Endotoxin-binding Proteins Modulate the Susceptibility of Bacterial Endotoxin to Deacylation by Acyloxyacyl Hydrolase*

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Acyloxyacyl hydrolase (AOAH) is an eukaryotic lipase that partially deacylates and detoxifies Gram-negative bacterial lipopolysaccharides and lipooligosaccharides (LPSs or LOSs, endotoxin) within intact cells and inflammatory fluids. In cell lysates or as purified enzyme, in contrast, detergent is required for AOAH to act on LPS or LOS (Erwin, A. L., and Munford, R. S. (1990) J. Biol. Chem. 265, 16444–16449 and Katz, S. S., Weinrauch, Y., Munford, R. S., Elshach, P., and Weiss, J. (1999) J. Biol. Chem. 274, 36579–36584). We speculated that the sequential interactions of endotoxin (E) with endotoxin-binding proteins (lipopolysaccharide-binding protein (LBP), CD14, and MD-2) might produce changes in endotoxin presentation that would allow AOAH greater access to its substrate, lipid A. To test this hypothesis, we measured the activity of purified AOAH against endotoxin complexes. Up to 100-fold differences in the efficiency of endotoxin deacylation by AOAH were observed, with the following rank order of susceptibility to AOAH: Es:CD14 ±endotoxin aggregates (Eagg):LBP (molar ratio of E/LBP 100:1) \( \gg \) Eagg: LBP (E/LBP \( \sim 1\), mol/mol), or E:MD-2. AOAH treatment of LOS-sCD14 produced partially deacylated LOS still complexed with sCD14. The underacylated LOS complexed to sCD14 transferred to MD-2 and thus formed a complex capable of preventing TLR4 activation. These findings strongly suggest that LBP- and CD14-dependent extraction and transfer of endotoxin monomers are accompanied by increased exposure of fatty acyl chains within lipid A and that the acyl chains are then sequestered when LOS binds MD-2. The susceptibility of the monomeric endotoxin-CD14 complex to AOAH may help constrain endotoxin-induced TLR4 activation when endotoxin and membrane CD14 are present in excess of MD-2/TLR-4.

Tissue invasion by even minute quantities of many Gram-negative bacteria (GNB) initiates rapid mobilization of the innate immune responses of the host. In these circumstances, both GNB recognition and many responses depend upon activation of the exquisitely sensitive Toll-like receptor 4 (TLR4) by endotoxins, structurally unique and abundant glycolipids that occupy much of the outer leaflet of the GNB outer membrane. Maximal TLR4-dependent host responses to endotoxin are orchestrated through a sequential set of interactions of endotoxin with lipopolysaccharide-binding protein (LBP), membrane or soluble CD14, and soluble or TLR4-associated MD-2. Although timely mobilization of host responses is essential, equally important is the regulation of the duration and intensity of host responses to endotoxin to prevent over-exuberant and sustained responses that can result in severe pathological consequences.

One mechanism of dampening host responses to endotoxin is for the host to modify endotoxin itself, converting it from a potent TLR4 agonist to a much weaker agonist with antagonistic properties. To date, the best described host endotoxin-detoxifying enzyme is acyloxyacyl hydrolase (AOAH) (6). AOAH reduces endotoxin activity by catalyzing the release of secondary fatty acyl chains that are attached to primary 3-hydroxy fatty acyl chains within the bioactive lipid A region (7, 8). AOAH thus converts hexaacylated endotoxin species that are potent TLR4 agonists to pentaacylated or tetraacylated forms that have reduced or no agonist properties (9–13) and, at least in vitro, possess the ability to inhibit TLR4 activation by intact, hexaacylated species (10, 11, 13, 14). Recent studies indicate that hexaacylated and partially deacylated or underacylated endotoxin react similarly with LBP, CD14, and MD-2 to form monomeric complexes with MD-2 (13). However, only hexaacylated endotoxin-human MD-2 is a potent TLR4 agonist (13, 14).

