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Involvement of SHIP in TLR2-Induced Neutrophil Activation and Acute Lung Injury

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The SHIP converts phosphatidylinositol 3,4,5 triphosphate to phosphatidyl 3,4 biphosphate. SHIP has negative regulatory functions on PI3K-dependent signaling pathways, which occupy important roles in modulating neutrophil functions. We used neutrophils from transgenic SHIP−/− and SHIP+/+ mice that were stimulated with peptidoglycan (PGN) to examine the role of SHIP in TLR2-induced neutrophil activation. SHIP−/− neutrophils demonstrated significantly increased activation of the PI3K-dependent kinase Akt after exposure to PGN. Release of cytokines and chemokines, including TNF-α, IL-1β, IL-6, IL-10, and MIP-2, was also increased in SHIP−/− compared with SHIP+/+ neutrophils. There was no difference in the nuclear translocation of the transcriptional factor NF-κB between PGN-stimulated SHIP−/− and SHIP+/+ neutrophils. However, phosphorylation of the p65 subunit of NF-κB, an event essential for optimal transcriptional activity of NF-κB, was increased in TLR2-activated SHIP−/− neutrophils. SHIP−/− neutrophils demonstrated greater activation of ERK1/2 and p38 MAPKs than did SHIP+/+ neutrophils after exposure to PGN. The severity of acute lung injury induced by PGN was greater in SHIP−/− as compared with SHIP+/+ mice. These results demonstrate that SHIP has a negative regulatory role in TLR2-induced neutrophil activation and in the development of related in vivo neutrophil-dependent inflammatory processes, such as acute lung injury. The Journal of Immunology, 2005, 174: 8064–8071.

The PI3K pathway plays an important role in multiple neutrophil functions, including expression of proinflammatory cytokines, activation of the transcriptional factor NF-κB, generation of reactive oxygen species (ROS), and chemotaxis (1–6). PI3K-regulated events catalyze the addition of a phosphate molecule to the inositol ring of phosphatidylinositol 4,5-biphosphate, generating phosphatidylinositol 4,5-trisphosphate (PIP3)3. PIP3 binds to the pleckstrin homology domain of Akt/protein kinase B, permitting association of the phosphatidylinositol-dependent kinases PDK1 and PDK2, that then results in activation of Akt through phosphorylation of Thr308 and Ser473 (7). The accumulation of activated neutrophils into the lungs and other sites contributes to morbidity and mortality associated with severe infection (8). The importance of neutrophils in acute lung injury (ALI) is shown by studies in which the severity of lung injury is decreased when neutrophils are eliminated (8, 9). Neutrophils that are present in the lungs during ALI produce increased amounts of proinflammatory cytokines and demonstrate enhanced nuclear accumulation of NF-κB, a transcriptional factor that participates in the regulation of many cytokines and other mediators of acute inflammatory responses (8–10). Activation of kinases, including Akt, is increased in neutrophils recovered from patients suffering from sepsis-induced ALI (11).

Engagement of Toll receptors, including TLR2 and TLR4, results in activation of Akt (3, 12–17). TLR2 are more abundant than TLR4 on neutrophils (18), activate Akt to a greater extent than TLR4 (3), and use different signaling mechanisms (19, 20). In particular, although PI3K and Akt participate in enhancing nuclear accumulation of NF-κB in neutrophils cultured with LPS (21), such a role for PI3K/Akt is not found after stimulation with peptidoglycan (PGN), a TLR2 ligand (3). Rather, in neutrophils activated through TLR2, the mechanism by which PI3K participates in increasing NF-κB-dependent transcription appears to be through regulation of phosphorylation of the p65 NF-κB subunit and not through affecting nuclear translocation of NF-κB (3). Studies in other cell populations have also found that the positive effects of Akt on the transcriptional activity of NF-κB are independent of NF-κB translocation but dependent on transactivation of p65 via phosphorylation of Ser326 (22–24).

