Antiinflammatory Effects of Reconstituted High-density Lipoprotein during Human Endotoxemia

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Summary

High-density lipoprotein (HDL) has been found to neutralize LPS activity in vitro and in animals in vivo. We sought to determine the effects of reconstituted HDL (rHDL) on LPS responsiveness in humans in a double-blind, randomized, placebo-controlled, cross-over study. rHDL, given as a 4-h infusion at 40 mg/kg starting 3.5 h before endotoxin challenge (4 ng/kg), reduced flu-like symptoms during endotoxemia, but did not influence the febrile response. rHDL potently reduced the endotoxin-induced release of TNF, IL-6, and IL-8, while only modestly attenuating the secretion of proinflammatory cytokine inhibitors IL-1ra, soluble TNF receptors and IL-10. In addition, rHDL attenuated LPS-induced changes in leukocyte counts and the enhanced expression of CD11b/CD18 on granulocytes. Importantly, rHDL infusion per se, before LPS administration, was associated with a downregulation of CD14, the main LPS receptor, on monocytes. This effect was biologically relevant, since monocytes isolated from rHDL-treated whole blood showed reduced expression of CD14 and diminished TNF production upon stimulation with LPS. These results suggest that rHDL may inhibit LPS effects in humans in vivo not only by binding and neutralizing LPS but also by reducing CD14 expression on monocytes.

The systemic toxicity of Gram-negative sepsis is in large part mediated by endotoxin which induces an extensive inflammatory response characterized by cytokine release and activation of leukocytes. Once in the circulation, endotoxin is bound by lipopolysaccharide-binding protein (LBP)[1] which can transfer LPS to either cell-bound CD14 (causing activation of these cells), to soluble CD14 (facilitating activation of cells not expressing CD14 on their surface), or to lipoproteins (1–7). Binding of LPS to lipoproteins results in inactivation of LPS (8–11). Preincubation of endotoxin with low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) and chylomicrons reduced endotoxin-induced lethality (12), whereas hypolipidemic animals were more sensitive to endotoxin (13). Further, transgenic mice with elevated apolipoprotein A-1 (apoA-1) and HDL levels are protected against LPS-induced mortality (14).

The endotoxin-neutralizing capacity of lipoproteins is dependent on the lipid composition. Reconstituted human HDL (rHDL), containing purified apoA-1, phosphatidyl choline and cholesterol, neutralized endotoxin in whole blood more effectively than LDL, VLDL, and natural HDL (15, 16). Pretreatment of animals with rHDL reduced endotoxin-induced TNF production, leukopenia and lethality (17–19). The present study was designed to investigate the endotoxin-neutralizing properties of rHDL (40 mg/kg) in humans in vivo.

[1] *Abbreviations used in this paper:* apoA-1, apolipoprotein A-1; HDL, high-density lipoprotein; LBP, lipopolysaccharide-binding protein; LDL, low-density lipoprotein; rHDL, reconstituted human HDL; VLDL, very low-density lipoprotein.

Materials and Methods

Human Endotoxemia. Eight healthy male volunteers (mean age 24, range 20–28 yr) were enrolled in this double-blind, cross-over, randomized, placebo-controlled study. Written informed consent was obtained from all participants. Medical history, physical and routine laboratory examination, chest x-ray and electrocardiogram were normal. The volunteers did not smoke, did not use any medication and did not have any febrile illness in the month preceding the study. Each participant was studied on two occasions, separated by a wash-out period of six weeks. On one occasion the subject was challenged with endotoxin in combination with placebo, on the other in combination with rHDL. The study was approved by the research and ethical committees of the Academic Medical Center.

The study drug rHDL Lot no. 4.955.006.0 (ZLB Central Laboratory, Bern, Switzerland) was supplied as a pyrogen-free lyophilized product with 91% apoA-1 purity (20). The appropriate

amount of rHDL , in a solution containing 2% protein and 10% saccharose, was aspirated into dark-colored Amberlite syringes (Plastipak, Becton-Dickinson, Mountain View, CA) and administered as a 4-h infusion through an intravenous line at a dose of 40 mg/kg. The placebo solution consisted of isotonic saline and was administered in an identical manner.

The *Escherichia coli* endotoxin preparation used in this study, lot EC-6 (D. Hochstein, Bureau of Biologics, Food and Drug Administration, Bethesda, MD) was administered over one minute in an antecubital vein of the contralateral arm at a dose of 4 ng/kg, 3.5 h after the initiation of the placebo or rHDL infusion. The study was performed at a special research unit under continuous supervision of at least two physicians with emergency and resuscitation equipment immediately available. Blood pressure and heart rate were assessed every 30 min using a Dinamap device (Criticon, Tampa, FL) during the first 8 h after endotoxin challenge; oral temperature and respiratory rate were assessed at the same time points. Adverse events were registered throughout the confinement periods by a clinical symptom score. Adverse events were scored by incidence and severity (0 as absent, 1 as weakly, 2 as moderately, and 3 as severely present).

