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Role for Circulating Lipoproteins in Protection from Endotoxin Toxicity

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Previous studies have shown that endotoxin (lipopolysaccharide [LPS])-induced death can be prevented by preincubating LPS with lipoproteins in vitro or by infusing large quantities of lipids into animals prior to LPS administration. In the present study we determined whether physiological levels of lipids also provide protection. Serum lipid levels were decreased by two different mechanisms: administration of 4-aminopyrolo-(3,4-D)pyrimide, which prevents the hepatic secretion of lipoproteins, and administration of pharmacological doses of estradiol, which increases the number of hepatic low-density lipoprotein receptors, leading to increased lipoprotein clearance. In both hypolipidemic models, LPS-induced mortality is markedly increased compared with that of controls with normal serum lipid levels. In both hypolipidemic models, administration of exogenous lipoproteins, which increase levels of serum lipids into the physiological range, reduces the increased mortality to levels similar to that seen in normal animals. In normal lipidemic animals, 63% of ^{125}I-LPS in plasma is associated with lipoproteins, where it would not be capable of stimulating cytokine production. In contrast, in hypolipidemic animals, very little LPS (12 to 17%) is associated with lipoproteins. Rather, more LPS is in the lipoprotein-free plasma compartment, where it could exert biological effects. In both hypolipidemic models, LPS produces a greater increase in serum tumor necrosis factor levels than it does in controls (three- to fivefold increase), and administration of exogenous lipoproteins prevents this increase. Cytokines, in particular tumor necrosis factor, are responsible for most of the toxic effects of LPS. These data provide evidence that physiological levels of serum lipids protect animals from LPS toxicity. Thus, lipoproteins, in addition to playing a role in lipid transport, may have protective functions. Moreover, as part of the immune response, cytokine-induced increases in serum lipid levels may play a role in host defense by decreasing the toxicities of biological and chemical agents.

Cytokines, such as tumor necrosis factor (TNF), the interleukins, and the interferons, are the hormones that mediate the host response to infection and inflammation (1, 8, 37). In addition to their classic immune regulatory functions, these cytokines also alter a wide array of metabolic processes. For example, TNF, interleukin-1, and interleukin-6 stimulate hepatic synthesis and secretion of acute-phase proteins such as C-reactive protein, α2-macroglubulin, α1-antitrypsinase, and fibrinogen, etc. (4, 7, 31, 40). These proteins are thought to be beneficial through a variety of different mechanisms, including enhancing the opsinization of bacteria and other foreign particles, limiting proteolysis to the sites of inflammation, and restoring or maintaining levels of clotting factors in serum (28, 41).

The administration of cytokines, such as TNF or interleukin-1, results in a rapid increase in serum triglyceride levels (peaks at 2 h) followed by a later rise in serum cholesterol levels (11, 15). The initial increase in serum triglyceride levels is due to increased amounts of very-low-density lipoprotein (VLDL), and the late increase in serum cholesterol is accounted for by increased amounts of low-density lipoprotein (LDL) (27). The hypertriglyceridemia is primarily due to increased hepatic secretion of VLDL, while the hypercholesterolemia is associated with a marked increase in hepatic cholesterol synthesis (6, 11, 12, 14). In addition, cytokines have effects on tissues other than the liver which could contribute to the hyperlipidemia. For example, a variety of different cytokines decrease adipose tissue lipoprotein lipase activity, which could slow the clearance of triglyceride-rich lipoproteins from the circulation (12, 26, 38, 39). The cytokine-induced alterations in lipid metabolism which result in hyperlipidemia can be considered to be part of the acute-phase response and therefore may also be beneficial to the host.

Numerous in vitro studies have demonstrated that lipoproteins bind endotoxin (lipopolysaccharide [LPS]) (21, 32, 35, 36, 51–54). Moreover, the ability of LPS to cause death can be reduced by preincubating LPS with either high-density lipoprotein (HDL), LDL, VLDL, or chylomicrons prior to administration (21). Furthermore, the infusion of large quantities of chylomicrons (which markedly increase serum triglyceride levels) or reconstituted HDL (to double the HDL concentration) prior to LPS administration also protects animals from LPS-induced death (22, 25, 30). Similarly, transgenic mice which overproduce apolipoprotein A1 and have elevated HDL levels also are protected from LPS-induced toxicity (30).

