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Lipoprotein-bound endotoxin exerts an immunomodulatory effect on hepatocytes through the lipid A domain of LPS

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Background: We have previously shown that chylomicron (CM)-bound lipopolysaccharide (LPS) inhibits the host innate immune response by rendering hepatocytes tolerant to pro-inflammatory cytokine stimulation. However, LPS is a complex macromolecule containing both lipid and carbohydrate domains. We hypothesized that just as lipid A confers the toxicity of LPS, it is also responsible for the immunoregulatory effect on hepatocytes.
Methods: We pretreated primary rat hepatocytes for 2 h with a series of CM-LPS complexes in which the endotoxin moiety varied in its structure and/or toxicity. Subsequently, the cells were stimulated with a mixture of pro-inflammatory cytokines. Nitric oxide production was measured as an indicator of hepatocellular activation.
Results: All pretreatments wherein the CM-bound complex contained the lipid A moiety readily inhibited the hepatocellular cytokine response, including CM bound to lipid A alone. In contrast, CM-LPS complexes containing detoxified LPS, which lacks the lipid A domain, had no effect on the hepatocellular response to cytokines.
Conclusions: The lipid A domain of the LPS macromolecule is both sufficient and essential for the CM-mediated induction of cytokine tolerance in hepatocytes. However, this process is independent of the specific endotoxic activity of the lipid A moiety.

Keywords: Lipoproteins, LPS, cytokine tolerance, rat, sepsis

INTRODUCTION

Sepsis is a life-threatening clinical condition and a major cause of death in surgical intensive care units. Each year, more than 750,000 Americans develop sepsis, resulting in 215,000 fatalities.[1,2] Most cases of sepsis are caused by endotoxin (LPS) present in the outer cell membrane of Gram-negative bacteria. When introduced into the circulation, LPS induces a strong inflammatory reaction, potentially leading to multiple organ failure and death.[3] In addition to these pro-inflammatory responses, the body also initiates a series of anti-inflammatory responses.[4,5] Ultimately, the balance between these pro-inflammatory and anti-inflammatory responses determines the outcome in patients with sepsis.[5]

Although hepatocytes do not respond directly to circulating LPS, the liver plays a major role in the host response to sepsis. In addition to facilitating the removal of LPS from the circulation during sepsis and the acute phase response, the liver also contributes to mobilizing the body's fat stores through the synthesis and secretion of triglyceride (TG)-rich lipoproteins, a process which is clinically known as lipemia of sepsis.[6,7]

We have previously demonstrated that TG-rich lipoproteins (chylomicrons [CMs], very low density lipoproteins], as well as a TG-rich lipid emulsion (Soyacal®) effectively protected mice against LPS-induced mortality.[8] We have also shown that in endotoxemic rats, chylomicrons increased the clearance of LPS from the

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circulation by increasing its delivery to the liver, specifically to hepatocytes, and decreased the rate of TNF-α production.[9] These results were paralleled by studies in humans in which hyperchylomicronemia reduced LPS-induced toxicity, TNF-α production, and activation of the acute phase response.[10] Moreover, we have shown that hepatocytes exposed to CM-LPS complexes demonstrated a markedly reduced response to subsequent stimulation by a mixture of pro-inflammatory cytokines, as measured by decreased nitric oxide (NO) production,[11] a phenomenon we termed cytokine tolerance. Further studies have shown that cytokine tolerance in hepatocytes is a receptor-dependent, transient process that directly correlates with internalization of the CM-LPS complexes via low density lipoprotein (LDL) receptors.[12] On the basis of these findings, we have postulated that the lipemia of sepsis, in addition to its homeostatic function, plays a protective role during sepsis.[13] Specifically, by mobilizing lipid stores, the increased level of TG-rich lipoproteins not only fuels the increased energy demands of the body, it may also simultaneously help protect the host from the deleterious effects of LPS by inducing cytokine tolerance in hepatocytes.