Whole Blood Incubation and In Vitro PBMC Stimulation. In separate in vitro experiments, blood was collected into pyrogen-free tubes (Falcon 2063; Becton Dickinson, Mountain View, CA), containing pyrogen-free heparin (Thromboliquine®; Organon, Oss, the Netherlands, final concentration 30 IU/ml). After incubation of whole blood with rHDL (final concentration 0.5 or 2.0 mg/ml) for 1 h in a 5% CO_2 incubator at 37°C, blood was diluted 1:1 in PBS and subsequently human PBMC were isolated by centrifugation over a Lymphopaque density gradient (Ficoll Paque; Pharmacia, Woerden, the Netherlands) at room temperature for 25 min at 1,000 *g*. After three washes, PBMC were brought to a concentration of 5×10^6 cells/ml with HBSS and CD14 expression on monocytes was measured using FACScan® analysis as described below. In parallel experiments, PBMC (5×10^6 cells/ml) were stimulated with LPS (*E. coli* 0111:B4; Sigma Chem. Co., St. Louis, MO; final concentration 10 ng/ml) for 4 h at 37°C in HBSS containing 10% sterile non-acute human serum (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service CLB, Amsterdam, the Netherlands).

After incubation, samples were centrifuged and stored at −20°C until TNF analysis. All experiments were performed four times.

Assays. For leukocyte and differential counts, blood was collected in tubes containing EDTA (K_3) (15%) and counted by flow cytometry (Technicon H1 system, Technicon Instruments, Tarrytown, NY). Blood for LPS measurements was collected in pyrogen-free plastic tubes (model 2063; Falcon, Oxnard, CA) containing pyrogen-free heparin (final concentration 30 IU/ml, Thromboliquine®; Organon). For the Limulus assay, platelet rich plasma was prepared by centrifugation of heparinized blood at 180 *g* for 10 min at 4°C and subsequently stored at −20°C. The Limulus assay was performed as described previously (21). Inhibitors and activated clotting factors were removed by dilution and heating at 37°C for 5 min. Standard curves were made with *E. coli* O55: B5 (Mallinckrodt Inc., St. Louis, MO). This assay had a detection limit in plasma of 36 EU/l. Cytokines were determined in serum using specific ELISAs (TNF, IL-6, IL-8, IL-10, soluble TNF receptors I and II [sTNFr I and II], and soluble CD14 [sCD14]; Medgenix Diagnostic, Brussels, Belgium; IL-1 receptor antagonist [IL-1ra]; R&D Systems, Abingdon, United Kingdom).

LBP was measured in serum using reagents kindly provided by Dr. S. Carroll (Xoma Corporation, Berkeley, CA), as described previously (22). HDL cholesterol concentrations were assayed using nephelometry (Boehringer Mannheim, Mannheim, Germany).

FACScan® Analysis. At −3.5, 0, 3, and 24 h relative to endotoxin challenge blood was drawn into tubes containing 15% EDTA (K_3) and placed on ice. After lysis of the erythrocytes with isotonic NH_4Cl solution (155 mmol/l NH_4Cl, 10 mmol/l $KHCO_3$, 0.1 mmol/l EDTA, pH 7.4), samples were centrifuged at 300 *g* for 10 min and residual erythrocytes were lysed for 5 min. The remaining cells were washed twice in PBS and subsequently fixed in PBS, containing 1% BSA, 0.3 mmol/l EDTA, 0.01% sodium azide and 0.1% paraformaldehyde (final concentration 5×10^6 cells/ml). All procedures were performed at 4°C. The following antibodies were used: FITC anti-human CD11b, FITC anti-human CD14, and FITC anti-human CD18 (CLB-mon-gran/1,B2, CLB-mon/1,8G3, CLB-LFA-1/1,5D7, respectively; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service CLB, Amsterdam, The Netherlands). As a control FITC IgG_1 (Becton Dickinson) was used. Cells were incubated for 30 min at 4°C after addition of the mAb to the cell suspension and washed twice in ice-cold PBS, containing 0.1% BSA, 0.3 mmol/L EDTA, 0.01% sodium azide. The mean fluorescence intensities (MFI) of labeled granulocytes and monocytes were recorded using FACS®, after gating the cells using their forward and side scatter properties. Per time point 10,000 granulocytes and 2,500 monocytes were counted. After subtracting control IgG_1 fluorescence, specific antibody binding was expressed as percent changes from pretreatment values obtained at 3.5 h before endotoxin challenge. Antibodies against apoA-1 or rHDL in the sera of the volunteers were determined by ELISA before and three months after rHDL infusion. Either rHDL or apoA-1 were used as coats on microtiter plates, and were incubated with volunteer's serum. The assay developed with a sheep antiserum against human apoA-1 and an anti-sheep IgG alkaline phosphatase conjugate. Positive controls included tests of the individual sera with a tetanus toxoid coat, and a test with rabbit anti-human apoA-1 on the apoA-1 coat with the corresponding second antibody.

Statistical Analysis. Values are given as mean ±SEM. Differences between placebo and rHDL treatment periods in the human endotoxemia model were tested by analysis of variance (ANOVA) for repeated measures using SPSS for Windows. Changes of parameters in time were tested using one-way ANOVA. Differences in CD14 expression and TNF production in the in vitro experiments were analyzed using the Wilcoxon test. A two-sided P value <0.05 was considered significant. Incidence and mean severity of clinical symptoms associated with endotoxemia were tabulated; summary results are given in a descriptive way.