AOAH-dependent deacylation of endotoxin has been demonstrated in vivo (1, 15, 16) and in complex in vitro settings that roughly simulate the extracellular and intracellular conditions of inflammatory fluids (1, 2, 15, 17–19). In contrast, either as purified enzyme or as part of a cell lysate, AOAH is virtually

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‡ The abbreviations used are: GNB, Gram-negative bacteria; AOAH, acyloxyacyl hydrolase; BPI, bacterial permeability increasing protein; Eagg, endotoxin aggregate; HBSS, Hank’s buffered salt solution; HSA, human serum albumin; LBP, lipopolysaccharide-binding protein; LOS, lipooligosaccharide; LPS, lipopolysaccharide; sCD14, soluble CD14; TLR4, Toll-like receptor 4; PBS, phosphate-buffered saline.
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inactive against purified or membrane-bound endotoxin in buffered salt solutions in the absence of detergent (20, 21). This suggests that AOAH alone is unable to act efficiently on endotoxin when the fatty acyl chains of the amphiphatic endotoxin are buried within pure supramolecular aggregates or the bacterial outer membrane. In contrast, the ability of AOAH to function physiologically suggests that other endotoxin binding molecules may act as “physiological detergents” to facilitate access of AOAH to the acyloxyacyl bonds that link the non-hydroxylated fatty acyl chains to lipid A.

The delivery of endotoxin to MD-2, as is needed for TLR4 activation, is markedly enhanced by LBP- and CD14-dependent extraction and transfer of endotoxin monomers from purified endotoxin aggregates (Eagg) (4) or the bacterial outer membrane (22). These steps are also albumin-dependent (23), suggesting that Eagg (or membrane-bound endotoxin)—LBP and monomeric endotoxin—CD14 intermediates present endotoxin in a manner that partially exposes the fatty acyl chains and so require albumin to maintain endotoxin solubility in an aqueous environment. By making use of metabolically radiolabeled endotoxin from Neisseria meningitidis serotype B (24) and from Escherichia coli, we now show that AOAH can act on endotoxin—LBP aggregates and, to an even greater extent, on monomeric endotoxin—sCD14 complexes, but much less on monomeric endotoxin—MD-2. These findings strongly support a model in which LBP- and CD14-dependent extraction and transfer of endotoxin expose fatty acyl chains that then become less accessible when endotoxin binds MD-2. The ability of AOAH to act on these protein—endotoxin intermediates provides a mechanism by which partial deacylation of endotoxin can take place before transfer to MD-2 and thus down-regulate TLR4 activation by endotoxin.

EXPERIMENTAL PROCEDURES

LBP, BPI, and sCD14 were provided by Xoma (US) LLC (Berkeley, CA). Recombinant soluble human MD-2 containing a hexapolyhistidine tag on the carboxyl-terminal end was prepared using infection of High Five insect cells with baculovirus containing the cDNA for this protein derived from recombination with the vector pBAC11 (Novagen) using the procedure previously described for soluble MD-2 (25). Insect supernatant containing sCD14 (1–156) was dialyzed against 20 mM phosphate, pH 7.4, 0.5 M NaCl before incubation at 37 °C for 30 min of LBP with LOSagg (usual molar ratio 1:100) and either equimolar sCD14 (23, 24) or purified sCD14 (1–156)–His6, before incubation by gel filtration chromatography on Sephacryl HR S200 in HBSS+ at 4 °C and analyzed by gel filtration chromatography on Sephacryl S200 as described under “Experimental Procedures” for formation of LOS-sCD14, Mw ∼ 60,000. Results are reported as % of LOS converted to LBP-sCD14 complexes. In each experiment, recovery of applied radioactivity was ≥90% as judged by liquid scintillation spectroscopy. Data shown represent either the mean ± S.E. of three or more independent determinations (A and B) or mean of two determinations (C).