LPS is an amphipathic macromolecule composed of three domains: (i) an extremely variable outer polysaccharide chain, called the O specific chain, which is the main criterion for serological classification of these bacteria; (ii) a more structurally preserved middle polysaccharide domain called the core region; and (iii) the lipid A domain, which has a disaccharide backbone with a variable number of acyl side chains.[14] The lipid A domain possesses the endotoxic and immunomodulating activities of the LPS, which depend on phosphorylation of the carbohydrate backbone and the number and orientation of the acyl side chains. Varying the structure of LPS and lipid A yields molecules with different endotoxic activities.[3] As most of the biological and immunological activities of the LPS macromolecule depend on lipid A endotoxicity, we hypothesized that the capacity of CM-LPS to induce cytokine tolerance in hepatocytes would be dependent on the endotoxic activity of this moiety. To test this hypothesis, we pretreated primary rat hepatocytes with CM-LPS complexes containing different LPS moieties, as well as different natural and synthetic lipid A structures, and compared the cytokine tolerance induced in hepatocytes with that following pretreatment with chylomicron bound to LPS from wild-type *Escherichia coli*.

Materials and Methods

Isolation and preparation of hepatocytes

All procedures involving rats were conducted in accordance with the National Institutes of Health guidelines regarding the care and use of laboratory animals and approved by the UCSF Institutional Animal Care and Use Committee. Hepatocytes were isolated from male Sprague-Dawley rats (225–300 g, Simonson, Gilroy, CA, USA) by Liberase® perfusion (Boehringer-Mannheim, Indianapolis, IN, USA), as previously described,[15] and purified via centrifugal elutriation (Beckman Coulter, Avanti J-20, Fullerton, CA, USA).[16] Hepatocyte purity was > 95% and viability > 90% as assessed by direct microscopic examination and trypan blue dye exclusion, respectively. Hepatocytes were grown as spheroids (multicellular aggregates) by plating them in 100-mm culture dishes coated with poly-HEMA (Sigma, St Louis, MO, USA) in M199 medium (Cell Culture Facilities, UCSF, San Francisco, CA, USA) supplemented with bovine serum albumin (2 g/l), dexamethasone (0.4 mg/l), insulin (60 U/l), and antibiotics.[17,18]

Preparation of synthetic chylomicron remnants

Synthetic chylomicron remnants were made and combined with recombinant human apoE_3, as described by Redgrave *et al*.[19,20] Recombinant apoE_3 was produced in bacteria by using a thioredoxin-fusion protein-expression vector as previously described.[21] The synthetic chylomicron emulsion was incubated with different LPS/lipid A analogues: smooth LPS from *E. coli* O111:B4, rough LPS from *E. coli* J5 devoid of the O-specific side chain, detoxified LPS from *E. coli* O111:B4, LPS from *Salmonella abortus equi*, diphosphorylated lipid A from *E. coli* F583, monophosphorylated lipid A from *E. coli* F583 (all from Sigma), compound 506 (a synthetic analog of lipid A from *E. coli* F515), compound 406 (the synthetic analog of lipid A precursor lipid IVa), and lipid X (the synthetic monosaccharide precursor of lipid A), all generous gifts from Dr Shoichi Kusumoto, Osaka University, Japan. All compounds were added to CM at a concentration of 1 μg/20 mg TG in the presence of 10% (v/v) lipoprotein-free fetal bovine serum at 37°C for 3 h.

The resulting complexes were kept under nitrogen at 4°C and used within 5 days of their preparation. Synthetic remnant particles measured 108 ± 45 nm in diameter as determined by electron microscopy.

Effect of different CM-LPS complexes on hepatocyte response

Hepatocyte spheroids were pretreated with different CM-LPS complexes (5 mg TG/ml), or the equivalent LPS or lipid A compounds alone for 2 h. Cells were subsequently washed 3 times with phosphate-buffered saline and incubated with fresh medium for 16 h to allow them time to recover. Hepatocytes were then stimulated with a mixture of pro-inflammatory cytokines (TNF-α 500 U/ml, IL-1β 100 U/ml, and IFN-γ 100 U/ml; R&D

Systems, Minneapolis, MN, USA).[22] Twenty-four hours later, NO production, an indicator of the hepatocellular pro-inflammatory response, was measured via the Griess reaction,[23] and normalized to total hepatocellular DNA.

Determination of endotoxicity

To determine the endotoxic activity of the different LPS or LPS moieties used, macrophage cell line (RAW 264.7) cells were stimulated with each compound (2 ng/ml), and the amount of NO production was measured after 24 h. The response of RAW 264.7 cells to the smooth LPS moiety served as the control. Values comparable to smooth LPS were considered to be strong (+++), less than 50% of the smooth LPS were considered to be moderate (++), and values less than 10% were considered to be mild (+) endotoxic activity.

