Effect of lipid-bound apoA-I cysteine mutants on lipopolysaccharide-induced endotoxemia in mice[S]

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Abstract HDL has been shown to be able to neutralize the toxicity of lipopolysaccharide (LPS). Our previous study (*J. Lipid Res.* 2005. 46: 1303–1311) characterized the properties of secondary structure and in vitro functions of different cysteine mutants of apolipoprotein A-I. Here, we reconstituted recombinant HDLs (named rHDLwt, rHDL52, rHDL74, rHDL107, rHDL129, rHDL173, rHDL195, and rHDL228) by mixing wild type or those mutants with dipalmitoyl phosphatidylcholine and examined their in vivo effects on LPS-induced endotoxemia in mice. Our results showed that 24 h after injection, mice receiving rHDL74 or rHDL52 had a significant decrease of plasma tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β), compared with control mice receiving either saline or rHDLwt ($P < 0.05$). Administration of rHDL74 to mice injected with LPS also led to a decrease of plasma IL-6, protection of lung against acute injury, and attenuation of endotoxin-induced clinical symptoms in mice, compared with controls injected with LPS only. However, injection of rHDL228 significantly increased plasma concentration of TNF-α and exacerbated LPS-induced lung injury. In summary, compared with rHDLwt, rHDL74 and rHDL52 exhibit higher anti-inflammation capabilities, whereas rHDL228 shows hyper-proinflammation by exacerbating LPS-induced endotoxemia in mice.—Wang, Y., X. Zhu, G. Wu, L. Shen, and B. Chen. **Effect of lipid-bound apoA-I cysteine mutants on lipopolysaccharide-induced endotoxemia in mice. *J. Lipid Res.* 2008.** 49: **1640–1645.**

Supplementary key words apolipoprotein A-I • cysteine mutant • rHDL • inflammatory cytokines • lung injury

Endotoxin or lipopolysaccharide (LPS) is the primary constituent of the leaflet of the outer surface membrane of Gram-negative bacteria. The structure of LPS includes a lipid portion (lipid A) that anchors LPS into the membrane, a polysaccharide core, and an oligo- or polysaccharide extending from the core beyond the bacterial surface (1). The lipid A domain can also anchor LPS in the phospholipid monolayers, liposomes, the surface of discoidal reconstituted HDL (rHDL), and spherical plasma HDL (2–4). After entering the bloodstream, LPS will bind to LPS binding protein, which helps in transfering LPS to co-receptor CD14, leading to the formation of receptor clusters of Toll-like receptor 4 (TLR4), CD14, and other adaptors, resulting in the activation of the nuclear factor-κB (NF-κB) pathway and production of a cascade of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6, which mediate the cellular inflammatory response to Gram-negative infection (2, 5–8). In addition, LPS can also bind to plasma HDL, resulting in inactivation of endotoxin (9–12).

Apolipoprotein A-I (apoA-I) is the major protein of HDL, containing a 43-residue N-terminal globular region, followed by a series of 10 repeating units that comprise approximately 80% of the protein (residues 44–243). Eight of these repeats contain 22 amino acids and two contain 11 amino acids, most of which are separated by helix-breaking amino acids such as proline (13, 14). According to the Edmundson wheel (15) and the structures of two natural apoA-I mutants, apoA-I_{Milano} and apoA-I_{Paris}, with the cysteine mutation position at the polar-nonpolar interface on the helix, we designed and constructed six cysteine mutants, with each cysteine residue located in each of the various helical domains at the same helical wheel position as for the substitution in A-I_M: A-I(S52C), A-I(N74C), A-I(K107C), A-I(G129C), A-I(K195C), and A-I(S228C) (16). Although A-I(S228C) was not shown in our previous data (16), it was generated using a method similar to that used for the other mutants, except for the mutant site. These variants exhibited different structural features regarding their secondary structure and biological activities of binding lipid, promoting cholesterol efflux from THP-1 macrophages, and anti-peroxidation (16). In

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this study, we examined the in vivo effect of these cysteine mutants and wild-type human apoA-I in the lipid-bound states (rHDL52, rHDL74, rHDL107, rHDL129, rHDL173, rHDL195, rHDL228, and rHDLwt) on LPS-induced endotoxemia in mice.

MATERIALS AND METHODS

Materials

LPS (from *Escherichia coli* 055:B5), dimyristoylphosphatidylcholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Sigma. The bicinchoninic acid protein assay kit and Detoxi-Gel™ endotoxin-removing gel were purchased from Pierce. ELISA kits were purchased from Boster Biological Technology, Ltd. Phospholipid assay kits were purchased from Beijing Kinghawk Pharmaceutical Co., Ltd. Male BALB/c mice (18–20 g) were purchased from the laboratory animal department of Peking Union Medical College. All animal experiments were approved by the animal care committee of the Chinese Academy of Medical Sciences.

