial peptidoglycan. J Infect Dis 1996; 174: 777–785.

Zachos G, Clements B, Conner J. Herpes simplex virus type 1 infection stimulates p38/c-Jun N-terminal mitogen-activated protein kinase pathways and activates transcription factor AP-1. J Biol Chem 1997; 272: 5097–5103.

Lee JC, Laydon JT, McConnell PC, Gallagher TF, Kumar S, Green D et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 1994; 372: 739–746.

Marriott JB, Clarke IA, Dalgleish AG. Inhibition of p38 MAP kinase during cellular activation results in IFN-gamma-dependent augmentation of IL-12 production by human monocytes/macrophages. Clin Exp Immunol 2001; 125: 64–70.

Peregrín S, Jurado-Pueyo M, Campos PM, Sanz-Moreno V, Ruiz-Gomez A, Crespo P et al. Phosphorylation of p38 by GRK2 at the docking groove unveiled a novel mechanism for inactivating p38MAPK. Curr Biol 2006; 16: 2042–2047.

Li X, Ma B, Malik AB, Tang H, Yang Y, Sun B et al. Bidirectional regulation of neutrophil migration by mitogen-activated protein kinases. Nat Immunol 2012; 13: 457–464.

Nijboer CH, Heijnen CJ, Willemen HL, Groenendaal F, Dorn GW II, Van Bel F et al. Cell-specific roles of GRK2 in onset and severity of hyposmotic ischemic brain damage in neonatal mice. Brain Behav Immun 2010; 24: 420–426.

Subramanian H, Gupta K, Parameswaran N, Ali H. Regulation of FcRn signaling in mast cells by G protein-coupled receptor kinase 2 and its RH domain. J Biol Chem 2014; 289: 20917–20927.

Miller WE, Houtz DA, Nelson CD, Kolattukudy PE, Lefkowitz RJ. G protein-coupled receptor (GPCR) kinase-coupled receptor signaling and beta-arrestin recruitment regulate the constitutive signaling activity of the human cytomembralicytovascular GRK2 GPCRs. J Biol Chem 2003; 278: 21663–21671.

Willemen HL, Eijkelkamp N, Wang H, Dantzer R, Dorn GW II, Kelley KW et al. Microglial/macrophage GRK2 determines duration of peripheral IL-1beta-induced hyperalgesia: contribution of spinal cord CX3CR1, p38 and E1 signal. Pain 2010; 150: 550–560.

Eijkelkamp N, Heijnen CJ, Carabajal AJ, Willemen HL, Wang H, Minett MS et al. G protein-coupled receptor kinase 6 acts as a critical regulator of cytokine-induced hyperalgesia by promoting phosphatidylinositol 3-kinase and inhibiting p38 signaling. Mol Med 2012; 18: 556–564.

Liu Z, Jiang Y, Li Y, Wang J, Fan L, Scott MJ et al. TLRA signaling augments monocyte chemotaxis by regulating G protein-coupled receptor kinase 2 translocation. J Immunol 2013; 191: 857–864.

Knight RJ, Buxton DB. Stimulation of c-Jun kinase and mitogen-activated protein kinase kinase by ischemia and reperfusion in the perfused rat heart. Biochem Biophys Res Commun 1996; 231: 83–88.

Komuro I, Kudo S, Yamazaki T, Zou Y, Shiojima I, Yazaki Y. Mechanical stretch activates the stress-activated protein kinases in cardiac myocytes. FASEB J 1996; 10: 631–636.

Liu Y, Gorospe M, Yang C, Holbrook NJ. Role of mitogen-activated protein kinase phosphorylation during the cellular response to genotoxic stress. Inhibition of c-Jun N-terminal kinase activity and AP-1-dependent gene activity. J Biol Chem 1995; 270: 8377–8380.

Dabrowski A, Grady T, Logsdon CD, Williams JA. Jun kinases are rapidly activated by cholecystokinin in rat pancreas both in vitro and in vivo. J Biol Chem 1996; 271: 5686–5690.
77 Gomez del Arco P, Martinez-Martinez S, Calvo V, Armessila AL, Redondo JM. JNK (c-Jun NH2-terminal kinase) is a target for antioxidants in T lymphocytes. J Biol Chem 1996; 271: 26335–26340.

78 Jung S, Yaron A, Alkalay I, Hatzubai A, Avraham A, Ben-Neria Y. Costimulation requirement for AP-1 and NF-kappa B transcription factor activation in T cells. Ann NY Acad Sci 1995; 766: 245–252.

