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In vivo protection against endotoxin by plasma high density lipoprotein

(lipopolysaccharide/tumor necrosis factor α/infectious disease/transgenic/photoaffinity labeling)

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ABSTRACT **Overwhelming bacterial infection is accompanied by fever, hypotension, disseminated intravascular coagulation, and multiple organ failure leading to death in 30–80% of cases. These classical symptoms of septic shock are caused by potent cytokines that are produced in response to endotoxin released from Gram-negative bacteria. Treatments with antibodies and receptor antagonists to block endotoxin or cytokine mediators have given mixed results in clinical trials. High density lipoprotein (HDL) is a natural component of plasma that is known to neutralize endotoxin *in vitro*. We report here that raising the plasma HDL concentration protects mice against endotoxin *in vivo*. Transgenic mice with 2-fold-elevated plasma HDL levels had more endotoxin bound to HDL, lower plasma cytokine levels, and improved survival rates compared with low-HDL mice. Intravenous infusion of HDL also protected mice, but only when given as reconstituted HDL prepared from phospholipid and either HDL apoprotein or an 18-amino acid peptide synthesized to mimic the structure of apolipoprotein A-I of HDL. Intact plasma HDL was mildly toxic, and HDL apoprotein was ineffective. The effectiveness of the reconstituted peptide renders very unlikely any significant contribution to protection by trace proteins in apo-HDL. These data suggest a simple leaflet insertion model for binding and neutralization of lipopolysaccharide by phospholipid on the surface of HDL. Plasma HDL may normally act to protect against endotoxin; this protection may be augmented by administration of reconstituted HDL or reconstituted peptides.**

The positive association of high density lipoprotein (HDL) with low rates of cardiovascular disease is thought to be due to HDL-mediated reverse cholesterol transport: binding of excess cholesterol from cells, neutralization by conversion to surface-inactive cholesterol ester, and transport to the liver for elimination (1–3). We propose that HDL is a vehicle for reverse transport of biologically active lipids, in general. *In vivo* studies of the binding and neutralization of endotoxin by HDL reported here support this hypothesis.

Endotoxin is a lipopolysaccharide (LPS) that is released from the outer surface membrane of Gram-negative bacteria (4). In plasma, LPS can form complexes with lipoproteins or with LPS-binding-protein (5, 6). Binding of the LPS–LPS-binding protein complex to the CD14 receptor on cells of the monocyte/macrophage lineage (7) triggers production and release of a cascade of cytokines including tumor necrosis factor α (TNF-α), interleukin 1, and interleukin 6 (8). These cytokines mediate the inflammatory response to Gram-negative infection (9). Systemic release of these mediators during bacteremia or overwhelming Gram-negative infection can lead to septic shock and death (10).

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In vitro studies of mediator production by monocyte/macrophages show that binding of LPS to HDL and other lipoproteins greatly reduces production and release of TNF-α, interleukin 1, and interleukin 6 (11–16). Several lines of evidence indicate that the dissociation rate of LPS from the LPS–lipoprotein complex is substantially slower than the clearance rate of the lipoprotein. LPS–HDL or LPS–LDL complexes are cleared with half-lives the same as, or slightly longer than, that of the natural lipoprotein (17). Tissues that clear LPS–lipoprotein complexes are those that express the appropriate lipoprotein receptors, and clearance is regulated by hormones that affect lipoprotein-receptor expression (18, 19). When administered as a preformed complex, LPS does not exchange from LPS–LDL or LPS–HDL onto other lipoproteins (20), and the complexes are substantially less toxic than free LPS *in vivo* (16, 21, 22).

Several authors have pointed out that the capacity of HDL to bind LPS is 10- to 1000-fold above the LPS concentrations reported in studies of septic patients (6, 20, 23). Given this excess binding capacity, it seemed unlikely that the 2- to 3-fold changes in plasma HDL concentration that can be achieved *in vivo* could increase the fraction of LPS bound to HDL or provide additional protection. We have studied HDL-mediated endotoxin resistance in mice *in vivo* when LPS is given directly without prior *ex vivo* incubation with HDL. We found increased survival, increased LPS binding to HDL, and decreased TNF-α production in transgenic mice that express human apolipoprotein A-I (Hu-A-I) at high levels and have elevated plasma HDL concentrations. These observations were extended in experiments with i.v. infusion of synthetic HDL into nontransgenic mice. Natural HDL contains traces of endotoxin (0.1–15 pg/ml) and oxidized lipids that are eliminated during the preparation of chemically defined, endotoxin-free reconstituted HDL (R-HDL). R-HDL resembles newly secreted hepatic HDL and is rapidly incorporated into the circulating HDL pool after i.v. infusion. Intravenous administration of R-HDL, made with either human apo-HDL or an 18-amino acid synthetic peptide, provided immediate protection against a lethal dose of endotoxin.