Statistical analysis

Data are reported as mean ± SEM. Differences between groups were analyzed using 2-tailed, unpaired *t*-tests. Probability values less than 5% were considered significant ($P < 0.05$).

RESULTS

Endotoxicity

Smooth and rough LPS, natural diphosphorylated lipid A, the synthetic diphosphorylated lipid A, and compound 506 each stimulated the RAW 264.7 cells by the same amount (Fig. 1). Lipopolysaccharide from *S. abortus equi* had a moderate effect on RAW 264.7 cells (42 ± 3.1% compared to smooth LPS, $P < 0.01$). Monophosphorylated lipid A mildly stimulated the RAW cells (1.8 ± 2.1% compared to smooth LPS, $P < 0.01$). Detoxified LPS, compound 406 and lipid X did not induce any detectable response in RAW cells in terms of NO production.

Induction of cytokine tolerance

Hepatocytes pretreated with CM-LPS complexes containing either smooth LPS, rough LPS, or LPS from *S. abortus equi* showed a significant reduction in NO production after stimulation with pro-inflammatory cytokines compared to unpretreated control cells (Fig. 2). Pretreatment with complexes containing detoxified LPS, which lacked lipid A domain, did not induce cytokine tolerance in hepatocytes. Pretreatment with either of these LPS molecules alone did not result in any change in the hepatocellular response to pro-inflammatory cytokines.

Chylomicron complexes containing diphosphorylated lipid A, monophosphorylated lipid A, compound 506, or compound 406 significantly attenuated the hepatocellular response to subsequent stimulation with pro-inflammatory cytokines (Fig. 3). However, compound 406 was much less potent in this respect, as it resulted in only about a 12% reduction in the hepatocellular response to pro-inflammatory cytokines. Complexes containing lipid X did not induce cytokine tolerance in the hepatocytes. In the unbound form, none of the LPS or lipid A compounds studied induced cytokine tolerance in hepatocytes.

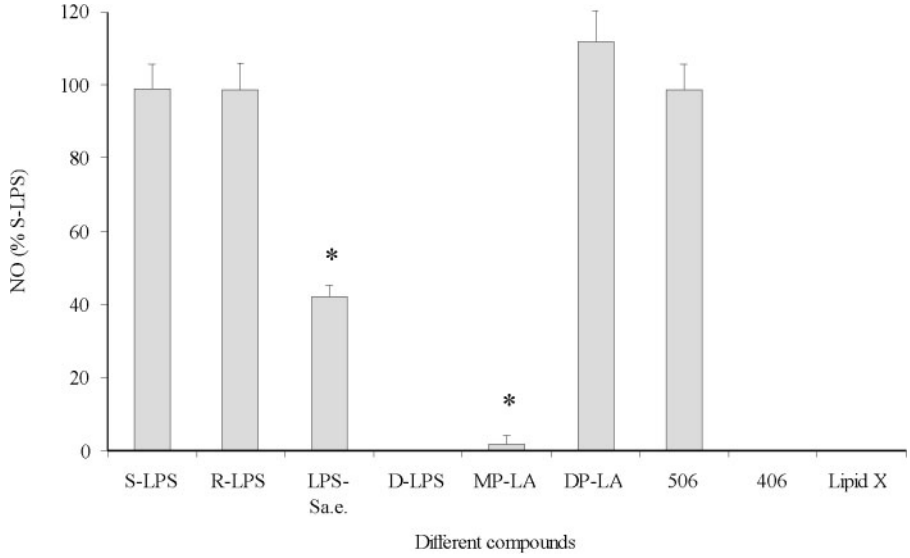

Fig. 1. Relative endotoxic activity of different LPS or LPS-related compounds. Data shown are mean ± SEM of six samples from 2 different experiments, *$P < 0.01$ according to *t*-test. S-LPS, smooth LPS from *E. coli* O111:B4; R-LPS, rough LPS from *E. coli* J5; LPS-Sae, LPS from *S. abortus equi*; D-LPS, detoxified LPS from *E. coli* O111:B4; MP-LA, monophosphorylated lipid A from *E. coli* F583; DP-LA, natural diphosphorylated lipid A from *E. coli* F583; 506, synthetic diphosphorylated lipid A from *E. coli* O55:B5; 406, synthetic lipid IVa.

