Protein Kinase C δ (PKCδ)-Extracellular Signal-regulated Kinase 1/2 (ERK1/2) Signaling Cascade Regulates Glycogen Synthase Kinase-3 (GSK-3) Inhibition-mediated Interleukin-10 (IL-10) Expression in Lipopolysaccharide (LPS)-induced Endotoxemia*§

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Background: GSK-3 inhibitor attenuates lipopolysaccharide (LPS)-induced endotoxemia.

Results: PKCδ-induced ERK1/2 activation by the inhibition of GSK-3 under LPS-stressed conditions provokes interleukin (IL)-10 production and attenuates endotoxemia.

Conclusion: IL-10 expression produced by GSK-3 inhibition-induced ERK1/2 activation via PKCδ relieves LPS-mediated endotoxemia.

Significance: IL-10 hyper-expression resulted from GSK-3 inhibition-induced ERK activation could be a new therapeutic pathway for endotoxemia.

Glycogen synthase kinase-3 (GSK-3) modulates a wide array of cellular processes, including embryonic development, cell differentiation, survival, and apoptosis. Recently, it was reported that a GSK-3 inhibitor attenuates lipopolysaccharide (LPS)-induced septic shock and regulates the mortality of endotoxemic mice. However, the detailed mechanism of reduced mortality via GSK-3 inhibition is not well defined. Herein, we showed that GSK-3 inhibition induces extracellular signal-regulated kinase 1/2 (ERK1/2) activation under LPS-stressed conditions via protein kinase C δ (PKCδ) activation. Furthermore, PKCδ-induced ERK1/2 activation by the inhibition of GSK-3 provoked the production of interleukin (IL)-10, playing a crucial role in regulating endotoxemia. Using a mitogen-activated protein kinase kinase-1 (MEK-1) and PKCδ inhibitor, we confirmed that GSK-3 inhibition induces PKCδ and subsequent ERK1/2 activation, resulting in increased IL-10 expression under LPS-treated conditions. We verified that septic shock caused by LPS is attenuated by GSK-3 inhibition using a GSK-3 inhibitor. This relieved endotoxemia induced by GSK-3 inhibition was restored in an ERK1/2-dependent manner. Taken together, IL-10 expression produced by GSK-3 inhibition-induced ERK1/2 activation via PKCδ relieved LPS-mediated endotoxemia. This finding suggests that IL-10 hyperexpression resulting from GSK-3 inhibition-induced ERK activation could be a new therapeutic pathway for endotoxemia.

Sepsis caused by Gram-negative and -positive bacteria, viruses, and fungi is defined as a complex clinical syndrome that is induced by a damaging host response to microbial infection (1). High mortality rates for adult hospital patients are mainly due to sepsis (2–4). This dysregulated host innate immune reaction is accompanied by a storm of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-12, IL-6, and IL-1β (5). This cytokine storm can induce secondary inflammatory cascades, including lipid mediators, reactive oxygen species, and the hyperregulation of cell adhesion molecules, such as vascular cell adhesion molecule (VCAM), intercellular cell adhesion molecule (ICAM), and monocyte chemotactic protein-1 (MCP-1) (6, 7). Proinflammatory cytokines and nitric oxide (NO) production by inducible NO synthase (iNOS) can lower systemic vascular resistance, leading to severe hypotension (8, 9). Furthermore, these cytokines activate the procoagulation pathway, resulting in microvascular thrombosis and damaged tissue perfusion. The combination of hypotension and microvascular closure leads to tissue ischemia and multiple organ dysfunction (10, 11). In contrast, an anti-inflammatory cytokine IL-10 plays a protective role against proinflammatory cytokine production, organ dysfunction, and mortality in the endotoxemic state (12–16). Toll-like receptors (TLRs) recognize microbial infection and initiate innate immune responses, and their response to lipopolysaccharide (LPS) is mainly orchestrated by TLR4. Docking of
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LPS to TLR4 recruits the adaptor protein MyD88, which initiates downstream signaling pathways, such as the nuclear factor κB (NF-κB) signaling pathway and mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 (17–19). MAPKs, a group of serine/threonine kinases, play a pivotal role in diverse cellular processes, including cell growth, differentiation, and apoptosis, in response to various extracellular stimuli (20, 21). The ERKs (ERK1 and ERK2) that are activated by MAPK kinase 1/2 (MEK-1/2) or protein kinase C (PKC) mainly regulate cell growth and differentiation (22), whereas JNK and p38 are generally involved in the regulation of stress-induced cellular apoptosis (23). Recently, PKC, an upstream kinase of ERKs, has been found to be deeply involved in the IL-1β signaling cascade (24, 25).