Results

Human Endotoxemia

Clinical Symptoms and Vital Signs. Endotoxin administration elicited clinical symptoms similar to those reported previously (23, 24). Briefly, flu-like symptoms were observed including headache, chills, nausea, vomiting, myalgia and backache. All volunteers were symptom free within 24 h after endotoxin challenge.

Infusion of rHDL did not cause any side-effects or changes in routine laboratory parameters. After rHDL treatment circulating HDL cholesterol concentrations increased

Table 1. *Effect of rHDL on LPS-induced Clinical Symptoms*

Treatment/ symptoms	Placebo and endotoxin (n = 8)	rHDL and endotoxin (n = 8)
Headache	8 (1.5)	8 (1.1)
Chills	5 (0.6)	2 (0.4)
Myalgia	5 (0.9)	3 (0.4)
Backache	5 (0.6)	1 (0.3)
Nausea	5 (0.8)	3 (0.3)
Vomiting	3 (0.8)	0 (0)

Incidence and mean severity of clinical symptoms in human endotoxemia as scored during the time of confinement. The total of volunteers suffering from a specific event during placebo/endotoxin treatment and rHDL/endotoxin (40 mg/kg) treatment is given, with in parentheses the mean severity (0 = absent, 1 = mild, 2 = moderate, and 3 = severe).

from 1.16 ± 0.06 mmol/l at t = −3.5 h to 1.64 ± 0.07 mmol/l at t = 6 h. HDL levels remained elevated until the end of the study period, 1.48 ± 0.05 mmol/l at t = 24 h (P <0.001 in time).

During the 3-mo follow up period none of the participants developed antibodies against Apo A-1 or rHDL. Furthermore, no seroconversion in HIV 1/2, hepatitis A, hepatitis B surface, hepatitis B core, hepatitis C, and parvovirus B19 status was observed within the 3-mo observation period.

As is shown in Table 1, rHDL reduced the endotoxin-induced clinical symptoms. In contrast, rHDL did not influence the febrile response to endotoxin. Peak temperatures were 37.8 ± 0.3°C and 37.6 ± 0.3°C after injection of endotoxin in conjunction with placebo and rHDL, respectively.

Endotoxin Activity in Plasma. Peak endotoxin plasma levels were observed 5 min after endotoxin injection. rHDL infusion significantly increased endotoxin activity as measured by the Limulus assay (21.8 ± 8.7 pg/ml versus 46.0 ± 5.7 pg/ml, P = 0.001) (Fig. 1).

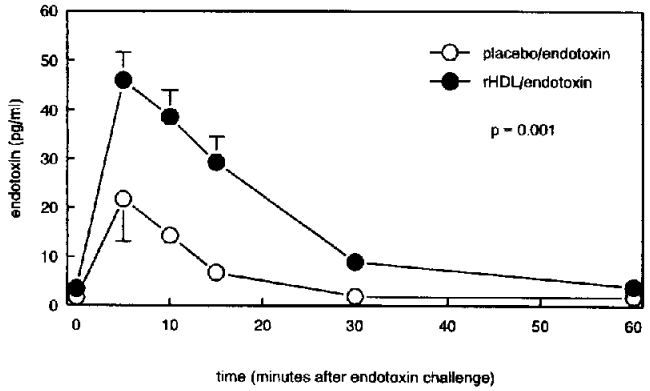

Figure 1. Mean (±SEM) endotoxin concentrations in human endotoxemia. Control saline (*open circles*) or rHDL (40 mg/kg, *closed circles*) was given as a 4-h infusion, starting 3.5 h before endotoxin challenge (P value indicates difference between treatment groups).

