Toll-like Receptor 4 Signaling Regulates Cytosolic Phospholipase A\textsubscript{2} Activation and Lipid Generation in Lipopolysaccharide-stimulated Macrophages* 

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Inflammatory lipid mediators such as prostaglandins and leukotrienes play crucial roles in the pathogenesis of bacterial lipopolysaccharide (LPS)-induced inflammation. Cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}) is a key enzyme in the generation of pro-inflammatory lipid mediators. Here, we found that Toll-like receptor 4 (TLR4) is essential for LPS-induced cPLA\textsubscript{2} activation and lipid release. Inhibition of TLR4 protein expression by TLR4 small interfering RNA or neutralization of TLR4 by the specific antibody against TLR4/MD2 blocked cPLA\textsubscript{2} phosphorylation and cPLA\textsubscript{2}-hydroyzed arachidonic acid release. Furthermore, activation of the TLR4 signaling pathway by LPS regulated cPLA\textsubscript{2} activation and lipid release. cPLA\textsubscript{2} phosphorylation and cPLA\textsubscript{2}-hydroyzed lipid release were significantly impaired when TLR4 adaptor protein, either MyD88 or TRIF, was knocked down in LPS-stimulated macrophages. Similarly, LPS-induced arachidonate release was inhibited in cells transfected with a dominant-negative MyD88 or TRIF construct. Subsequently, cPLA\textsubscript{2} activation could be suppressed by inhibition of the TLR4 adaptor protein-directed p38 and ERK MAPK pathways. These findings suggest that, in LPS-induced inflammation, the TLR4-mediated MyD88- and TRIF-dependent MAPK pathways result in cPLA\textsubscript{2} activation and production of pro-inflammatory lipid mediators.

Since the identification of the Toll-like receptor (TLR) family, the pathways of pro-inflammatory cytokine production in response to LPS and other pathogen-associated molecular patterns from invading microbes have been well demonstrated (9, 10). In mammals, the TLR family comprises at least 11 members (TLR1–TLR11) expressed on the surface of macrophages and other innate immune cells (11, 12). TLRs act as primary innate immune sensors, each of which specifically recognizes distinct pathogen-associated molecular patterns (10, 12–14). Stimulation of TLRs by TLR ligands triggers the recruitment of the cytoplasmic adaptor protein MyD88 and accordingly culminates in the activation of two distinct downstream signaling pathways, the transcription factor NF-κB and MAPK pathways, which induce the expression of inflammatory cytokines (15). TLR4 is the signaling receptor of LPS (16–18). After recognizing LPS in the presence of LPS-binding protein, CD14, and MD2 protein, TLR4 activates the common MyD88-dependent signaling pathway as well as a MyD88-independent pathway that is unique to TLR3 and TLR4 signaling pathways, leading to interferon-β production (15, 19–21). In the MyD88-independent pathway, TLR4 interacts with the adaptor protein TRIF, instead of MyD88, to activate NF-κB and MAPK as well as interferon-β induction (20, 21). Thus, the LPS signaling pathways show a bipartite nature. Both TRIF and MyD88 are independently capable of initiating signaling events that lead to pro-inflammatory cytokine production (19, 21).

However, the mechanism by which LPS induces the production of pro-inflammatory lipid mediators in macrophages remains to be further determined. PAF and eicosanoids, including leukotrienes, prostaglandins, and thromboxanes, are the major constituents of pro-inflammatory lipid mediators, which play a critical role in inflammation and host defense (22–26). They have been implicated in the pathogenesis of asthma, sepsis, and other inflammatory diseases as well (22, 27–29). When LPS and other inflammatory stimuli activate macrophages, the lipid mediators are synthesized de novo from membrane phospholipid through a cascade of enzymes (22). The initial step of eicosanoid biosynthesis, which is thought to be the rate-limiting step, is arachidonic acid release from membrane phospholipids by activation of cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}). The liberated arachidonic acid is the common precursor molecule of all eicosanoids and is in turn converted to prostaglandins and thromboxanes by the cyclooxygenase pathway and to leukotrienes by the 5-lipoxygenase pathway (22, 23). Concomitant with the release of arachidonate, lysophospholipid is formed and can be enzymatically converted to PAF (30).