79 Eckhart AD, Demaurex N, Penen RB, Benovic JL, Lefkowitz RJ, Koch WJ. Hybrid transgenic mice reveal in vivo specificity of G protein–coupled receptor kinases in the heart. Circ Res 2000; 86: 43–50.

80 Cotton M, Clangi A. G protein–coupled receptors stimulation and the control of cell migration. Cell Signal 2009; 21: 1045–1053.

81 Insall R. The interaction between pseudopods and extracellular signalling during epithelial cell motility. Curr Adh Migr 2012; 6: 495–501.

82 Penela P, Nogues L, Mayor Jr F. Role of G protein-coupled receptor kinases in cell migration. Curr Opin Cell Biol 2014; 27: 10–17.

83 Lafarga V, Mayor Jr F., Penela P. The interplay between G protein-coupled receptor kinase 2 (GRK2) and histone deacetylase 6 (HDAC6) at the crossroads of epithelial cell motility. Cell Adh Migr 2012; 6: 163–171.

84 Arnon Ti, Xu Y, Lo C, Pham T, An J, Coughlin S et al. GRK2-dependent S1PR1 desensitization is required for lymphocytes to overcome their attraction to blood. Science 2011; 333: 1899–1903.

85 Aragay AM, Mellado M, Frade JM, Martin AM, Jimenez-Sainz MC, Martinez AC et al. Monocyte chemokine receptor-1–produced CCR2B receptor desensitization mediated by the G protein-coupled receptor kinase 2. Proc Natl Acad Sci USA 1998; 95: 2985–2990.

86 Olbrich H, Proudfoot AE, Oppermann M. Chemokine–induced phosphorylation of CCR2 chemokine receptor (CCR2). J Leukoc Biol 1999; 65: 281–285.

87 Raghunwanshi SK, Su Y, Singh V, Haynes K, Richmond A, Richardson RM. The chemokine receptors CXCR1 and CXCR2 couple to distinct G protein-coupled receptor kinases to mediate and regulate leukocyte functions. J Immunol 2012; 189: 2824–2832.

88 Vroon A, Heijnen CJ, Lombardi MS, Cobelens PM, Mayor F Jr, Caron MG et al. Reduced GRK2 level in T cells potentiates chemotaxis and signaling in response to CCL2. J Leukoc Biol 2004; 75: 901–909.

89 Otten JJ, de Jager SC, Kavelaars A, Seijens T, Bot I, Wijnands E et al. Hematopoietic G-protein-coupled receptor kinase 2 deficiency decreases atherosclerotic lesion formation in LDL receptor-knockout mice. FASEB J 2013; 27: 265–276.

90 Leorratti FM, Trevelin SC, Cunha FQ, Rocha BC, Costa PA, Gravina HD et al. Neutrophil paralysis in Fls2l–/– mice. PLoS Negl Trop Dis 2012; 6: e1710.

91 Alves-Filho JC, Sonego F, Souto FO, Freitas A, Verri Jr WA, Auxiliadora-Martins M et al. Interleukin-33 attenuates sepsis by enhancing neutrophil infiltration to the lungs. Ann NY Acad Sci 2012; 1265: 526–539.

92 Kang SH, Pitcher JA. G protein-coupled receptor kinase 2-mediated phosphorylation of moesin and regulates cell migration. J Biol Chem 2012; 287: 13489–13497.

93 Penela P, Ribas C, Aymerich I, Eijkelkamp N, Barreiro O, Heijnen CJ et al. Prostaglandin E2–dependent Met receptor desensitization by CRK5 in a retinal pigment epithelial cell line. Mol Vis 2008; 14: 1532–1541.

94 Ferrari LF, Bogen O, Alessandri-Haber N, Levine E, Gear RW, Levine JD. Transient overexpression of the G protein-coupled receptor kinase-2 determines myocardial ischemia/reperfusion injury via pro- and anti-apoptotic mechanisms. Circ Res 2010; 107: 1140–1149.

95 Kahsai AW, Zhu S, Fenteany G. G protein-coupled receptor kinase 2 activates (c-Jun NH2-terminal kinase) is a target for antioxidants in T lymphocytes. J Biol Chem 2008; 283: 26512–26520.

96 Liu J, Rasul I, Sun Y, Wu G, Li L, Premont RT et al. GRK2 deficiency leads to reduced hippocampal acetylcholine level via impaired presynaptic M2/M4 autoreceptor desensitization. J Biol Chem 2009; 284: 19564–19571.