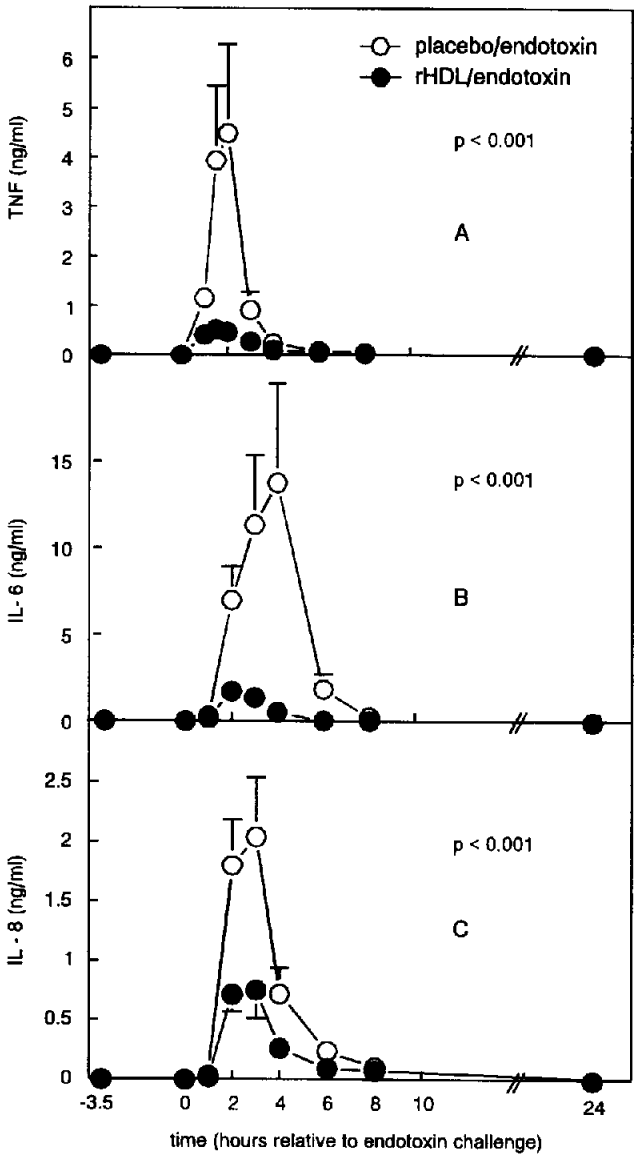

Figure 2. Mean (±SEM) circulating concentrations of TNF (*A*), IL-6 (*B*) and IL-8 (*C*) after endotoxin administration in humans. Endotoxin was given in combination with control (*open circles*) or rHDL (40 mg/kg, *closed circles*), P value indicates difference between treatment groups.

Cytokines. Endotoxin administration resulted in transient increases in serum levels of TNF (peak level 4.50 ± 1.79 ng/ml at t = 2 h), IL-6 (peak level 13.79 ± 5.75 ng/ml at t = 4 h), and IL-8 (peak level 2.05 ± 0.50 ng/ml at t = 3 h). rHDL treatment importantly reduced the release of all three cytokines (P <0.001 versus placebo): TNF (peak level 0.52 ± 0.11 ng/ml at t = 1.5 h), IL-6 (peak level 1.75 ± 0.45 ng/ml at t = 2 h), and IL-8 (peak level 0.75 ± 0.25 ng/ml at t = 3 h) (Fig. 2).

rHDL had a less pronounced inhibiting effect on the release of antagonist cytokines during endotoxemia. During endotoxin/placebo administration IL-1ra levels increased

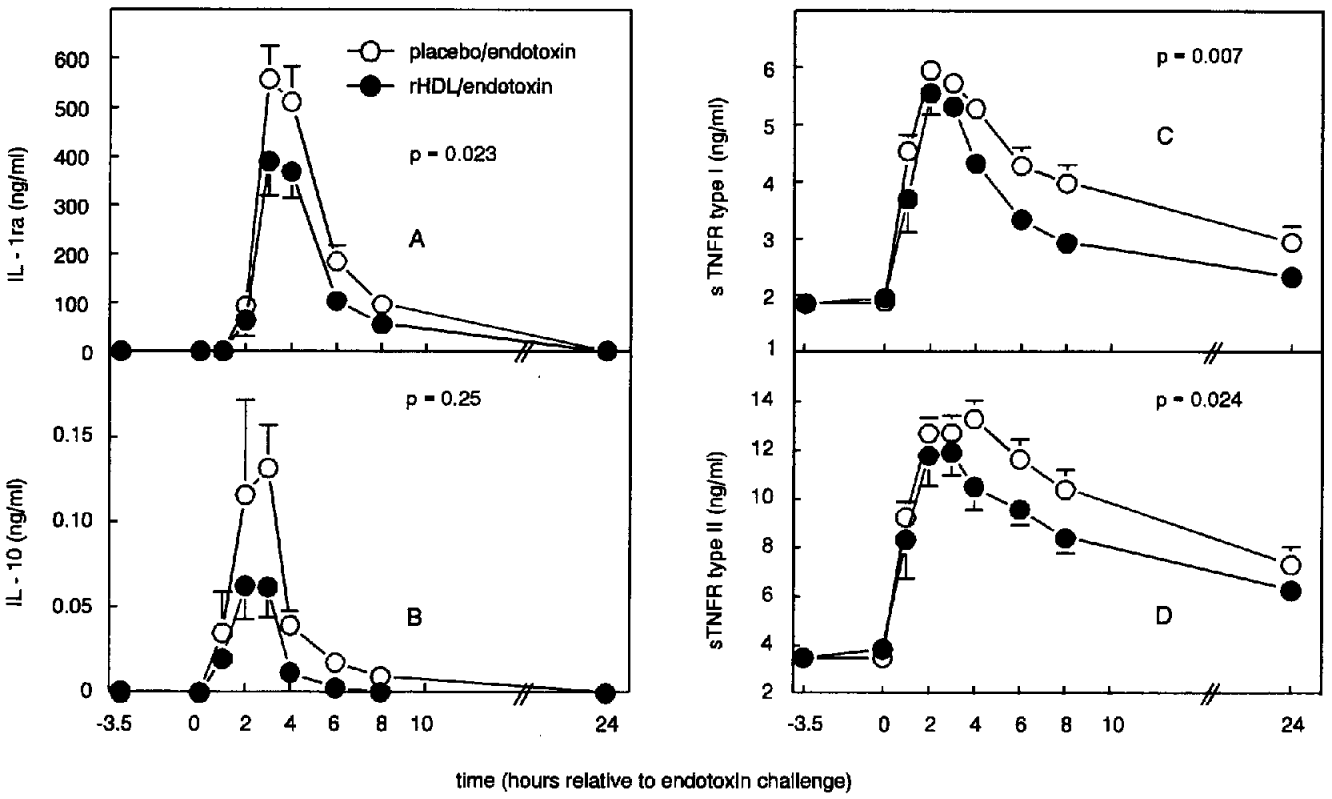

Figure 3. Mean (±SEM) circulating concentrations of IL-1ra (*A*), IL-10 (*B*), sTNFr I (*C*), and sTNFr II (*D*) in human endotoxemia. Endotoxin was given in combination with control (*open circles*) or rHDL (40 mg/kg, *closed circles*). *P* value indicates difference between treatment groups.

