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Authors

Zhong, Jian
Gavrilescu, L Cristina
Molnár, Arpád
et al.

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Retraction

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Retraction for "GCK is essential to systemic inflammation and pattern recognition receptor signaling to JNK and p38," by Jian Zhong, L. Cristina Gavrilescu, Árpád Molnár, Lauren Murray, Stephen Garafalo, John H. Kehrl, Amy R. Simon, Richard A. Van Etten, and John M. Kyriakis, which appeared in issue 11, March 17, 2009, of *Proc Natl Acad Sci USA* (106:4372–4377; first published February 25, 2009; 10.1073/pnas.0812642106).

The undersigned authors wish to note the following: "Upon rederivation of the *gck*-/- mouse line, we have been unable to reproduce the data showing that disruption of *gck* impairs PAMP activation of JNK and p38. We also cannot reproduce the finding that administration of PAMPs leads to the signal-dependent stabilization of endognous GCK. Inasmuch as these signal transduction findings supported a central conclusion of the above article, we wish to retract this article in its entirety."

L. Cristina Gavrilescu
Árpád Molnár
Lauren Murray
Stephen Garafalo
John H. Kehrl
Amy R. Simon
Richard A. Van Etten
John M. Kyriakis

GCK is essential to systemic inflammation and pattern recognition receptor signaling to JNK and p38

Jian Zhong[a,b], L. Cristina Gavrilescu[c,d], Árpád Molnár[a,b], Lauren Murray[e,f], Stephen Garafalo[e,f], John H. Kehrl[g], Amy R. Simon[e,f], Richard A. Van Etten[c,d], and John M. Kyriakis[a,b,1]

[a]The Molecular Cardiology Research Institute, [c]The Molecular Oncology Research Institute, and [e]The Division of Pulmonology, Critical Care and Sleep Medicine, Tufts Medical Center; Departments of: [b]Medicine, [d]Hematology/Oncology, and [f]Cellular and Molecular Physiology, Tufts University School of Medicine, Boston, MA 02111; and [g]National Institute of Allergy and Infectious Disease, Bethesda, MD 20892

Systemic inflammation arising from the organismal distribution of pathogen-associated molecular patterns is a major cause of clinical morbidity and mortality. Herein we report a critical and previously unrecognized in vivo role for germinal center kinase (GCK, genome nomenclature: *map4k2*), a mammalian *Sterile 20* (*STE20*) orthologue, in PAMP signaling, and systemic inflammation. We find that disruption of *gck* in mice strongly impairs PAMP-stimulated macrophage cytokine and chemokine release and renders mice resistant to endotoxin-mediated lethality. Bone marrow transplantation studies show that hematopoietic cell GCK signaling is essential to systemic inflammation. Disruption of *gck* substantially reduces PAMP activation of macrophage Jun-N-terminal kinase (JNK) and p38 mitogen-activated protein kinases (MAPKs) via reduced activation of the MAPK-kinase-kinases (MAP3Ks) mixed lineage kinases (MLKs)-2 and -3. Extracellular signal-regulated kinase (ERK) and nuclear factor-κB (NF-κB) activation are largely unaffected. Thus, GCK is an essential PAMP effector coupling JNK and p38, but not ERK or NF-κB to systemic inflammation.

MAPK | sepsis | innate immunity | toll-like receptor | germinal center kinase

The systemic dissemination of pathogenic microorganisms triggers sepsis via the whole-body induction of excess proinflammatory cytokines. This cytokine excess is triggered by pathogen-associated molecular patterns (PAMPs)—conserved molecular moieties produced by invading microbial pathogens. PAMPs engage cells of the innate immune system, stimulating an inflammatory response. This systemic inflammatory response syndrome (SIRS) is marked by fever, shock, multiorgan failure, disseminated intravascular coagulation, and death. SIRS/sepsis is responsible for >200,000 deaths per year in the United States.

The innate immune system uses pattern recognition receptors (PRRs) to detect PAMPs and implement responses to invading microbial pathogens (1–7). The PRRs include the transmembrane toll-like receptors (TLRs) and the cytosolic PRRs: the nuclear binding/oligomerization domain-leucine-rich repeat proteins (NOD-LRRs) and the retinoic acid-inducible gene (RIG)-like-helicases (RLHs) (1–7). Upon engagement with a ligand, PRRs activate inflammatory and antimicrobial host defense responses via the recruitment of multiple signaling cascades, including the Jun-N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathways; the nuclear factor-κB (NF-κB) pathway; and the IFN response factor (IRF)-3 pathway (1–7).

