Platelet-activating Factor Mediates Endotoxin Tolerance by Regulating Indoleamine 2,3-Dioxygenase-dependent Expression of the Suppressor of Cytokine Signaling 3*

Kyung Tae Noh†1, In Duk Jung‡1, Gil Sun Cha§1, Myung-Kwan Han¶1, and Yeong-Min Park¶2

From the †Department of Infectious Diseases, Armed Forces Medical Research Institute, 90bun, Jaunro, Yuseong-gu, Daejeon 305-878, South Korea, the ‡Department of Immunology, College of Medicine, Konkuk University, Seoul 143-701, South Korea, and the ¶1Department of Microbiology, Chonbuk National University Medical School, Jeonju 561-182, South Korea

Edited by Dennis R. Voelker

Indoleamine 2,3-dioxygenase (IDO) mediates immune tolerance, and suppressor of cytokine signaling 3 (SOCS3) negatively regulates the JAK/STAT signal transduction pathway. We determined previously that platelet-activating factor (PAF) protects mice against LPS-induced endotoxic shock, but its detailed mechanism of action was unknown. We performed survival experiments in IDO+/− and IDO−/− mice using an LPS-induced endotoxemia model and rated organ injury (neutrophil infiltration). Using ELISA and Western blotting, we also investigated the mechanism of PAF-mediated endotoxin tolerance during endotoxemia. PAF-mediated endotoxin tolerance was dependent on IDO in vivo and in vitro and was not observed in IDO−/− mice. JAK/STAT signaling, crucial for SOCS3 expression, was also impaired in the absence of IDO. In an IDO- and STAT-dependent manner, PAF mediated a decrease in IL-12 and a dramatic increase in IL-10 and reduced mouse mortality. In addition, PAF attenuated LPS-mediated neutrophil infiltration into the lungs and interactions between neutrophil-like (THP-1) and endothelial cells (human umbilical vein endothelial cells). These results indicate that PAF-mediated endotoxin tolerance is initiated via IDO- and JAK/STAT-dependent expression of SOCS3. Our study has revealed a novel tolerogenic mechanism of IDO action and an important association between IDO and SOCS3 with respect to endotoxin tolerance.

Sepsis represents the systemic inflammatory response to infection by microorganisms such as Gram-negative and -positive bacteria, viruses, and fungi (1) and is the main cause of in-hospital deaths in adult patients (2–4). Each year, more than 200,000 people die from sepsis in the United States (5). Specifically, the proinflammatory endotoxin LPS, an abundant glycolipid in the outer membrane of Gram-negative bacteria, induces monocytes and macrophages to produce proinflammatory cytokines, reactive oxygen and nitrogen species, and other inflammatory mediators (1, 6, 7).

Pathophysiological adaptations to attenuate this hyperinflammatory response include a defense mechanism in the host against endotoxemia (7), such as endotoxin tolerance (8). In this phenomenon, cells or organisms pre-exposed to a low concentration of endotoxin enter a transient hyporesponsiveness state and do not respond to subsequent LPS stimulation. Instead, they respond with an attenuated production of proinflammatory cytokines (e.g. IL-12, TNF-α, IL-1, IL-6, and IFN-γ) and improved protection against endotoxic lethality (8). A previous study revealed that activation of NF-κB and MAPKs, such as ERK, JNK, and p38, was impaired in endotoxin-tolerized cells (9).

Platelet-activating factor (PAF, 1-O-alkyl-2-(R)-acyethyl-sn-glyceryl-3-phospho nocholine), a potent phospholipid mediator of leukocyte activation, plays a pivotal role in various physiological conditions (10). For example, PAF is involved in endotoxic shock pathogenesis; thus, researchers have explored the therapeutic effects of PAF antagonists against sepsis. Previous studies observed increased PAF levels in the blood during endotoxemia and that PAF antagonist administration attenuated the endotoxin effect in animal models (11–12). However, clinical trials utilizing anti-PAF therapy for treating septic shock have been unsuccessful (13). Moreover, other experimental studies present conflicting information regarding the classic functions of PAF. We and other researchers have proposed that PAF may sometimes play a suppressive role in the immune response (14, 15).