Lipoproteins diminish the ability of LPS to stimulate macrophage cytokine production in vitro (5, 18). LPS bound to lipoproteins does not interact with the cellular receptors on macrophages that induce cytokine production and secretion. It is now well recognized that many of the adverse effects of LPS, such as septic shock, are mediated by the overproduction of cytokines, in particular TNF (2, 9). It is therefore possible that lipoproteins may protect against LPS toxicity in vivo by binding

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LPS, thereby decreasing the uptake of LPS by macrophages, which results in a diminished stimulation of cytokine production and reduced toxicity.

Previous studies of lipoprotein protection in intact animals have examined the protective effects only of markedly elevated serum lipid levels on LPS toxicity. A crucial question is whether physiological levels of circulating lipids also provide protection. Therefore, in the present study, we have lowered serum lipid levels by the administration of two agents that work by different mechanisms: 4-aminopyrolo-(3,4-D)pyrimide (4APP), which prevents the secretion of lipoproteins by the liver (23, 46), or pharmacological doses of estradiol, which increase the number of hepatic LDL receptors, leading to the increased clearance of lipoproteins (17, 55). In both hypolipidemic models, LPS administration led to higher levels of non-lipoprotein-bound LPS in the plasma, increased secretion of TNF, and increased mortality. Furthermore, the increased secretion of TNF and the increased toxicity found in hypolipidemic animals could be prevented by administering exogenous lipoproteins just prior to LPS administration.

MATERIALS AND METHODS

Animal procedures. Male Sprague-Dawley rats (approximately 200 to 220 grams) were purchased from Simonsen Animal Laboratories (Gilroy, Calif.). The animals were maintained in a reverse-light-cycle room (dark from 3:00 a.m. to 3:00 p.m., light from 3:00 p.m. to 3:00 a.m.) and were provided with Simonsen rat chow and water ad libitum. 17α-Ethynylestradiol (5 mg/kg) (Sigma Chemical Company, St. Louis, Mo.) in propylene glycol was administered subcutaneously daily for 4 days prior to study and the morning of the study. Control animals were injected with vehicle alone. 4APP (Sigma Chemical Company) (20 mg/kg) in a 0.025 M phosphate buffer (pH 4) was administered intraperitoneally 16 and 40 h prior to the study. Control animals were injected with phosphate buffer alone. Because 4APP causes anorexia and decreased food intake, both the treatment and control groups were not fed beginning immediately after the first injection of 4APP. At the time of study, neither the 4APP- nor the estradiol-treated animals appeared to be ill. Where indicated, animals were injected intravenously (i.v.) with 1 ml of a lipoprotein solution (triglycerides, 304 to 570 mg/dl; cholesterol, 750 to 1300 mg/dl), prepared as described below, 45 min prior to LPS administration. Control animals were injected with saline containing 0.01% EDTA.

Serum TNF levels were assayed with serum samples obtained 90 min after the i.v. administration of 1 μg of LPS (*Escherichia coli* O55:B5; purchased from Difco Laboratories, Detroit, Mich.) per kg. The LPS was freshly diluted to the desired concentration in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Inc., Irvine, Calif.).

Survival after LPS administration was studied with several protocols utilizing pretreatment with 4APP or estradiol followed by administration of LPS, which in some cases was accompanied by galactosamine sensitization. Galactosamine was administered because it sensitizes the animal to LPS toxicity (21). Galactosamine has also been noted to alter lipoprotein composition and structure by decreasing plasma lecithin:cholesterol acyltransferase activity (43). However, this effect takes hours, and given that in our experiments LPS and galactosamine were administered simultaneously, it is very unlikely that this effect would influence our results. The interaction of LPS with lipoproteins occurs soon after LPS administration, before the galactosamine-induced lipoprotein changes. For galactosamine sensitization, survival of the estradiol-treated animals was determined 48 h after the i.v. administration of 9 μg of LPS per kg and 375 mg of galactosamine per kg. Survival in the 4APP experiments was determined after the i.v. administration of 12 μg of LPS per kg and 375 mg of galactosamine per kg. In experiments in which galactosamine was not administered, survival was determined after the administration of either 400 μg or 20 mg of LPS per kg.

