Lipoproteins inhibit macrophage activation by lipoteichoic acid

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Abstract **Regulation of lipid metabolism during infection is thought to be part of host defense, as lipoproteins neutralize endotoxin (LPS) and viruses. Gram-positive infections also induce disturbances in lipid metabolism. Therefore, we investigated whether lipoproteins could inhibit the toxic effects of lipoteichoic acid (LTA), a fragment of gram-positive bacteria. LTA activated RAW264.7 macrophage cells, stimulating production of tumor necrosis factor (TNF) in a dose-dependent matter, but produced less TNF than that seen after LPS activation. High density (HDL) or low density lipoprotein (LDL) alone inhibited the ability of LPS to stimulate TNF production, but had little effect on the activation by LTA. When a maximally effective dose of LTA was mixed with lipoproteins and 10% lipoprotein-depleted plasma (LPDP), the ability of LTA to stimulate macrophage production of TNF was inhibited. HDL, LDL, and the synthetic particle, Soyacal, when mixed with LPDP, were able to inhibit the ability of LTA to activate macrophages. Lipopolysaccharide-binding protein (LBP) substituted for LPDP in catalyzing lipoprotein neutralization of LTA by HDL. Antibody to LBP inhibited the ability of LPDP to induce LTA neutralization by HDL. Thus, lipoproteins can prevent macrophage activation by fragments from both gram-positive and gram-negative microorganisms.—**Grunfeld, C., M. Marshall, J. K. Shigenaga, A. H. Moser, P. Tobias, and K. R. Feingold. **Lipoproteins inhibit macrophage activation by lipoteichoic acid. *J. Lipid Res.* 1999.** 40: **245–252.**

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Multiple disturbances of lipid metabolism, including increased hepatic lipoprotein production and decreased peripheral clearance of lipoproteins, occur during infection (1, 2). It is now generally recognized that cytokines, the hormones that mediate the host immune response to infection and inflammation (3), mediate these changes in lipid metabolism (3). For example, changes in lipid metabolism occur rapidly after cytokine release or exogenous cytokine administration and are induced at very low doses of cytokines, similar to those that induce fever (2). Among the cytokines that mediate systemic changes in lipid metabolism are tumor necrosis factor (TNF), interleukin-1 (IL)-1 and IL-6, which play a critical role in host defense including the acute phase response of the liver (2, 3). Given the prominent role of increased hepatic lipoprotein production, these changes in lipid metabolism may be viewed as part of the acute phase response (2, 4).

The host responses that occur during infection can be reproduced by administration of bacterial fragments, the most extensively studied of which is endotoxin (LPS) from gram-negative bacteria. LPS, which is found in the circulation during sepsis (5), induces cytokine release, hypotension, and death (6). LPS also induces the metabolic responses seen during infection. For example, changes in hepatic lipoprotein production or clearance can be seen within 2 h of LPS administration (7). These metabolic changes are among the most sensitive host responses to LPS.

The rapid and sensitive coupling of these changes in lipid metabolism to the host immune response to LPS suggests that such changes in lipid metabolism may serve a protective function. Indeed, lipoproteins have been shown to bind LPS (8–13); protection by lipoproteins from LPS toxicity has been demonstrated using both in vitro and in vivo models (14–25). For example, LPS activation of macrophages, as manifested by secretion of cytokines such as TNF, can be blocked by incubation of LPS with lipoproteins (14–17). Similarly, incubation of LPS with lipoproteins decreases the ability of LPS to induce death in animals (18, 19). Pharmacological agents that decrease circulating lipoprotein levels increase sensitivity to LPS, while infusion of exogenous lipoproteins returns sensitiv-

Abbreviations: LTA, lipoteichoic acid; LPDP, lipoprotein-depleted plasma; LBP, lipopolysaccharide-binding protein; HDL, high density lipoprotein; LDL, low density lipoprotein; HSA, human serum albumin; TNF, tumor necrosis factor; IL, interleukin.

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ity to normal (20). Additionally, in transgenic mice that overexpress apolipoprotein A-I and have elevated HDL levels, the toxicity of LPS is reduced. Multiple other experiments indicate a role for lipoproteins in protection from the toxic effects of LPS (21–25). Likewise, a wide variety of viruses and even parasites are neutralized by lipoproteins (26–33).

Gram positive infections induce cytokine cascades similar to those of LPS and can also lead to septic shock (34–39). Although less well studied than gram-negative infections, there is evidence that gram positive infection leads to similar disturbances in lipid metabolism (40–45). Gram-positive organisms can affect both lipoprotein production and lipoprotein clearance (43–45).