As an important rate-limiting enzyme in the hydrolysis of arachidonic acid release, cPLA\textsubscript{2} plays a key role in initiating and regulating the multistage biosynthetic process of eicosanoid production (22, 31). cPLA\textsubscript{2} is a member of a diverse superfamily of PLA\textsubscript{2} enzymes that hydrolyze fatty acid from the sn-2 position of phospholipids (32). However, cPLA\textsubscript{2} is the only well characterized PLA\textsubscript{2} that is highly selective for phospholipids containing arachidonic acid at the sn-2 position (33–
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35), cPLA2 is activated by phosphorylation by MAPKs and translocated to the membrane in response to submicromolar concentrations of Ca2+ (33, 36–38). cPLA2-deficient mice have provided the most definitive evidence for the central role of cPLA2 in eicosanoid and PAF production (39–42) as well as in the pathogenesis of several inflammatory diseases such as adult respiratory distress syndrome due to bacterial sepsis (43, 44). Peritoneal macrophages derived from cPLA2 knockout mice have diminished ability to generate leukotrienes, prostaglandins, and PAF (40). Furthermore, the bone marrow-derived mast cells from these mice fail to produce eicosanoids in either immediate or delayed phase responses (42). Animals that lack cPLA2 are resistant to bronchial hyper-reactivity and have significantly reduced pulmonary edema, polymorphonuclear leukocyte sequestration, and deterioration of gas exchange caused by LPS administration (43). The role of cPLA2 as an important mediator of inflammatory diseases has made it a therapeutic target (41, 45–47).

Because cPLA2 is essential for production of lipid mediators, to address the mechanism by which cPLA2 is activated and leads to lipid production in macrophage responses to LPS will be a key to understanding the production of pro-inflammatory lipid mediators in LPS-induced inflammation. Here, we report that cPLA2 activation and lipid release are regulated by TLR4 signaling in LPS-activated macrophages. The regulation is through TLR4-mediated MyD88- and TRIF-dependent MAPK signaling pathways.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Thioetheramide-PC and methyl arachidonoyl fluorophosphonate were purchased from Cayman Chemical Co. (Ann Arbor, MI), and SB 203580 and U0126 were purchased from Calbiochem. LY-294,002 and wortmannin were from Sigma. LPS from Escherichia coli serotype O111:B4 was purchased from List Biological Laboratories (Campbell, CA), and LPS and lipid A from Escherichia coli serotype R515 were purchased from Alexis Biochemicals (San Diego, CA). The monoclonal antibody against mouse TLR4/MD2 (MTS510, serotype R515, O111:B4) was purchased from BioXCell. MyD88 were from Apotech Corp. (Epalinges, Switzerland). Antibodies against phospho-p38, p38, phospho-ERK1/2, ERK1/2, phospho-Ser505 are regulated by TLR4 signaling in LPS-activated macrophages. Antibodies against TLR4, IL6, β-actin, Akt, and phospho-Akt were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The antibodies against TRIF and TRLR3 were from R&D Systems. Cells Culture—The mouse macrophage cell line RAW264.7 was obtained from American Type Culture Collection (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium (BIOSOURCE, Camarillo, CA) supplemented with 10% fetal calf serum (Invitrogen). Expression Plasmids and Transient Transfection—pE-BOS-TRIFANAC for dominant-negative TRIF was a gift from Dr. S. Akira (Osaka University, Osaka, Japan). TRIF was a gift from Dr. Douglas Golenbock (University of Massachusetts Medical School). MyD88 was a gift from Dr. David Segal (NCI, National Institutes of Health). Dominant-negative MyD88 and TRLR3 were purchased from InvivoGen (San Diego, CA). The expression plasmids were transfected into the mouse macrophage cell line RAW264.7 by Nucleofection electroporating transfection (Amaxa Inc., Gaithersburg, MD) following the manufacturer’s directions. [3H]Arachidonic acid release and Western blot analysis were performed after 36 h of transfection.