Indoleamine 2,3-dioxygenase (IDO), an immunoregulatory enzyme, triggers T cell tolerance by degrading tryptophan via the kynurenine pathway (16, 17). According to various reports, IDO regulates tumor-mediated immune tolerance by causing T

* This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (2014R1A1A2054999, 2014R1A1A10054634, 2015R1A2A1A13001713, 2013R1A1A1069575, and 2016R1A5A2012284). The authors declare that they have no conflicts of interest with the contents of this article.

† Both authors contributed equally to this work.

‡ To whom correspondence may be addressed: Dept. of Microbiology, Chonbuk National University Medical School, Jeonju 561-182, South Korea. Tel.: 82-63-270-3106; Fax: 82-63-274-9833; E-mail: iamton@chonbuk.ac.kr.

§ To whom correspondence may be addressed: Dept. of Immunology, College of Medicine, Konkuk University, 120 neungdong-ro Kwangjin-gu, Seoul 143-701, South Korea. Tel.: 82-2-2049-6158; Fax: 82-2-2049-6192; E-mail: immun3023@kku.ac.kr.

¶ The abbreviations used are: PAF, platelet-activating factor; IDO, indoleamine 2,3-dioxygenase; BMDC, bone marrow-derived dendritic cell; MPO, myeloperoxidase; ALT, alanine aminotransferase; PI, propidium iodide; PMN, polymorphonuclear leukocyte; rmGM-CSF, recombinant mouse GM-CSF; HUVEC, human umbilical vein endothelial cell.
cell suppression (16, 18, 19). Suppressor of cytokine signaling 3 (SOCS3), a negative modulator of the JAK/STAT signaling cascade, is a feedback inhibitor that down-regulates activated cytokine receptor signaling (20). Recent studies found that forced expression of SOCS3 negatively regulates proinflammatory cytokines produced by LPS-induced signaling pathways (21, 22). Both IDO and SOCS3 appear to possess tolerogenic characteristics, but the relationship between IDO and SOCS3 has not been addressed. Here we demonstrate that IDO-dependent SOCS3 expression via the JAK/STAT signaling cascade plays a pivotal role in PAF-mediated endotoxin tolerance.

Results

**PAF-mediated Endotoxin Tolerance Modulates Inflammatory Cytokine Production in an IDO-dependent Manner**—We previously determined that PAF plays a protective role against LPS-mediated endotoxemia (15), but its detailed mechanism of action was not fully characterized. Because IDO, a representative immunosuppressive molecule, functions as an attenuator against hyperimmune responses via tryptophan breakdown in the kynurenine pathway, we investigated whether PAF-mediated endotoxin tolerance occurs in an IDO-dependent manner. Consistent with our previous results, in the presence of IDO, PAF protects mice against LPS-mediated endotoxemia. However, in the absence of IDO, its protective action was not observed (Fig. 1A). Endotoxin-mediated mortality is mainly due to a cytokine storm. Consistent with *in vivo* survival data, PAF-injected *IDO*+/+ mice showed reduced levels of the proinflammatory cytokine IL-12p70 and increased levels of the anti-inflammatory cytokine IL-10 during subsequent LPS stimulation. However, this pattern of PAF-mediated cytokine regulation was not observed in *IDO*−/− mice (Fig. 1B). We observed this same phenomenon in our *in vitro* ELISA experiments as well (Fig. 1C). From these results, we determined that PAF protects mice via modulation of pro- and anti-inflammatory cytokines in an IDO-dependent manner.

![FIGURE 1. PAF-mediated endotoxin tolerance attenuates mouse mortality and the cytokine storm in an IDO-dependent manner. A, the survival of *IDO*+/+ and *IDO*−/− mice after intraperitoneal injection with LPS (40 mg/kg) following PAF (5 μg/mouse) was monitored for 6 days. Shown is one representative of three independent experiments (n = 8 mice/group; *, p < 0.05). B, PAF-mediated attenuation of the proinflammatory cytokine IL-12p70 and enhancement of the anti-inflammatory cytokine IL-10 after LPS stimulation. *IDO*+/+ and *IDO*−/− BMDCs were treated with PAF (200 nM), followed by treatment with LPS (100 ng/ml) for 18 h. The concentrations of IL-12p70 and IL-10 were measured in the culture supernatants by ELISA. The mean ± S.E. values shown represent three independent experiments (***, p < 0.001). C, *IDO*+/+ and *IDO*−/− mice were injected with LPS (20 mg/kg) immediately following control solution (PBS containing 0.25% BSA) or PAF (2.5 μg/mouse). Blood was collected at 4 h for IL-12p70 and IL-10 measurements. The mean ± S.E. values shown represent three independent experiments (*, p < 0.05; **, p < 0.01).
Hyperexpression of SOCS3 Is IDO-dependent and Occurs via JAK/STAT Signaling—