MATERIALS AND METHODS

Reagents. LPS, *Escherichia coli* strain 0111:B4 and *Salmonella minnesota* strain Re595 were purchased from List Biological Laboratories (Cambell, CA). *Salmonella* LPS was from Immuno AG (batch H1271; Vienna). Phosphatidylcho-

Abbreviations: HDL, high density lipoprotein; LPS, lipopolysaccharide (endotoxin); TNF-α, tumor necrosis factor α; Mo-A-I, mouse apolipoprotein A-I; Hu-A-I, human apolipoprotein A-I; R-HDL, reconstituted preparation with apo-HDL; ASD, 2-(*p*-azidosalicylamido)-1,3′-dithiopropionate; R-peptide, reconstituted preparation with peptide 18A.

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line (99% from egg yolk) was from Avanti Polar Lipids. Human plasma was obtained from the blood bank at The New York Hospital—Cornell Medical Center.

Mice. The derivation of transgenic mice lines [Tg(OHSA-A-I)179 and Tg(OHSA-A-I)427, which carry 11.5- or 2.2-kb constructs of the Hu-A-I gene, respectively] has been described (24, 25). Heterozygous transgenic C57BL6/CBA males from each line were harem-bred with nontransgenic C57BL6/CBA females to produce litters that contained nontransgenic controls and transgenic mice in roughly equal numbers. Outbred male Swiss–Webster mice (30 g) were purchased from Taconic Farms.

Preparation of Human Apo-HDL. HDL was purified from human plasma by sequential density-flotation ultracentrifugation between 1.063 g/ml and 1.21 g/ml. The remaining traces of plasma proteins were removed by density flotation through a layer of NaBr at 1.21 g/ml in a Beckman Ti60 rotor (26). Five-gram batches of purified HDL were dialyzed against EDTA at 1 mg/ml, lyophilized, and extracted three times with 2 liters of cold chloroform/methanol, 2:1 (vol/vol) and twice with 500 ml of anhydrous diethyl ether. Lipid-free, apo-HDL was collected by filtration through Whatman no. 4 paper, dried under vacuum, and stored at −70°C. Composition was estimated by scanning of SDS/3–27% PAGE gels or by direct measurement of protein in fractions separated by fast protein liquid chromatography through columns of Superose 6 and 12 operated in series. The final HDL preparation contained 85% apolipoprotein A-I with 15% apolipoprotein A-II and apolipoprotein Cs, <0.5% albumin, and no detectable endotoxin.

Synthetic Peptide. An 18-amino acid peptide (DWLKAFYDKVAEKLKEAF), designated 18A (27), was prepared by the solid-phase peptide-synthesis core facility at the State University of New York at Stony Brook. Purified peptide was reconstituted with phospholipid by the same method described below for apo-HDL.

Reconstitution. Apo-HDL (for R-HDL) and peptide 18A (for R-peptide) were reconstituted with 99% pure egg phosphatidylcholine, 2:1 (wt/wt), as described by Matz and Jonas (28) except that the last cholic acid traces were removed by adsorption to Bio-Rad SM2 beads as described by Bonomo and Swaney (29). The compositions of R-HDL and R-peptide preparations were protein (apo-HDL or the 18-aa peptide) phospholipid/cholic acid, 1:100: < 0.2 (mole ratio).

^{125}I-Labeled-2-(*p*-azidosalicylamido)-1,3′-dithiopropionamide Re595 LPS (^{25}I-ASD-LPS). Re595 LPS (*S. minnesota*) was derivatized with sulfosuccinimidyl-2-(*p*-azidosalicylamido)-1,3′-dithiopropionate (ASD; Pierce) and radioiodinated with Na^{125}I using chloramine-T as described by Wollenweber and Morrison (30). The specific activity of ^{125}I-labeled ASD-LPS was adjusted to 0.1 μCi/μg (1 Ci = 37 GBq) by addition of unlabeled Re595 LPS before i.p. administration to mice.