The distribution of endotoxin was determined by using endotoxin radiolabeled with ^{125}I by the method of Ulevitch (50). Briefly, *E. coli* (O55:B5) endotoxin was first derivatized by being reacted with *p*-OH-methylbenzimidate at an alkaline pH and then labeled with Na^{125}I. The ^{125}I-endotoxin had a specific activity of 1.96 to 2.30 μCi/μg. The quantities of ^{125}I-endotoxin in circulating lipoproteins, plasma, and livers of control, 4APP-treated and estradiol-treated animals were determined by gamma counting at 40 min following administration of labeled endotoxin. Lipoproteins in the plasma, including VLDL, LDL, and HDL subfractions, were precipitated by a modification of the method described by Burstein et al. (3). Briefly, dextran sulfate and $MnCl_2$ were added to plasma at final concentrations of 0.65% and 0.2 M respectively. Precipitation began immediately and was complete by 2 h. The mixture was centrifuged at 20,000 × *g* for 30 min, and the pellet and supernatant were separated. The supernatant did not have detectable cholesterol in our assay system. The addition of ^{125}I-endotoxin to lipoprotein-deficient serum in vitro resulted in very little precipitation of ^{125}I-endotoxin, indicating that the dextran sulfate and $MnCl_2$ did not cause the precipitation of either endotoxin itself or endotoxin-protein complexes. The plasma volume was determined by administering ^{14}C-methylated albumin and measuring plasma ^{14}C by liquid scintillation counting 10 min after injection.

TABLE 1. Effects of 4APP and estradiol on serum lipid levels[a]

Treatment (*n*)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control (22)	43.3 ± 4.5	83.0 ± 3.9
4APP (21)	2.8 ± 0.9 ($P < 0.001$)	3.9 ± 0.8 ($P < 0.001$)
4APP + lipoproteins (14)	11.6 ± 1.4 ($P < 0.001$)	75.9 ± 4.5 (NS)
Control (14)	39.7 ± 2.4	61.3 ± 3.8
Estradiol (13)	15.6 ± 3.6 ($P < 0.001$)	12.3 ± 3.9 ($P < 0.001$)
Estradiol + lipoproteins (8)	34.2 ± 5.6 (NS)	32.3 ± 5.5 ($P < 0.001$)

[a] Sprague-Dawley rats were injected intraperitoneally with either 20 mg of 4APP per kg or buffer alone 16 and 40 h prior to study or subcutaneously with either estradiol (5 mg/kg) in propylene glycol or propylene glycol alone daily for 4 days prior to study and the morning of the study. At 45 min prior to study, animals were injected i.v. with either 1 ml of a lipoprotein solution prepared as described in Materials and Methods or 1 ml of saline containing 0.01% EDTA. LPS (1 μg/kg) was administered i.v., and 90 min later serum triglyceride and cholesterol levels were determined. In separate experiments serum triglyceride and cholesterol levels in control and estradiol-treated animals were determined prior to LPS administration (control triglyceride, 51.0 ± 4.5 mg/dl; estradiol triglyceride, 23.7 ± 3.1 mg/dl [$P < 0.01$]; control cholesterol, 115.8 ± 4.8 mg/dl; estradiol cholesterol, 11.5 ± 2.1 mg/dl [$P < 0.001$] [$n = 5$ in each group]). Results are expressed as means ± standard errors of the mean. All *P* values are versus controls.

Lipoprotein isolation. To avoid contamination with exogenously derived LPS, all heat-stable materials used in the isolation and processing of lipoproteins were rendered sterile and free of LPS by steam autoclaving followed by dry heating at 180°C for a minimum of 4 h. Lipoproteins were isolated in depyrogenated stainless-steel ultracentrifugation tubes (Beckman Instruments, Palo Alto, Calif.) with custom-crafted silicone O-rings. To remove any adherent LPS, the dialysis tubing (Spectropor 3; Spectrum Medical Instruments Inc., Los Angeles, Calif.) was autoclaved in 3% H_2O_2, rinsed with a saline solution containing 0.01% EDTA (pH 7.4), and used immediately.