Preparation of recombinant apolipoproteins

The expression and purification of recombinant apolipoproteins were performed as described previously (16). The purified proteins were lyophilized and stored at −70°C and used in the assays described below.

DMPC clearance assay

In order to assess the abilities of apoA-I and its mutants to associate with lipid, interactions of seven cysteine mutants and wild-type apoA-I with DMPC were monitored as previously described (16).

Construction of recombinant HDLs

The lyophilized proteins were dissolved in physiologic saline. To minimize endotoxin contamination in recombinant rHDL particles, we pretreated apolipoproteins before preparing HDLs, as described previously (17–19), then treated them with Detoxi-Gel™ endotoxin removing gel to remove any residual endotoxin. Recombinant HDLs were prepared using the sodium cholate dialysis technique (20). Briefly, DPPC powder was dissolved in 725 mM sodium cholate solution in Tris-HC1 buffer, mixed on a Vortex mixer for 1 min at room temperature and added to the recombinant apolipoproteins. After ultrasound, the mixture was incubated in a 24°C shaking water bath for 20 min and then dialyzed extensively against physiologic saline for 20 h at 4°C to remove cholate.

Ratios of DPPC to apoA-I or apoA-I mutants in rHDLs

The DPPC was measured by phospholipid assay kits according to the manufacturer's instructions.

The effect of recombinant HDL on the septic mice

In vivo LPS-induced septic shock and the protection of rHDL against septic shock in mice were performed according to the methods described in the literature (2, 21). Animals were divided into 18 experimental groups as shown in **Table 1**, where N denotes the number of animals in each group. Two control groups were used in this study: the LPS group, in which mice received only LPS by tail vein injection (4 mg/kg) to induce endotoxemia; and the saline group, in which mice received only 300 μl of physiologic saline through the tail vein. In the experimental rHDLs groups, LPS was injected through the tail vein, then after the anal temperature increased 0.5°C (11 min 32 s ± 30 s after LPS injection), each of the rHDLs prepared above was slowly injected into mice through the tail vein within 60 s. Three and twenty-four hours after injection, mice were euthanized and bled by extirpating eyeballs, and plasma was isolated by centrifugation at 12,000 *g* for 10 min and stored at −80°C until analyses were performed. The plasma levels of IL-1β, L-6, and TNF-α were measured by ELISA kits according to the manufacturer's instructions. Twenty-four hours after LPS injection, lungs were isolated and fixed in 10% formaldehyde solution at room temperature, sectioned, then stained with hematoxylin-eosin (H and E) and examined under light microscopy (2, 21).

TABLE 1. Experimental groups used in LPS and rHDL injection study

Group	Injection	LPS	rHDL	3 h	24 h
	300 μl	*mg/kg*		*N*	
Saline	Physiologic saline	—	—		5
LPS	LPS	4	—	4	5
WT	LPS/rHDLwt	4	80	4	6
WT control	rHDLwt	—	80	4	4
Mut52	LPS/rHDL52	4	80	4	6
Mut52 control	rHDL52	—	80	4	4
Mut74	LPS/rHDL74	4	80	4	6
Mut74 control	rHDL74	—	80	4	4
Mut107	LPS/rHDL107	4	80	4	6
Mut107 control	rHDL107	—	80	4	4
Mut129	LPS/rHDL129	4	80	4	6
Mut129 control	rHDL129	—	80	4	4
Mut195	LPS/rHDL195	4	80	4	6
Mut195 control	rHDL195	—	80	4	4
Mut173	LPS/rHDL173	4	80	4	6
Mut173 control	rHDL173	—	80	4	4
Mut228	LPS/rHDL228	4	80	4	6
Mut228 control	rHDL228	—	80	4	4

LPS, lipopolysaccharide; rHDL, reconstituted HDL; WT, wild-type. The mutant or WT control groups were only injected with rHDL; 3 h and 24 h represent the time after rHDL injection. In the rHDL treatment groups, 3 h and 24 h represent the time after LPS injection.

Statistical analysis

Values were expressed as mean ± SD, and differences between groups were examined for significance using one-way ANOVA for multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Expression and purification of recombinant apolipoproteins

The recombinant apolipoproteins were expressed mainly in soluble form in BL21 (DE3) cells. As shown in **Fig. 1**, most of the purified proteins were present in monomeric form under nonreducing conditions, (Fig. 1A) or reducing conditions (Fig. 1B) except A-I (S228C). The degrees of dimerization and the migration rates of the dimers were variable, depending on the sites of cysteine substitution. Different from the other mutants, A-I (S228C) easily forms dimers, and this may be due to the specific mutant site of A-I (S228C). According to the model shown by Bhat et al. (22), the mutant site of A-I (S228C) lies in the C-terminal region, closer to the end of the peptide chain than the other mutants, and so may produce more-stabile interchain disulfide bonds to stabilize its dimer form.