Lipid, Lipoprotein, and Apolipoprotein Measurements. All measurements were done on plasma using a Roche COBAS FARA II clinical chemistry analyzer (Roche Diagnostics). Plasma total cholesterol, HDL cholesterol (plasma HDL concentrations are expressed as mg of HDL cholesterol per dl), and acylglycerols (commonly called triglycerides) were measured by enzymatic methods (31); Hu-A-I was measured by an immunoturbidimetric method (INCSTAR, Stillwater, MN).

Experiments with Transgenic Mice. A total of 305 nontransgenic controls and transgenic mice were screened from both transgenic lines in roughly equal numbers. Mice were identified by ear tattoos and weighed, and a sample of blood was taken for measurement of total cholesterol, acylglycerols, HDL, and Hu-A-I. After a minimum of 3 days to recover from bleeding, the mice were challenged with LPS at 30 mg/kg (*E. coli* 0111:B4) given by i.p. injection. Survivors were counted at 12, 24, 36, and 48 hr by observers blinded to plasma Hu-A-I levels.

Photoaffinity Labeling of Apolipoprotein A-I by ^{125}I-Labeled ASD-LPS. A second group of 300 mice were screened as above to identify high-expressors (plasma Hu-A-I > 250 mg/dl; HDL ≥ 120 mg/dl) and low expressors (90 < Hu-A-I < 150; HDL ≤ 70 mg/dl). ^{125}I-labeled ASD-LPS was administered i.p. at 30 mg/kg, and the mice were bled at 0.5, 1, 1.5, and 3 hr. Plasma ^{125}I was measured in a Packard 5210 scintillation spectrometer, and samples were irradiated with UV light (4 W at 254 nm Mineralight; Ultra-Products, San Gabriel, CA) in borosilicate tubes for 10 min at 20°C. Photoaffinity-labeled proteins (2 μl of mouse plasma per lane) and ^{14}C-labeled M_r standards (Amersham) were reduced with 200 mM dithioerythritol/4 M urea/3% SDS/10% (vol/vol) glycerol/0.02% bromphenyl blue/62.5 mM Tris·HCl buffer, loaded onto a 3% stacking gel, and separated by SDS/15% PAGE gels according to Laemmli (32). Radioautography was done on fixed and dried gels as described by Wollenweber and Morrison (30).

Plasma TNF-α. Another group of five high- and five low-expressor mice was selected as described above. Blood was drawn from each mouse through the orbital plexus into heparinized capillary tubes 0.5 hr before and 1.5, 2.0, 2.5, and 3 hr after LPS (*E. coli* strain 0111:B4; 30 mg/kg) was given i.p. The separated plasma fraction was diluted for measurement of mouse TNF-α in a bioassay using the WEHI 164 clone 13 murine fibrosarcoma cell line (33). All measurements were done in triplicate; data are means ± SDs in units/ml relative to recombinant human TNF-α.

Intravenous Administration of R-HDL and R-Peptide. Outbred, male Swiss-Webster mice weighing between 25 and 35 g were randomized to control and treatment groups. Saline (n = 20), R-HDL (n = 40), or R-peptide (n = 20) was administered through the tail vein at a dose of 80 mg/kg (2.4 mg of protein or peptide per 30-g mouse) to raise the circulating apolipoprotein A-I concentration [mouse apolipoprotein A-I (Mo-A-I) plus Hu-A-I] ≈2-fold from ≈140 to ≈300 mg/dl. Preparations of reconstituted HDL were free of endotoxin by the United States Pharmacopeia rabbit pyrogen test. Endotoxin (*Salmonella* LPS) was given i.p. at a dose of 10 mg/kg within 15 min of the HDL. This dose of *Salmonella* LPS was lethal to 80% of Swiss–Webster control mice and nonlethal in endotoxin-resistant C3H/HEJ mice (four of four). All mice were kept in a temperature- and humidity-controlled environment; survivors were counted at 24 and 48 hr by observers that were blinded to treatment.