Lipoteichoic acid (LTA), a heat-stable component of the cell membrane and wall of most gram-positive bacteria, has structural similarities to LPS (46, 47). Furthermore, LTA induces circulatory shock and treatment of macrophages or adherent mononuclear cells with LTA has been shown to induce TNF, IL-1, and IL-8, the mediators of septic shock (35, 48–51). We have also shown that LTA treatment induces hyperlipidemia in rodents similar to that seen with LPS (52). As a consequence, we asked whether lipoproteins could likewise protect against the toxicity associated with LTA. In this paper we demonstrate that lipoproteins inhibit the ability of LTA to stimulate macrophage production of TNF, a marker of macrophage activation.

MATERIALS AND METHODS

LTA from *Staphylococcus aureus* and the Limulus lysate were obtained from Sigma (St. Louis, MO); the Limulus chromogenic substrate S-2222 was obtained from Helena Laboratories (Beaumont, TX); LPS (*E. coli* 055:B5) was obtained from Difco Laboratories (Detroit, MI); polymyxin B from Pfizer Inc. (New York, NY); tissue culture plates from Flow Laboratory (McLean, VA); Dulbecco's modified Eagle's minimal essential medium (DMEM) from Media Tech (Herndon, VA); fetal calf serum (FCS) from Hyclone (Logan, UT). WEHI 164 clone 13 cells were kindly provided by Dr. M. Palladino of Genentech Inc. (South San Francisco, CA). Soyacal was from Abbott Laboratories (North Chicago, IL). LBP was cloned, expressed, and prepared as described previously (53). Antibodies were raised against LBP using standard methods (54).

Lipoprotein preparations

To avoid contamination with exogenous LPS, lipoproteins were isolated using special precautions previously described in detail (18). All materials used including chemical solutions and glassware were either obtained in apyrogenic form commercially or were depyrogenated by steam autoclaving followed by dry heating at 180°F for a minimum of 4 h. Blood was drawn from healthy volunteers using anticoagulation with EDTA at a final concentration of 1 mg/ml. This protocol was approved by the Committee on Human Research at UCSF. Lipoproteins were then prepared by ultracentrifugation using depyrogenated stainless-steel tubes (Beckman Instruments, Palo Alto, CA) with custom-crafted silicone O-rings. Total plasma lipoproteins were obtained by centrifuging at d 1.21 g/ml with KBr. LDL was isolated at d 1.006–1.063 g/ml and HDL was isolated at d 1.063–1.21 g/ml using KBr by density ultracentrifugation methods of Havel, Eder, and Bragdon (55). Lipoproteins were extensively dialyzed against normal saline containing 0.01% EDTA, pH 7.4. Lipoprotein-depleted plasma (LPDP) was prepared from the infranatant of centrifugations performed at density 1.21 g/ml[3], then was dialyzed and handled in a manner identical to that for lipoproteins.

Incubation of LTA and LPS with lipoproteins and LPDP

The assay for the ability of lipoproteins and LPDP to inhibit the toxicity of LTA and LPS was based on methods that we have previously described for the inactivation of LPS (18). The indicated amount of LTA or LPS and/or the specified concentrations of lipoproteins and/or LPDP were incubated at 37°C for 3 h before addition to RAW264.7 cells. LTA was also pre-incubated with polymyxin B (5 μg/ml) for 1 h at room temperature by the method of Wakabayashi et al. (35) before incubation with lipids and/or LPDP. For experiments with LBP antibody, the antibody was pre-incubated with LBP or LPDP at room temperature for 30 min prior to addition of other components of the treatment medium.

Cells and cell cultures

The murine macrophage cell line, RAW 264.7, was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown at 37°C in 5% CO_2 in DMEM that was low in LPS (LPS $<$ 0.03 ng/ml) supplemented with 10% FCS. For experiments, cells were plated in 96-well tissue culture plates in DMEM with 10% FCS overnight. Prior to addition of LTA, LPS, lipid and/or plasma fractions, cells were washed with DMEM without FCS, then medium whose content is detailed in figure captions was added.

Assays

Lipoprotein cholesterol and triglycerides were determined using enzymatic assays (Sigma Kit No. 352 and 339, St. Louis, MO).

TNF was measured by an ELISA using reagents obtained from Genzyme (Cambridge, MA). For several experiments the biological activity of TNF was confirmed using WEHI 164 clone 13, a cell line that is highly sensitive to TNF, in a cytotoxicity assay which was developed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium blue (56).