Because of the necessity of IDO for PAF-mediated endotoxin tolerance, we first examined its expression and potential tolerogenic characteristics under PAF-conditioning circumstances using IDO knockout dendritic cells. As shown in Fig. 2A, PAF did not influence IDO expression under LPS-stimulated conditions. Thus, we focused on another tolerogenic protein, SOCS3, a cytokine-induced regulator of hyperimmune responses that down-regulates activated cytokine receptor signaling. SOCS3 was overexpressed by PAF in the presence of IDO in response to LPS, but the expression level of SOCS3 by PAF against LPS was not enhanced in the absence of IDO (Fig. 2B).

Various reports indicate that the JAK/STAT signaling cascade contributes to SOCS3 expression (20), so we examined whether the JAK/STAT pathway is necessary for IDO-dependent SOCS3 expression during LPS exposure. Although LPS provokes the activation of multiple JAK/STAT signaling cascade components, including JAK1, JAK2, STAT1, and STAT3, in IDO+/+ BMDCs, the activation of these molecules was impaired in IDO−/− BMDCs (Fig. 2C). Because of the blockade of JAK/STAT signaling in the absence of IDO, we investigated whether PAF-mediated hyperexpression of SOCS3 is dependent on STAT signaling under LPS-stimulated conditions. We confirmed that IDO-dependent, PAF-mediated SOCS3 expression is indeed attenuated by a STAT3 inhibitor (Fig. 3). Furthermore, PAF induces the reduction of IL-12 and elicits dramatic overproduction of IL-10 compared with the LPS-treated control (Fig. 3). This PAF-mediated cytokine modulatory pattern is dependent on both IDO and STAT3, suggesting that the PAF-mediated, LPS-induced hyperexpression of SOCS3 is IDO-dependent and regulated via components of the JAK/STAT signaling pathway.

PAF Attenuates Neutrophil Infiltration in an IDO- and STAT3-dependent Manner—

Excess levels of endotoxins, such as LPS, stimulate neutrophil infiltration into major organs, provoke organ dysfunction, and lead to septic shock. Therefore, we explored the influence of PAF on neutrophil infiltration in the lungs. LPS-injected mice showed massive neutrophil infiltration (increase in black dot-shaped cells) into the interstitial space and remarkable thickening of alveolar septa. Consistent with previous reports, PAF decreased LPS-induced neutrophil infiltration, but not in the absence of IDO (Fig. 4A). Furthermore, this attenuation phenomenon was also not observed in STAT3 inhibitor-injected mice (Fig. 4B). We also confirmed organ injury using the MPO ELISA method. MPO is a peroxidase enzyme of neutrophils and is widely used as an indicator of polymorphonuclear leukocyte activation under endotoxemic conditions. Consistent with our histological data from tissue,
PAF protected LPS-induced activation of MPO in the lung and liver in an IDO- and STAT3-dependent manner (Fig. 4, B and C). To confirm this in vivo neutrophil infiltration, we performed in vitro assays to observe binding between HUVECs and THP-1 cells and verified that PAF inhibits the LPS-induced binding between these two cell types (Fig. 5A). In addition, we found that HUVECs are crucial for LPS-mediated binding between both cell types (Fig. 5B). Therefore, IDO and STAT3 are required for PAF-mediated attenuation of LPS-induced neutrophil infiltration.
PAF-mediated Modulation of Liver Function Is Dependent on IDO and STAT3 under Endotoxemic Conditions—We investigated the influence of PAF on hepatic function by measuring levels of alanine aminotransferase (ALT) in blood serum, a parameter of liver function that represents the degree of liver damage. LPS-induced elevated levels of ALT were lowered by PAF, but only in the presence of IDO (Fig. 6A). In addition, PAF did not modulate the levels of ALT under STAT3-inhibited conditions (Fig. 6A). Furthermore, to prove liver injury, we performed an Annexin V/PI double staining assay (Fig. 6B). Here we also observed a similar result, consistent with ALT data. PAF prevented LPS-induced liver cell death in an IDO- and STAT3-dependent manner. Thus, we inferred that PAF improves liver function by modulating IDO and STAT3 under LPS-stimulated circumstances.