PRRs recruit downstream targets through an array of intracellular adapter proteins that bind to the receptors in a stimulus-dependent manner. For example, TLR4 (the receptor for bacterial lipopolysaccharide [LPS]) recruits myeloid differentiation factor-88 (MyD88) and MyD88 adapter-like (Mal, also called TIR-associated protein [TIRAP]). These, in turn, bind additional adapter proteins including IL-1 receptor-associated kinases (IRAKs) and TNF-receptor-associated factor-6 (TRAF6). TRAF6 is required for activation of NF-κB and the MAPK pathways by all PAMPs tested thus far. The mechanisms coupling these adapters to MAPK pathways are not well understood (8–12).

MAPK pathways are pivotal to a competent innate immune response. These pathways always include 3-tiered diverse MAPK-kinase-kinase (MAP3K) → MAPK-kinase (MKK) → MAPK core pathways (13).

The biological roles of the various MAP3Ks in PRR and proinflammatory signaling are still quite nebulous, and are likely complex and cell-specific. Thus, tumor progression locus-2 (Tpl-2) is required for tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) activation of the ERKs (14). B lymphocyte-specific disruption of *TGF-β-activated kinase-1* (*tak1*) impairs JNK and NF-κB activation by TLR ligands, TNF-α, and interleukin 1 (IL-1) and impairs cell survival (15–17). By contrast, embryonic fibroblasts isolated from *MAPK/ERK kinase-kinase-3−/−* (*mekk3−/−*) mice manifest impaired LPS, IL-1-β, and TLR8 activation of JNK, p38, and NF-κB (18–21). Recent studies indicate that in B-lymphocytes, TRAF2 and MEKK1 are key to CD40 activation of MAPKs (22).

The activation mechanisms of MAP3Ks recruited by inflammatory stimuli also remain unclear, although some insight into PRR and proinflammatory receptor activation of MAP3Ks has emerged recently. Ubiquitination is key to the activation of many of these MAP3Ks. TRAF6 (as well as TRAF2) is an E3 ubiquitin ligase that autoubiquitinates via Lys-63-linked polyubiquitin chains. TRAF6 autoubiquitination is a precursor to TAK1 activation (23). In resting cells, the Tpl-2 is associated with NF-κB1/p105. Engagement of PRRs results in inhibitor of NF-κB-kinase (IKK)-catalyzed NF-κB1/p105 phosphorylation, ubiquitination, and degradation, freeing Tpl-2 and enabling its activation (24).

We have shown that germinal center kinase (GCK), a mammalian STE20p orthologue, is recruited by PAMPs and can activate MAP3Ks in vitro and in situ (25–27). Our biochemical and RNAi studies indicate that GCK itself is inherently unstable—subject to constitutive degradation by the ubiquitin proteasome. Activating stimuli do not increase GCK's kinase activity per se. Instead, GCK agonists trigger a transient association between GCK and TRAF6—an association that functions to
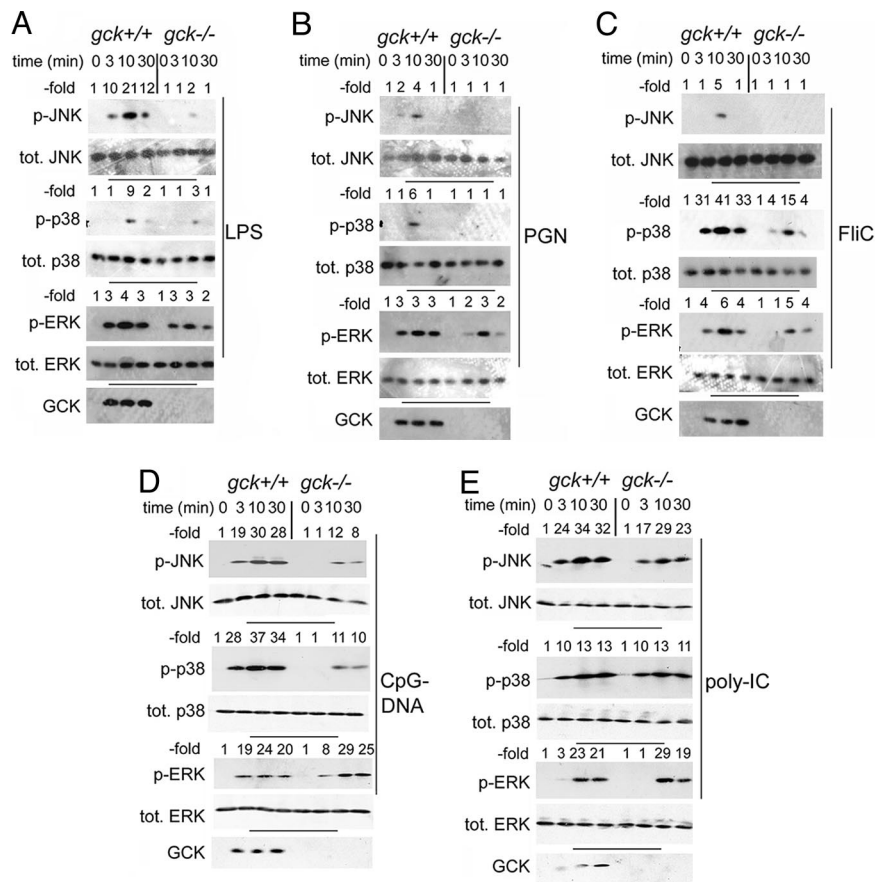
Fig. 1. Disruption of *gck* impairs LPS, PGN, FliC, CpG DNA, and OxLDL, but not polyIC activation of macrophage JNK and p38. ERK activation is largely unaffected. BMMs are prepared from *gck*+/+ and −/− mice and treated with LPS (*A*), PGN (*B*), FliC (*C*), CpG-DNA (*D*), or polyIC (*E*) for the indicated times. WCEs are subjected to SDS/PAGE and immunoblotting with the indicated antibodies. MAPK activation (*p*-JNK, *p*-p38, or *p*-ERK) is quantitated with ImageJ and is indicated. Results are from a representative experiment.