Statistical Analysis. Results for plasma levels of Hu-A-I, total cholesterol, acylglycerols, and HDL are reported as the mean ± SD; between-group differences in plasma total cholesterol, acylglycerols, and HDL were analyzed by the χ^2 linear-trend test. Survival data were analyzed by Kaplan–Meier analysis (34). Preliminary analysis of the data from the group of 305 transgenic and nontransgenic mice revealed a strong inverse relationship between body weight and survival among mice that weighed <20 g. This result was expected from preliminary experiments in which smaller animals had been observed to be far more susceptible to the effects of dehydration. After exclusion of the 129 animals weighing <21 g, there was no significant relationship between survival and body weight, sex, or strain (lines 179 and 427). The remaining 178 animals were divided into expressor and nonexpressor groups according to plasma Hu-A-I concentration. Expressors were further divided into quartiles.

RESULTS

Transgenic High-HDL Mice. Two transgenic mice lines [Tg(OHSA-A-I)179 (24) and Tg(OHSA-A-I)427 (25)], which carry 11.5- or 2.2-kb constructs of the Hu-A-I gene and have

plasma HDL concentrations of ≈90 and ≈120 mg/dl, respectively, were used to obtain animals with a range of plasma HDL concentrations. Plasma concentrations of Hu-A-I, total cholesterol, acylglycerols, and HDL cholesterol are shown in Table 1. In nontransgenic littermates, the Mo-A-I plasma concentration averaged 137 ± 12 mg/dl (35), and HDL averaged 60 ± 7 mg/dl. Expression of Hu-A-I in these mice causes plasma Mo-A-I concentrations to fall to very low levels (< 20 mg/dl) (35, 36). Consequently plasma HDL does not differ significantly between groups O and I. The largest differences in plasma HDL were in comparisons of groups O and I vs. group IV. However, within the four quartile groups of transgenic animals, plasma HDL concentrations were linearly related to Hu-A-I.

The same trend is apparent in the survival data shown in Fig. 1. Less than 12% of nonexpressors and 8% of the lowest quartile of expressors survived 48 hr compared with 37% of mice in the highest quartile of Hu-A-I concentration. Kaplan–Meier analysis demonstrated that survival time was significantly improved among mice in the highest quartile of Hu-A-I compared with nonexpressors ($P < 0.04$). Among expressors, the plasma concentration of Hu-A-I predicted survival ($P = 0.059$; $n = 101$; Cox covariates model). The data suggest further increases in survival rate at higher HDL levels; survival rates among the 10 highest and 5 highest expressors were 50% and 60% with HDL levels of 127 ± 11 and 136 ± 19 mg/dl, respectively. After adjustment for Hu-A-I, there was no significant relationship between survival and total cholesterol or acylglycerols or transgene construct [Tg(OHSA-A-I)179 vs. Tg(OHSA-A-I)427].

Binding of LPS to HDL in Transgenic Mice. To determine whether changes in survival were related to increased binding of LPS to HDL, low- and high-HDL transgenic mice were given ^{125}I-labeled ASD-LPS (*S. minnesota* Re595) at 30 mg/kg as above. Re595 LPS was used because the photoaffinity reagent attaches to a free aminoethyl group, just above the lipid-A domain (see refs. 37, 38 and *Discussion*), making it available to crosslink to a protein at the surface of HDL in LPS–HDL complexes. The time course of appearance of ^{125}I in plasma is shown in Fig. 2 *Upper*. ^{125}I-Labeled ASD-LPS was detected in plasma within 5 min, and concentrations peaked at ≈30 min after i.p. administration. Laser densitometry revealed that significantly more ^{125}I was incorporated into apolipoprotein A-I in high- vs. low-HDL mice (high/low = 2.3 ± 0.7; $P < 0.02$, paired t test; $n = 4$ pairs; Fig. 2 *Upper Inset*).

Inhibition of TNF Release. Plasma TNF-α was measured in another set of high- and low-HDL mice to test the hypothesis that binding of LPS to HDL would reduce plasma TNF-α levels in endotoxin-treated mice. The time course of plasma TNF-α concentration after challenge with endotoxin is shown in Fig. 2 *Lower*. Plasma TNF-α concentrations were lower at all times in the high-HDL group; the area under the TNF-α concentration vs. time curve in mice with high plasma HDL was only 17% of that measured in the mice with a low plasma HDL (194 ± 132 vs. 1126 ± 582 units/ml per hr; $P < 0.03$).