Discussion

Sepsis is a pathological condition characterized by a cytokine storm, occasional organ dysfunction, and high mortality by septic shock in severe cases. Numerous proinflammatory cytokines produced by immune cells in response to infection contribute to these physiological responses. In a previous report, we revealed the role of PAF as an attenuator of the LPS-mediated hyperinflammatory response (15). However, we did not define the mechanism of PAF-mediated endotoxin tolerance at that time. Thus, in this study, we focused on the endotoxin tolerance mechanism of PAF and examined which signaling pathway is involved in the protective mechanism of PAF.

The general biological function of PAF during inflammation is to mediate the activation of immune cells involved in this process. Contrary to this role, however, our previous results demonstrated that PAF-injected mice showed resistance against LPS-induced endotoxic shock. This result challenges the current paradigm of PAF as a crucial mediator of endotoxemia. Here we performed various molecular experiments to confirm our previous results and further investigate the activity of PAF during endotoxin exposure. We found that PAF-mediated attenuation of mouse mortality was not observed in knock-out mice of IDO, a tolerogenic protein, so we surmised that PAF elicited endotoxin tolerance via regulation of IDO expression. However, PAF-mediated modulation of IDO under LPS-stimulated conditions was not detected. Thus, we focused on another tolerogenic protein, SOCS3, a negative regulator of
cytokine receptor signaling, and observed LPS-induced SOCS3 expression. To verify the relevance of SOCS3 in PAF-mediated, IDO-dependent endotoxin tolerance, we examined PAF-mediated hyperexpression of SOCS3 in response to LPS in the absence of IDO and found that PAF-mediated potentiation of SOCS3 expression is dependent on IDO.

Typically, SOCS3 expression is controlled by the JAK/STAT signaling pathway, so we examined the influence of this pathway in the presence or absence of IDO. In \( \text{IDO}^{-/-} \) BMDCs, activation of multiple JAK/STAT signaling cascade components was inhibited compared with that in \( \text{IDO}^{+/+} \) BMDCs. Although IDO is known to mediate immune tolerance via tryptophan starvation, we elucidated a novel tolerantergic characteristic of IDO involving its role in SOCS3 expression. Therefore, it is worthwhile to define the link between these two tolerogenic proteins. Walterscheid \textit{et al.} (14) first provided evidence for a new role of PAF whereby it could activate immunosuppressive mechanisms in response to cellular damage (14). Additionally, we further confirmed its suppressive role under endotoxemia conditions (15), showing that exogenous PAF can prevent the series of events leading to onset of severe endotoxin shock. Therefore, characterizing the molecular mechanism of PAF-mediated endotoxin tolerance became the main objective of this study.

We observed several PAF-mediated endotoxin tolerance phenomena, including attenuation of the proinflammatory
cytokine IL-12p70, increased levels of the anti-inflammatory cytokine IL-10, hyperexpression of SOCS3, and reductions in neutrophil infiltration into lung tissue, liver function, and LPS-mediated mortality. Furthermore, all of these phenomena were dependent on IDO and STAT3. Because PAF is involved in juxtacrine mechanism-mediated activation and adhesion of leukocytes, we also examined adherence of polymorphonuclear leukocytes (PMNs) and neutrophil-like THP-1 cells to endothelial cells under endotoxemic conditions and observed neutrophil-dependent, LPS-induced adhesion of leukocytes to endothelial cells in vivo and in vitro. However, PAF blocked the binding between neutrophils and endothelial cells, thus attenuating neutrophil-mediated inflammation. Excessive endotoxin-induced PMN infiltration into the endothelium evokes tissue hyperinflammation, which promotes organ dysfunction. Therefore, we inferred that neutrophil manipulation could be a possible therapeutic approach against sepsis. Although the detailed mechanism of neutrophil-mediated inflammation during endotoxemia was not specifically investigated in this study, we did demonstrate the influence of PAF on neutrophil infiltration into the endothelium under endotoxemic conditions.