sequester GCK from the proteasome. Accordingly, "activation" of GCK involves its stimulus-dependent stabilization and accumulation in the cell (28). Once stabilized, GCK interacts with and activates MAP3Ks (29, 30).

Our findings suggested that GCK might be an important PAMP effector upstream of JNK and p38; and that GCK may have a critical role in systemic inflammation. Thus, GCK is recruited by PAMPs and our RNAi studies suggested that GCK was necessary for activation of JNK and p38 by PAMPs (28–30). However, given the complexity and cell specificity of the proximal mechanisms that regulate MAPKs in response to proinflammatory stimuli, we sought to determine the in vivo role of GCK and its effectors. To that end, we generated *gck*−/− mice. Our studies of these mice indicate that GCK is critical to JNK/p38 activation by PAMPs and to a competent innate immune response.

Results and Discussion

The *gck* gene disruption strategy used (supporting information (SI) Fig. S1) produced a null allele that expresses no fragments of the GCK polypeptide (Fig. S2 *A* and *B*). This was an important consideration inasmuch as ectopic expression of some GCK fragments can trigger JNK activation (25, 31). C57/BL6 *gck*−/− mice are viable and fertile, manifesting no obvious unevoked phenotype. Lymphocytes and in vitro-prepared bone marrow macrophages (BMMs) from *gck*−/− mice manifest, respectively, normal lymphoid and myeloid developmental markers (Fig. S2).

We wished to examine GCK-dependent signaling by using a cell type relevant to innate immune responses. We prepared BMMs from *gck*+/+ and −/− mice. Cells were treated for various times with either LPS, bacterial peptidoglycan (PGN), flagellin (FliC), unmethylated CpG DNA oligonucleotide (CpG-DNA), or polyinosine/cytosine (polyIC). Activation of the JNK, p38, and ERK pathways was assessed by immunoblotting by using phospho-specific antibodies that selectively recognize the activated forms of each MAPK. GCK activation was measured as accumulation of the endogenous polypeptide (28, 30). Under the conditions used, we detect no endogenous GCK in resting cells; however, all 5 PAMPs tested elicited the characteristic (28, 30) stabilization and accumulation of endogenous GCK, and stimulated a robust activation of each *gck*+/+ BMM MAPK group. Disruption of *gck* substantially impairs activation of BMM JNK and p38 by LPS, PGN, FliC, and CpG DNA, but not that by polyIC (Fig. 1). PRRs respond both to PAMPs and to danger-associated molecular patterns (DAMPs), molecular entities produced at sites of tissue injury or in situations of chronic stress (1–7, 32). Oxidized low-density lipoprotein (oxLDL) is a DAMP implicated in the pathogenesis of inflammatory atherosclerosis, and is a potential PRR agonist (33–35). oxLDL triggers robust MAPK activation, with JNK and p38 activation impaired in *gck*−/− BMMs (Fig. S3).