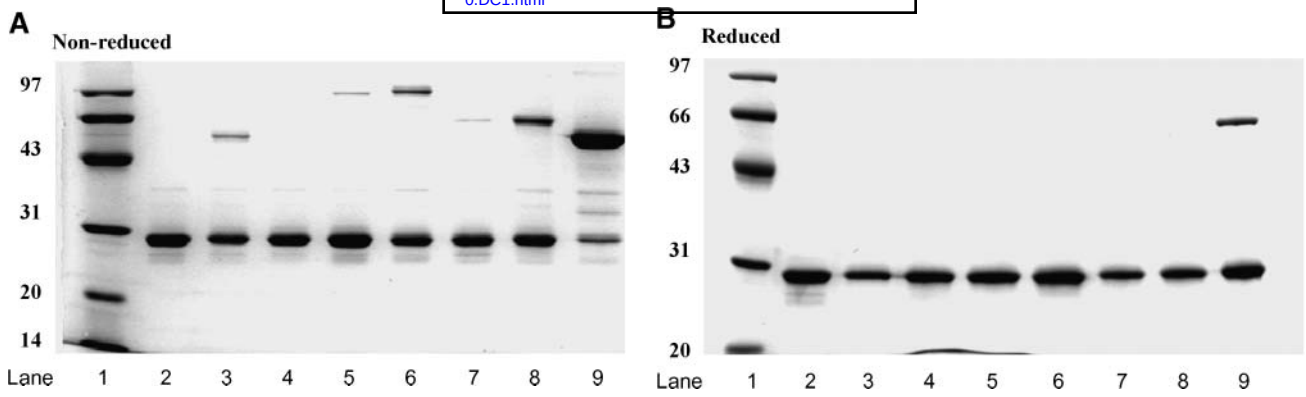

Fig. 1. Recombinant purified wild-type and Cys mutant apolipoprotein A-I (apoA-I) were examined by 12% SDS-PAGE. A: Cysteine substitution mutants are covalently linked dimers in nonreducing SDS-PAGE. B: Under reducing conditions (100 mmol/l DTT), these proteins migrated as single bands of the same molecular mass as wild-type apoA-I. Each gel was loaded with approximately 2 μg of protein per lane. Lanes 1–9 represent low-molecular-mass wild-type apoA-I, A-I(S52C), A-I(N74C), A-I(K107C), A-I(G129C), A-I(R173C), A-I(K195C), and A-I(S228C).

DMPC clearance assay

Compared with wild-type apoA-I, the ability of Mut228 to clear the lipid turbidity (determined by rate constant $k_{1/2}$) decreased by 90.8% ($P < 0.05$) (see supplementary Table I). However, Mut74 had about 33.9% higher efficiency to bind DMPC liposome than wild-type apoA-I (0.079 ± 0.023 vs.0.059 ± 0.002).

Weight ratios of DPPC to apoA-I or apoA-I mutants in rHDL assay

As shown in **Table 2**, the weight ratio of DPPC to apoA-I in rHDL74 was slightly higher than in rHDLwt (1.93 ± 0.06 vs. 1.75 ± 0.11). However, the ratios in rHDL228 (0.82 ± 0.05 vs. 1.75 ± 0.11, $P < 0.05$), rHDL107 (1.49 ± 0.12 vs. 1.75 ± 0.11, $P < 0.05$), and rHDL129 (1.47 ± 0.04 vs. 1.75 ± 0.11, $P < 0.05$) were significantly lower than in rHDLwt. No significant alteration was observed in the other rHDL groups.

Detection of level of inflammatory cytokines in plasma of mice

To examine the ability of our recombinant HDLs to neutralize LPS, we intravenously injected different HDL particles immediately following LPS injection to induce in vivo septic shock, as described in Materials and Methods. Two time points (3 h and 24 h post LPS injection) were checked to better assess the effect of the rHDLs.