In conclusion, we illustrated the immunosuppressive effects of PAF during endotoxemia and determined its ability to regulate the tolerogenic proteins IDO and SOCS3 (Fig. 8). Furthermore, we identified a novel tolerogenic pathway of IDO related to SOCS3 expression. Highlighting the attenuative pathophysiological role of PAF during endotoxemia will contribute to our understanding of other complex functions of PAF as a mediator of immune responses. We will also investigate the effectiveness of PAF for treating patients experiencing endotoxic shock. We are also interested in defining the physiological role of PAF in various immune disorders, including asthma, atopy, and autoimmune diseases.

**Experimental Procedures**

**Mice**—Four 8-week-old C57BL/6 (H-2Kb and I-Ab) mice were purchased from the Korean Institute of Chemical Technology (Daejeon, Korea). Indo−/− C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice with wild-type (+/+ or homozygous null (−/−) genotypes for the targeted disruption of the IDO gene were selected from offspring of heterozygous/homozygous mating and verified by tail PCR. Age-matched wild-type (+/+ ) littermates were used as controls. Mice were housed in a pathogen-free environment in our animal facility for at least 1 week before use. All experiments were performed in accordance with the guidelines of the Committee of Ethics of Animal Experiments of Konkuk University.

**Reagents and Antibodies**—Recombinant mouse GM-CSF (rmGM-CSF), rmIL-4, and cytokine ELISA kits for murine IL-12p70 and IL-10 were purchased from R&D Systems (Minneapolis, MN). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* O127:B8 was obtained from Sigma-Aldrich (St. Louis, MO).

**LPS-induced Endotoxemia Model**—This model was utilized as described previously (23). Briefly, mice were injected i.p. with the designated dose of LPS (*E. coli* O127:B8, Sigma-Aldrich).
dissolved in PBS containing 0.25% bovine serum albumin. General health conditions and mortality were recorded for up to 6 days after injection to ensure that no later deaths occurred. The STAT3 inhibitor Stattic (10 mg/kg) and PAF (5 μg/mouse) were injected i.p. 2 h before LPS injection. A solution containing the same concentration of DMSO was used as a control.

**Generation and Culture of Dendritic Cells**—Primary culture of bone marrow-derived dendritic cells (BMDCs) was performed as described previously (24). Briefly, BM was flushed from the tibiae and femora of 6- to 8-week-old male C57BL/6 mice and depleted of red blood cells using red blood cell lysing buffer (Sigma-Aldrich). The cells were plated in 6-well culture plates (1 × 10⁷ cells/ml, 2 ml/well) in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, 20 ng/ml rmGM-CSF, and 10 ng/ml rmIL-4 at 37 °C with 5% CO₂. On days 3 and 5, 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 DC aggregates were either harvested for subsequent analysis according to the instructions of the manufacturer. The purity of the selected cell fraction was >90%.

**Cell Culture**—Low passage (passages 3–6) human umbilical vein endothelial cells (HUVECs) were cultured in complete M199 medium containing 10% FBS, 100 μg/ml heparin, and 50 μg/ml pituitary growth factor. THP-1 monocytes were cultured in RPMI 1640 medium containing 10% FBS and 0.05 mM β-mercaptoethanol. These cells were generously provided by Dr. Jae Ho Kim (Pusan National University, Busan, South Korea).

**Cell Adhesion Assay**—HUVECs were cultured to 80% confluency in 24-well plates. THP-1 cells (5 × 10⁶ cells/ml) were labeled with the fluorescent dye calcein acetoxymethyl ester (Sigma-Aldrich) for 30 min at 37 °C. Unincorporated calcein was removed, and the cells were added to microwells containing HUVECs. Co-cultured cells were treated with LPS (100 ng/ml) and incubated for 12 h in the presence or absence of 200 nM PAF. Non-adherent monocytes were then removed by washing with PBS, and the fluorescence emission of HUVEC-bound THP-1 cells was monitored at 485-nm excitation and 538-nm emission. All images were captured with a fluorescence microscope (D80i, Nikon, Tokyo, Japan). Cell counts were performed by viewing the images. The error bars represent the mean ± S.D. of three experiments.