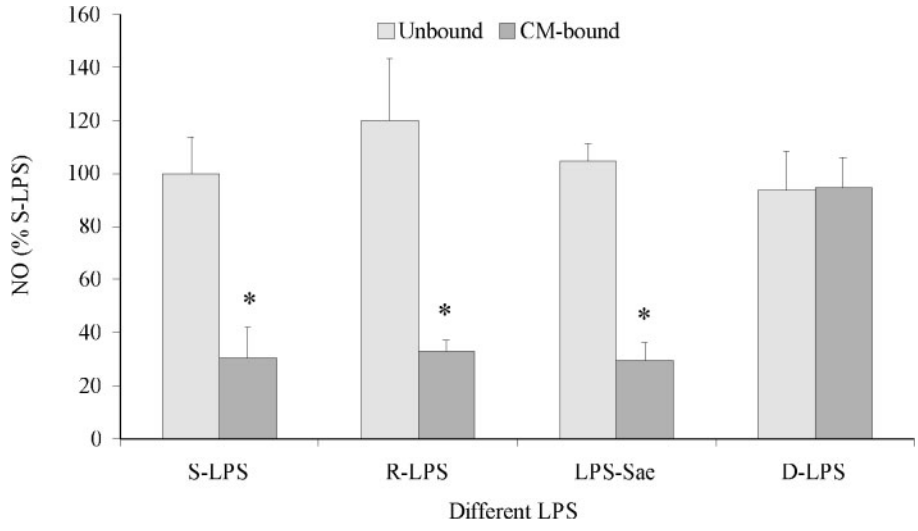

Fig. 2. Cytokine tolerance in hepatocytes pretreated with different CM-LPS complexes. Light-gray bars represent pretreatment with different unbound compounds alone. Darker-gray bars represent pretreatment with compounds bound to chylomicron. Data shown are mean ± SEM of 6 samples of two different experiments, *$P < 0.01$ compared to their corresponding unbound compounds, according to *t*-test. For abbreviations, see caption to Figure 1.

DISCUSSION

This study shows that the induction of cytokine tolerance in primary rat hepatocytes is dependent upon the lipid A moiety of the LPS macromolecule. Moreover, we found that the induction occurs independently of the endotoxic activity of the LPS moiety (Table 1). The magnitude of cytokine tolerance in hepatocytes pretreated with CM-LPS complexes that contained LPS from *S. abortus equi*, which has much lower endotoxicity than other LPS moieties, was the same as that in hepatocytes pretreated with complexes containing natural smooth or rough LPS molecules. In contrast, complexes containing detoxified LPS, in which the lipid A moiety is removed, did not induce cytokine tolerance in hepatocytes. Inhibition of the hepatocellular response to pro-inflammatory cytokines after pretreatment with complexes containing solely the lipid A moiety of the LPS molecule confirmed the essential role of lipid A in inducing cytokine tolerance in hepatocytes.

Lipid A is responsible for endotoxic activity of LPS both *in vivo* and *in vitro*.[14] The specific requirements for