As shown in **Fig. 2**, 24 h post LPS injection, compared with controls injected with rHDLwt (135.28 ± 12.84 pg/ml), mice receiving either rHDL74 or rHDL52 exhibited significantly lower plasma levels of TNF-α (rHDL74: 24.47 ± 3.96 pg/ml, $P = 0.002 < 0.05$ vs. rHDLwt; rHDL52: 39.96 ± 2.44 pg/ml, $P = 0.009 < 0.05$ vs. rHDLwt). The plasma concentration of TNF-α in rHDL74-treated mice was reduced down to the baseline level (24.16 ± 1.63 pg/ml). Alternatively, mice treated with rHDL195 or rHDL228 had much higher plasma concentrations of TNF-α compared with the LPS single-injection groups (rHDL195: $P = 0.037 < 0.05$, rHDL228: $P < 0.001$, respectively). Treatment with recombinant HDL containing wild-type apoA-I also resulted in a significant decrease of plasma TNF-α (135.28 ± 12.84 pg/ml, $P = 0.045 < 0.05$ vs. LPS group). However, no statistical differences were observed in groups treated with rHDL107, rHDL129, or rHDL173, compared with the LPS–only group. We also observed that the treatment of rHDLwt and all the other rHDL mutants except rHDL228 resulted in a decrease of plasma IL-1β ($P < 0.001$ vs. LPS). Mice treated with rHDL74 and rHDL52 had significantly lower secretion of IL-1β than those treated with rHDLwt (rHDL74: $P < 0.001$; rHDL52: $P = 0.021 < 0.05$, respectively). Other rHDLs (rHDL107, rHDL129, rHDL173, or rHDL195, $P < 0.001$, compared with LPS) also showed the ability to decrease plasma IL-1β induced by LPS injection. We also found that treating mice with rHDL74 significantly attenuated plasma IL-6 production induced by in vivo LPS injection, compared with the single-LPS-injection group ($P = 0.006 < 0.05$); however, treatments with rHDL52 and rHDL107 led to an increase of plasma IL-6 (rHDL52: $P = 0.009 < 0.01$; rHDL107: $P = 0.02 < 0.05$, respectively).

Similar to our observations at 24 h after LPS injection, at 3 h after LPS injection, mice treated with rHDLwt, rHDL74, and rHDL52 had lower levels of TNF-α than mice that received LPS injection alone (rHDLwt: $P = 0.049 < 0.05$; rHDL74: $P = 0.012 < 0.05$; rHDL52: $P = 0.048 <$

TABLE 2. Weight ratios of DPPC to apoA-I or apoA-I mutants in rHDLs

rHDLs	DPPC:apoA-I
rHDLwt	1.75 ± 0.11
rHDL52	1.66 ± 0.11
rHDL74	1.93 ± 0.06
rHDL107	1.49 ± 0.12[a]
rHDL129	1.47 ± 0.04[a]
rHDL173	1.63 ± 0.06
rHDL195	1.56 ± 0.04
rHDL228	0.82 ± 0.05[a]

Each value represents means ± SD of at least three independent determinations.
[a] $P < 0.05$ versus rHDLwt.