Membrane recruitment of the SHIP initiates negative feedback pathways, inhibiting PI3K-dependent signaling (25). SHIP converts PIP3 to phosphatidyl 3,4 biphosphate, thereby reducing the concentration of PIP3 and deactivating phosphorylated members of the PI3K/Akt pathway. Although most reports describe a negative regulatory role for SHIP in NF-κB activation (16, 26–28), there are recent studies showing that SHIP positively affects NF-κB-dependent transcription, as well as the release of NF-κB-dependent cytokines, such as TNF-α, in macrophages stimulated with LPS (29). Those results contrast with experiments showing an inhibitory role for SHIP in macrophage activation induced by immune receptor ligation and growth factor receptor signaling (30). In addition, a recent article by Sly et al. (16) found that SHIP−/− mice were more sensitive to LPS-induced mortality and that SHIP−/− macrophages and mast cells are hyperresponsive to LPS, particularly in terms of cytokine release (TNF-α, IL-6, and IL-1β). Therefore, it appears that the role of SHIP, as well as its effects on PI3K and Akt, in the positive or negative regulation of cellular activation may depend both on the stimulus and cell population.

The involvement of SHIP in TLR2 signaling has not been described previously. In the present experiments, we show that SHIP...
has a negative regulatory role on TLR2-induced neutrophil functions, which results in effects on the intensity and duration of Akt activation, generation of cytokines and chemokines, activation of p38 MAPK (p38) and ERK 1/2, and phosphorylation of the p65 subunit of NF-κB. In addition, the severity of TLR2-induced ALI is worse in the absence of SHIP. Such results demonstrate that SHIP participates in neutrophil activation and is important in modulating neutrophil-dependent proinflammatory responses.

Materials and Methods

Mice

SHIP−/− and SHIP+/− mice, previously described (16), 8–12 wk of age, were obtained from Dr. G. Krystal (Terry Fox Laboratory, Vancouver, British Columbia, Canada) and were bred in the University of Colorado Health Sciences Center animal facility. The mice were kept on a 12-h light/dark cycle with free access to food and water. All experiments were conducted in accordance with institutional review board-approved protocols.

Isolufurane was obtained from Abbott Laboratories. PGN was purchased from InvivoGen. RPMI 1640/25 mM HEPES/τ-glutamine was obtained from BioWhittaker, and FBS and penicillin/streptomycin were purchased from Gemini Bio-Products. Activation-specific Abs for phospho-Thr202/Tyr204 Erk1, phospho-Thr180/Tyr182 ERK2, phospho-Thr358/Tyr367 Akt, phospho-Ser536 p65, and total Erk1/2, p38, Akt and p56 NF-κB were purchased from Cell Signaling Technologies. HRP-labeled anti-rabbit Abs and chemiluminescence reagents were purchased from Bio-Rad. SB203580, U0126, and LY294002 were purchased from Calbiochem. All other reagents were purchased from Sigma-Aldrich unless otherwise noted in the text. Custom mixture Abs and columns for neutrophil isolation were purchased from StemCell Technologies.

Isolation and culture of bone marrow-derived mouse neutrophils

Mouse neutrophils were isolated from bone marrow as previously described, using negative immunoselection (3). To obtain the bone marrow cell suspension, the femur and tibia of a mouse were flushed with RPMI 1640. Tissue fragments were removed by rapid filtration through a glass wool column and cells collected by centrifugation. The cell pellets were resuspended in RPMI 1640 and 1% FCS and then incubated with primary Abs specific for cell surface markers F4/80, CD4, CD45R, CD5, and TER119 for 15 min at 4°C. This custom mixture (StemCell Technologies) is specific for T and B cells, RBC, monocytes, and macrophages. After a 15-min incubation, 100 μl of antimouse tetrameric Ab complexes were added, and the cells were incubated for 15 min at 4°C. Following this, 60 μl of colloidal magnetic dextran iron particles were added to the suspension and incubated for 15 min at 4°C. The entire cell suspension was then placed into a column surrounded by a magnet. The T cells, B cells, RBC, monocytes, and macrophages were captured in the column, allowing the neutrophils to pass through by negative-selection methods. Bone marrow neutrophils (2 × 107/0.5 ml) were cultured in RPMI 1640/0.2% FCS. The p38 inhibitor SB203580 (0.4 and 12 μM), the MEK 1/2 inhibitor U0126 (3 and 12 μM), and the isoform nonspecific PI3K inhibitor LY294002 (5 and 25 μM) were used at concentrations previously shown by our laboratory and others to inhibit selectively the kinase of interest (31, 32).

Neutrophil purity, as determined by Wright’s-stained cytopsin preparations, was consistently >97%. Flow cytometry was also used to analyze the purity of neutrophils. Neutrophils were stained with PE-labeled Gr1 and FITC-labeled CD11b mAbs (obtained from BD Pharmingen), according to the manufacturer’s instructions. On analysis, 99.62% of the CD11b+ population also stained positively for Gr1.