Glycogen synthase kinase-3 (GSK-3), a basally active kinase, was first identified as a key regulator of glycogen metabolism; GSK-3 regulates various cellular processes, including apoptosis, differentiation, growth, cell motility, and embryonic development by modulating a broad range of substrates (26–28). According to recent studies, GSK-3 inhibition protected mice against endotoxin shock through modulating the inflammatory response and provoked interferon-β-mediated IL-10 production (29–31). GSK-3 was recently shown to negatively regulate AP-1, which inhibits the MEK kinase 1 (MEKK-1), an activator of JNK, thereby provoking apoptosis (25, 32). However, the function of GSK-3 in regulating MAPK/ERK in the endotoxemia has not yet been elucidated.

In this study, we investigated the regulation of IL-10 by GSK-3 inhibition via the PKC-ERK1/2 signaling pathway and showed that the inhibition of GSK-3 induced ERK1/2 activation via PKC-ERK activation under LPS-stressed conditions. Finally, PKC-ERK1/2 activation that resulted from GSK-3 inhibition induced IL-10 expression, which plays a crucial role in negatively regulating endotoxemia.

**EXPERIMENTAL PROCEDURES**

**Animals**—Eight- to ten-week-old male C57BL/6 (H-2Kb and I-Ab) mice were purchased from the Korean Institute of Chemistry Technology (Daejeon, Korea). The animals were housed in a specific pathogen-free environment within our animal facility and used in accordance with the institutional guidelines for animal care. All experiments were done in accordance with the guidelines of the committees of Ethics of Animal Experiments, Pusan National University.

**Reagents and Antibodies**—Recombinant mouse GM-CSF, IL-4, and cytokine ELISA kits for murine IL-12 p70, TNF-α, nitrite, and IL-10 were purchased from R&D Systems (Minneapolis, MN). LPS (from *Escherichia coli* O127:B8) was obtained from Sigma-Aldrich. To detect protein levels, anti-phospho-ERK, anti-ERK, anti-phospho-PKCδ, anti-PKCθ, and anti-tubulin antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and GSK-3 (SB415286) and MEK inhibitors (PD98059) were purchased from Tocris Bioscience (Ellisville, MO). GSK-3β and PKCθ siRNAs were purchased from Santa Cruz Biotechnology, Inc. DOTAP liposomal transfection reagent was purchased from Roche Applied Science.

**LPS-induced Endotoxemia Model**—For LPS-mediated endotoxemia models, mice were injected intraperitoneally with the designated dose of LPS dissolved in PBS containing 0.25% bovine serum albumin (BSA). The general conditions and mortality were recorded for up to 6 days after injection to ensure that no additional late deaths occurred. MEK inhibitor PD98059 (10 mg/kg) was injected intraperitoneally 24 h before LPS injection. A GSK-3 inhibitor (25 mg/kg) was injected intraperitoneally 2 h before LPS insult. The solution containing the same concentration of DMSO was used as a control vehicle.

**Generation and Culture of Bone Marrow-derived Dendritic Cells (BMDCs)**—Briefly, bone marrow was flushed from the tibiae and femurs of 6–8-week-old male C57BL/6 mice and was depleted of red blood cells using Red Blood Cell Lysing Buffer (Sigma-Aldrich). The cells were plated in 6-well culture plates (1 × 10^6 cells/ml; 2 ml/well) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 mg/ml streptomycin, 20 ng/ml recombinant murine GM-CSF, and 10 ng/ml recombinant murine IL-4 at 37 °C at 5% CO_2_. On days 3 and 5, the floating cells were gently removed from the cultures, and fresh medium was added. On day 6 of culture, non-adherent cells and loosely adherent proliferating dendritic cell aggregates were harvested and re-plated in 60-mm dishes (1 × 10^6 cells/ml; 5 ml/dish) for stimulation and analysis. On day 7, 80% or more of the non-adherent cells expressed CD11c. In certain experiments, BMDCs were labeled with bead-conjugated anti-CD11c mAb (Miltenyi Biotech, Gladbach, Germany) and subjected to positive selection through paramagnetic columns (LS columns; Miltenyi Biotech, Auburn, CA) to obtain highly purified populations for subsequent analysis according to the manufacturer’s instructions. The purity of the selected cell fraction was >90%.