Table 1. Plasma Hu-A-I, lipids, and HDL in normal and transgenic mice

Group (n)	A-I	TC	AC	HDL
O (131)		81 ± 20	95 ± 42	60 ± 7
I (26)	168 ± 38	88 ± 19	92 ± 52	69 ± 11
II (25)	235 ± 14	96 ± 18	97 ± 44	88 ± 4
III (26)	269 ± 12	111 ± 26	108 ± 49	97 ± 3
IV (24)	389 ± 30	138 ± 20	105 ± 43	120 ± 11

Plasma Hu-A-I, lipid, and HDL-cholesterol concentrations in transgenic mice. Transgenic males from lines Tg(OHSA-A-I)179 and Tg(OHSA-A-I)427 were harem-bred with nontransgenic C57BL6/CBA females to produce roughly equal numbers of normal and transgenic mice. Animals were divided for statistical analysis into five groups: O, nonexpressors (Hu-A-I below detection limit of the assay; <26 mg/dl); and groups I, II, III, IV—the four quartiles of Hu-A-I expressors. Total cholesterol (TC), HDL cholesterol (HDL), acylglycerols (AC), and Hu-A-I (mg/dl) are presented as means ± SDs. Between-group differences in TC and HDL are significant (χ^2 linear-trends test, $P < 0.01$).

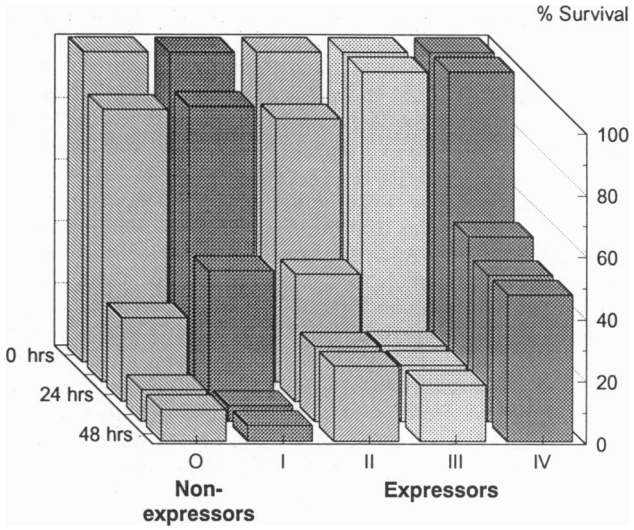

FIG. 1. Percentage survival of transgenic mice after endotoxin challenge. Groups are described in Table 1. Survival (%) is shown at $t = 0$, 12, 24, 36, and 48 hr. Kaplan–Meier analysis revealed significant differences in survival between nonexpressors and the top quartile of transgenic Hu-A-I expressors (groups O vs. IV; $P < 0.04$).

Intravenous Administration of Reconstituted HDL and Reconstituted Synthetic Peptide. To address the possibility that disruptive insertions of the transgenes might have affected TNF-α production or that chronic exposure to elevated plasma HDL might be immunosuppressive, we studied the effect of raising plasma HDL immediately before endotoxin challenge. In preliminary experiments in rabbits, we found that natural HDL contained endotoxin traces (50–1000 pg of endotoxin per mg of HDL protein; five preparations) and was mildly pyrogenic when given i.v. to rabbits. This endogenous LPS was removed (below level of detection, <5 pg of LPS per mg of HDL protein by extraction of HDL lipids with chloroform/methanol. Intravenous administration of apo-HDL had little or no effect on plasma HDL concentration. Apo-HDL reconstituted with phospholipid (R-HDL) was not pyrogenic in rabbits. R-HDL rapidly incorporated cholesterol and cholesterol ester and was converted to spherical plasma HDL in agreement with previous reports (27 and the references therein).

Nontransgenic male Swiss–Webster mice were administered saline, R-HDL, or R-peptide at 80 mg/kg through the tail vein to double the plasma HDL concentration. Endotoxin was administered by i.p. injection 15 min later. R-HDL significantly improved survival compared with saline-treated controls (Fig. 3; Kaplan–Meier analysis, $P < 0.01$). Particles reconstituted with a synthetic peptide that contained only the helical lipid-associating domain of apolipoprotein A-I were also effective compared with saline-treated controls ($P < 0.05$).