from 0.64 ± 0.11 ng/ml (baseline) to 557.75 ± 68.29 ng/ml (t = 3 h); IL-10 to 0.13 ± 0.03 ng/ml at t = 3 h; sTNFr I from 1.86 ± 0.12 ng/ml to 5.96 ± 0.20 ng/ml at t = 2 h; sTNFr II from 3.52 ± 0.25 ng/ml to 13.30 ± 0.73 ng/ml at t = 4 h. rHDL infusion modestly decreased the endotoxin-induced release of IL-1ra (390.50 ± 70.42 ng/ml at t = 3 h, P = 0.023); sTNFr I (5.56 ± 0.38 ng/ml at t = 2 h; P = 0.007); sTNFr II (11.92 ± 0.94 ng/ml at t = 3 h; P = 0.024). The rHDL-induced inhibition of IL-10 release did not reach statistical significance (Fig. 3).

Leukocyte Responses. After endotoxin challenge leukocyte counts decreased from 4.5 ± 0.4 × 10^9/l at −3.5 h to 1.9 ± 0.3 × 10^9/l at t = 1 h followed by an increase up to 12.8 ± 1.8 × 10^9/l at t = 8 h. rHDL blunted both the early leukopenia (3.1 ± 0.5 × 10^9/l (t = 1 h) and the subsequent rise in leukocyte counts (10.8 ± 1.2 × 10^9/l at t = 10 h) (P <0.001). Neutrophil counts closely followed total leukocyte counts; rHDL attenuated both the initial neutropenia and the subsequent neutrophilia. After endotoxin challenge, the monocyte count dropped from 0.4 ± 0.04 × 10^9/l (baseline) to 0.02 ± 0 × 10^9/l (t = 1 h) followed by a rise to 1.0 ± 0.3 × 10^9/l (t = 24 h). rHDL administration reduced both endotoxin-induced monopenia and monocytosis (0.05 ± 0.01 × 10^9/l at t = 1 h and 0.5 ± 0.07 × 10^9/l at t = 24 h) (P = 0.01) (Fig. 4).

CD11b expression on granulocytes obtained from endotoxin challenged volunteers increased to 401.2 ± 116.5% at t = 3 h (P <0.001 in time) and CD18 to 133.7 ± 13.2% (P = 0.004 in time). rHDL treatment significantly reduced the endotoxin-induced increase of both CD11b and CD18 expression to 168.4 ± 45.9% (CD11b) and to 94.8 ± 13.1% (CD18) at t = 3 h (P = 0.02 and P = 0.04 versus placebo, respectively) (Fig. 5).

Fig. 6 shows that endotoxin upregulated CD14 expression on monocytes in vivo, reaching a peak of 160.2 ± 13.7% at 3 h after endotoxin challenge (P <0.001 in time). Before endotoxin administration, rHDL treatment resulted in a significant reduction of CD14 expression on monocytes of 71.5 ± 13.7% at t = 0 h (P = 0.016 in time); after endotoxin challenge, CD14 expression only increased to 106.2 ± 10.6% at 3 h (P = 0.025 versus placebo). Circulating granulocytes exhibited an increase in CD14 expression during endotoxin treatment to 343.0 ± 25.3% at t = 24 h (P <0.001 in time), and rHDL partially prevented this increase (202.3 ± 25.2% at t = 24 h) (P <0.001 versus placebo).

Endotoxin-binding Proteins. Endotoxin challenge slightly decreased sCD14 concentrations from 2.71 ± 0.07 (g/ml at t = −3.5 h to 2.43 ± 0.24 (g/ml at t = 2 h (P = 0.02 in time), which was not influenced by rHDL (P = 1.0) (Fig. 6). LBP levels rose from 2.8 ± 0.2 (g/ml at t = −3.5 h to 12 ± 1.9 (g/ml 24 h after endotoxin administration, which was reduced by rHDL (9.9 ± 1.1 (g/ml at t = 24 h; P = 0.03 versus placebo) (Fig. 7).