RNA Interference—For the plasmid constructs of mouse TLR4, MyD88, and TRIF knockdown RAW264.7 cells were grown on 6-well plates with stimuli or other treatment as indicated. Cells were lysed in cell lysis buffer (0.3 ml/well; 20 mM Hepes (pH 7.4), 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM Na3VO4, 1% Triton X-100, and 10% glycerol) containing protease inhibitor mixture (Roche Applied Science, Basel, Switzerland). The cell lysates were centrifuged, and equivalent amounts of lysate protein were loaded for nolucetidial membranes. The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline/Tween (20 mM Tris-HCl (pH 7.4), 137 mM NaCl, and 0.1% Tween 20). The blots were incubated primary antibodies overnight at 4°C and washed with Tris-buffered saline/Tween. The blots were developed with the ECL chemiluminescence detection kit (Amersham Biosciences), and the signals were captured on x-ray films or an Image Station 440CF (Eastman Kodak Co.).

[^H]Arachidonic Acid Release—RAW264.7 cells; TLR4, MyD88, or TRIF knockdown RAW264.7 cells; or cells transfected with the plasmid with or without wild-type or dominant-negative mutant TRIF and MyD88 were incubated overnight in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 0.5 µCi of [5,6,8,9,11,12,14,15-3H]arachidonic acid (150–230 Ci/mmol/ml; Amersham Biosciences). The medium was removed, and the cells were washed thoroughly and incubated in fresh medium with or without the various treatments as indicated. At the time points indicated, the media were collected and centrifuged, and the radioactivity was determined in the supernatants. The data are presented as the mean ± S.D. of -fold stimulation compared with the control in triplicate experiments.

Dual-Luciferase Reporter Assay for NF-κB Activation—RAW264.7 cells or TRIF knockdown RAW264.7 cells were cultured until they reached 70–85% confluence. The NF-κB luciferase reporter construct (a gift from the laboratory of Dr. Ulrich Siebenlist, NIAID, National Institutes of Health) was cotransfected with a Renilla luciferase reporter gene (Promega Corp., Madison, WI) into the cells by Nucleofection electroporating transfection. The cells were seeded into 12-well plates at a density of 6 × 10^5 cells/well. The following day, the cells were treated
as indicated. After 6 h of stimulation, the cells were lysed in passive lysis buffer (Promega), and reporter gene activity was measured using the Dual-Luciferase assay reporter system (Promega Corp.) and a Victor2 plate reader luminometer (PerkinElmer Life Sciences). In all cases, the data were normalized for transfection efficiency and are presented as the means ± S.D. of -fold stimulation in triplicate experiments.

RESULTS

LPS Induces cPLA₂ Activation in Mouse RAW264.7 Macrophages—To examine whether TLR4 is required for cPLA₂ activation, we used LPS preparations to stimulate the mouse macrophages for various times. We then tested cPLA₂ activation first by measuring phosphorylation of cPLA₂ by Western blot analysis with the antibody against phospho-cPLA₂ or total cPLA₂. Fig. 1A shows that phosphorylation of cPLA₂ became evident at 30 min after LPS stimulation, peaked at 2 h, and slowly declined thereafter. However, total cPLA₂ did not show any change in expression at the different time points. As a positive control, LPS-induced IκBα degradation, which results in the release and activation of NF-κB, was observed at 30 min and returned to the base-line level by 2–3 h (Fig. 1A), suggesting that the LPS preparation used to induce the activity of cPLA₂ in our experiments activates the TLR4 signaling pathway. We next determined the activation of cPLA₂ by measuring the release of its catalytic product arachidonic acid from [3H]arachidonic acid-labeled RAW264.7 cells. After stimulation with different types of LPS and lipid A (which is the core component of LPS),
the arachidonic acid release was significantly increased in an LPS dose-dependent manner (Fig. 1B). Although cPLA₂ is a major enzyme of arachidonic acid release from membrane phospholipids, secretory PLA₂, another member of the PLA₂ superfamily with a low molecular mass, may also mediate arachidonic acid release (48). To determine whether LPS-induced arachidonic acid release is specifically catalyzed by cPLA₂, the specific chemical inhibitors of cPLA₂ and secretory PLA₂ were used to treat RAW264.7 cells. As shown in Fig. 1C, the cPLA₂ inhibitor methyl arachidonyl fluorophosphonate markedly blocked arachidonic acid release by LPS. In contrast, the secretory PLA₂ inhibitor thioetheramide-PC did not inhibit the LPS response, suggesting that arachidonic acid release in response to LPS is due mainly to activation of cPLA₂.