Blood was obtained from healthy volunteers who had fasted for 12 to 14 h. Plasma was separated from cells by centrifugation, and the density of the plasma was adjusted to 1.21 g/ml with KBr. The plasma samples were centrifuged at 100,000 × *g* for 18 h at 19°C. Aliquots from the upper layer of each tube were pooled and dialyzed against a saline solution containing 0.01% EDTA (pH 7.4) at 4°C. Immediately prior to use the lipoprotein was filtered through a 0.80-μm filter (Schleicher and Schuell, Keene, N.H.).

Serum assays. Serum cholesterol levels were measured with Sigma Diagnostic Kit no. 351 (Sigma Chemical Company). Serum triglyceride levels were measured with Sigma Diagnostic Kit no. 337-B. Serum TNF levels were measured with the TNF-sensitive cell line WEHI 164 clone 13 in a cytotoxic assay which was developed with 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrozolium blue (10). Concentrations were calculated by comparison with a recombinant murine TNF standard (Genentech, South San Francisco, Calif.).

Statistics. Results are presented as means ± standard errors of the mean. Statistical significance was determined by Student's *t* test or chi-square analysis.

RESULTS

As observed in previous studies, 4APP administration markedly reduced serum cholesterol and triglyceride levels (Table 1) (13, 16). High-dose estradiol treatment also reduced serum lipid levels (Table 1). As shown in Table 1, the administration of exogenous lipoproteins produced a significant increase in serum cholesterol and triglyceride levels in both 4APP- and estradiol-treated animals.

We next examined whether LPS administration causes greater mortality in hypolipidemic animals than in control animals. As shown in Table 2, after 4APP treatment, LPS administration to galactosamine-sensitized animals resulted in an 80% mortality rate, whereas in control animals the mortality rate for the same dose of LPS was 0% ($P < 0.001$). Similarly, after estradiol treatment (Table 2), the mortality rate following LPS administration to galactosamine-sensitized animals was

TABLE 2. Effect of hypolipidemia on endotoxin-induced mortality and TNF secretion[a]

Treatment	Mortality (%)		Serum TNF levels (nmol/ml)	
Control	0	$P < 0.001$	5.2 ± 1.2	$P < 0.02$
4APP	80	$P < 0.001$	24.6 ± 7.8	$P < 0.02$
4APP + lipoproteins	0		3.4 ± 1.0	
Control	28	$P < 0.001$	6.8 ± 1.2	$P < 0.001$
Estradiol	100	$P < 0.001$	22.1 ± 2.5	$P < 0.001$
Estradiol + lipoproteins	30		3.9 ± 1.2	

[a] Sprague-Dawley rats were injected intraperitoneally with either 20 mg of 4APP per kg or buffer alone 16 and 40 h prior to study or subcutaneously with either estradiol (5 mg/kg) in propylene glycol or propylene glycol alone daily for 4 days prior to study and the morning of the study. At 45 min prior to study, animals were injected i.v. with either 1 ml of a lipoprotein solution prepared as described in Materials and Methods or 1 ml of saline containing 0.01% EDTA. In the mortality studies, 375 mg of galactosamine per ml and LPS (12 μg/kg in the 4APP experiments and 9 μg/kg in the estradiol experiments) were administered i.v., and survival was determined over 48 h. In the TNF studies, 1 μg of LPS per ml was administered i.v., and 90 min later serum TNF levels were determined. For the TNF studies results are presented as means ± standard errors of the mean. For the 4APP mortality studies, $n = 12$ for controls, $n = 15$ for 4APP, and $n = 11$ for 4APP plus lipoproteins. For the estradiol mortality studies, $n = 18$ for controls, $n = 18$ for estradiol, and $n = 17$ for estradiol plus lipoproteins. For the 4APP TNF studies, $n = 22$ for controls, $n = 21$ for 4APP, and $n = 14$ for 4APP plus lipoproteins. For the estradiol TNF studies, $n = 14$ for controls, $n = 13$ for estradiol, and $n = 8$ for estradiol plus lipoproteins.

also markedly increased (estradiol, 100%; control, 28%; $P < 0.001$). For both 4APP- and estradiol-treated animals, studies were also carried out without the simultaneous administration of galactosamine. The i.v. administration of 20 mg of LPS per kg to hypolipidemic 4APP- or estradiol-treated animals resulted in 100% mortality (8 of 8 4APP-treated animals and 7 of 7 estradiol-treated animals died), whereas in control animals the mortality was 13% (2 of 15 animals died) ($P < 0.001$ for both 4APP and estradiol).