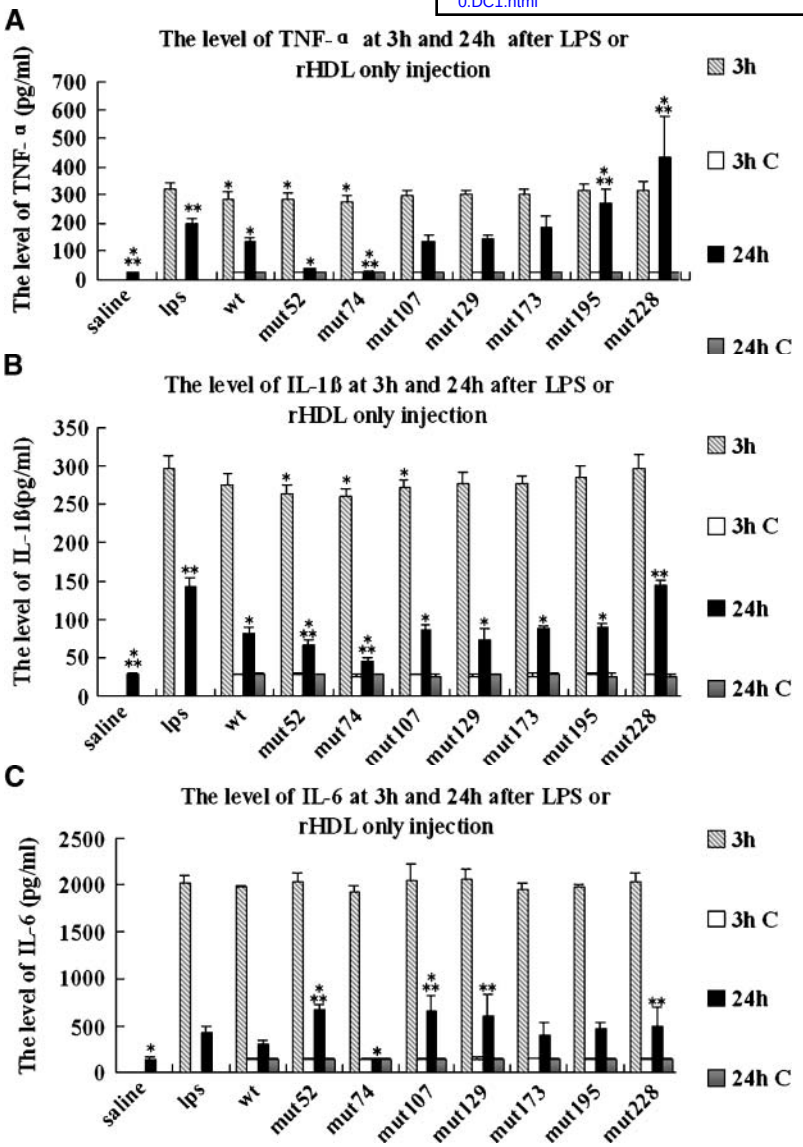

Fig. 2. Plasma cytokine concentration levels at 3 h and 24 h after lipopolysaccharide (LPS)- or reconstituted HDL (rHDL)-only injection. Saline, LPS, wt, mut52, mut74, mut107, mut129, mut173, mut195, and mut228 represent the groups that were treated separately by physiologic saline, LPS, rHDLwt, rHDL52, rHDL74, rHDL107, rHDL129, rHDL173, rHDL195, and rHDL228, respectively. A: Levels of TNF-α. B: Levels of IL-1β. C: Levels of IL-6. wt, wild-type apoA-I. * $P < 0.05$ vs. LPS; ** $P < 0.05$ vs. wt. Error bars indicate SD.

0.05, respectively). No significant alteration was observed in other groups of mice treated with rHDL107, rHDL129, rHDL173 and rHDL228, compared with the LPS-only group. Recombinant HDL74, rHDL52, and rHDL107 decreased the level of IL-1β, compared with the LPS-only group (rHDL74, $P = 0.003 < 0.05$; rHDL52, $P = 0.007 < 0.05$; rHDL107, $P = 0.034 < 0.05$), but we did not see any significant effects of other rHDLs in reducing plasma IL-1β. None of the rHDLs showed a reduction of plasma IL-6 at 3 h post LPS injection.

Compared with 24 h of LPS injection, the effect of rHDL on cytokine levels at 3 h after LPS was biologically very small, suggesting a better neutralization of residual LPS in the circulation by rHDL injection for a longer time.

Histological sections of lung tissue

Pretreatment with rHDL was shown to have a significant effect in inhibiting endotoxin-induced organ damage, such as the lowered neutrophil infiltration in lung seen in histological examination (23, 24). Hubsch, Casas, and Doran (25) found that both prophylactic and therapeutic rHDL administration could improve clinical outcome, with a significant attenuation of acidosis in the rabbit Gram-negative bacteremia model. Here, we have shown that our rHDLs also had therapeutic function. In our study, the lung of mice receiving only LPS (**Fig. 3C, D**) had significant pathological changes: *1*) congestion, *2*) broadening of pulmonary interstitial tissue, and *3*) leukocyte infiltration, including monocytes and neutrophils. As shown in Fig. 3E, F, we observed that the mice treated with rHDLwt exhibited only slight pathological lung changes, such as the moderate broadening of pulmonary interstitial tissue, compared with the saline group (Fig. 3A, B). In addition, the ability of rHDL74 to protect lungs against LPS-induced injury in this septic mice model is strongly supported by its histological results (Fig. 3I, J), which were very close to those of the saline group, and there was almost no pathological change in the Mut74 group. However, in lung sections (Fig. 3K, L) from mice treated with rHDL228, we did not see an effect of reduced histological tissue injury, but saw instead aggravation of the pathological changes, compared with the LPS-only group. In addition, the histological sections of the