Cytokine ELISA

Immunoreactive TNF-α, IL-1β, IL-6, IL-10, MIP-2, and KC were quantified using commercially available ELISA kits (R&D Systems), according to the manufacturer’s instructions and as described previously (3).

EMSA

Nuclear extracts were prepared and assayed by EMSA as described previously (3). For the analysis of NF-κB, the κB-DNA consensus sequence of the Ig gene was used. Synthetic κB double-stranded sequences (with enhancer motifs underlined) were filled in and labeled with [α-32P]ATP using Sequenase DNA polymerase as follows: 5′-TTTTTCAGGCTCGG GACTTTCCAGCC-3′ and 3′-GCTTCGAGCCTGAAGCCTGTATT-5′.

Western blot analysis

Western blots for phosphorylated and total kinases were performed as described previously (3). Western blots for phosphorylated p65 (S536) and total p65 NF-κB were performed as previously described (3), using whole cell extracts. Parallel samples for total protein kinase were run with samples used for activation-specific phosphorylation analysis. Densitometry was performed using chemiluminescence system and analysis software (Bio-Rad) to determine the ratio between phosphorylated and total kinase.

Myeloperoxidase assay

Myeloperoxidase activity was assayed as reported previously (9). In brief, excised lungs from three to four mice per treatment group were frozen in liquid nitrogen, weighed, and stored at −86°C until used for the assay. Lungs were homogenized for 30 s in 1.5 ml of 20 mM potassium phosphate (pH 7.4) and centrifuged at 4°C for 30 min at 40,000 × g. The pellet was resuspended in 1.5 ml of 50 mM potassium phosphate (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide, sonicated for 90 s, then incubated at 60°C for 2 h and centrifuged. The supernatant was assayed for peroxidase activity corrected to lung weight.

Wet-to-dry lung weight ratios

Wet-to-dry ratio was determined as reported previously (9). All mice used for lung wet-to-dry weight ratios were of identical ages. Lungs were excised, rinsed briefly in PBS, blotted, and then weighed to obtain the “wet” weight. Lungs were then dried in an oven at 80°C for 7 days to obtain the “dry” weight.

IKK kinase (IKK) assay

The IKK assay was performed as described previously (33). In brief, cells were washed in PBS, then placed in lysis buffer (Cell Signaling Technologies) and rocked for 30 min on ice. After the cell extracts were centrifuged at 14,000 × g for 15 min at 4°C, the protein concentration of the supernatant was determined by bicinchoninic acid protein assay (Pierce), and 500 μg of protein were incubated overnight at 4°C in 300 μl of cell lysate buffer and protease inhibitor mixture (P-8383–40; Sigma-Aldrich) with 1.5 μg of mouse anti-IKKα or anti-IKKβ mAbs (Cell Signaling Technologies). Fifty microliters of 50% protein A-Sepharose were then added to each sample and incubated for 2 h at 4°C. The samples were centrifuged, and the beads were washed twice with lysis buffer (Cell Signaling Technologies), followed by three washings with kinase buffer (Cell Signaling Technologies). After this, the beads were incubated at 30°C in a final volume of 20 μl of kinase buffer with 1 μg of IκBα expressed in Escherichia coli as a 70-kDa tagged fusion protein (Santa Cruz Biotechnology), 100 μM ATP, and 0.5 μl of [γ-32P]ATP (5 μCi/sample). SDS sample buffer was then added to the protein A beads. The samples were boiled for 5 min and resolved on 8% SDS-PAGE. The gels were dried, and the intensity of the radioactive signal was quantified using a phospho imager and ImageQuant (Bio-Rad).

Statistical analysis

To limit variability and provide appropriate controls, for each experimental condition, the entire group of animals was prepared and studied at the same time. Mice in experimental groups were male, had the same birth date, and had been housed together. Separate groups of mice (n = 3–9/group) were used for wet-to-dry lung weight ratios, myeloperoxidase assay, Western blotting, IKK assays, ELISA, and EMSA. Data are presented as mean ± SEM for each experimental group. One-way ANOVA and the Tukey-Kramer multiple comparisons test (for multiple groups) or Student’s t test (for comparisons between two groups) were used. Values of p < 0.05 were considered significant.