Of note, not only is polyIC activation of MAPKs unimpaired by disruption of *gck*, but, in contrast with the other PAMPs tested, polyIC-stimulated GCK stabilization is weak and occurs
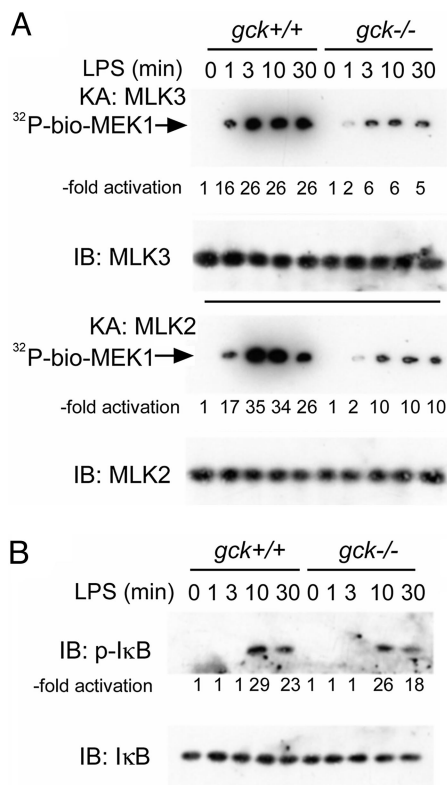
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Fig. 2. Disruption of *gck* impairs LPS activation of macrophage MLKs-2 and -3, but not NF-κB. BMMs are prepared from *gck+/+* and −/− mice and treated with LPS for the indicated times. (*A*) MLK2 or MLK3 is immunoprecipitated, as indicated, from crude cell extracts and assayed by using biotinylated-MEK1 and γ[32]ATP as substrates. The −fold activation is shown. (*B*) Extracts are prepared from BMMs stimulated as above and subjected to SDS/PAGE and immunoblotting with the indicated antibodies. Levels of phospho-IκB are quantitated by ImageJ. IB: immunoblot; KA: kinase assay.

with kinetics that lag well behind those of MAPK activation. ERK activation is largely unaffected by disruption of *gck*.

Consistent with the results in Fig. 1, disruption of *gck* also impairs LPS and PGN-stimulated phosphorylation of c-Jun (Fig. S4). Thus, BMM GCK appears to be activated/stabilized by several PAMPs, but is only rate-limiting to the activation—by a subset of PAMPs— of JNK and p38, but not ERK.

Ectopically expressed GCK can activate the MAP3Ks mixed lineage kinases (MLKs)-2 and -3 in situ and in vitro (29, 30). Disruption of *gck* blunts LPS activation of BMM MLKs-2 and -3 (Fig. 2*A*). The indolocarbazole analogue CEP-11004 is a potent and specific inhibitor of MLK family kinases—especially MLK3, in vitro and in vivo (36, 37). Treatment of *gck+/+* BMMs with CEP11004 substantially impairs LPS activation of JNK and p38 but is without effect on ERK activation (Fig. S5).

GCK appears to be selective for MAPK (JNK and p38) signaling. Thus, disruption of *gck* is without effect on the key initiating event in LPS activation of NF-κB: phosphorylation of inhibitor of κB (IκB), a reaction catalyzed by the IKKs (Fig. 2*B*) (38).

A functional inflammatory response involves the PAMP-stimulated production of cytokines and chemokines. An initial and critically important step in the macrophage inflammatory response to PRR engagement is the expression of the proinflammatory cytokine TNF-α. TNF-α, in turn, marshals the innate immune system and participates in mounting an effective inflammatory response. We measured the levels of LPS-stimulated *gck+/+* and *gck−/−* macrophage *tnf-α* mRNA by reverse transcriptase-PCR. As is shown in Fig. 3*A*, disruption of
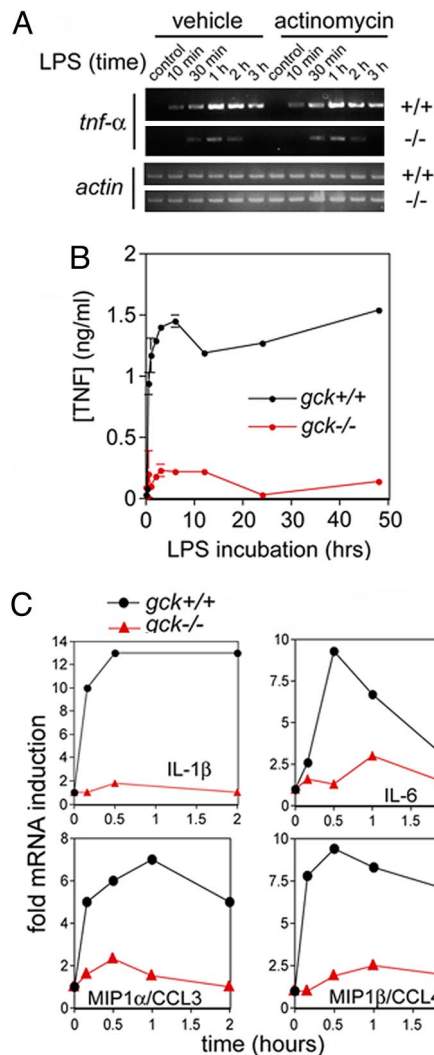