TLR4 Mediates LPS-induced cPLA₂ Activation and Lipid Release—Two lines of evidence suggest that TLR4 is the receptor that mediates the LPS signal to cPLA₂ activation and lipid release. First, RAW264.7 cells were stably transfected with a vector expressing MyD88 siRNA or nonspecific siRNA. Western blot analysis indicated the silencing of TLR4 expression in RAW264.7 cells by MyD88 siRNA (Fig. 2C). To further confirm the knockdown of TLR4 signaling in macrophages, we measured LPS-induced NF-κB promoter activity using the luciferase reporter plasmid in TLR4-siRNA-treated RAW264.7 cells. As shown in Fig. 2D, NF-κB promoter activity was suppressed after LPS stimulation, suggesting the inhibition of TLR4-mediated NF-κB activation by TLR4 siRNA. We next examined LPS-induced arachidonic acid release and cPLA₂ phosphorylation in TLR4 knockdown RAW264.7 cells. The results in Fig. 2E show that the arachidonic acid release induced by LPS was suppressed in TLR4 siRNA-treated cells compared with control siRNA-treated cells. In addition, Western blotting showed reduced cPLA₂ phosphorylation in TLR4 knockdown RAW264.7 cells after LPS stimulation (see Fig. 4).

Both the TRIF and MyD88 Adaptor Proteins of the TLR4 Signaling Pathway Are Involved in LPS-induced cPLA₂ Activation and Lipid Release—Both MyD88- and TRIF-dependent pathways in TLR4 signaling are capable of activating downstream NF-κB and MAPK pathways (20, 21). We next asked whether TRIF and MyD88 adaptor proteins are required for cPLA₂ activation and lipid release. We generated MyD88 and TRIF knockdown RAW264.7 cells by transfection of MyD88 and TRIF siRNA constructs into the macrophages, respectively. Western blot analysis using antibodies against MyD88 and TRIF showed that MyD88 protein expression was knocked down by MyD88 siRNA, but not by TRIF siRNA. Likewise, TRIF siRNA inhibited TRIF protein expression, but did not affect MyD88 protein expression (Fig. 3A). Subsequently, cPLA₂-hydrolyzed arachidonic acid release and cPLA₂ phos-
phorylation were assessed in the MyD88 and TRIF knockdown macrophages after LPS stimulation. Arachidonic acid release was suppressed in both the MyD88 and TRIF knockdown macrophages. However, no inhibition of arachidonic acid release was observed in the cells treated with control siRNA (Fig. 3B). cPLA₂ phosphorylation was significantly reduced in both adaptor protein knockdown macrophages as well (Fig. 4). In addition, overexpression of wild-type TRIF and MyD88 in the macrophages slightly induced cPLA₂-hydrolyzed lipid release (Fig. 3C). In contrast, overexpression of the dominant-negative form of MyD88 or TRIF suppressed LPS-stimulated arachidonic acid release (Fig. 3D). To further study whether TRIF or MyD88 is capable of independently transmitting the TLR ligand signal from the cell surface to the cytoplasm for cPLA₂ activation, we used the TLR3 ligand poly(I:C) RNA, which specifically activates the TRIF adaptor protein, and the TLR9 ligand CpG DNA, which activates only the MyD88 adaptor protein, to stimulate macrophages. Because there is a lower expression of endogenous TLR3 compared with TLR4 in the RAW264.7 cell line (64, 65), a TLR3-expressing construct was transfected into the macrophages. Poly(I:C) only slightly induced cPLA₂-hydrolyzed lipid release before TLR3 transfection (Fig. 3E), but significantly induced arachidonic acid release and cPLA₂ phosphorylation after TLR3 overexpression in the macrophages (Fig. 3F). Fig. 3(G and H) shows CpG DNA ligand-induced arachidonic acid release and cPLA₂ phosphorylation. Therefore, either TRIF or MyD88 is able to independently activate cPLA₂.