DISCUSSION

In two independent model systems, we have tested the hypothesis that raising the plasma HDL concentration can protect mice against LPS-induced endotoxic shock. Intravenous infusion of reconstituted protein or peptide and expression of Hu-A-I transgenes protected mice from a lethal dose

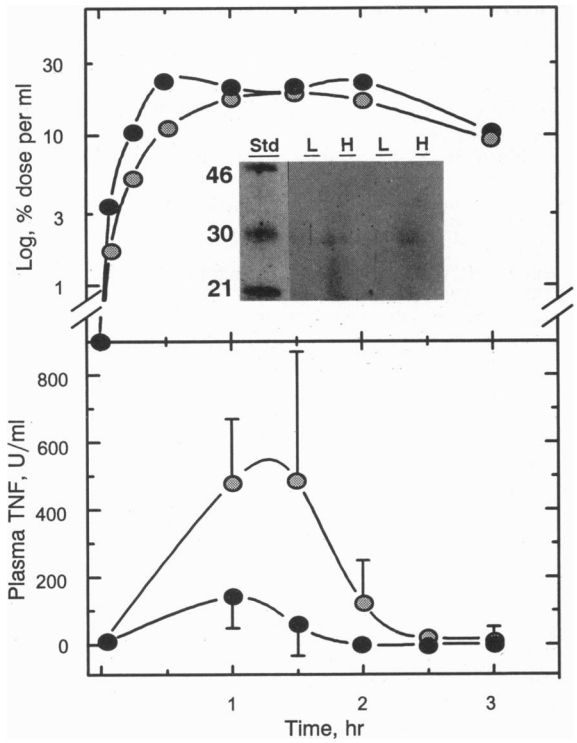

FIG. 2. (*Upper*). Transfer of ^{125}I-labeled ASD-LPS from the peritoneum to the plasma compartment and increased binding to apolipoprotein A-I of HDL in high- vs. low-HDL mice (Hu-A-I > 250 and < 150 mg/dl, respectively). Data are presented as percentage of total administered dose per ml of plasma for high- (●) and low- (○) HDL mice. (*Inset*) Apoprotein A-I photoaffinity-labeled with ^{125}I from ^{125}I-labeled ASD-LPS at 120 and 180 min in high (H) and low (L) HDL mice, respectively. Laser densitometry of exposed films revealed significant increase of ^{125}I-labeled apolipoprotein A-I (28-kDa) in the high-HDL animals (2.3 ± 0.7-fold; $P < 0.02$, paired t test). U, units; Std, standards. (*Lower*) Plasma TNF-α concentration in high- and low-HDL mice. Data are means ± SD of triplicate measurements in units/ml relative to recombinant human TNF-α; ●, high HDL, $n = 5$; ○, low HDL, $n = 5$.

of LPS. Doubling the plasma HDL level resulted in a 3- to 4-fold increase in survival. An alternative hypothesis of immunosuppression by a disruptive insertion of the transgenes or by chronic exposure to elevated plasma HDL was rejected because i.v. administration of R-HDL and R-peptide provided immediate protection against LPS. In isolated peripheral blood mononuclear cells R-HDL selectively blocked TNF-α production in response to LPS, but not in response to phytohemagglutinin. Moreover, R-HDL only modestly reduced TNF-α production in response to heat-killed *Staphylococcus aureus* in the whole human blood system (T. S. Parker, D. M. Levine, A. L. Rubin, The Rogosin Institute, New York; J. C. Chang, J. A. Laxer, and C. C. Coffin, Immune Response Corporation, Carlsbad, CA; unpublished data). Finally, immunosuppression by HDL is inconsistent with the positive association between plasma HDL concentration and low morbidity and mortality in epidemiological studies.

The structural model of the LPS–HDL complex shown in Fig. 4 illustrates how the lipid-A domain of LPS can be masked by insertion into the phospholipid bilayer of discoidal R-HDL or the phospholipid monolayer on the surface of spherical plasma HDL. The lipid-A domain anchors LPS in the phospholipid bilayer of the outer membrane of Gram-negative bacteria (4), phospholipid monolayers and liposomes (39, 40), and LDL (41). We propose that the fatty acyl chains of lipid A become anchored to the surface of discoidal