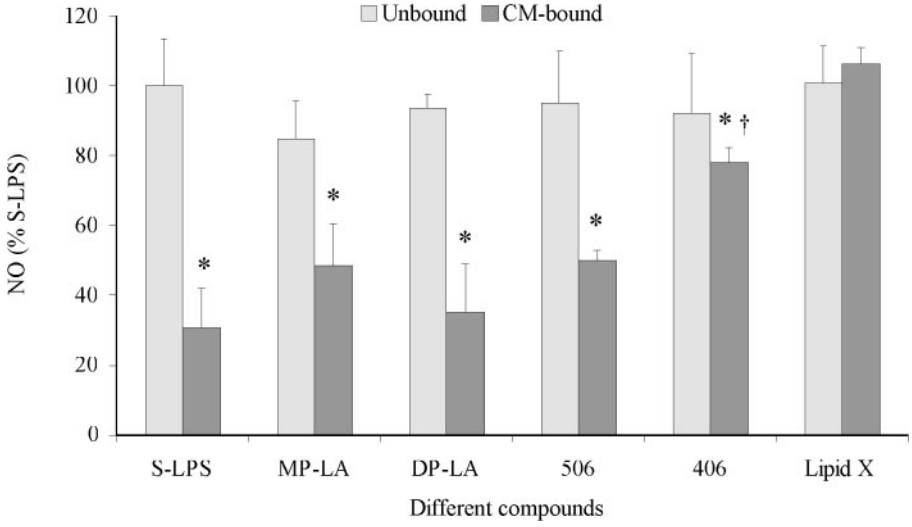

Fig. 3. Cytokine tolerance in hepatocytes after pretreatment with different LPS-related compounds. Light-gray bars represent pretreating with unbound compounds alone. Darker-gray bars represent pretreatment with the compound bound to chylomicron. Data shown are mean ± SEM of 6 samples of two different experiments. *$P < 0.01$ compared to the corresponding unbound groups; dagger, $P < 0.01$ compared to bound S-LPS group, according to *t*-test. For abbreviations, see caption to Figure 1.

Table 1. Effect of different LPS or LPS compounds on the hepatocellular response to pro-inflammatory cytokines

LPS or LPS compound	Toxicity	Tolerance
S-LPS	+++	Yes
R-LPS	+++	Yes
LPS-Sae	+	Yes
D-LPS	–	No
DP-LA	+++	Yes
MP-LA	+	Yes
Compound 506	+++	Yes
Compound 406	–	Yes
Lipid X	–	No

S-LPS, smooth LPS from *E. coli* O111:B4; R-LPS, rough LPS from *E. coli* J5; LPS Sae, LPS from *S. abortus equi*; D-LPS, detoxified LPS from *E. coli* O111:B4; MP-LA, monophosphorylated lipid A from *E. coli* F583; DP-LA, natural diphosphorylated lipid A from *E. coli* F583; 506, synthetic diphosphorylated lipid A from *E. coli* O55:B5; 406, synthetic lipid IVa.
+++ strong, ++ moderate, and + mild toxicity.

this biological activity are not fully known; however, it has been shown that for full expression of the 'endotoxic principle', lipid A must possess a particular chemical composition and primary structure.[24] The most potent known lipid A comes from a deep, rough, mutant strain of *E. coli* F 515 consisting of a β-1,6-linked D-glucosamine (GlcN) disaccharide backbone carrying two negatively charged phosphates, and six saturated fatty acids, which are bound in a defined asymmetric distribution, four at the non-reducing and two at the reducing GlcN molecule. Because of the phosphate groups and the asymmetric position and length of the acyl side chains, this lipid A moiety has a conical or wedge-shaped cross section. When lipid A with this cross section intercalates into the cell membrane, the conical shape exerts a mechanical stress on the CD14 molecule. A change in the molecular structure of lipid A, such as removal of a phosphate group, changes the conical cross section to a more cylindrical form and significantly reduces its endotoxic activity.[3] Because the cylindrical LPS or lipid A occupies the CD14 molecule on the membrane, it cannot exert the effective mechanical stress required to activate the signaling pathway.

In addition to endotoxic activity, lipid A can induce most of the biological properties of the intact LPS molecule, specifically the early phase of endotoxin tolerance, immune modulation, and preconditioning. Interestingly, most of these biological activities are independent of the endotoxic properties of lipid A.[25] Monophosphorylated lipid A has been shown to induce delay preconditioning in rats, and inhibit the TNF-α related myocardial damage observed in ischemia-reperfusion injury.[26] In our study, although monophosphorylated lipid A was significantly less potent in stimulating RAW 264.7 cells than diphosphorylated lipid A, it induced the same amount of cytokine tolerance in hepatocytes.

Studies of the biological activities of the lipid A moiety of LPS were unreliable because of the complicated structure of the LPS macromolecule and the difficulty in purifying it; thus, artificial synthesis of lipid A was a major milestone. Ishida *et al*.[27] have shown that the synthetic lipid A analogue ONO-4007 effectively induced endotoxin tolerance in mice and prevented plasma leakage when LPS was subsequently administered. Furthermore, the biological activities of lipid A are not limited to endotoxin signaling pathways. It can also interfere with other intracellular signaling mechanisms,[28] and intervene with and inhibit the glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) signaling pathway.[29] This inhibition is a specific process that is probably due to binding of the lipid A structure at the substrate-binding site of the enzyme.