The chromogenic Limulus assay was performed as described in detail elsewhere (17). In brief, test solutions (0.1 ml) were added to 0.1 ml Limulus lysate reconstituted to 20-fold less than the manufacturer's recommended concentration with endotoxin-free H_2O and then incubated in a water bath at 37°C for 60 min. Next, 0.4 ml of 0.5 mM chromogenic substrate S-2222 reconstituted using endotoxin-free PBS was added, and the mixture was incubated at 37°C for an additional 30 min before the reaction was quenched by the addition of 0.4 ml 60% glacial acetic acid. Absorption of the final mixture was measured at 405 nm using a spectrophotometer with distilled H_2O as a blank. The assay was linear over a range of 10–100 pg endotoxin/ml.

Statistical analysis

Results are expressed as mean ± SEM. Significance was calculated using two-tailed Student's *t*-test for comparisons of two groups, Dunnet multiple comparison test for comparison of dose response to control, and Student-Neuman-Keuls for comparison of multiple groups.

RESULTS

We first compared the ability of LTA and LPS to induce TNF secretion from macrophages (RAW264.7 cells). LTA

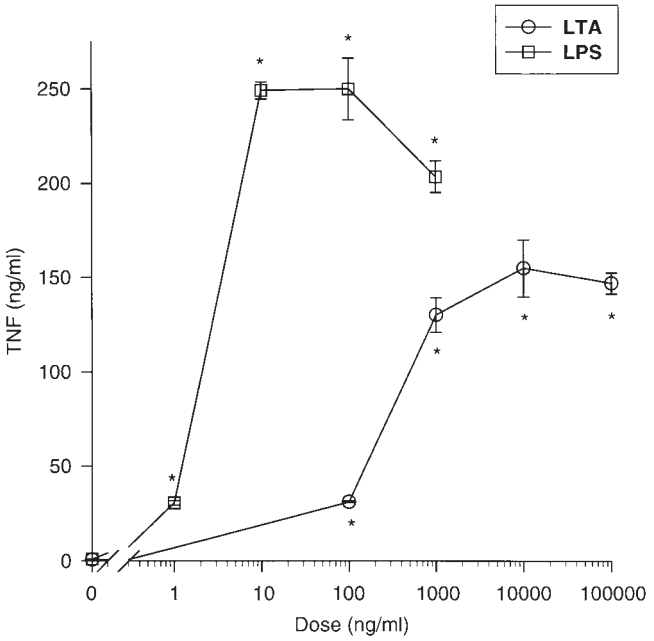

Fig. 1. LTA and LPS Stimulate TNF secretion. RAW 264.7 macrophage cells were incubated for 16 h in 10% LPDP and increasing concentrations of LTA (in the presence of 5 μg/ml polymyxin B) or LPS as indicated on the abscissa. Media were collected and assayed for TNF as described under Methods; n = 3 for each point. Values are mean ± SEM. Where error bars are not visible, they were smaller than the symbol. *$P < 0.01$ compared to control (no LTA or LPS) using Dunnett Multiple Comparison Test.

stimulated RAW cells to produce TNF, but the maximal levels of TNF produced were consistently lower than that seen with LPS (**Fig. 1**). At maximum doses, LTA induced only 62 ± 6.1% of the TNF induced by maximal doses of LPS. More than 1 μg/ml of LTA was needed for maximal activation, with doses of 100 μg/ml having no additional effect. In contrast, maximal activation with LPS occurred at much lower doses (10 ng/ml). When the concentrations for half maximal activation were compared, LTA was 100-fold less effective than LPS (1/2 maximal activation for LTA was 280 ng/ml and for LPS was 2.7 ng/ml). These data should be compared to the differential activity of LTA and LPS in the Limulus assay. As much as 1000 to 5000 times of LTA was needed to give equivalent reactivity as LPS in the Limulus assay. If the Limulus assay solely measured LPS contamination rather than cross-reactivity of LTA, then the amount of LPS found in 1 mg/ml of LTA (an LTA dose that gives near maximal activation) would be 0.2 to 1 ng/ml, an amount of LPS which would only minimally, if at all, stimulate TNF secretion in RAW cells.