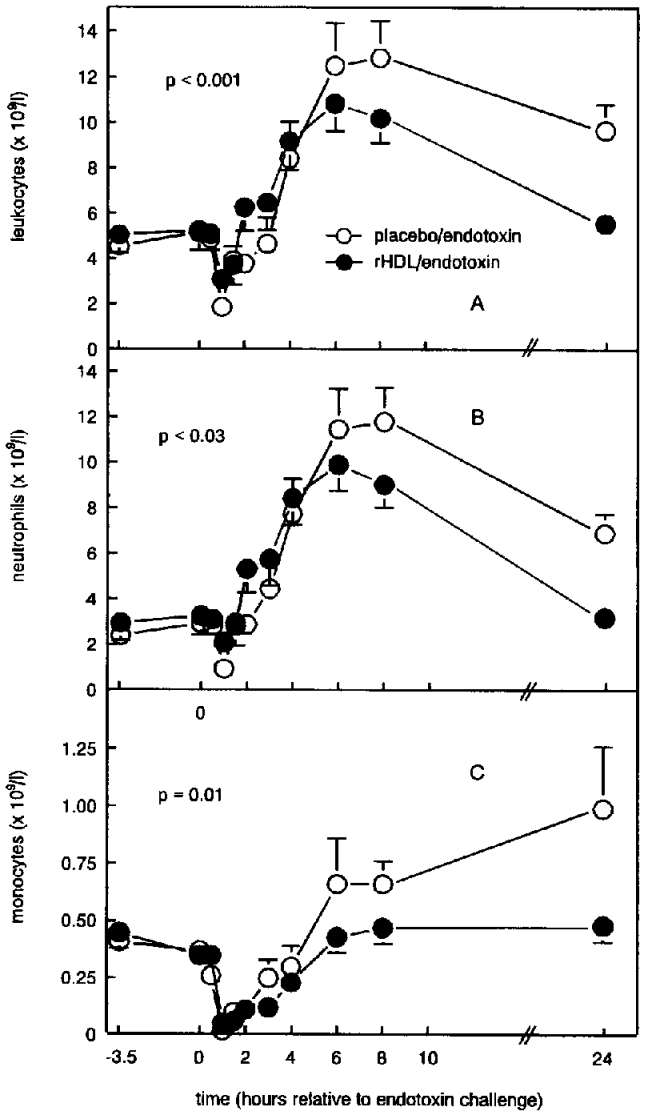

Figure 4. Mean (±SEM) leukocyte counts and differential counts in human endotoxemia. Endotoxin was given in combination with control saline (*open circles*) or rHDL (40 mg/kg, *closed circles*). Leukocyte (*A*), granulocytes (*B*), monocytes (*C*), *P* value indicates difference between treatment groups.

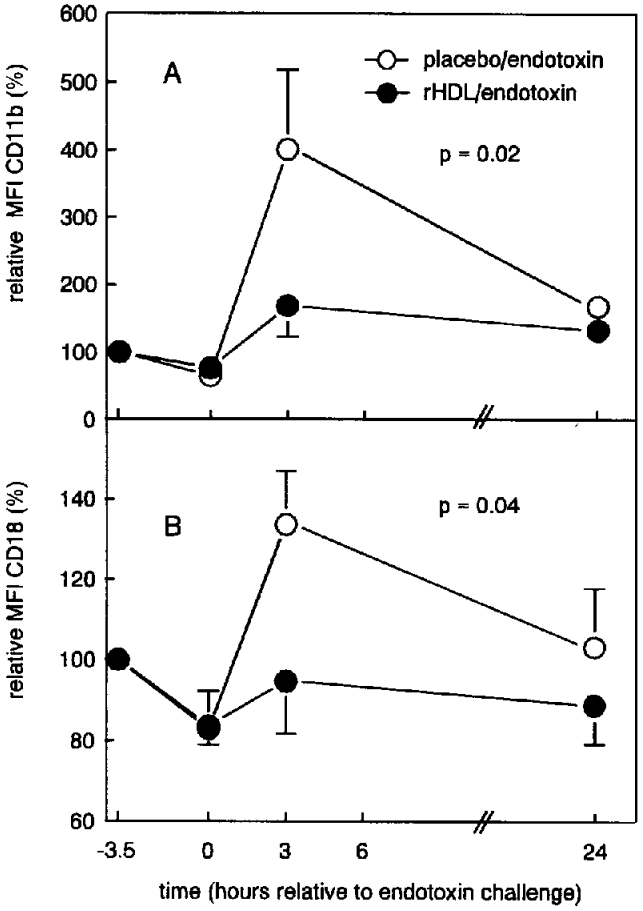

Figure 5. Mean (±SEM) relative mean fluorescence intensity (MFI) of CD11b (*A*) and CD18 (*B*) on circulating granulocytes in human endotoxemia. Control saline (*open circles*) or rHDL (40 mg/kg, *closed circles*) was given as a 4-h infusion in combination with endotoxin (4 ng/kg), *P* value indicates difference between treatment groups.

In Vitro Whole Blood Incubation and PBMC Stimulation

After incubation of whole blood with 0.5 mg/ml rHDL, a dose corresponding to the in vivo concentrations achieved in this study, CD14 expression on monocytes was reduced to 72.5 ± 1.0% ($P < 0.001$). The reduction of CD14 expression on monocytes was even greater after incubation of whole blood with 2.0 mg/ml rHDL (36.0 ± 0.3%, $P < 0.001$) (Fig. 8 *A*). PBMC isolated from rHDL-treated whole blood did not produce detectable TNF levels spontaneously. As shown in Fig. 8 *B*, a dose-dependent reduction in TNF production by endotoxin-stimulated PBMC (isolated from rHDL-treated whole blood) was seen from 3.9 ± 0.2 ng/ml (control, no rHDL added) to 2.5 ± 0.2 ng/ml (0.5 mg/ml rHDL) and 1.4 ± 0.3 ng/ml (2.0 mg/ml rHDL) ($P = 0.01$ for each).