Although at first it was believed that structures similar to the natural lipid A moiety were required for this inhibitory effect, recent studies have shown that even monosaccharide lipid A analogs are capable of interfering with GPI-PLD activity.[30] In our study, CM-LPS complexes containing compound 506, which is the synthetic analog of *E. coli* F515, induced the same amount of cytokine tolerance in hepatocytes as complexes containing natural lipid A or intact smooth LPS molecules. Complexes containing compound 406, which is the synthetic analogue of lipid IVa, but has only four acyl side chains, also induced cytokine tolerance in hepatocytes, but by a much lower amount than the natural or synthetic lipid A structures. However, in our study, complexes containing lipid X, which is the synthetic counterpart of the monosaccharide precursor of lipid A and has only two acyl side chains, could not attenuate the hepatocellular response to pro-inflammatory cytokines.

The mechanism of this inhibitory effect of CM-lipid A complexes on the cytokine signaling mechanism of hepatocytes is not clear. We have previously shown that to induce cytokine tolerance, complexes should be internalized into the hepatocytes, and that the rate of tolerance is directly proportional to the amount of internalization.[31] Furthermore, we have shown that once the complexes are internalized, they follow the same intracellular trafficking pathway of the chylomicrons from the early to the late endosomal compartment and finally, to the lysosomal compartment, where they are apparently enzymatically digested.[32] However, the fact that CM[25] increase the biliary excretion of intact LPS[33] indicates that some fraction of the internalized LPS can escape lysosomal degradation and, either directly or indirectly, interfere with cytokine signaling pathways.

Conclusions

This study shows that the lipid A domain of the LPS macromolecule is both sufficient and essential for the CM-LPS-mediated induction of cytokine tolerance in hepatocytes. Although this induction process depends on the molecular structure of lipid A, it is independent of the specific endotoxic activity of the lipid A domain. The cytokine tolerance-inducing ability of complexes containing modified lipid A moieties, which have much lower endotoxic activities than the native LPS structure, could lead to a novel therapeutic strategy for sepsis by modulating the response of cells or organs to pro-inflammatory mediators.