Fig. 3. Disruption of *gck* blunts in vitro LPS induction in BMMs of critical proinflammatory cytokines and chemokines. (*A*) LPS induction of BMM *tnf-α* occurs primarily through a posttranscriptional mechanism that is strongly dependent on GCK. Top, BMMs from *gck+/+* or −/− mice, as indicated, are prepared and pretreated with actinomycin. Cells are then treated with LPS for the indicated times. Either the *tnf-α* or the actin mRNA (loading control) is amplified by RT-PCR. (*B*) *gck−/−* BMMs show reduced LPS-stimulated TNF-α protein production. BMMs from *gck+/+* (black circles) or −/− (red circles) mice are treated with LPS for the indicated times. TNF-α secretion into the culture medium is detected by ELISA and quantitated versus a standard curve. Shown are mean ± SD for triplicate samples. (*C*) LPS induction of BMM cytokine mRNA induction is impaired in *gck−/−* cells. BMMs from *gck+/+* (black circles) or −/− (red triangles) mice are treated with LPS for the indicated times. RT-PCR to amplify the mRNAs of the indicated cytokines is performed on total cellular RNA; samples are separated on agarose gels and the amplified bands quantitated with ImageJ.

gck substantially reduces LPS-stimulated increases in macrophage TNF-α mRNA and protein. Of particular note, the robust LPS induction of *tnf-α* mRNA in *gck+/+* and the substantially reduced induction of *tnf-α* in *gck−/−* macrophages, are resistant to the transcriptional inhibitor actinomycin (Fig. 3*A*)(Fig. S6). Thus, the GCK-dependent induction by LPS of *tnf-α* is likely due to mRNA stabilization. The impairment of LPS-stimulated *tnf-α* mRNA stabilization in *gck−/−* BMMs results in a profound reduction in LPS-stimulated macrophage TNF-α release (Fig. 3*B*). The link between GCK and p38 signaling (Fig. 1) provides a potential explanation for these results. LPS treatment strongly

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inhibits the rapid degradation of mRNAs, such as that for *tnf-α*, which contain AU-rich elements (AREs) in their 3′ untranslated regions. This process requires p38α-catalyzed phosphorylation and activation of the kinase MAPK-activated protein kinase-2 (MK2), which, in turn, phosphorylates and modulates the functions of several ARE-binding proteins (39).

In addition to TNF-α, the induction of a suite of additional cytokines and chemokines is central to a coordinated inflammatory response. These cytokines act to recruit circulating leukocytes to sites of injury to enable leukocyte extravasation and to trigger organismal physiologic responses to infection, including fever, shock, and activation of the acquired immune system. The induction of these cytokines is, at least in part, MAPK-dependent (40); and we find that disruption of *gck* substantially reduces LPS induction, in BMMs, of the mRNAs for *interleukin (IL)-1β, IL-6, IL-10, IL-12, monocyte chemoattractant protein-1 (mcp1/C-C chemokine ligand-2 [ccl2]), macrophage inflammatory protein (mip)-1α/ccl3, mip1β/ccl4,* and *mip2/ C-X-C chemokine ligand-2 (cxcl2)* (Fig. 3C) (Fig. S7).

There is considerable variability in the degree to which the LPS induction of different cytokine mRNAs is susceptible to disruption of *gck* (Fig. 3B, S7). MAPKs can influence transcriptional, posttranscriptional, and translational events, and GCK may affect cytokine induction at multiple points. Both BMM and plasma (below) cytokine protein induction in response to LPS are reduced in *gck*−/− mice coincident with a considerably reduced systemic inflammatory response.

We next wished to determine if the effects on signaling and cytokine induction that we observed upon disruption of *gck* could be attributed specifically to GCK per se. To test this, we infected *gck*−/− BMMs with a recombinant lentiviral vector that expresses *gck* and looked for restoration of LPS-stimulated JNK and p38 signaling and cytokine production. From Fig. 4A, top, it is evident that the conditions of infection were sufficient to enable expression of ectopic *gck* at levels that produce the characteristic stimulus-induced stabilization of the GCK polypeptide. Under these circumstances, expression of *gck* restores LPS activation of *gck*−/− BMM JNK and p38 to levels seen in *gck*+/+ BMMs (Fig. 4A, middle and bottom).

We also examined the effect of *gck* disruption and "rescue" on the LPS-stimulated production by BMMs of key cytokine proteins. ELISA analysis indicated that disruption of *gck* substantially impairs LPS induction of the TNF-α, IL-1-β, MCP1, IL-6, and IL-10 proteins, and that this process is substantially reversed upon ectopic expression, in *gck*−/− BMMs, of recombinant GCK (Fig. 4B). Thus, the observed effects of *gck* disruption appear to be attributable specifically to GCK itself.