**Measurement of Cytokines**—Serum and culture supernatants of BMDCs were collected and assayed for various cytokine levels using ELISAs according to the instructions of the manufacturer.

**Measurement of PMN Infiltration**—Lungs were first perfused with 10% formalin at a constant distending pressure of 25 cm H₂O for 10 min. Lungs were then 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 H&E.

**MPO Activity Assay**—Myeloperoxidase (MPO) activity was measured in tissue using a procedure similar to that documented by Hillegass et al. (25). The tissue homogenates were incubated in a 96-well plate in buffer containing 50 mM potassium phosphate buffer (pH 6.0), 0.167 mg/ml odaniisidine, and 0.0005% hydrogen peroxide. The plate was read at an absorbance of 460 nm at regular intervals for 2 min.

**Isolation of Liver Cells and Determination of Cell Viability**—Removed livers were cut into small pieces and digested in 2 ml of prewarmed digestion solution (RPMI 1640 medium containing 5% FBS, 2 mg/ml collagenase IV, and 0.2 mg/ml DNase I) for 25 min at 37 °C with constant shaking at 250 rpm. Suspended single cells were collected by passing through a mesh. Isolated liver cells were harvested using a syringe, with 1 × 10⁶ cells being collected for each sample, and washed three times with ice-cold 1× PBS. The viability of PECs was analyzed by double-staining with 5 μg/ml propidium iodide (PI) and Annexin-V and then analyzed by flow cytometry.

**Statistical Analysis**—All experiments were performed at least in duplicate. Unless otherwise stated, data are expressed as the mean ± S.E. Analysis of variance was used to compare experimental groups with control values, and comparisons between multiple groups were made using Tukey’s multiple comparison tests (Prism 3.0, GraphPad). p < 0.05 indicated statistical significance.

**Author Contributions**—K. T. N. designed, performed, and analyzed the experiments shown in Figs. 1–6 and wrote the paper. I. D. J. designed, performed, and analyzed the experiments shown in Figs. 4, 5, and 7. G. S. C. provided technical assistance and contributed to the design and preparation of the figures. M. K. H. and Y. M. P. conceived and coordinated the study and wrote the paper. All authors reviewed the results and approved the final version of the manuscript.

**References**

1. Sriskandan, S., and Altmann, D. M. (2008) The immunology of sepsis. J. Pathol. 214, 211–223
2. Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clermont, G., Carcillo, J., and Pinney, M. R. (2001) Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit. Care Med. 29, 1303–1310
3. Dombrovskiy, V. Y., Martin, A. A., Sunderram, J., and Paz, H. L. (2007) Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. Crit. Care Med. 35, 1244–1250
4. Martin, G. S., Mannino, D. M., Eaton, S., and Moss, M. (2003) The epidemiology of sepsis in the United States from 1979 through 2000. N. Engl. J. Med. 348, 1546–1554
5. Martin, G. S. (2012) Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. Expert Rev. Anti Infect. Ther. 10, 701–706
6. Bozza, F. A., Salluh, J. I., Japiassu, A. M., Soares, M., Assis, E. F., Gomes, R. N., Bozza, M. T., Castro-Faria-Neto, H. C., and Bozza, P. T. (2007)
Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit. Care 11, R49

7. Castellheim, A., Brekke, O. L., Espevik, T., Harboe, M., and Mollnes, T. E. (2009) Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. Scand. J. Immunol. 69, 479–491

8. Biswas, S. K., and Lopez-Collazo, E. (2009) Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol. 30, 475–487

9. Peroval, M. Y., Boyd, A. C., Young, J. R., and Smith, A. L. (2013) A critical role for MAPK signalling pathways in the transcriptional regulation of toll like receptors. PLoS ONE 8, e51243