**TLR4-mediated MAPK Pathways Are Required for LPS-induced cPLA₂ Activation and Lipid Release**—We have demonstrated that LPS induced cPLA₂ activity through TLR4 and its adaptor proteins. The MAPK pathway is one of the major downstream pathways in TLR4 signaling. As shown in Fig. 4, knockdown of TLR4 or TLR4 adaptor proteins MyD88 and TRIF diminished LPS-induced phosphorylation of p38 and ERK MAPKs. In addition, it has been shown that cPLA₂ activation requires phosphorylation by MAPKs and that cPLA₂ is a substrate of p38 and ERK MAPKs (36, 37). Therefore, we next studied whether the TLR4-mediated MAPK pathway is required for LPS-induced cPLA₂ activation and lipid release. Fig. 5A shows that inhibitors of p38 (SB 203580) and ERK (U0126) inhibited LPS-induced phosphorylation of p38 and ERK1/2, respectively, suggesting that these inhibitors block TLR4-mediated MAPK pathways. Similarly, LPS-stimulated cPLA₂ phosphorylation (Fig. 5B) and cPLA₂-hydrolyzed arachidonic acid release (Fig. 5C) were markedly inhibited by these MAPK inhibitors as well. Thus, the TLR4-mediated MyD88- and TRIF-dependent MAPK pathways are involved in LPS-induced cPLA₂ activation.

The Phosphoinositide 3-Kinase (PI3K)/Akt Pathway Is Not Required for TLR4-mediated cPLA₂ Activation by LPS—It has been reported that the PI3K/Akt pathway participates in LPS-induced TLR4 signaling, leading to NF-κB activation (55–57). To determine whether the PI3K/Akt pathway affects TLR4-mediated cPLA₂ activation and lipid release by LPS, RAW264.7 macrophages were treated with LPS with or without two specific pharmacological inhibitors (LY-294,002 and wortmannin) that block the activation of PI3K by different mechanisms. PI3K activation was evaluated by immunoblot analysis of phosphorylated Akt. As shown in Fig. 6A, LPS stimulation of the cells resulted in phosphorylation of Akt. Preincubation with either LY-294,002 or wortmannin completely abolished LPS-induced activation of Akt. However, blockade of...
The evidence shows that LPS induces cPLA₂ activation and lipid release. Furthermore, direct evidence was obtained by treating the macrophages with TLR4 siRNA or antibody MTS510 (raised against TLR4/MD2). Antibody MTS510, which is capable of neutralizing the TLR4-MD2 complex on the cell surface, blocked cPLA₂-hydrolyzed arachidonate release induced by LPS and lipid A. On the other hand, LPS-induced arachidonate release and cPLA₂ phosphorylation were significantly inhibited in TLR4 knockdown macrophages by TLR4 siRNA. Together, these data strongly support the conclusion that TLR4 is required for cPLA₂ activation and lipid release in response to LPS stimulation. In addition, our data have shown that not only LPS, but also the ligands of TLR3 and TLR9 are capable of inducing cPLA₂ activation, suggesting that cPLA₂ activation can be regulated by different members of the TLR family.

As described above, the LPS-induced signaling pathway has a bipartite nature. Mouse macrophages with defective or missing TRIF or MyD88 protein exhibit severely impaired production of inflammatory cytokines in response to LPS (20, 21). MyD88 and TRIF might each be capable of independently initiating the major signaling pathway for production of inflammatory cytokines. The reason that a cell needs two branches of the pathway responsible for signals that emanate from the LPS receptor remains unclear. To determine which branch of the TLR4 signaling pathway is responsible for cPLA₂ activation and lipid release, we examined cPLA₂ phosphorylation and cPLA₂-hydrolyzed arachidonic acid release in MyD88 or TRIF protein knockdown macrophages by siRNAs and dominant-negative mutants after LPS challenge. LPS-induced arachidonate release and cPLA₂ phosphorylation were impaired in either adaptor protein-deficient macrophages. In addition, cPLA₂ activation and lipid release could be induced by poly(I:C) RNA and CpG DNA ligands, which specifically activate the TRIF and MyD88 pathways through TLR3 and TLR9, respectively, indicating that MyD88 and TRIF may independently mediate LPS-induced cPLA₂ activation and pro-inflammatory lipid production.

cPLA₂ is essential for both the immediate and delayed phases of eosinophil generation in the mouse bone marrow-derived mast cell response to stimuli (42). Activation of cPLA₂ for the rapid generation of leukotrienes is thought to be through the post-translational mechanism of cPLA₂ phosphorylation (53), whereas in a more delayed response (such as in response to inflammatory cytokines and certain growth factors), cPLA₂ activity may be regulated by its expression level (34). Thus, cPLA₂ activation can be regulated by both transcriptional and post-translational mechanisms. Our data show that activation of cPLA₂ was accompanied by its phosphorylation in macrophages exposed to LPS. However, no increased expression of cPLA₂ was observed during 6 h of stimulation by LPS, suggesting that, in immediate response to LPS stimulation, cPLA₂ is activated through the post-translational mechanism leading to rapid generation of pro-inflammatory lipid mediators.