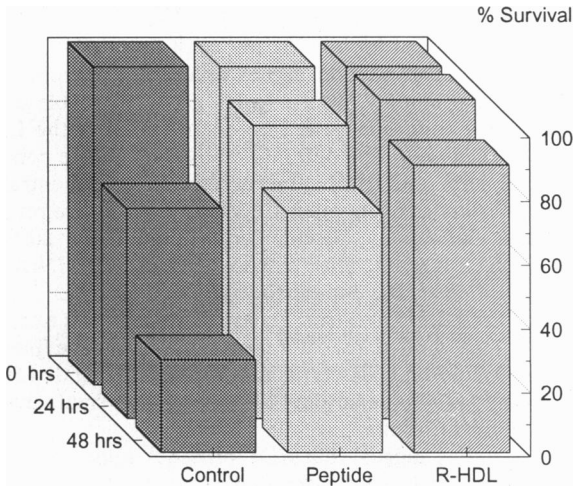

FIG. 3. Protection from LPS by i.v. R-HDL and R-peptide. *Salmonella* LPS (batch H1271 from Immuno AG, Vienna) was given i.p. at 10 mg/kg within 15 min of the HDL. Survival was significantly improved by i.v. administration of R-HDL ($P < 0.01$) and R-peptide ($P < 0.05$; Kaplan–Meier analysis).

R-HDL and spherical plasma HDL in the same orientation; this effectively masks the biological activity of LPS, which resides entirely within the lipid-A domain (4). Although further studies of the LPS–HDL complex are needed, this working model is consistent with the ability of HDL to neutralize LPS from rough (*E. coli* 0111:B4) and smooth (*S. minnesota* Re595) Gram-negative bacteria that contain a competent membrane-anchoring domain independent of the structure of the core and O-antigen domains. This orientation places ^{125}I-labeled ASD-LPS in a position that allows it to crosslink proteins on the surface HDL. The model is also consistent with changes in the mobility of HDL on native gradient PAGE after *in vitro* incubation with LPS or recovered from mice that have received LPS (data not shown).

Several authors have pointed out that the capacity of HDL to bind LPS is 10- to 1000-fold above the LPS concentrations reported in studies of septic patients (6, 20, 42), yet our data show clearly that further increases of plasma HDL provide additional protection. It is possible that only a small subfrac-

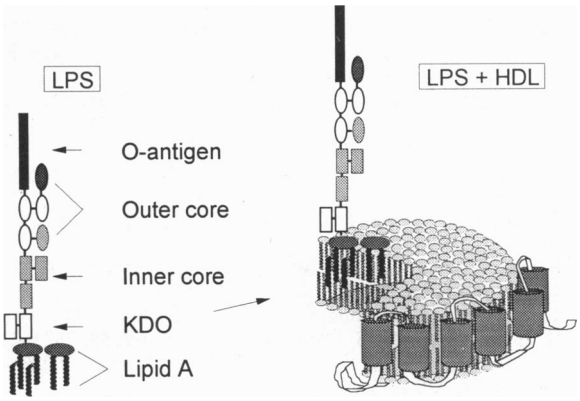

FIG. 4. Structural model of LPS binding and neutralization by HDL. R-HDL is drawn to scale to illustrate the insertion of the membrane-anchoring (Lipid A) domain of LPS into the phospholipid bilayer of discoidal, reconstituted HDL. The complete disk contains 120 mol of phospholipid per leaflet, 240 mol of total, and 3 mol of apolipoprotein A-I per particle and has a diameter of ≈103 Å. Reconstituted disks with this composition are rapidly converted to spherical HDL in plasma. One of the three apolipoprotein A-I peptides is shown with six amphipathic helices (II–VII) as suggested by the energy minimization modeling of Brasseur *et al.* (44, 45). KDO, 3-deoxy-D-*manno*-octulosonic acid.

tion of HDL is active in binding LPS. Similar mechanisms have been proposed for binding free cholesterol (43), but the effectiveness of R-peptide argues against this hypothesis. Assuming that binding of LPS to HDL is irreversible under physiological conditions, the rate of formation of the LPS–HDL complex would be determined by the relative concentration of LPS and HDL. Increased HDL concentration increases binding, even when LPS binding sites are present in excess. Conversely, recent studies show lower survival rates among transgenic mice with low plasma HDL [Nishina, P. M. and Paigen, B., unpublished work; these studies used congenic C57BL6/J and transgenic C57BL6/J mice expressing the human cholesterol ester transfer protein gene (plasma HDL of 64 and 34 mg/dl, respectively)], suggesting that HDL substantially affects sensitivity to LPS in the normal range of plasma concentrations.

These studies suggest that HDL provides some protection against acute endotoxemia for individuals with high plasma HDL levels. HDL or reconstituted HDL may be useful clinically in the prevention or treatment of septic shock by acting broadly against endotoxins to block production of at least one mediator (TNF-α) of septic shock.

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