10. Imaizumi, T. A., Stafforini, D. M., Yamada, Y., McIntyre, T. M., Prescott, S. M., and Zimmerman, G. A. (1995) Platelet-activating factor: a mediator for clinicians. J. Intern. Med. 238, 5–20

11. Casals-Stenzel, J. (1987) Protective effect of WEB 2086, a novel antagonist of platelet activating factor, in endotoxin shock. Eur. J. Pharmacol. 135, 117–122

12. Giral, M., Balsa, D., Ferrando, R., Merlos, M., Garcia-Rafanell, J., and Forn, J. (1996) Effects of UP-12633, a new antagonist of platelet-activating factor, in rodent models of endotoxic shock. Br. J. Pharmacol. 118, 1223–1231

13. Mathiak, G., Szewczyk, D., Abdullah, F., Ovadia, P., and Rabinovici, R. (1997) Platelet-activating factor (PAF) in experimental and clinical sepsis. Shock 7, 391–404

14. Walterscheid, J. P., Ullrich, S. E., and Nghiem, D. X. (2002) Platelet-activating factor, a molecular sensor for cellular damage, activates systemic immune suppression. J. Exp. Med. 195, 171–179

15. Jeong, Y. I., Jung, I. D., Lee, C. M., Chang, J. H., Chun, S. H., Noh, K. T., Jeong, S. K., Shin, Y. K., Lee, W. S., Kang, M. S., Lee, S. Y., Lee, J. D., and Park, Y. M. (2009) The novel role of platelet-activating factor in protecting mice against lipopolysaccharide-induced endotoxic shock. PLoS ONE 4, e6503

16. Moller, A. L., and Munn, D. H. (2004) IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat. Rev. Immunol. 4, 762–774

17. Löb, S., Königsrainer, A., Rammensee, H. G., Opelz, G., and Ternes, P. (2009) Inhibitors of indoleamine-2,3-dioxygenase for cancer therapy: can we see the wood for the trees? Nat. Rev. Cancer 9, 445–452

18. Muller, A. J., and Prendergast, G. C. (2007) Indoleamine 2,3-dioxygenase in immune suppression and cancer. Curr. Cancer Drug Targets 7, 31–40

19. Munn, D. H., and Mellor, A. L. (2007) Indoleamine 2,3-dioxygenase and tumor-induced tolerance. J. Clin. Invest. 117, 1147–1154

20. Shuai, K., and Liu, B. (2003) Regulation of JAK-STAT signalling in the immune system. Nat. Rev. Immunol. 3, 900–911

21. Yan, C., Cao, J., Wu, M., Zhang, W., Jiang, T., Yoshimura, A., and Gao, H. (2010) Suppressor of cytokine signaling 3 inhibits LPS-induced IL-6 expression in osteoblasts by suppressing CCAAT/enhancer-binding protein (26) activity. J. Biol. Chem. 285, 37227–37239

22. Yan, C., Ward, P. A., Wang, X., and Gao, H. (2013) Myeloid depletion of SOCS3 enhances LPS-induced acute lung injury through CCAAT/enhancer binding protein δ pathway. FASEB J. 27, 2967–2976

23. Noh, K. T., Son, K. H., Jung, I. D., Kang, T. H., Hwang, S. A., Lee, W. S., You, J. C., and Park, Y. M. (2012) Protein kinase C δ (PKCδ)-extracellular signal-regulated kinase 1/2 (ERK1/2) signaling cascade regulates glycoprotein synthase kinase-3 (GSK-3) inhibition-mediated interleukin-10 (IL-10) expression in lipopolysaccharide (LPS)-induced endotoxemia. J. Biol. Chem. 287, 14226–14233

24. Noh, K. T., Son, K. H., Jung, I. D., Kang, T. H., Choi, C. H., and Park, Y. M. (2015) Glycogen synthase kinase-3β (GSK-3β) inhibition enhances dendritic cell-based cancer vaccine potency via suppression of interferon-γ-induced indoleamine 2,3-dioxygenase expression. J. Biol. Chem. 290, 12394–12402

25. Hillegass, L. M., Grisswold, D. E., Brickson, B., and Albrightson-Winslow, C. (1990) Assessment of myeloperoxidase activity in whole rat kidney. J. Pharmacol. Methods 24, 285–295