Discussion

The present study is the first to demonstrate the LPS-neutralizing capacity of rHDL in humans in vivo. rHDL, given as an intravenous infusion starting before induction of endotoxemia in healthy volunteers, markedly reduced TNF, IL-6, and IL-8, while only modestly reducing the release of the proinflammatory cytokine inhibitors, IL-1ra, sTNFr I, sTNFr II, and IL-10. Further, rHDL attenuated endotoxin-induced clinical symptoms and leukocyte activation. These LPS-inhibiting effects appeared in part to be mediated by a rHDL-induced downregulation of monocyte-bound CD14, the predominant receptor for LPS.

During the rHDL treatment period, endotoxin levels, as measured with the LAL assay, were significantly higher than during the endotoxin/placebo treatment period and endotoxin remained detectable in the circulation longer. These findings are in accordance with a previous study in which patients with high HDL levels exhibited a higher LPS recovery upon ex vivo stimulation with LPS in comparison with patients with low HDL levels (25). It should be noted that the LAL assay includes a dilution and heating

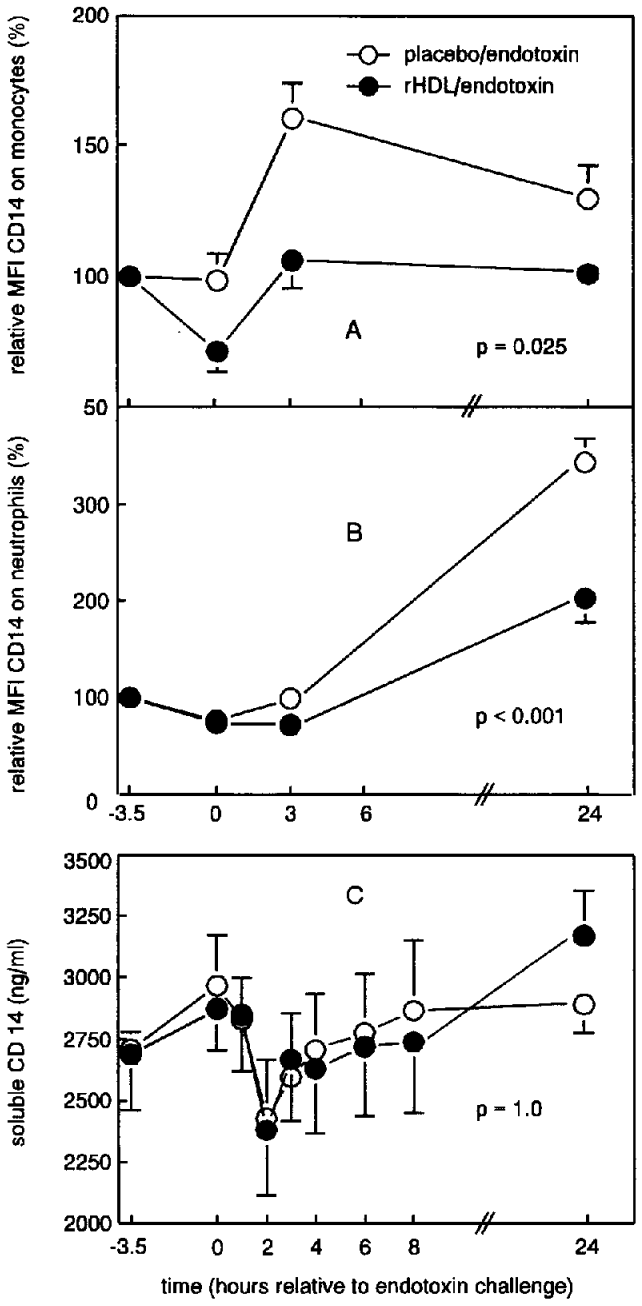

Figure 6. Mean (±SEM) relative mean fluorescence intensity (MFI) of CD14 on circulating monocytes (*A*) and neutrophils (B) and mean (±SEM) concentrations of soluble CD14 in human endotoxemia. rHDL (40 mg/kg, *closed circles*) treatment was compared with control saline (*open circles*), *P* value indicates difference between treatment groups.

step in which endotoxin is recovered from LPS-binding proteins such as LBP and presumably also from HDL. The increased LAL activity after rHDL/endotoxin treatment merely indicates that rHDL indeed bound LPS in vivo. Thus, LAL activities do not necessarily represent bioactive endotoxin levels in humans in vivo.

The reduction in the release of proinflammatory cytokines by rHDL was larger than the reduction in IL-1ra,

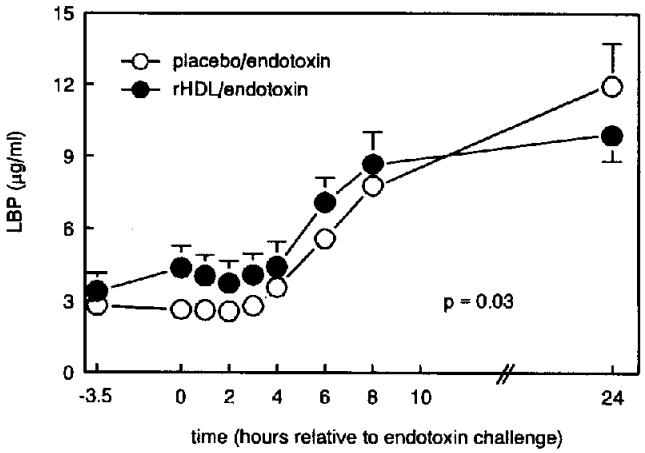

Figure 7. Mean (±SEM) concentrations of LBP after endotoxin challenge in humans. Open circles represent the endotoxin/placebo treatment period, closed circles represent endotoxin/rHDL (40 mg/kg) treatment period.