**Western Blot Analysis**—In brief, cell lysates were subjected to SDS-PAGE and transferred to PVDF membranes. The PVDF membranes were then blocked with 5% nonfat milk in a washing buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween 20) and incubated with the indicated antibodies for 1 h at room temperature. The membranes were washed and incubated for 1 h at room temperature with the appropriate secondary antibodies conjugated with horseradish peroxidase (Amersham Biosciences). Protein bands were visualized using an enhanced chemiluminescence system (Amersham Biosciences).

**Measurement of Cytokines**—Serum and culture supernatants of BMDCs were collected and assayed for various cytokine levels using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions. ELISA kits were purchased from R&D Systems (Minneapolis, MN).

**Measurement of Polymorphonuclear Leukocyte Infiltration**—Lungs were first perfused with 10% formalin at a constant distending pressure of 25 cm H_2O for 10 min. Lungs were excised from the animals and placed in 10% formalin overnight at 4 °C. The tissue was embedded in paraffin, and 4-μm sections were prepared and stained with hematoxylin and eosin.
Myeloperoxidase (MPO) Estimation—Neutrophil sequestration in lung, liver, and kidney was quantified by measuring tissue MPO activity. Tissue samples for MPO analysis were frozen in liquid nitrogen immediately after removal from the animal and were thawed and homogenized in the following lysis buffer: 200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml leupeptin, and 28 mg/ml aprotinin (pH 7.4). Samples were centrifuged twice (6,000 × g at 4 °C for 15 min) to avoid contamination of cell debris, and the cleared supernatant was used for the MPO assay. MPO activities were measured using a mouse MPO ELISA kit (Hycult Biotechnology b.v., Uden, The Netherlands) according to the manufacturer’s instructions. Protein concentration in the supernatants was measured by a protein dye binding assay (Protein Assay, Bio-Rad). The levels of MPO in organ extracts were expressed as ng/mg of protein.

Measurement of Mean Arterial Blood Pressure (MABP)—We performed cannulation of the carotid artery under urethane anesthesia, and MABP of mice was monitored by laser-doppler flowmetry (FLO-N1, Omegawave, Tokyo, Japan).

Statistics—Experiments were repeated at least three times with consistent results. Unless otherwise stated, data are expressed as the mean ± S.E. Analysis of variance was used to compare experimental groups to control values. Comparisons between multiple groups were done using Tukey’s multiple comparison test. Statistical significance was determined as p < 0.05.

RESULTS

ERK1/2 Activation Resulting from GSK-3 Inhibition Induces IL-10 Expression under LPS-stressed Conditions—Recently, MEK/ERK activation via GSK-3 inhibition in human colon cancer cell lines was reported (25). To assess GSK-3 inhibition-induced MEK/ERK regulation under LPS-stressed conditions, we examined the influence of GSK-3 inhibition on LPS-induced ERK1/2 activation using a GSK-3-specific inhibitor, SB415286, in Bone marrow-derived dendritic cells (BMDCs). In this experiment, we observed the potentiated ERK1/2 activity by LPS in GSK-3 inhibitor-pretreated cells, compared with non-treated cells (Fig. 1A). In addition, GSK-3 inhibition-induced ERK1/2 activation under LPS-stressed conditions was decreased by a MEK-1 (upstream kinase of ERK1/2) inhibitor (PD98059), indicating that enhanced LPS-induced ERK activation via GSK-3 inhibition is MEK-1-dependent (Fig. 1A).