Results

Neutrophils from SHIP−/− mice demonstrate increased TLR2-stimulated Akt activation

We previously demonstrated that stimulation of neutrophils through TLR2 produced prolonged activation of Akt (3). In the present experiments, we found a similar pattern of Akt activation in SHIP−/− neutrophils cultured with the TLR2 ligand PGN (Figs. 1A and 2).

Ser536 phosphorylation of Akt was at least 3-fold greater in PGN-stimulated SHIP−/− neutrophils at all time points during the
SHIP−/− neutrophils demonstrate increased TLR2-stimulated Akt activation. A, SHIP−/− and SHIP+/+ neutrophils were cultured with 50 μg/ml PGN for the times shown, and Western blots were used to determine levels of Ser473-phosphorylated (phospho-Akt(S473)) and total Akt. B, SHIP−/− and SHIP+/+ neutrophils were cultured with increasing concentrations of PGN for 4 h, and Western blots were used to determine levels of Tyr1020 phosphorylated (phospho-SHIP(Y1020)). In both A and B, densitometry results showing the fold increase in phosphorylated Akt or SHIP are presented. In B, only the results for the SHIP+/+ neutrophils are presented because, as expected, no SHIP or phosphorylated SHIP was detected in the SHIP−/− neutrophils. The data shown are representative of three independent experiments.

Enhanced cytokine release in TLR2-stimulated SHIP−/− neutrophils

In previous experiments (3), we found that release of proinflammatory cytokines, such as TNF-α, was dependent on PI3K and Akt in TLR2-stimulated neutrophils. The studies presented in Figs. 1A and 2, showing that SHIP−/− neutrophils cultured with PGN exhibit increased activation of Akt, suggested that such neutrophils might produce greater amounts of cytokines than those from control SHIP+/+ mice. To examine this hypothesis, we measured the secretion of IL-1β, IL-6, IL-10, TNF-α, KC, and MIP-2 in SHIP−/− and SHIP+/+ neutrophils stimulated with PGN.

As shown in Figs. 3 and 4, SHIP−/− neutrophils not only produced more IL-1β, IL-6, IL-10, TNF-α, and MIP-2 after culture with PGN than did SHIP+/+ cells, but also were more sensitive to PGN than the SHIP+/+ neutrophils. This increase in the response of SHIP−/− neutrophils was not found for all proinflammatory mediators because there were no differences in the release of KC between PGN-stimulated SHIP−/− and SHIP+/+ neutrophils (Fig. 4). Of note, whereas release of TNF-α, IL-6, and IL-10 was increased for all concentrations of PGN in SHIP−/− neutrophils, enhanced secretion of IL-1β and MIP-2 was only found for the highest concentrations of PGN in SHIP−/− as compared with SHIP+/+ neutrophils. Such results indicate that SHIP participates in a variable manner in modulating cytokine release in neutrophils stimulated via TLR2.

Phosphorylation of the p65 subunit of NF-κB is increased in SHIP−/− neutrophils

We have previously shown (3) that blockade of PI3K inhibits PGN-induced Ser328 phosphorylation of the NF-κB p65 subunit, a critical step in optimizing the transcriptional efficacy of NF-κB. In those studies, there were no effects of PI3K and Akt inhibition on TLR2-induced nuclear translocation of NF-κB, suggesting that the...
mechanism through which Akt modulated NF-κB-dependent transcription and expression of proinflammatory cytokines was by affecting phosphorylation of p65.

As shown in Fig. 5A, the absence of SHIP did not affect nuclear translocation of NF-κB in PGN-stimulated neutrophils. Similarly, there were no differences in activation of IKKα and IKKβ in SHIP−/− and SHIP+/+ neutrophils after culture with PGN (Fig. 5B). However, culture of SHIP−/− neutrophils with PGN resulted in greater phosphorylation of p65Ser536 than that found in SHIP+/+ neutrophils (Fig. 5C). The increased levels of Ser53-phosphorylated p65 occurred earlier and were more persistent in SHIP−/− compared with in SHIP+/+ neutrophils.

SHIP−/− neutrophils demonstrate increased activation of ERK1/2 and p38 after TLR2 stimulation

In previous studies (3), we found that inhibition of PI3K in TLR2-stimulated neutrophils resulted in decreased activation of ERK1/2 and p38 kinases. To determine whether there were also interactions between SHIP and these kinases, we examined activation of ERK1/2, p38, and JNK in PGN stimulated SHIP−/− and SHIP+/+ neutrophils. As shown in Fig. 6, SHIP−/− neutrophils demonstrated increased activation of ERK1/2 and p38, but not JNK, after culture with PGN.