Preparation of Metabolically Labeled Endotoxin and Endotoxin-Protein Complexes—[3H]LOS (specific activity 25,000 cpm/pmol) was isolated from an acetate auxotroph of N. meningitidis serogroup B after metabolic labeling as previously described (24). LOSagg (apparent Mw > 20 million) used as start material to generate complexes was prepared using Sephacryl HR S500 or by ultracentrifugation (24). Aggregates containing various concentrations of LBP (LOSagg·LBP) or BPI (LOS-BPIagg) to be used in AOAH assays were prepared in situ (0.1 ml volume) by incubation of 1 nM [3H]LOSagg in Hanks’ buffered salt solution with Ca2+ and Mg2+ (HBSS+), pH 7.4, 10 mM HEPES, 0.1% HSA for 20 min with the indicated concentration of either LBP or BPI (range from 0.1 to 10 nM) before addition of AOAH.

[3H]LOS-sCD14, [3H]LOS-sCD14-(1–156), and [3H]LOS-MD-2 complexes were isolated by gel filtration chromatography using columns of Sephacryl HR S200 (1.6 × 30 cm or 1.6 × 70 cm) equilibrated in HBSS+ (pH 7.4, 10 mM HEPES, ± 0.1% HSA). Isolated [3H]LOS-sCD14 (Mw ∼ 60,000) or [3H]LOS-sCD14-(1–156)–His6 (Mw ∼ 25,000) complexes were generated by incubation at 37 °C for 30 min of LBP with LOSagg (usual molar ratio 1:100) and either equinmolar sCD14 (23, 24) or purified sCD14 (1–156)–His6, before isolation by gel filtration chromatography on Sephacryl HR S200 in HBSS+ (pH 7.4, 10 mM HEPES, 0.03% HSA). In experiments where the effects of LBP concentration on formation of [3H]LOS-sCD14 were evaluated, the concentration of LBP ranged from 0.2 pmol to 1 nmol with a concentration of 1 nM [3H]LOS. Evaluation of the formation...
of monomeric [3H]LOS-sCD14 was monitored by gel filtration chromatography on Sephacryl HR S200 in PBS, pH 7.4, and the fractions corresponding to [14C]LOS-MD-2 were subjected to 14C-fatty acid analysis. [14C]Lipopolysaccharide (LPS) (specific activity ~6,000 cpm/pmol) was isolated from an acetate auxotroph generated in E. coli BW25113 (BW25113 aceE). This strain was created by deletion-insertion mutagenesis according to the method of Dat-senko and Wanner (26) resulting in insertion of a chloramphenicol acetyltransferase (cat) gene cassette within the aceE gene. Disruption of the aceE gene and insertion of the chloramphenicol acetyltransferase cassette was confirmed by PCR analysis and growth was shown to be acetate-dependent. Analysis of the 14C-fatty acids released by acid and alkaline hydrolysis of isolated LPS was performed as described below for LOS to determine the specific activity and composition of 14C-fatty acids in the isolated LPS. [14C]LPS-aggregate, [14C]LPS-sCD14, and [14C]LPS-MD-2 complexes were prepared as described above for [3H]LOS.

In gel filtration chromatography, fractions (1 ml) were collected (flow rate 0.5 ml/min) at room temperature using AKTA Purifier™ FPLC. Aliquots of the collected fractions were analyzed by liquid scintillation spectroscopy using a Beckman LS liquid scintillation counter to detect radioactive LOS. Recoveries of LOS and LPS were >70%. All solutions used were pyrogen-free and sterile-filtered. After chromatography, selected peak fractions to be used in bioassays were pooled and passed through sterile syringe filters (0.22 or 0.45 μm) with greater than 90% recovery of radiolabeled material in the sterile filtrate. Fractions were stored under sterile conditions at 4 °C until needed. Sephacryl S200 columns were calibrated with Bio-Rad gel filtration standards that included thyroglobulin (Vo), IgG, human serum albumin, ovalbumin, myoglobin, and vitamin B12 (Vj).