IL-10, a representative anti-inflammatory cytokine, is a protective regulator against proinflammatory cytokine production and mortality in the LPS-induced endotoxin shock model. A recent study showed that the IL-10 storm induced by GSK-3 inhibition plays an attenuator role in modulating the inflammatory response (29). Thus, we investigated whether ERK1/2 activation by GSK-3 inhibition regulates the production of the anti-inflammatory cytokine IL-10. As shown in Fig. 1B, the increase of LPS-mediated IL-10 production by GSK-3 inhibition was significantly diminished by a MEK-1 (upstream kinase of ERK1/2) inhibitor (PD98059), indicating that enhanced LPS-induced ERK activation via GSK-3 inhibition is MEK-1-dependent (Fig. 1A).

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FIGURE 1. ERK1/2 activation by GSK-3 inhibition induces IL-10 expression under LPS-stressed conditions. A, Bone marrow-derived dendritic cells (BMDCs) were pretreated with or without the indicated MEK inhibitor (PD98059) concentration (10 μM) for 30 min, followed by treatment with a GSK-3 inhibitor (SB415286) (25 μM) alone or in combination for 30 min and harvested after incubating with LPS (200 ng/ml) for 30 min. Cell lysates were directly subjected to immunoblot analysis (IB) with the indicated antibodies. B and C, BMDCs were pretreated with or without the indicated MEK inhibitor concentration for 30 min followed by treatment with a GSK-3 inhibitor (SB415286) alone or in combination for 30 min and harvested after incubating with LPS (200 ng/ml) for 24 h. Cytokine concentrations in culture supernatants were measured in triplicate by ELISA. The mean ± S.E. values (error bars) shown represent three independent experiments. *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared with LPS challenge.

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kine IL-12 was unaffected by LPS-induced ERK1/2 activity modulation via the inhibition of GSK-3 (Fig. 1C). This result indicates that the activation of the MEK/ERK1/2 signaling pathway by the inhibition of GSK-3 is crucial for the production of IL-10 rather than IL-12.

LPS-induced ERK1/2 Activation Is Potentiated by GSK-3 Inhibition via PKCδ—Because previous reports document that PKCδ is an upstream regulator of the MEK/ERK signaling pathway, we examined the kinase activity and the influence of PKCδ on the LPS-stimulated ERK1/2 hyperactivation mediated by GSK-3 inhibition. In the enhanced activation of LPS-induced ERK1/2 mediated by the inhibition of GSK-3, PKCδ activity is also activated, compared with that treated with LPS alone (Fig. 2A). PKCδ was not inhibited by PD98059, indicating that PKCδ is not downstream of the MEK/ERK signaling pathway (Fig. 2A). In addition, we observed that the abnormal LPS-stimulated ERK1/2 hyperactivation induced by treatment with a GSK-3 inhibitor was abolished by PKCδ inhibition using a selective PKCδ inhibitor, rottlerin. These results indicate that PKCδ functions as an upstream regulator in the LPS-induced hyperactivation of ERK1/2 mediated by GSK-3 inhibition (Fig. 2B). To examine IL-10 expression produced by GSK-3 inhibition-induced ERK activation via PKCδ under LPS-stressed conditions, we performed ELISA using rottlerin. In rottlerin-treated cells, LPS-mediated IL-10 production by GSK-3 inhibition was notably reduced (Fig. 2C). Also, we found that LPS-induced ERK1/2 activity and IL-10 production is attenuated under PKCδ-reduced conditions using the PKCδ siRNA system (supplemental Fig. 3). This observation suggests that PKCδ is an important upstream regulator of LPS-stimulated IL-10 hyperproduction by GSK-3 inhibition-induced ERK activation.

GSK-3 Regulates LPS-stimulated ERK1/2 Activation via PKCδ but Not PKCa/β—The above results suggest that PKCδ is involved in LPS-stimulated IL-10 hyperproduction mediated by the inhibition of GSK-3. Thus, we examined the influence of other PKC isoforms on IL-10 production mediated by the GSK-3 inhibition-regulation of the MEK/ERK signaling pathway. Whereas the LPS-induced hyperactivation of ERK1/2 mediated by GSK-3 inhibition was reduced in rottlerin-treated BMDCs, this phenomenon was not observed after treatment with Gö6976, a PKCa/β isoenzyme inhibitor (Fig. 3A). Moreover, treatment with a PKCδ inhibitor resulted in the inhibition of LPS-stimulated IL-10 hyperproduction mediated by GSK-3 inhibition, but this phenomenon was not observed in Gö6976-treated BMDCs (Fig. 3B). These results indicate that PKCδ, but not PKCa/β, specifically regulates LPS-stimulated IL-10 hyperproduction mediated by the inhibition of GSK-3 in BMDCs.