To determine if this enhanced mortality following LPS treatment was due to the hypolipidemia, we next determined the effect of administering exogenous lipoproteins prior to LPS treatment. As shown in Table 2, the administration of exogenous lipoproteins to 4APP-treated animals markedly reduced mortality following LPS administration in galactosamine-sensitized animals (4APP, 80% mortality; 4APP plus lipoproteins, 0% mortality; $P < 0.001$). Similarly, among estradiol-treated animals, the administration of lipoproteins also reduced the mortality rate in galactosamine-sensitized animals (estradiol, 100% mortality; estradiol plus lipoproteins, 30% mortality; $P < 0.001$) (Table 2). In fact, the mortality rate in hypolipidemic animals treated with exogenous lipoproteins was similar to that observed in control animals.

Additionally, as shown in Table 3, the administration of exogenous lipoproteins also reduced mortality in estradiol-treated animals that were not sensitized with galactosamine. In hypolipidemic estradiol-treated animals, the mortality was 88%, whereas in estradiol-treated animals that were administered lipoproteins, the mortality was only 12%. Given the very different mechanisms by which 4APP and estradiol decrease serum lipoprotein levels and the reversal of the increased toxicity in both hypolipidemic models achieved by administration of exogenous lipids, it is likely that hypolipidemia per se results in an increase in LPS toxicity.

TABLE 3. Effect of hypolipidemia on endotoxin-induced mortality in the absence of galactosamine treatment[a]

Treatment	Mortality		
	No. died/total no.	%	
Control	0/8	0	$P < 0.005$
Estradiol	7/8	87.5	$P < 0.025$
Estradiol + lipoproteins	1/8	12.5	

[a] Sprague-Dawley rats were injected subcutaneously with either estradiol (5 mg/kg) in propylene glycol or propylene glycol alone daily for 4 days prior to study and the morning of the study. At 45 min prior to study, animals were injected i.v. with either 1 ml of a lipoprotein solution prepared as described in Materials and Methods or 1 ml of saline containing 0.01% EDTA. LPS (400 μg/kg) was administered i.v., and survival was determined over 48 h.

To determine the mechanism for the increased mortality in the hypolipidemic animals, we first examined the distribution of labeled LPS. In previous studies, we observed that the level of ^{125}I-endotoxin in plasma was relatively constant during the period from 2.5 to 45 min following administration of endotoxin to control animals (22). In control animals, 40 min after ^{125}I-endotoxin administration we found that 63% ± 2.7% of the radioactive counts in the plasma were associated with lipoproteins, while 37% ± 2.7% of the label was in the lipoprotein-free plasma compartment. In contrast, in both 4APP- and estradiol-treated animals, the major portion of the labeled LPS in the plasma was in the lipoprotein-free plasma compartment (4APP, 83.1% ± 4.8%; estradiol, 87.4% ± 0.9%), with very little associated with the lipoprotein fraction (4APP, 16.9% ± 4.8%; Estradiol, 12.6% ± 0.9%). Thus, the proportion of circulating LPS that is not bound to lipoproteins and is potentially capable of interacting with macrophages and stimulating cytokine production is greatly enhanced in the hypolipidemic animals.

The distribution of labeled endotoxin as a percentage of the total dose administered is shown in Table 4. As expected, the percentage of injected label associated with lipoproteins was markedly decreased in both the 4APP (decreased 87%)- and estradiol (decreased 76%)-treated animals compared with control animals. Conversely, the percentage of injected label in the lipoprotein-free plasma compartment was increased in the 4APP (increased 97%)- and estradiol (increased 51%)-treated groups. Additionally, the percentage of injected LPS localized in the liver was increased in 4APP- and estradiol-treated animals. Previous studies in our laboratory and by others have shown that labeled endotoxin is taken up primarily by Kupffer cells, i.e., hepatic macrophages (19, 22, 33, 57). The percentage of injected label in the spleen and lungs is very small and is similar in control and hypolipidemic animals (data not shown). It should be noted that the total recovery of label is less in the estradiol-treated animals than in either the control or 4APP-treated animals, raising the possibility that estradiol treatment alters the metabolism of LPS. In normal lipidemic animals, a substantial proportion of injected LPS in plasma is associated with lipoproteins, where it would not be capable of stimulating cytokine production by macrophages. In contrast, in hypolipidemic animals, a higher percentage of LPS is in the lipoprotein-free plasma compartment, where it can exert its biological effects; in parallel, more LPS is taken up by the liver.