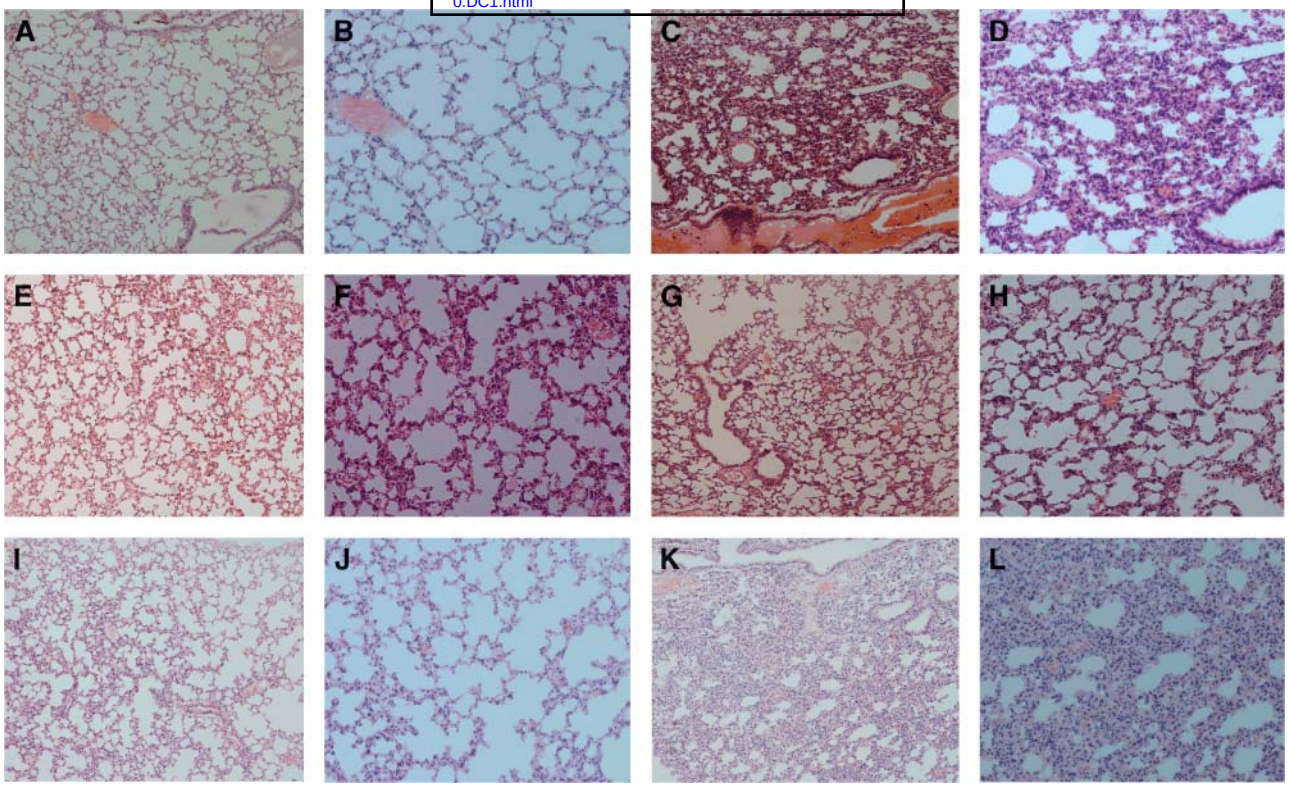

Fig. 3. Photomicrographs of representative histological sections of lung. (A, C, E, G, I, K: magnification ×100; B, D, F, H, J, L: magnification ×200). A, B: Saline group (control group), treated by physiologic saline only. C, D: Septic group without any other treatment. E, F: Group treated by rHDLwt. G, H: Group treated by rHDL52. I, J: Group treated by rHDL74. K, L: Group treated by rHDL228.

other four rHDLs (see supplementary Fig. I), compared with the saline group (Fig. 3A, B), showed many pathological changes.

DISCUSSION

In this study, we reconstituted eight recombinant HDLs (rHDL52, rHDL74, rHDL107, rHDL129, rHDL173, rHDL195, rHDL228, and rHDLwt) by mixing DPPC with either wild-type or mutant apoA-I containing one cysteine mutation at the indicated residue. Then, we examined the in vivo effects of different rHDLs on LPS-induced endotoxemia in mice.

Our results showed that 24 h post LPS injection, mice injected with rHDL74 or rHDL52 showed significantly decreased plasma concentrations of TNF-α and IL-1β, compared with control mice injected with saline or controls injected with rHDLwt ($P < 0.05$). rHDL74 injection also led to a decrease of plasma IL-6, lung protection from LPS-induced acute injury, and as well as attenuation of endotoxin-induced clinical symptoms in mice, compared with controls injected with LPS only. We observed that only rHDL74 could decrease the level of plasma IL-6, perhaps because of the different activation pathways or cells of these inflammatory cytokines. However, rHDL228 treatment increased plasma concentrations of TNF-α ($P < 0.05$ vs. LPS or rHDLwt injection group) and exacerbated LPS-induced lung injury. Taken together, these data indicate that the site of cysteine mutation on apoA-I dramatically alters the endotoxin neutralization capability of HDLs. rHDL74 may be a potential clinical candidate for therapy for endotoxin-induced septic shock.

In 1957, Rall, Gaskins, and Kelly (26) first reported that preincubation of the serum with LPS could decrease the febrile response of rabbits to bacterial LPS. At the end of the 1970s, LPS was shown to be able to interact with HDL to form HDL-LPS complexes (27, 28). Later, more evidence emerged showing that lipoproteins, especially HDL, were able to bind bacterial cell wall components, including lipoteichoic acid (12, 29, 30). H that in gram-negative arris et al. (31) reported that preincubation of endotoxin with HDL or other lipoproteins before injection could protect animals against LPS-induced mortality. In 1995, Hubsch, Casas, and Doran (25) showed in septicemia models that rHDL infusion, by either prophylactic or therapeutical administration, could significantly lower plasma LPS levels and attenuate acidosis. Taken together, these data indicate a therapeutic function of rHDL. In our study, consistent with these findings, we also observed similar therapeutic functions of rHDLwt, as shown in Results.