To address the relative importance of p38 and ERK1/2 in increased cytokine generation from SHIP−/− neutrophils, we used selective inhibitors for each of these kinases in PGN-stimulated cultures. As shown in Fig. 7, whereas inhibition of p38 decreased cytokine production to a similar extent in SHIP−/− and SHIP+/+ derived neutrophils, blockade of MEK 1/2, the kinase upstream to ERK1/2, appeared to have a greater effect on cytokine generation by SHIP−/− neutrophils, particularly at higher concentrations (12 μM).

The severity of ALI is increased in SHIP−/− mice

The above experiments demonstrated increased activation of Akt as well as p38 and ERK1/2, phosphorylation of the NF-κB p65 subunit, and proinflammatory cytokine release in TLR2-stimulated SHIP−/− neutrophils. Because neutrophils play a central role in the
development of ALI (8, 9), we hypothesized that the severity of TLR2-induced ALI might be more severe in SHIP\textsuperscript{−/−} mice (Fig. 8).

To examine this issue, we exposed SHIP\textsuperscript{−/−} and SHIP\textsuperscript{+/+} mice to intratracheal PGN and then examined measures of lung injury.

Intratracheal PGN administration did not affect the degree of interstitial lung edema (lung leak) in SHIP\textsuperscript{+/+} mice but resulted in a significant increase in this parameter of lung injury in SHIP\textsuperscript{−/−} mice (Fig. 8A). Similarly, the degree of neutrophil infiltration,
measured by lung myeloperoxidase levels, showed minimal increase in SHIP\(^{+/−}\) mice after exposure to PGN but was increased by \(>12\)-fold from baseline values in SHIP\(^{−/−}\) mice (Fig. 8B).

**FIGURE 7.** Involvement of p38 and ERK 1/2 MAPK in PGN-induced cytokine responses of SHIP\(^{+/+}\) and SHIP\(^{−/−}\) neutrophils. SHIP\(^{+/+}\) and SHIP\(^{−/−}\) neutrophils were cultured for 4 h with 50 \(\mu\)g/ml PGN, in the presence and absence of LY294002 (LY) at 25 and 5 \(\mu\)M, SB203580 (SB) at 12 and 0.4 \(\mu\)M, and U0126 (U0) at 12 and 3 \(\mu\)M, then levels of TNF-\(\alpha\) (A), IL-10 (B), and MIP-2 (C) in the culture supernatants were measured. The data shown are representative of three independent experiments; means ± SEM are presented.

**FIGURE 8.** PGN-induced lung injury is more severe in SHIP\(^{−/−}\) mice. SHIP\(^{+/+}\) and SHIP\(^{−/−}\) mice were subjected to intratracheal administration of 10 mg/kg PGN in PBS or PBS alone, and 24 h later, lung wet-to-dry ratios (A) and myeloperoxidase concentrations (B) were measured. The increases above values present in mice receiving PBS alone are shown in each figure. Each group contained three mice. Means ± SEM are presented. *, \(p < 0.05\) for control vs PGN treated; and #, \(p < 0.05\) for SHIP\(^{+/+}\) vs SHIP\(^{−/−}\) PGN treated.

**Discussion**

In the present studies, we used neutrophils lacking the SHIP to address its role in TLR2-induced neutrophil activation. These experiments demonstrated that SHIP played a negative regulatory role in this setting. In particular, in neutrophils lacking SHIP, stimulation through TLR2 resulted in increased proinflammatory cytokine release, phosphorylation of the p65 NF-\(\kappa\)B subunit, and activation of the kinases Akt, p38, and ERK 1/2. In addition, ALI was more severe in transgenic SHIP\(^{−/−}\) mice, indicating that the negative regulatory role of SHIP found in vitro translated into relevant in vivo effects in neutrophil driven acute inflammatory responses.