AOAH Deacylation Assay—AOAH was stored at -80 °C, diluted in storage buffer (20 mM sodium acetate, pH 6, 0.9%...
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**RESULTS**

*LBP and sCD14 Increase LOS Susceptibility to AOAH*—To determine whether LBP- and/or CD14-dependent alterations of endotoxin presentation render endotoxin more sensitive to AOAH, we examined the ability of AOAH to deacylate purified meningococcal LOS when LOS was presented either alone, as purified LOSagg or after treatment with LBP to produce LOSagg-LBP aggregates, or after sequential treatment with LBP and sCD14 to yield monomeric LOS-sCD14 complexes. All assays were performed in HBSS, 10 mM HEPES, 0.1% HSA, pH 7.4. The susceptibility of LOS to AOAH was markedly increased by pretreatment of LOSagg with LBP + sCD14 to convert LOSagg to monomeric LOS-sCD14 complex (Fig. 1A) (23). Incubation of LOSagg with LBP alone also markedly increased the susceptibility of LOS to AOAH at specific LBP:LOS ratios (Fig. 1B). The dose-dependence of LBP alone on AOAH susceptibility of LOSagg showed a complex bell-shaped curve (Fig. 1B) with maximum AOAH activity at a molar ratio of ~1 mol LBP/100 mol LOS. At the concentration of LBP most favorable for AOAH activity, there was no detectable change in the gel sieving profile of the LOSagg (i.e. no detectable disaggregation of LOSagg) (28). The bell-shaped LBP dose-dependence of AOAH activity on LOSagg closely paralleled the bell-shaped LBP dose-dependence for extraction and delivery of LOS monomers to sCD14 (Fig. 1C). This suggests that LBP-catalyzed extraction and delivery of endotoxin monomers to sCD14 produce alter-
endotoxin-MD-2 complex interacts with TLR4 without efficiently inducing TLR4 activation (13). Thus, provided the partially deacylated LOS remains bound to CD14 during and after AOAH treatment, deacylation of monomeric endotoxin-sCD14 complex (e.g. LOS-sCD14) could provide substrate for generation of AOAH-treated endotoxin-MD-2 complex (e.g. LOS\(^{\text{AOAH}}\)-MD-2) that can then act as a TLR4 antagonist.

Whether or not deacylated LOS remained associated with sCD14 could not be tested readily by comparing the gel sieving properties of the LOS-sCD14 complex before and after treatment with AOAH because of the closely similar \( M \) of sCD14 and AOAH. Therefore, we made use of a truncated form of recombinant CD14 (amino acid residues 1–156) containing a carboxyl-terminal hexahistidine tag (sCD14-(1–156)-His\(_6\)). This recombinant sCD14-(1–156)-His\(_6\) fully retains the reactivity of full-length sCD14 (356 residues) toward LOS-sCD14-LBP and MD-2 (data not shown). LOS-sCD14-(1–156)-His\(_6\) was also a good substrate for AOAH (Fig. 3A). After AOAH treatment, the majority of the \(^{[3]}\text{H}\)LOS cpm remained associated with sCD14-(1–156)-His\(_6\) as judged by gel sieving analysis (\( M_\ell \sim 25,000 \)) (Fig. 3B) and co-capture of \(^{[3]}\text{H}\)LOS\(^{\text{AOAH}}\) contained in the \( M_\ell \sim 25,000 \) peak by metal chelation chromatography (Fig. 3C). The earlier eluting peaks containing \(^{[3]}\text{H}\)LOS-derived cpm may represent dimers and larger aggregates of \(^{[3]}\text{H}\)LOS\(^{\text{AOAH}}\)-CD14-(1–156)-His\(_6\), as well as released \(^{3}\text{H}\)-free fatty acids complexed with albumin.