Our previous study (16) showed that the ability of Mut129 and Mut195 to clear lipid turbidity decreased by 83% and 76%, respectively, relative to wild-type apoA-I ($P < 0.05$). Here, we can see that compared with wild-type apoA-I, the ability of mut228 to clear lipid turbidity decreased by 90.8% (see supplementary Table II) (0.0054 ± 0.002 vs.0.059 ± 0.002, $P < 0.05$). In addition, we also saw that the ratio of DPPC to apoA-I in rHDL74 was much higher, compared to other rHDLs (1.93 ± 0.06), and the ra-

tio of DPPC to apoA-I in rHDL228 was the lowest among all the recombinant HDLs (0.82 ± 0.06). When rHDLs were injected in mice preinjected with LPS, rHDL228 could not attenuate LPS-induced endotoxemia, compared with rHDL52 or rHDL74. Instead, treatment using rHDL228 post LPS injection resulted in an increase of pro-inflammatory cytokine expression and exaggerated acute lung damage. We assume that this result may be due in part to the poor ability of Mut228 to bind DPPC, inasmuch as we showed that approximately 80% of the mut228 spontaneously formed into dimer in solution after expression by BL21 (DE3) (Fig. 1).

In addition, it was suggested that an increase of phospholipid-to-apoA-I ratios in reconstituted HDL particles could increase their ability to neutralize LPS (32). In our study, we found a higher phospholipid binding ability of mut74 (see supplementary Table II) and also a higher ratio of DPPC to lipoprotein in reconstituted rHDL74 (Table 2). This increased lipid binding capability of mut74 may explain, in part, the increased ability of rHDL74 to neutralize LPS and to decrease LPS-induced pro-inflammatory cytokine expression and protect lung from acute injury in our in vivo study.

In summary, our study compared, for the first time, the different effects of rHDL containing apoA-I cysteine mutants on in vivo LPS-induced endotoxemia in mice. Our data suggest that cysteine mutation not only induces the alteration of secondary structure and in vitro functions of apoA-I but also influences the capability of HDL to neutralize endotoxin or LPS. Because of its significant positive protection against LPS-induced endotoxemia in our study, rHDL74 may be a potential clinical candidate for therapy for endotoxin-induced septic shock.