cPLA₂ phosphorylation is an important mechanism for cPLA₂ activation (42). Extracellular ligands that activate cPLA₂ cause the phosphorylation of the catalytic domain at conserved serines 505 and 727 (36, 48). The mutation of Ser⁵⁰⁵ to Ala is known to block cPLA₂ activation by members of the MAPK family (36), but the functional relevance of Ser⁷²⁷ has not been reported. Previous reports have demonstrated that either one or multiple MAPK members are able to activate cPLA₂ upon extracellular stimulation and the ability of a specific receptor to trigger the different MAPK family members (48, 54). TLR4 signaling activates multiple members of the MAPK family, including ERK1/2, p38 kinase, and JNK (c-Jun NH₂-terminal kinase) (15). We have found that either a pH-8-specific or an ERK1/2-specific inhibitor was capable of blocking...
TLR4-mediated cPLA2 phosphorylation and cPLA2-hydrolyzed arachidonic acid release after LPS stimulation. Thus, both the ERK1/2 and p38 signaling pathways act to activate cPLA2, in response to LPS challenge. Whether cPLA2 phosphorylation is sufficient to account for prolonged activation of cPLA2 is uncertain. cPLA2 activation may also be enhanced by binding to lipids such as phosphatidylinositol bisphosphate (58) and ceramide 1-phosphate (59). LPS may induce intracellular release of ceramide (60). Ceramide 1-phosphate has been reported to bind to the C2 domain of cPLA2, and to increase its sensitivity to intracellular calcium.

PI3K and the downstream serine/threonine kinase Akt play important roles in host defense (61). It has been shown that LPS stimulation of inflammatory cells activates the PI3K/Akt pathway (55–57, 62). Although the signaling steps between TLR4 and activation of PI3K have not been characterized, it has been suggested that the activation of PI3K by LPS is TLR4- and MyD88-dependent (56, 57). PI3K and Akt participate in the TLR4 signaling pathway to regulate NF-kB activation induced by LPS (55, 62, 63). However it is not clear whether the LPS-activated PI3K/Akt pathway involves TLR4-mediated cPLA2 activation induced by LPS. We have found that incubation of macrophages with LPS resulted in phosphorylation of Akt. Addition of the PI3K inhibitors to the macrophage cultures blocked LPS-induced Akt phosphorylation. However, the blockade of the PI3K/Akt pathway did not impair LPS-induced cPLA2 phosphorylation and arachidonic acid release. Therefore, PI3K/Akt is not necessary for TLR4-dependent cPLA2 activation and lipid release.

TLR4 studies have focused on the mechanism by which TLR4 mediates the production of pro-inflammatory cytokines. Our data now suggest that TLR4 may also be involved in the generation of potent pro-inflammatory lipid mediators by regulating cPLA2 activation. This novel therapies for LPS-induced inflammation.

Acknowledgments—We thank Dr. Harris Bernstein for reading this manuscript and Sura Alsatou for DNA sequencing.

REFERENCES

1. Raetz, C. R., and Whitfield, C. (2002) Annu. Rev. Biochem. 71, 635–700C. R. H.
2. Cohen, J. (2002) Nature 416, 885–891
3. Edwin, S., Amersfoort, V., Theo, J. C., Berkel, V., and Kuiper J. (2003) Annu. Rev. Microbiol. 47, 379–414
4. Beutler, B., and Rietschel, E. T. (2003) Science 303, 1522–1526
5. Li, X., Tupper, J. C., Bannerman, D. D., Winn, R. K., Rhodes, C. J., and Harlan, J. M. (1997) J. Biol. Chem. 272, 27723–27729
6. Ulevitch, R. J., and Tobias, P. S. (1995) Science 279, 615–625
7. Janeway, C. A., Jr., and Medzhitov, R. (2002) Annu. Rev. Immunol. 20, 197–216
8. Serhan, C. N. (2000) J. Biol. Chem. 275, 18211–18218
9. Six, D. A., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1488, 1–19