To verify that treatment of LOS-sCD14 with AOAH yielded partially deacylated LOS-sCD14 complex capable of transferring the partially deacylated LOS to MD-2, \(^{[1]}\text{C}\)LOS-sCD14 (full-length sCD14) was deacylated with AOAH, subsequently treated with MD-2-His\(_6\) and the product(s) separated using Sephacryl S200 (Fig. 4A). Fatty acid analysis of the peak fractions of \(^{[1]}\text{C}\)LOS corresponding to \(^{[1]}\text{C}\)LOS-MD-2 by migration on Sephacryl S200 indicated that the recovered LOS-MD-2 contained partially deacylated LOS (Fig. 4B). Experimental conditions were used that yielded \( \sim 60\% \) release of the non-hydroxylated fatty acid (12:0) from LOS-sCD14 (data not shown). Because cleavage by AOAH of the two non-hydroxylated fatty acids present in each molecule of endotoxin normally follows in rapid succession (2, 19, 27), the major product under our experimental conditions is likely tetracylated LOS-sCD14 with \( \sim 40\% \) of the hexaacylated LOS-sCD14 exposed to

\[ 4 \quad T. \text{L. Gioannini, data not shown.} \]
AOAH remaining undigested. The close match in \(^{14}\text{C}\)-fatty acid composition of the AOAH-treated \[^{14}\text{C}\]LOS\(^{\text{AOAH}}\)-sCD14 and the recovered \[^{14}\text{C}\]LOS\(^{\text{AOAH}}\)-MD-2 suggests, therefore, essentially equal efficiency of transfer of undigested and partially deacylated LOS from sCD14 to MD-2, as was previously reported (13).

Monomeric Endotoxin-MD-2 Complex Is Relatively Resistant to AOAH—In contrast to LOS-sCD14, monomeric LOS-MD-2 complexes do not depend upon albumin to maintain aqueous solubility and induce TLR4-dependent cell activation. Therefore, we have speculated that the fatty acids of lipid A are more fully buried within a deeper hydrophobic cavity within MD-2 and so might be a poorer substrate for AOAH. To test this hypothesis, we compared the effect of increasing concentrations of AOAH on LOS-sCD14 and LOS-MD-2 and, for comparison, unmodified LOS\(_{agg}\). As predicted, LOS-MD-2, in comparison to LOS-sCD14, showed much reduced sensitivity to added AOAH (Fig. 5A). As illustrated in Fig. 5B, a closely similar pattern of endotoxin sensitivity to AOAH comparing purified endotoxin aggregates (LOS\(_{agg}\)) ± LBP and monomeric LPC-sCD14 or MD-2 was seen with a rough form of \(E\). coli \[^{14}\text{C}\]LPS isolated from \(E\). coli strain BW25113 aceE. Monomeric LPC-sCD14 complex and LPC\(_{agg}\)-LBP at substoichiometric ratios of LBP-LPS, which most readily resulted in the formation of LPC-sCD14, were deacylated most effectively by AOAH in HBSS \(^+\), 10 mM HEPES, 0.1% HSA, pH 7.4. As seen with LOS, LPS in the form of LPS\(_{agg}\) and LPC-MD-2 was much more resistant to deacylation by AOAH (Fig. 5B).

DISCUSSION

LBP and CD14 have important and complex roles in host interactions with endotoxin. The most potent activation of mammalian cells by many endotoxins requires sequential protein-endotoxin and protein-protein interactions involving LBP, C14, MD-2, and TLR4 (3–5). The combined action of LBP and CD14 results in extraction of endotoxin monomers from purified endotoxin aggregates (30–32) and the GNB outer membrane to yield a monomeric endotoxin-CD14 complex that is the preferred substrate of MD-2/TLR4 (4, 25, 33, 34). Maximal generation of endotoxin-CD14 requires a very low molar ratio of LBP to endotoxin and CD14 (Fig. 1C) (30–32). Higher molar ratios of LBP to endotoxin and CD14 reduce MD-2/TLR4-dependent cell activation by endotoxin (31, 32, 35, 36) and instead promote cellular and extracellular (e.g. plasma lipoprotein-dependent) clearance of endotoxin (32, 35, 37–39).