REFERENCES

1. Gunn, J. S., and R. K. Ernst. 2007. The structure and function of Francisella LPS. *Ann. N. Y. Acad. Sci.* **1105:** 202–218.
2. Levine, D. M., T. S. Parker, T. M. Donnelly, A. Walsh, and A. L. Rubin. 1993. In vivo protection against endotoxin by plasma high density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **90:** 12040–12044.
3. Romeo, D., A. Hinckley, and L. Rothfield. 1970. Reconstitution of a functional membrane enzyme system in a monomolecular film. II. Formation of a functional ternary film of lipopolysaccharide, phospholipid and transferase enzyme. *J. Mol. Biol.* **53:** 491–501.
4. Weiser, M. M., and L. Rothfield. 1968. The reassociation of lipopolysaccharide, phospholipid, and transferase enzymes of the bacterial cell envelope. Isolation of binary and ternary complexes. *J. Biol. Chem.* **243:** 1320–1328.
5. Schumann, R. R., S. R. Leong, G. W. Flaggs, P. W. Gray, S. D. Wright, J. C. Mathison, P. S. Tobias, and R. J. Ulevitch. 1990. Structure and function of lipopolysaccharide binding protein. *Science.* **249:** 1429–1431.
6. Beutler, B., I. W. Milsark, and A. C. Cerami. 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science.* **229:** 869–871.
7. Vesy, C. J., R. L. Kitchens, G. Wolfbauer, J. J. Albers, and R. S. Munford. 2000. Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from gram-negative bacterial membranes. *Infect. Immun.* **68:** 2410–2417.
8. Chow, J. C., D. W. Young, D. T. Golenbock, W. J. Christ, and F. Gusovsky. 1999. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* **274:** 10689–10692.
9. Emancipator, K., G. Csako, and R. J. Elin. 1992. In vitro inactivation of bacterial endotoxin by human lipoproteins and apolipoproteins. *Infect. Immun.* **60:** 596–601.
10. Flegel, W. A., A. Wolpl, D. N. Mannel, and H. Northoff. 1989. Inhibition of endotoxin-induced activation of human monocytes by human lipoproteins. *Infect. Immun.* **57:** 2237–2245.
11. Read, T. E., H. W. Harris, C. Grunfeld, K. R. Feingold, J. P. Kane, and J. H. Rapp. 1993. The protective effect of serum lipoproteins against bacterial lipopolysaccharide. *Eur. Heart J.* **14:** 125–129.
12. Baumberger, C., R. J. Ulevitch, and J. M. Dayer. 1991. Modulation of endotoxic activity of lipopolysaccharide by high-density lipoprotein. *Pathobiology.* **59:** 378–383.
13. Chan, L. 1989. The apolipoprotein multigene family: structure, expression, evolution, and molecular genetics. *Klin. Wochenschr.* **67:** 225–237.
14. Thomas, M. J., S. Bhat, and M. G. Sorci-Thomas. 2006. The use of chemical cross-linking and mass spectrometry to elucidate the tertiary conformation of lipid-bound apolipoprotein A-I. *Curr. Opin. Lipidol.* **17:** 214–220.
15. Segrest, J. P., D. W. Garber, C. G. Brouillette, S. C. Harvey, and G. M. Anantharamaiah. 1994. The amphipathic alpha helix: a multifunctional structural motif in plasma apolipoproteins. *Adv. Protein Chem.* **45:** 303–369.
16. Zhu, X., G. Wu, W. Zeng, H. Xue, and B. Chen. 2005. Cysteine mutants of human apolipoprotein A-I: a study of secondary structural and functional properties. *J. Lipid Res.* **46:** 1303–1311.
17. Reichelt, P., C. Schwarz, and M. Donzeau. 2006. Single step protocol to purify recombinant proteins with low endotoxin contents. *Protein Expr. Purif.* **46:** 483–488.
18. Bordier, C. 1981. Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem.* **256:** 1604–1607.
19. Liu, S., R. Tobias, S. McClure, G. Styba, Q. Shi, and G. Jackowski. 1997. Removal of endotoxin from recombinant protein preparations. *Clin. Biochem.* **30:** 455–463.
20. Chen, C. H., and J. J. Albers. 1982. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
21. Yan, Y. J., Y. Li, B. Lou, and M. P. Wu. 2006. Beneficial effects of ApoA-I on LPS-induced acute lung injury and endotoxemia in mice. *Life Sci.* **79:** 210–215.
22. Bhat, S., M. G. Sorci-Thomas, E. T. Alexander, M. P. Samuel, and M. J. Thomas. 2005. Intermolecular contact between globular N-terminal fold and C-terminal domain of apoA-I stabilizes its lipid-bound conformation: studies employing chemical cross-linking and mass spectrometry. *J. Biol. Chem.* **280:** 33015–33025.
23. McDonald, M. C., P. Dhadly, G. W. Cockerill, S. Cuzzocrea, H. Mota-Filipe, C. J. Hinds, N. E. Miller, and C. Thiemermann. 2003. Reconstituted high-density lipoprotein attenuates organ injury and adhesion molecule expression in a rodent model of endotoxic shock. *Shock.* **20:** 551–557.
24. Lee, R. P., N. T. Lin, Y. F. Chao, C. C. Lin, H. J. Harn, and H. I. Chen. 2007. High-density lipoprotein prevents organ damage in endotoxemia. *Res. Nurs. Health.* **30:** 250–260.
25. Hubsch, A. P., A. T. Casas, and J. E. Doran. 1995. Protective effects of reconstituted high-density lipoprotein in rabbit gram-negative bacteremia models. *J. Lab. Clin. Med.* **126:** 548–558.
26. Rall, D. P., J. R. Gaskins, and M. G. Kelly. 1957. Reduction of febrile response to bacterial polysaccharide following incubation with serum. *Am. J. Physiol.* **188:** 559–562.
27. Ulevitch, R. J., and A. R. Johnston. 1978. The modification of biophysical andendotoxic properties of bacterial lipopolysaccharides by serum. *J. Clin. Invest.* **62:** 1313–1324.
28. Ulevitch, R. J., A. R. Johnston, and D. B. Weinstein. 1979. New function for high density lipoproteins. Their participation in intravascular reactions of bacterial lipopolysaccharides. *J. Clin. Invest.* **64:** 1516–1524.
29. Murch, O., M. Collin, C. J. Hinds, and C. Thiemermann. 2007. Lipoproteins in inflammation and sepsis. I. Basic science. *Intensive Care Med.* **33:** 13–24.
30. Grunfeld, C., M. Marshall, J. K. Shigenaga, A. H. Moser, P. Tobias, and K. R. Feingold. 1999. Lipoproteins inhibit macrophage activation by lipoteichoic acid. *J. Lipid Res.* **40:** 245–252.
31. Harris, H. W., C. Grunfeld, K. R. Feingold, and J. H. Rapp. 1990. Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J. Clin. Invest.* **86:** 696–702.
32. Cué, J. I., J. T. DiPiro, L. J. Brunner, J. E. Doran, M. E. Blankenship, A. R. Mansberger, and M. L. Hawkins. 1994. Reconstituted high density lipoprotein inhibits physiologic and tumor necrosis factor alpha responses to lipopolysaccharide in rabbits. *Arch. Surg.* **129:** 193–197.