These experiments used mice and neutrophils that lacked the SHIP1 isoform of SHIP (25). SHIP1 acts as a negative regulator of PI3K and Akt activation by dephosphorylating the second messenger PIP\(_2\) to phosphatidyl 3,4 biphosphate, thereby reducing Akt activation (25). SHIP1 is expressed predominantly in leukocytes (25). However, it is not the only PIP\(_2\) phosphatase present in neutrophils because neutrophils express SHIP2, phosphatase and tensin homologue deleted on chromosome 10, and phosphoinositide-specific inositol polyphosphate 5-phosphatase IV, all of which also possess PIP\(_2\) phosphatase activity (34, 35). However, despite the existence of these other PIP\(_2\) phosphatases, the present results demonstrate a central role for SHIP1 in both in vitro and in vivo neutrophil responses to TLR2 stimulation. Whether SHIP1 has similar proinflammatory effects in other settings of neutrophil activation remains to be determined.

One recent study found that SHIP1 had a positive, rather than a negative, regulatory role in macrophages cultured with LPS, a TLR4 ligand (29). In that setting, SHIP\(^{−/−}\) macrophages demonstrated decreased release of IL-6 and TNF-\(\alpha\) after stimulation with LPS (29). Additionally, the same group reported that macrophages from mice deficient in phosphatase and tensin homologue deleted on chromosome 10 displayed similar responses of diminished cytokine release after exposure to LPS (14). However, a recent article by Sly et al. (16) found that SHIP\(^{−/−}\) mice were more sensitive to LPS-induced mortality and that SHIP\(^{−/−}\) macrophages were hyperresponsive to LPS, particularly in terms of cytokine release (TNF-\(\alpha\), IL-6, and IL-1\(\beta\)).

In previous experiments, we found that signaling through PI3K/Akt participated in TLR2-induced neutrophil activation (3). In particular, inhibition of PI3K resulted in decreased cytokine release and NF-\(\kappa\)B p65 phosphorylation in TLR2-stimulated neutrophils. The present studies are consistent with such results and confirm that signaling pathways involving PI3K and Akt are involved not only in modulating NF-\(\kappa\)B activation and proinflammatory cytokine release in neutrophils after TLR2 engagement but also contribute to the activation of other kinases, such as p38 and ERK 1/2. Although we and others have shown previously (3, 12, 21, 36, 37) a positive regulatory role for PI3K/Akt in NF-\(\kappa\)B activation and NF-\(\kappa\)B-dependent cytokine expression in neutrophils and other cell populations, this has not been a universal finding. In particular, several studies, although not in neutrophils, have reported a negative relationship between TLR4-activated NF-\(\kappa\)B and PI3K/Akt (13, 14, 29). Such findings would suggest that the role of PI3K and Akt in modulating NF-\(\kappa\)B activation may be cell type and stimulus dependent.

Although translocation of p50:p65 heterodimers into the nucleus is necessary for NF-\(\kappa\)B-dependent transcription, phosphorylation of the p65 subunit at serine residues, including 276, 529, and 536, is required for maximal transcripational activity (37–40). The molecular mechanism by which p65 phosphorylation increases
NF-κB activity is not well defined but may involve reversible acetylation, which is linked to the duration of NF-κB activation (41). Consistent with previous reports from our laboratory (3) and others (23, 42), the PI3K-Akt axis does not appear to be involved in nuclear translocation of NF-κB but does increase the duration and magnitude of p65 (Ser536) phosphorylation (22–24, 38, 41, 43, 44). This finding suggests a mechanism by which SHIP and its downstream effects on Akt activation might affect the generation of NF-κB-dependent cytokines and chemokines, such as TNF-α, MIP-2, IL-6, and IL-10. However, the present experiments, which found that SHIP and Akt are also involved in the TLR2-regulated induction of p38 and ERK 1/2, indicate that SHIP may participate in other mechanisms affecting cytokine release. In particular, although p38 and ERK 1/2 both participate directly in modulating NF-κB-dependent transcription (45), they also affect cytokine expression (46) through interactions with other transcriptional factors (47) and coactivators (48), as well as through translational and posttranslational mechanisms (49).

The present experiments, as well as previous studies by our laboratory and others (3, 16, 21), indicate that the PI3K/Akt signaling pathway participates in TLR2- and TLR4-induced proinflammatory pathways in neutrophils and macrophages, as well as in clinically relevant inflammatory processes, such as ALI (11). The involvement of SHIP in regulating Akt activation in such situations may provide therapeutic opportunities. Increasing SHIP activity in acute inflammatory conditions may result in down-regulation, but not elimination, of Akt activity, thereby decreasing deleterious inflammatory responses without inducing immunosuppression, such as would be expected with total blockade of Akt activation. Future experiments will be necessary to examine this hypothesis.

Disclosures

The authors have no financial conflict of interest.

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