We next determined the ability of LPS to increase serum TNF levels (Table 2). In control, 4APP-treated, and estradiol-treated animals, serum TNF was undetectable prior to LPS administration. In 4APP-treated animals, the ability of LPS to

TABLE 4. Distribution of labeled endotoxin[a]

Treatment (*n*)	% of administered labeled endotoxin		
	In lipoprotein-free plasma	Lipoprotein associated	In liver
Control (16)	17.6 ± 1.29	30.3 ± 1.70	22.9 ± 0.79
4APP (6)	34.6 ± 3.42 ($P < 0.001$)	7.28 ± 2.50 ($P < 0.001$)	26.7 ± 1.20 ($P < 0.02$)
Estradiol (5)	26.6 ± 1.39 ($P < 0.001$)	3.82 ± 0.25 ($P < 0.001$)	26.0 ± 1.53 ($P < 0.02$)

[a] Control, 4APP-treated, and estradiol treated animals were administered approximately 1.5×10^6 cpm of ^{125}I endotoxin i.v. Forty minutes later, the animals were killed, plasma was obtained, and the livers were removed. HDL, LDL, and VLDL fractions were isolated by precipitation as described in Materials and Methods. The percentage of label in the lipoprotein-free plasma represents that in plasma after the lipoproteins had been precipitated. ^{125}I was determined by gamma counting. Results are means ± standard errors of the means; all *P* values are versus controls.

increase serum TNF levels was greatly enhanced compared with controls (increased 4.7-fold). In estradiol-treated animals, the ability of LPS to increase serum TNF levels was also markedly enhanced (increased 3.3-fold). Thus, in two different models of hypolipidemia, LPS results in an increased stimulation of TNF production.

To determine whether the increased TNF response to LPS was due to low lipid levels per se or to other, nonspecific effects of the lipid-lowering drugs, we tested the effects of exogenous lipoproteins administered to 4APP- and estradiol-induced hypolipidemic animals. As shown in Table 1, administration of exogenous lipoproteins produced a significant increase in serum cholesterol and triglyceride levels in both types of hypolipidemic animals. Furthermore, in the 4APP- and estradiol-treated animals that were also treated with exogenous lipoproteins, the increase in serum TNF levels induced by LPS was much less than it was in the untreated hypolipidemic animals (Table 2). In fact, the increase in serum TNF levels following LPS administration in the hypolipidemic animals treated with lipoproteins was similar to that observed in control animals. These results with lipid restoration, combined with the two very different mechanisms by which 4APP and estradiol lower serum lipid levels, suggest that it is the decrease in circulating lipid levels that enhances the TNF response to LPS.

DISCUSSION

In the present study we obtained direct evidence that physiological levels of circulating lipoproteins protect against LPS toxicity. First, we demonstrated that LPS administration results in a greater increase in serum TNF levels in hypolipidemic animals than in control animals with normal serum lipid levels. It is well recognized that cytokines, in particular TNF, are responsible for most of the toxic effects of LPS (2, 9). Second, and more importantly, we demonstrated that following LPS administration, mortality is markedly increased in the hypolipidemic animals compared with control animals with normal serum lipid levels. In normal animals 20 mg of LPS per kg causes minimal mortality (13%). In contrast, in both 4APP- and estradiol-treated hypolipidemic animals, 20 mg of LPS per kg causes 100% mortality. Moreover, in estradiol-treated animals, a dose of LPS as low as 400 μg/kg produces an 87% mortality rate, demonstrating a marked increase (50-fold) in the sensitivity of hypolipidemic animals to LPS-induced toxicity.

The exaggerated increase in serum TNF levels and the increased toxicity induced by LPS administration are seen in two very different hypolipidemic models: 4APP treatment, which inhibits lipoprotein secretion by the liver (23, 46), and estradiol treatment, which increases the number of hepatic lipoprotein receptors, leading to the increased clearance of lipoproteins (17, 55). Because similar results are seen in two very different models of hypolipidemia, these data suggest that the increased toxicity following LPS administration is due to the hypolipidemia per se and not to nonspecific effects of either drug.