The studies described here demonstrate a new role for LBP and CD14 in negative regulation of the pro-inflammatory action of endotoxin: they can participate as cofactors in the catalytic detoxification of endotoxin by AOAH. Our findings clealy show that both LBP alone and LBP and sCD14 in concert (i.e. by yielding endotoxin-sCD14) substantially increase the sensitivity of both meningococcal LOS and \(E\). coli LPS to AOAH (Figs. 1 and 5). The stimulatory effects of LBP ± sCD14 on AOAH activity require substoichiometric concentrations of LBP (Figs. 1B and 5B) that correspond closely to the concentrations required for LBP-dependent extraction and delivery of endotoxin monomers from endotoxin aggregates to sCD14 (Fig. 1C). The increased sensitivity to AOAH of endotoxin aggregates treated with very low concentrations of LBP suggests that, under these conditions, LBP induces a rearrangement of endotoxin within the aggregates that increases exposure of the lipid A region of endotoxin, facilitating AOAH action as well as extraction and transfer of endotoxin monomers to CD14. The inhibitory effect of higher concentrations of LBP on AOAH action and formation of endotoxin-sCD14 monomers (Fig. 1, B and C) may reflect increased competition by added LBP on the interaction of endotoxin with CD14 or AOAH. The inability of BPI to significantly increase endotoxin sensitivity to AOAH (Fig. 1B) mirrors its inability to promote extraction and transfer of endotoxin to CD14 (28, 40). This may reflect the higher affinity of BPI for endotoxin and further illus-
brates differences in the interactions of LBP and BPI with endotoxin-rich interfaces (28, 41).

The sensitivity of monomeric endotoxin-sCD14 complex to AOAH is consistent with structural data derived from x-ray crystallography of mouse CD14 (42). Structural and functional data suggest that the binding site for endotoxin in CD14 includes a wide, flexible hydrophobic pocket near the amino terminus (42). The characteristics of this pocket predict that the fatty acyl chains of bound endotoxin will be partially exposed, explaining the need for albumin to maintain the stability of endotoxin-sCD14 in aqueous solution and rendering the acyloxyacyl linkages in lipid A accessible to AOAH. The relative resistance of LOS-MD-2 and LPS-MD-2 to AOAH (Fig. 5, A and B), by contrast, suggests a more complete sequestration of the fatty acyl chains within MD-2. This is consistent with models of MD-2 structure that suggest a deep hydrophobic cavity (43, 44) and with the solubility and stability of endotoxin-MD-2 in aqueous solution even in the absence of albumin (13).

AOAH action on endotoxin-CD14 yields a partially deacylated endotoxin-CD14 complex (Fig. 3, B–D). AOAH-treated endotoxin can be readily transferred from CD14 to MD-2 (see Fig. 4) (13), producing a complex of underacylated endotoxin with MD-2 that can occupy TLR4 without efficiently inducing receptor activation (13, 34). Thus, the underacylated endotoxin-MD-2 complex inhibits responsiveness of TLR4 by competing with the fully hexaacylated endotoxin-MD-2 complex (13). Soon after bacterial invasion, however, levels of extracellular AOAH are likely to be limiting. Moreover, comparison of the reactivity of LOS-sCD14 with AOAH (K_m ~ 5 nanomolar) versus MD-2 (apparent K_m for transfer ~ 100 picoMolar) (34) strongly suggests that at low (pM) concentrations of endotoxin-CD14, AOAH is unlikely to blunt MD-2/TLR4-dependent cell activation by endotoxin. This difference in reactivity between AOAH and MD-2 with endotoxin-CD14 may be necessary to permit induction of robust host innate and adaptive immune response to small numbers of invading GNB and psi endotoxin (15). However, under conditions when the levels of endotoxin-sCD14 produced substantially exceed those of MD-2/TLR4 and the concentrations of other potential host reagents with endotoxin-sCD14 (e.g. plasma lipoproteins) are limiting, AOAH activity against endotoxin-CD14 may help constrain potentially protracted endotoxin-driven inflammatory and immune responses.