The results in Figs. 1–4 suggest a central role for the GCK → MLK2/3 → JNK/p38 pathway in the macrophage responses characteristic of local and systemic inflammation. Systemic inflammatory response syndrome (SIRS) and sepsis are cytokine excess conditions triggered by the organismal dissemination of microbial PAMPs and are highly significant clinically. LPS treatment of animals is a robust SIRS/sepsis model. Intraperitoneal administration of LPS (10 mg/kg) to *gck*+/+ mice results in 70−80% lethality within 72 h. By contrast, and consistent with the observation that JNK and p38 activation and cytokine release are reduced in *gck*−/− BMMs, *gck*−/− mice are strongly resistant to LPS administration, manifesting between 20 and 30% lethality (Fig. S8).

As noted above, SIRS and sepsis trigger striking elevations in plasma proinflammatory cytokines and chemokines. Administration of LPS (10 mg/kg, i.p.) to *gck*+/+ mice leads to a substantial induction of plasma cytokines, a process that is significantly impaired in *gck*−/− mice (Figs. 5A, S7). One consequence of SIRS/sepsis-mediated cytokine production is multiorgan failure. The lungs are especially susceptible to PAMP-stimulated injury. Thus, LPS-treated *gck*+/+ mice manifest dra-
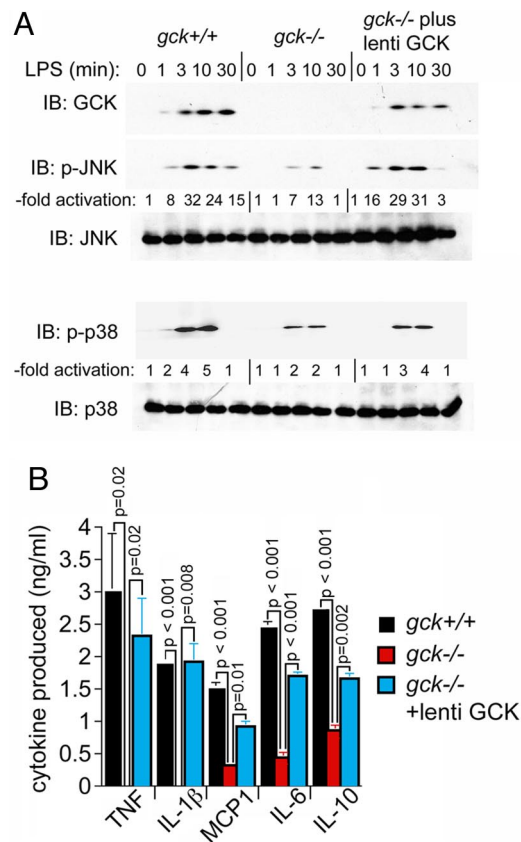

Fig. 4. Reintroduction of GCK, expressed from lentiviral vectors, which restores LPS activation of macrophage JNK and p38 and cytokine/chemokine secretion. (A) Reintroduction of ectopic GCK at levels that reproduce the characteristic agonist-induced stabilization of the GCK polypeptide, which restore JNK and p38 activation. BMMs from *gck*+/+ or −/− mice are prepared; and a portion of the *gck*−/− cells are infected with lentiviral GCK ("lenti GCK" in the figure). The remaining *gck*+/+ or −/− cells are infected with empty viral vector. Cells are treated with LPS for the indicated times and JNK or p38 activation is measured as in Fig. 2. (B) Expression of cytokine proteins. BMMs are prepared from *gck*+/+ (black bars) or −/− (red bars) mice. A portion of the −/− BMMs (blue bars) is infected with wt-GCK lentivirus as above. Cells are stimulated with LPS for 2 h. Secretion of the indicated cytokine into the culture medium is measured by ELISA and calibrated against the cognate cytokine's standard curve. In all cases, cytokine production by unstimulated cells is undetectable. Expression of TNF-α and IL-1-β is also undetectable in LPS-treated *gck*−/− cells that are not expressing lentiviral GCK. Shown is the mean ± SD (*n* = 3). Unpaired, 2-tailed Student's *t* test p values for the indicated sample groups are indicated above the brackets. *P* ≤ 0.05 indicates statistical significance.

matic lung injury marked by striking tissue edema, alveolar destruction, and leukocyte infiltration. By contrast, lung injury in LPS-treated *gck*−/− mice is markedly reduced (Fig. 5B).