LPS-stimulated ERK1/2 Hyperactivation Mediated by GSK-3 Inhibition Attenuates Endotoxemia—To assess the importance of ERK1/2 hyperactivation in GSK-3 inhibition-induced attenuation of endotoxemia, we examined the survival rate of LPS-induced endotoxemic mice using specific inhibitors. Indeed, mice injected intraperitoneally with a GSK-3 inhibitor before LPS challenge showed significantly reduced mortality (Fig. 4). Besides, this attenuated mortality mediated by GSK-3 inhibition was partially restored in MEK-1 inhibitor-preinjected mice (Fig. 4). In addition, GSK-3 inhibition-mediated attenuated...
mortality was restored in PKCδ inhibitor-preinjected mice (supplemental Fig. 4). These results demonstrate that GSK-3β-induced attenuation of the inflammatory response in the LPS-mediated endotoxin shock model is via PKCδ-mediated ERK1/2 hyperactivation.

**Enhanced IL-10 Production via ERK1/2 Hyperactivation Is Mediated by Inhibition of GSK-3 in Endotoxic Mice**—Endotoxin shock-induced death in mice is mediated by the excessive production of proinflammatory cytokines, including IL-12 and TNF-α (33, 34). The immune suppressive cytokine IL-10 is known as an attenuator of mice mortality and proinflammatory cytokines (IL-12 and TNF-α) production (12–14). As shown in Fig. 5, we confirmed that GSK-3 inhibition induced down-regulation of IL-12 and TNF-α and up-regulation of IL-10 in vivo endotoxemia. In addition, in MEK-1 inhibitor-injected mice, GSK-3 inhibition-induced down-regulation of IL-12 and TNF-α was not changed, but GSK-3 inhibition-induced up-regulation of IL-10 was reduced. These results indicate that ERK1/2 hyperactivation mediated by GSK-3 inhibition in endotoxemia modulates the production of IL-10 rather than IL-12 and TNF-α.

**LPS-stimulated ERK1/2 Hyperactivation Mediated by GSK-3 Inhibition Is Involved in Organ Injury**—LPS is a potent stimulus of neutrophil infiltration into major organs, such as the lung, and LPS-mediated neutrophil infiltration is closely related to endotoxemia. Therefore, we histologically examined the effect of ERK1/2 hyperactivation mediated by the inhibition of GSK-3 in endotoxemia on neutrophil infiltration in lung. Mice injected with LPS alone exhibited massive neutrophil infiltration into the interstitial space and remarkable thickening of alveolar septa in lung tissue (Fig. 6A). However, the above phenomena were dramatically attenuated by treatment with a GSK-3 inhibitor (Fig. 6A). Furthermore, this attenuated pattern mediated by GSK-3 inhibition was restored in MEK-1 inhibitor-preinjected mice (Fig. 6A).

Also, we observed the restoration of GSK-3 inhibition-mediated reduced neutrophil infiltration in PKCδ inhibitor-preinjected mice (supplemental Fig. 5). Next, we confirmed organ injury using the MPO ELISA method. MPO is a peroxidase enzyme present in neutrophil granulocytes, and its increase in plasma is often used as a marker of polymorphonuclear leukocyte activation in endotoxemia. Consistent with our histological observation in tissue, LPS-mediated increase of the MPO level was reduced by a GSK-3 inhibitor, and the down-regulation of the MPO level resulting from GSK-3 inhibition was restored by a MEK-1 inhibitor in various tissues, including lung, liver, and kidney (Fig. 6B).