sTNF receptors, and IL-10. Thus, the potency with which rHDL influenced various LPS responses differed. Conceivably, part of the LPS administered did not associate with rHDL. In this context, the threshold dose at which LPS induces fever and release of IL-1ra, sTNF receptors, and IL-10

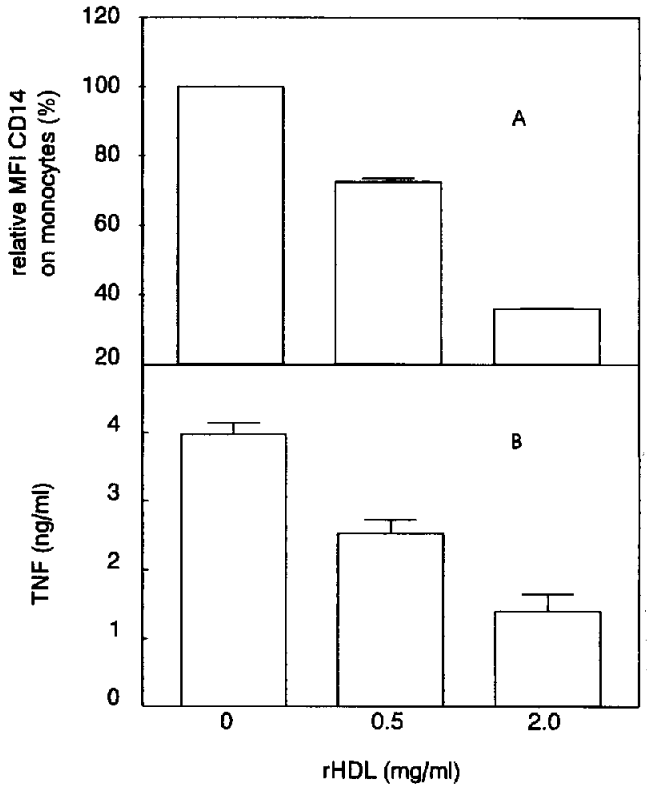

Figure 8. Effect of rHDL on monocyte CD14 expression and TNF production. Whole blood was incubated with rHDL (0, 0.5, or 2.0 mg/ml) for 1 h at 37°C. Thereafter, PBMC were isolated and CD14 expression on monocytes was determined by FACScan® (*A*), or isolated PBMC were stimulated with LPS for 4 h and TNF production was assessed (*B*).

may be lower than the threshold at which it elicits release of proinflammatory cytokines and leukocyte activation.

In this study we demonstrate for the first time that CD14 expression on monocytes was increased in vivo after LPS administration. Previous in vitro studies showed that LPS is able to increase CD14 expression on monocytes (26), while others found that LPS decreased CD14 expression (27). However, clear evidence is given that blocking CD14 on both monocytes and neutrophils attenuated LPS responses (4, 28). An important and new finding is that rHDL induced a downmodulation of CD14 at the surface of monocytes in vivo and in whole blood in vitro. We demonstrated that this reduction in CD14 expression may be biologically relevant, since monocytes isolated from rHDL-treated whole blood produced less TNF upon stimulation with LPS. The mechanism by which rHDL downregulates monocyte CD14 expression remains to be elucidated. If CD14 was shed from the monocyte cell surface by rHDL, one might expect an elevation in sCD14 levels. In our study sCD14 concentrations did not increase during rHDL treatment, but a small change in sCD14 levels by rHDL might have been masked by the already high concentrations of sCD14 constitutively present in the circulation. Nonetheless, our data suggest that it is conceivable that rHDL may reduce endotoxin-induced TNF production not only by binding and neutralizing endotoxin, but also by reducing CD14 monocyte surface expression.

A previous in vitro study demonstrated that activation of granulocyte β_2 integrin (CD11b/CD18) on PMN occurred by binding of LPS-LBP complexes on CD14 on PMN and could be blocked by anti-CD14 antibodies (4). Thus the reduction of CD11b/CD18 expression on neutrophils by rHDL in our study might be caused directly by reduced presentation of LPS-LBP complexes. It seems unlikely that the reduction in CD11b/CD18 is caused by rHDL-induced attenuation in CD14 expression on granulocytes because the reduction of LPS-induced increase of CD11b/CD18 expression by rHDL preceded the rHDL-induced reduction of CD14 expression on granulocytes. As β_2 integrins participate in the adhesion of neutrophils to endothelium, the reduction in β_2 integrin expression may prevent endotoxin-associated endothelial damage (29, 30).

In conclusion, the results of this study clearly demonstrate that rHDL, given as a 4-h infusion at a dose of 40 mg/kg, dramatically reduced the endotoxin-induced inflammatory response as measured by reduced inflammatory cytokines, cell activation, and clinical symptoms in humans. This is partly caused by neutralization of endotoxin by rHDL and by rHDL-induced reduction in CD14 expression on monocytes.

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