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References

1. The Veterans Administration Systemic Sepsis Cooperative Study Group. Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. *N Engl J Med* 1987; **317**: 659–665.
2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; **29**: 1303–1310.
3. Morrison DC, Silverstein R, Luchi M, Shnyra A. Structure-function relationships of bacterial endotoxins. Contribution to microbial sepsis. *Infect Dis Clin North Am* 1999; **13**: 313–340.
4. Kushner I, Mackiewicz A. Acute phase proteins as disease markers. *Dis Markers* 1987; **5**: 1–11.
5. Van Zee KJ, Coyle SM, Calvano SE *et al*. Influence of IL-1 receptor blockade on the human response to endotoxemia. *J Immunol* 1995; **154**: 1499–1507.
6. Feingold KR, Serio MK, Adi S, Moser AH, Grunfeld C. Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. *Endocrinology* 1989; **124**: 2336–2342.
7. Thieblemont N, Wright SD. Transport of bacterial lipopolysaccharide to the Golgi apparatus. *J Exp Med* 1999; **190**: 523–534.
8. Harris HW, Grunfeld C, Feingold KR, Rapp JH. Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J Clin Invest* 1990; **86**: 696–702.
9. Harris HW, Grunfeld C, Feingold KR *et al*. Chylomicrons alter the fate of endotoxin, decreasing tumor necrosis factor release and preventing death. *J Clin Invest* 1993; **91**: 1028–1034.
10. Harris HW, Johnson JA, Wigmore SJ. Endogenous lipoproteins impact the response to endotoxin in humans. *Crit Care Med* 2002; **30**: 23–31.
11. Harris HW, Rockey DC, Chau P. Chylomicrons alter the hepatic distribution and cellular response to endotoxin in rats. *Hepatology* 1998; **27**: 1341–1348.
12. Kasravi FB, Welch WJ, Peters-Lideu CA, Weisgraber KH, Harris HW. Induction of cytokine tolerance in rodent hepatocytes by chylomicron-bound LPS is low-density lipoprotein receptor dependent. *Shock* 2003; **19**: 157–162.
13. Harris HW, Gosnell JE, Kumwenda ZL. The lipemia of sepsis: triglyceride-rich lipoproteins as agents of innate immunity. *J Endotoxin Res* 2000; **6**: 421–430.
14. Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. *J Endotoxin Res* 2001; **7**: 167–202.
15. Bissell DM, Tilles JG. Morphology and function of cells of human embryonic liver in monolayer culture. *J Cell Biol* 1971; **50**: 222–231.
16. Knook DL, Sleyster EC. Isolated parenchymal, Kupffer and endothelial rat liver cells characterized by their lysosomal enzyme content. *Biochem Biophys Res Commun* 1980; **96**: 250–257.
17. Sakaguchi K, Koide N, Asano K *et al*. Promotion of spheroid assembly of adult rat hepatocytes by some factor(s) present in the initial 6-hour conditioned medium of the primary culture. *Pathobiology* 1991; **59**: 351–356.
18. Tong JZ, De Lagausie P, Furlan V, Cresteil T, Bernard O, Alvarez F. Long-term culture of adult rat hepatocyte spheroids. *Exp Cell Res* 1992; **200**: 326–332.
19. Redgrave TG, Maranhao RC. Metabolism of protein-free lipid emulsion models of chylomicrons in rats. *Biochim Biophys Acta* 1985; **835**: 104–112.
20. Martins IJ, Vilcheze C, Mortimer BC, Bittman R, Redgrave TG. Sterol side chain length and structure affect the clearance of chylomicron-like lipid emulsions in rats and mice. *J Lipid Res* 1998; **39**: 302–312.
21. Morrow JA, Arnold KS, Weisgraber KH. Functional characterization of apolipoprotein E isoforms overexpressed in *Escherichia coli*. *Protein Expr Purif* 1999; **16**: 224–230.
22. Kumwenda ZL, Wong CB, Johnson JA, Gosnell JE, Welch WJ, Harris HW. Chylomicron-bound endotoxin selectively inhibits NF-kappaB activation in rat hepatocytes. *Shock* 2002; **18**: 182–188.
23. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [^{15}N]-nitrate in biological fluids. *Anal Biochem* 1982; **126**: 131–138.
24. Seydel U, Oikawa M, Fukase K, Kusumoto S, Brandenburg K. Intrinsic conformation of lipid A is responsible for agonistic and antagonistic activity. *Eur J Biochem* 2000; **267**: 3032–3039.
25. Persing DH, Coler RN, Lacy MJ *et al*. Taking Toll: lipid A mimetics as adjuvants and immunomodulators. *Trends Microbiol* 2002; **10**: S32–S37.
26. He SY, Deng HW, Li YJ. Monophosphoryl lipid A-induced delayed preconditioning is mediated by calcitonin gene-related peptide. *Eur J Pharmacol* 2001; **420**: 143–149.
27. Ishida H, Irie K, Suganuma T *et al*. A lipid A analog ONO-4007 induces tolerance to plasma leakage in mice. *Inflamm Res* 2002; **51**: 38–43.
28. Reisser D, Pance A, Jeannin JF. Mechanisms of the antitumoral effect of lipid A. *Bioessays* 2002; **24**: 284–289.
29. Low MG, Huang KS. Phosphatidic acid, lysophosphatidic acid, and lipid A are inhibitors of glycosylphosphatidylinositol-specific phospholipase D. Specific inhibition of a phospholipase by product analogues? *J Biol Chem* 1993; **268**: 8480–8490.
30. Low MG, Stutz P. Inhibition of the plasma glycosylphosphatidylinositol-specific phospholipase D by synthetic analogs of lipid A and phosphatidic acid. *Arch Biochem Biophys* 1999; **371**: 332–339.
31. Kasravi FB, Brecht WJ, Weisgraber KH, Harris HW. Induction of cytokine tolerance requires internalization of chylomicron-bound LPS into hepatocytes. *J Surg Res* 2003; **115**: 303–309.
32. Harris HW, Brady SE, Rapp JH. Hepatic endosomal trafficking of lipoprotein-bound endotoxin in rats. *J Surg Res* 2002; **106**: 188–195.
33. Read TE, Harris HW, Grunfeld C *et al*. Chylomicrons enhance endotoxin excretion in bile. *Infect Immun* 1993; **61**: 3496–3502.