Further evidence for a specific effect of lipoproteins is the fact that in both hypolipidemic models, the administration of exogenous lipoproteins prevents the exaggerated increase in serum TNF levels and reduces the increased mortality to levels similar to that seen in animals with normal lipid levels. In the experiments presented here, triglyceride and cholesterol levels were restored to normal or less-than-normal levels. Given the very different mechanisms of action of the two hypolipidemic agents and the restoration of sensitivity to normal with administration of exogenous lipoproteins, it is likely that the increased susceptibility to LPS is secondary to the decrease in circulating lipoproteins per se and not due to nonspecific effects of the hypolipidemic agents. These data thus provide direct evidence that physiological levels of circulating lipoproteins protect animals from LPS toxicity. That this finding may be relevant to humans is suggested by our prior studies which demonstrated that LPS is bound to VLDL in the circulations of healthy subjects with normal circulating lipid levels (21). One can speculate that binding of LPS to lipoproteins reduces the toxicity that might occur when LPS intermittently enters the bloodstream.

Our studies of the distribution of labeled LPS in control and hypolipidemic animals suggest a possible pathophysiological mechanism which could contribute to the increased toxicity in the hypolipidemic animals. In control animals, a substantial portion (30.3%) of administered LPS is bound to lipoproteins. In vitro studies have demonstrated that LPS associated with lipoproteins is not capable of stimulating cytokine production by macrophages (5, 18). In contrast, in hypolipidemic animals, the quantity of administered LPS sequestered in lipoproteins is very small (4APP, 3.8%; estradiol 7.3%). Rather, in hypolipidemic animals, the proportion of LPS in the lipoprotein-free plasma compartment is increased. LPS in this plasma compartment would be available to bind to LPS-binding protein (LBP), which would facilitate its interaction with CD14 receptors on macrophages, stimulating the secretion of cytokines and increasing the toxicity of the administered LPS (32, 34, 48). Additionally, it should be recognized that there are other pathways by which macrophages take up LPS and that the ability of LPS to stimulate macrophage cytokine secretion is dependent on the route of uptake (56). The uptake of LPS into macrophages via the scavenger or CD18 receptor has been shown to elicit only a minimal cytokine-secretory response (56). In in vivo studies such as this, one cannot evaluate the effect of hypolipidemia on the route of uptake of LPS into macrophages, but on the basis of in vitro experiments, one would anticipate that a reduction in plasma lipoproteins would enhance binding to plasma LBP and thereby enhance delivery of LPS via the CD14 receptor, leading to increased cytokine secretion (32, 34, 48,

56). Conversely, increased levels of lipoproteins would compete with LBP for binding LPS and thereby diminish the toxic effects of LPS. Since LPS binding to LBP amplifies the biological response of macrophages to LPS, one would expect that relatively small differences in the binding of LPS to LBP versus that of LPS to lipoproteins could produce large differences in biological responses.

It should of course be recognized that in addition to binding LPS, lipoproteins are likely to have other beneficial roles during endotoxic shock. For example, lipoproteins could deliver lipids, which can be utilized as a fuel source or for forming membranes, to cells that are activated during the immune response and to cells involved in tissue repair. One could speculate that there are many important roles served by lipoproteins which would be advantageous to survival after administration of endotoxin.

In addition to binding LPS, lipoproteins have been shown to bind a variety of viruses; this binding can reduce the cytotoxicities of these viruses (24, 29, 44, 45, 47). Additionally, lipoproteins induce lysis of the parasite *Trypanosoma bruceii* (20, 42). Lastly, lipoproteins bind to urate crystals, reducing the inflammatory response induced by these crystals (49). Thus, it can be postulated that lipoproteins represent a nonspecific host defense mechanism (as opposed to a specific defense mechanism such as antibody production) that could play a role in decreasing the toxicities of a variety of biological and chemical agents. Considered in this light, it makes sense that multiple different cytokines, hormones of the immune response, are capable of altering lipid metabolism in a variety of tissues in a manner that leads to an increase in serum lipid levels (12). This increase in serum lipid levels induced by the immune response can be considered part of the acute-phase response and is likely to be beneficial in protecting the host. Our results also indicate that normal circulating levels of lipoproteins, in addition to playing a role in lipid transport, have other important functions in host defense.

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