It should be noted that the experimental conditions we have used roughly model extracellular AOAH action (pH 7.4) against extracellular endotoxin or, perhaps, cell surface-bound monomeric endotoxin-membrane CD14. However, within an inflammatory exudate, intracellular AOAH action within phagocytic cells (macrophages, dendritic cells, neutrophils) may predominate (2, 15, 45, 46). In these cells, most endotoxin that is internalized is taken up in the form of large aggregates or membrane remnants, distinct from the endotoxin-sCD14 complex that is the preferred substrate for MD-2/TLR4 and extracellular AOAH. The identities of the intracellular factors and conditions that promote intracellular AOAH action remain to be determined, as does the biological significance of the partially deacylated endotoxin that is released from phagocytes into their extracellular environment.

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REFERENCES

1. Erwin, A. L., and Munford, R. S. (1990) J. Biol. Chem. 265, 16444–16449
2. Katz, S. S., Weinrauch, Y., Munford, R. S., Elsbach, P., and Weiss, J. (1999) J. Biol. Chem. 274, 36579–36584
3. Beutler, B., and Rietschel, E. T. (2003) Nat. Rev. Immunol. 3, 169–176
4. Gioannini, T. L., Teghanemt, A., Zhang, D., Levis, E. N., and Weiss, J. P. (2005) J. Endotoxin Res. 11, 117–123
5. Ulevitch, R. J., and Tobias, P. S. (1999) Curr. Opin. Immunol. 11, 19–22
6. Munford, R. S. (2005) J. Endotoxin Res. 11, 69–84
7. Hall, C. L., and Munford, R. S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6671–6675
8. Munford, R. S., and Hall, C. L. (1989) J. Biol. Chem. 264, 15613–15619
9. Darveau, R. P. (1998) Curr. Opin. Microbiol. 1, 36–42
10. Kitchens, R. L., and Munford, R. S. (1995) J. Biol. Chem. 270, 9904–9910
11. Kitchens, R. L., Ulevitch, R. J., and Munford, R. S. (1992) J. Exp. Med. 176, 485–494
12. Riedo, F. X., Munford, R. S., Campbell, W. B., Reichs, J. S., Chien, K. R., and Gerard, R. D. (1990) J. Immunol. 144, 3506–3512
13. Teghanemt, A., Zhang, D., Levis, E. N., Weiss, J. P., and Gioannini, T. L. (2005) J. Immunol. 175, 4669–4676
14. Coats, S. R., Pham, T. T., Bainbridge, B. W., Reife, R. A., and Darveau, R. P. (2005) J. Immunol. 175, 4490–4498
15. Lu, M., Zhang, M., Takashima, A., Weiss, J., Apicella, M. A., Li, X. H., Yuan, D., and Munford, R. S. (2005) Nat. Immunol. 6, 989–994
16. Munford, R. S. (1986) Methods Find Exp. Clin Pharmacol. B, 63–65
17. Feulner, J. A., Lu, M., Shetlon, J. M., Zhang, M., Richardson, J. A., and Munford, R. S. (2004) Infect. Immun. 72, 3171–3178
18. Weinrauch, Y., Katz, S. S., Munford, R. S., Elsbach, P., and Weiss, J. (1999) Infect. Immun. 67, 3376–3382
19. Hagen, F. S., Grant, J. F., Kuiper, J. L., Slaughter, C. A., Moomaw, C. R., Orth, K., O’Hara, P. J., and Munford, R. S. (1991) Biochemistry 30, 8415–8423
20. Staab, J. F., Ginkel, D. L., Rosenberg, G. B., and Munford, R. S. (1994) J. Biol. Chem. 269, 23736–23742