To assess the relative contribution of hematopoietic cell GCK signaling in LPS-induced lethality, we performed bone marrow transplantation studies. Thus, *gck*+/+ mice were lethally irradiated and injected with bone marrow cells (BMCs) from *gck*−/− mice. This procedure resulted in chimeric mice bearing no detectable host hematopoietic cells (Fig. S9). Remarkably, these mice displayed a resistance to SIRS nearly commensurate to that of intact *gck*−/− mice. By contrast, intact *gck*+/+ mice were highly sensitive to LPS (70% lethality) (Fig. 5C). In a similar experiment, we lethally irradiated *gck*−/− mice and injected them with BMCs from *gck*+/+ mice. Complete reconstitution of the host animal hematopoietic system with the *gck*+/+ cells was achieved. As expected, and consistent with a
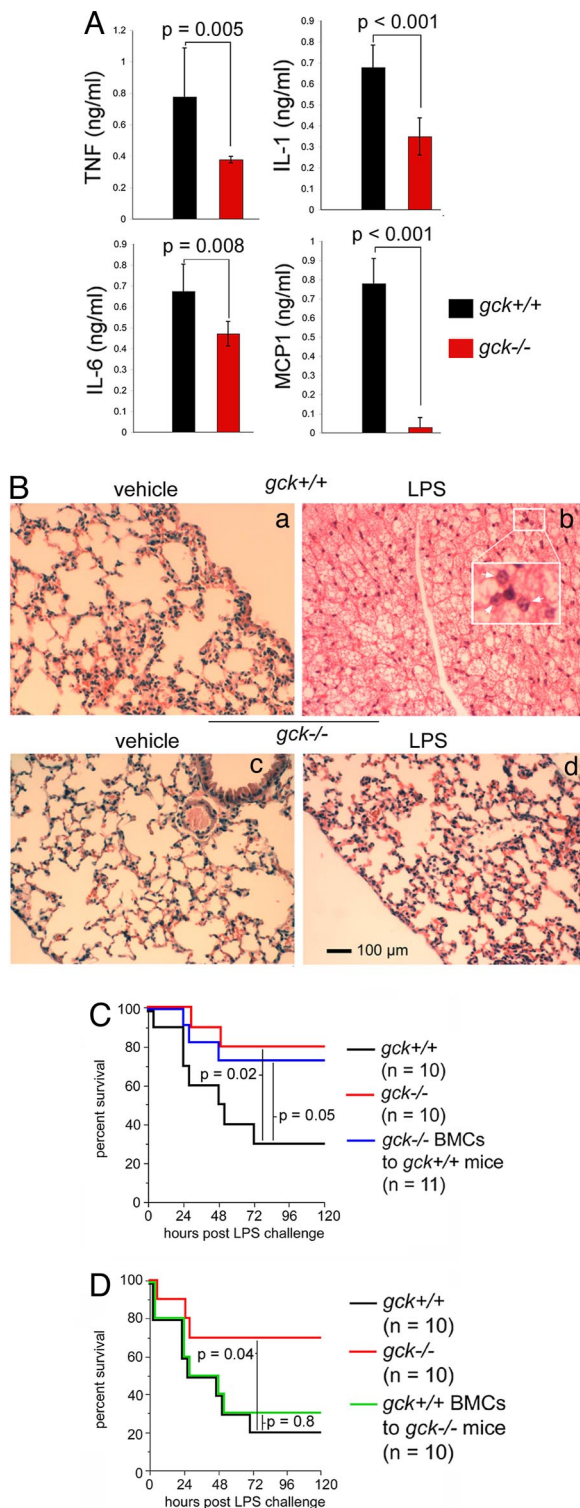
prominent role for hematopoietic cell GCK signaling in SIRS, the *gck*−/− mice bearing *gck*+/+ BMCs showed a greatly enhanced sensitivity to LPS toxicity (70% lethality) (Fig. 5*D*), approaching that of intact *gck*+/+ mice (80% lethality) and in striking contrast to that of intact *gck*−/− mice (30% lethality).

Our results identify GCK, likely acting through activation of MLKs-2 and -3, as an important and selective regulator of PRR activation of JNK and p38, and an essential component in innate immunity. Most notably, signaling through GCK appears to be indispensable for a robust systemic inflammatory response. We have identified a signaling pathway limited to a subset of MAPKs and not NF-κB that is critical to systemic inflammation.

Our findings indicate that the essential role of GCK in SIRS/sepsis is mediated almost entirely by cells from the hematopoietic compartment inasmuch as bone marrow transplantation of *gck*−/− BMCs to *gck*+/+ mice confers a substantial resistance to LPS challenge. Our observation that pharmacological inhibition of BMM MLK signaling also blocks PAMP activation of MAPKs indicates that targeting the BMM GCK → MLK2/3 pathway could prove useful in the treatment of early systemic inflammation.

The observation that oxLDL activation of JNK and p38 is also impaired in *gck*−/− BMMs suggests a wider role for GCK signaling, which may include inflammatory atherosclerosis. PRR signaling is emerging as a key component of atherogenesis; and atherogenic lipids may signal in part through PRRs, thereby triggering the inflammatory aspects of atherosclerosis (32–35). It will be important to determine if GCK is relevant to atherogenesis.

Cytokine induction by PAMPs is a pivotal trigger for innate immunity. The cytokines induced by LPS in a GCK-dependent manner (Figs. 3, 4) are well established as important regulators of innate immunity (41–44). The degree to which disruption of *gck* impairs PAMP (LPS) induction of different cytokines is quite variable. This, in turn, may affect the overall physiologic impact of *gck* disruption. Nevertheless, it is clear that disruption of *gck* has a profoundly suppressive effect on LPS-induced systemic inflammation.