These results demonstrate that GSK-3 inhibition-induced ERK1/2 activation in endotoxemia is related to organ injury. Sepsis accompanied by hypotension is defined as severe sepsis and plays a crucial role in the pathophysiology of septic shock and multiorgan failure syndrome. To illuminate the effect of GSK-3 inhibition-induced ERK1/2 activation on the physiological state of arterial blood pressure during endotoxemia, we measured the MABP in mice. Whereas a rapid drop of MABP was observed during endotoxic shock, the MABP was restored to normal by treatment with a GSK-3 inhibitor (Fig. 6C). However, in MEK-1 inhibitor-injected mice, GSK-3 inhibition-mediated restoration in the endotoxemial circumstance was partially abolished (Fig. 6C). These results indicate that GSK-3 inhibition-induced ERK1/2 activation attenuates multiorgan failure syndrome via hypotension.
Nitric oxide (NO), a potent hypotensive agent, was also analyzed in blood collected 20 h after administration of vehicle alone, LPS (20 mg/kg), SB415286 (25 mg/kg) + LPS, or PD98059 (10 mg/kg) + SB415286 (25 mg/kg) + LPS. Serum nitrite levels elevated with LPS challenge were decreased by a GSK-3 inhibitor, and GSK-3 inhibition-induced down-regulation of nitrite level was restored by a MEK-1 inhibitor (Fig. 6D). These data strongly indicate that GSK-3 inhibi-
tion-induced ERK1/2 activation modulates multiorgan failure syndrome via NO-induced hypotension.

**DISCUSSION**

 Toll-like receptor stimulation influences the production of pro- and anti-inflammatory cytokines and modulates the magnitude of the inflammatory response. In a recent study, it was revealed that GSK-3β plays a role as an attenuator in endotoxemia via the regulation of pro- and anti-inflammatory cytokines (29). In detail, it was elucidated that GSK-3β functions as a regulator of cAMP response element-binding (CREB) responsible for cytokine production. In this report, we demonstrate the alternative protective effect of a GSK-3 inhibitor via the production of the anti-inflammatory cytokine IL-10 by ERK1/2 hyperactivation in LPS-induced endotoxemia. Consistent with our results, it was previously reported that GSK-3 inhibition-dependent basal ERK1/2 activation via PKC8 plays a determinant role in the regulation of COX-2 and IL-8 expression in human colon cancer cells (25).

Sepsis is a pathologic condition associated with a cytokine storm; in other words, it is a circumstance in which highly increased levels of proinflammatory cytokines cause high mortality by septic shock. Clinical characteristics of severe sepsis include massive polymorphonuclear leukocyte infiltration, which plays a pivotal role in bacterial clearance, and hypotension, which plays a crucial role in the pathophysiology of septic shock and multiple organ dysfunction syndromes. Several reports have demonstrated that NO mediates the above-mentioned hypotension in sepsis (8, 9). In various reports, it was known that IL-10, a potent anti-inflammatory cytokine, attenuates the above mentioned septic symptoms. In detail, it blocks the release of various proinflammatory cytokines and prevents endotoxin-mediated mortality (13, 14, 35). IL-10−/− mice show an accelerated onset of mortality compared with IL-10+/+ mice in the cecal ligation and puncture mouse model (36). In the experiment using blocking antibodies to IL-10, the rate of lethality is increased (37, 38). Thus, the level of IL-10 has a determinant role in sepsis, and it is crucial for sepsis therapy. From this perspective, we focused on the regulatory mechanism of IL-10 in septic environment. Here, we revealed that IL-10 expression via the GSK-3 inhibition-induced ERK1/2 activation signaling cascade plays a determinant role in the attenuation of the pathophysiology of septic shock and multiple organ dysfunction syndrome. Also, we showed that GSK-3 cross-talks with MAPK in sepsis. In detail, GSK-3 inhibition provokes IL-10 expression via up-regulating ERK1/2 kinase activity in LPS-stimulated conditions.

In conclusion, we provide an immunosuppressive mechanism of GSK-3 inhibition via the ERK1/2-IL10 pathway in an endotoxemia mouse model. Our findings may accelerate the development of selective drugs for sepsis. In addition, vaccine-based immunotherapy via these drugs for sepsis might be possible. Further studies are required to establish the pivotal role of IL-10 regulation via GSK-3-induced ERK1/2 activation in various disease models induced by clinical immune imbalances, such as asthma and autoimmune diseases.

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