21. Post, D. M., Zhang, D., Eastvold, J. S., Teghanemt, A., Gibson, B. W., and Weiss, J. P. (2005) J. Biol. Chem. 280, 38383–38394
22. Gioannini, T. L., Zhang, D., Teghanemt, A., and Weiss, J. P. (2002) J. Biol. Chem. 277, 47818–47825
23. Giardina, P. C., Gioannini, T., Buscher, B. A., Zaleski, A., Zheng, D. S., Stoll, L., Teghanemt, A., Apicella, M. A., and Weiss, J. (2001) J. Biol. Chem. 276, 5883–5891
24. Gioannini, T. L., Teghanemt, A., Zhang, D., Coussens, N. P., Dockstader, W., Ramaswamy, S., and Weiss, J. P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 4186–4191
25. Datsenko, K. A., and Wanner, B. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6640–6645
26. Munford, R. S., and Erwin, A. L. (1992) Methods Enzymol. 209, 485–492
27. Iovine, N., Eastvold, J., Elsbach, P., Weiss, J. P., and Gioannini, T. L. (2002) J. Biol. Chem. 277, 7970–7978
28. Saitoh, S., Akashi, S., Yamada, T., Tanimura, N., Kobayashi, M., Konno, K., Matsumoto, F., Fukase, K., Kusumoto, S., Nagai, Y., Kusumoto, Y., Kosugi, A., and Miyake, K. (2004) Int. Immunol. 16, 961–969
29. Gnegner, J. A., Ulevitch, R. J., and Tobias, P. S. (1995) J. Biol. Chem. 270, 5320–5325
30. Gioannini, T. L., Teghanemt, A., Zarember, K. A., and Weiss, J. P. (2003) J. Endotoxin Res. 9, 401–408
31. Gutmann, T., Haberer, N., Carroll, S. F., Seydel, U., and Wiese, A. (2001) Biol. Chem. 382, 425–434
32. Kennedy, M. N., Mullen, G. E., Leifer, C. A., Lee, C., Mazzoni, A., Dileespan,
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K. N., and Segal, D. M. (2004) J. Biol. Chem. 279, 34698–34704
34. Prohinar, P., Re, F., Widstrom, R., Zhang, D., Teghanemt, A., Weiss, J. P., and Gioannini, T. L. (2007) J. Biol. Chem. 282, 1010–1017
35. Hamann, L., Alexander, C., Stamme, C., Zahringer, U., and Schumann, R. R. (2005) Infect. Immun. 73, 193–200
36. Thompson, P. A., Tobias, P. S., Viriyakosol, S., Kirkland, T. N., and Kitchens, R. L. (2003) J. Biol. Chem. 278, 28367–28371
37. Lamping, N., Dettmer, R., Schroder, N. W., Pfeil, D., Hallatschek, W., Burger, R., and Schumann, R. R. (1998) J. Clin. Investig. 101, 2065–2071
38. Vesy, C. J., Kitchens, R. L., Wolfbauer, G., Albers, J. J., and Munford, R. S. (2000) Infect. Immun. 68, 2410–2417
39. Wurfel, M. M., Kunitake, S. T., Lichenstein, H., Kane, J. P., and Wright, S. D. (1994) J. Exp. Med. 180, 1025–1035
40. Weiss, J. (2003) Biochem. Soc. Trans. 31, Pt. 4, 785–790
41. Tobias, P. S., Soldau, K., Iovine, N. M., Elsbach, P., and Weiss, J. (1997) J. Biol. Chem. 272, 18682–18685
42. Kim, J. I., Lee, C. J., Jin, M. S., Lee, C. H., Paik, S. G., Lee, H., and Lee, J. O. (2005) J. Biol. Chem. 280, 11347–11351
43. Gangloff, M., and Gay, N. J. (2004) Trends Biochem. Sci. 29, 294–300
44. Gruber, A., Mancek, M., Wagner, H., Kirschning, C. J., and Jerala, R. (2004) J. Biol. Chem. 279, 28475–28482
45. Lu, M., Zhang, M., Kitchens, R. L., Fosmire, S., Takashima, A., and Munford, R. S. (2003) J. Exp. Med. 197, 1745–1754
46. Luchi, M., and Munford, R. S. (1993) J. Immunol. 151, 959–969