We have shown that GCK can form a PAMP-stimulated complex with TRAF6 and with MLK3. The GCK-TRAF6 interaction does not require TRAF6's E3 ubiquitin ligase activity. Instead, stimulus-dependent TRAF6 binding to GCK sequesters GCK from the proteasome leading to the accumulation of GCK in the cell (28, 30). Our results from experiments using primary *gck*+/+ and −/− macrophages or intact animals point to a previously unknown central role for the GCK pathway in PRR signaling. Our findings underscore the complex cell-stimulus and tissue-specificity of MAPK regulation by proinflammatory stimuli (15, 16, 18–22, 24).

Although TRAF6 appears to be essential for stimulus-induced GCK stabilization/activation (28), the mechanisms by which PRRs recruit this pathway remain to be determined. Our studies indicate that GCK is necessary for optimal JNK and p38 activation by PGN, LPS, FliC, CpG-DNA, and oxLDL, but not

Fig. 5. *gck*−/− mice are resistant to LPS-stimulated systemic inflammation. (*A*) Disruption of *gck* reduces LPS-induced plasma cytokines. *gck*+/+ (black bars) or −/− (red bars) mice are administered vehicle (pyrogen-free water) or LPS (10 mg/kg, i.p.). After 24 h, mice are killed and plasma samples obtained and analyzed by ELISA for the indicated cytokines/chemokines. Shown are the mean ± SD (*n* = 9 per study arm); *P* ≤ 0.05 indicates statistical significance. Data are for LPS-treated animals only; samples from vehicle-treated animals contained no detectable cytokine. (*B*) Disruption of *gck* reduces LPS-induced lung injury. Mice (*gck*+/+, a and b; *gck*−/−, c and d) are treated with vehicle (pyrogen-free water, a and c) or LPS (b and d, 10 mg/kg, i.p.). Animals are killed after 24 h and lungs are removed, fixed, and stained with hematoxylin-eosin. Staining conditions are the same for all samples. The scale bar in the lower left corner of d indicates 100 μm—for all panels. The insert in b is a magnification of the indicated area and the arrows indicate infiltrating leukocytes. (*C* and *D*) Hematopoietic cell GCK signaling is essential to LPS-induced systemic inflammation. *gck*+/+ (black) or −/− (red) mice are left unirradiated. Alternatively, *gck*−/− (blue in *C*) or +/+ (green in *D*) mice are lethally irradiated and transplanted with, respectively, *gck*+/+ or −/− bone marrow cells. After recovery from the transplantation, mice are treated with LPS (10 mg/kg, i.p.) and monitored for the times indicated. The percentage surviving mice, as a function of time post LPS injection, is measured and is shown on the indicated Kaplan–Meier curves. Survivability data are subjected to nonparametric analysis (Mantel–Cox), with *P* ≤ 0.05 indicating significance; p values are shown for the indicated datasets.

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by polyIC. These GCK-dependent stimuli all use the common adapter protein MyD88 for recruitment of downstream targets (8–10, 34, 35, 45). However, LPS can also signal through MyD88-independent mechanisms (46, 47). By contrast, polyIC, which does not signal prominently through GCK, solely utilizes the MyD88-independent TRIF pathway to couple to downstream effectors (11). Future studies will dissect which of these upstream mediators, in response to specific PAMPs or microbial pathogens, couples to GCK.

To conclude, our results implicate GCK as a PAMP/DAMP effector required for efficient JNK and p38 activation. GCK is not centrally involved in the more familiar NF-κB proinflammatory pathway and at most contributes trivially to ERK activation. Although several ERK and NF-κB signaling networks are clearly required for efficient proinflammatory responses in vivo (13–23), our results highlight the importance—in a relevant cellular and physiological setting—of the GCK → MLK2/3 → JNK/p38 pathway to proinflammatory signaling. Thus, each of these pathways contributes pivotal elements to the inflammatory response. Our findings provide another potential target for antiinflammatory therapeutic intervention.

Methods

All methods are described in detail in *SI*.

Mice. Disruption of *gck* was performed by using standard methods (see SI for details) and produced animals that expressed no detectable GCK fragments.

BMM Preparation and Treatment, Kinase Assays, ELISA. BMMs were prepared from bone marrow cells by incubation with L929 cell medium. PAMP and CEP11004 treatments were as in SI. Kinase assays and cytokine reverse transcriptase PCR were performed as in references 28 and 29. Reverse transcriptase PCR primers are indicated in Table S1. ELISA was performed with the eBioscience kit according to the manufacturer's instructions (*SI*).

In Vivo Studies. All studies with animals conformed to IACUC regulations and are described in *SI*.

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