Lipoproteins have been reported to neutralize the effects of LPS, both in vivo and in vitro, by blocking LPS activation of macrophages. We therefore next tested the ability of HDL to block LPS or LTA stimulation of TNF secretion. As reported by others (16), when LPS was incubated in vitro with HDL before addition to RAW cells, HDL inhibited LPS stimulation of TNF secretion by >85% (**Fig. 2A**). The half maximal concentration for HDL inhibition of LPS stimulation was 0.3 μg/ml. In contrast, much higher doses of HDL

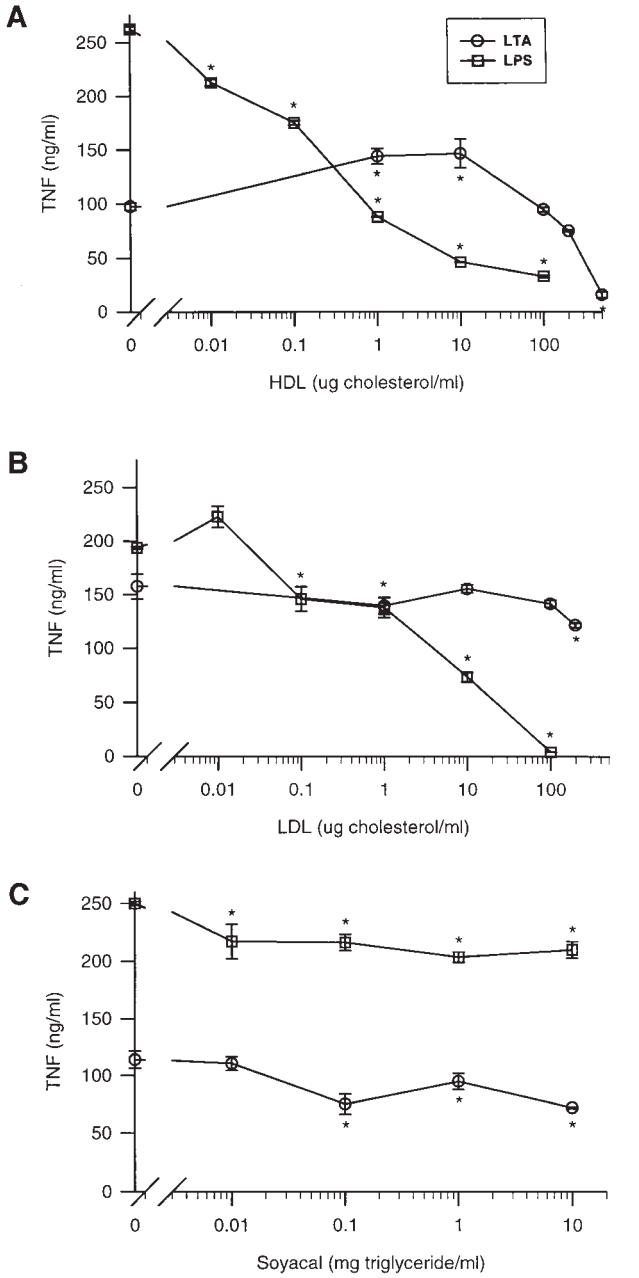

Fig. 2. Concentration dependence for lipoprotein and lipid particle inhibition of LTA and LPS-induced TNF secretion. Macrophages were incubated for 16 h in the presence of LTA (10 μg/ml) or LPS (10 ng/ml) that had been pre-incubated as described under Methods with increasing concentrations of lipoprotein or lipid particles as indicated in the abscissa (A: HDL, B: LDL, C: Soyacal). Media were collected and assayed for TNF by ELISA; n = 3 for each point. Values are mean ± SEM. Where error bars are not visible, they are smaller than the symbol. *$P < 0.01$ compared to no lipid. Asterisks for LTA curve are below and for LPS curve are above their respective symbols.

were needed to neutralize LTA (Fig. 2A). For example, 100 μg/ml of HDL (a dose which greatly inhibited LPS stimulation of RAW cells) had no effect on the ability of LTA to stimulate TNF secretion by RAW cells. The concentration for half maximal inhibition of LTA was 210 μg/ml of HDL, 700 times that needed to inhibit LPS.

Similar studies were carried out with LDL (Fig. 2B). As reported by others (16, 17), when LPS was incubated with LDL before addition to RAW cells, LDL inhibited LPS stimulation of TNF secretion by >95%. The half maximal concentration for LDL inhibition was approximately 5 μg/ml. In contrast, LDL had only very minimal effects on the ability of LTA to stimulate TNF secretion by macrophages.

Soyacal is a triglyceride- and phospholipid-containing particle used for intravenous nutrition that mimics the effects of triglyceride-rich lipoproteins such as chylomicrons. Soyacal alone had little effect on either LPS or LTA activation of macrophages (Fig. 2C). Soyacal was only able to inhibit LPS activation of RAW cells by 16%. Increasing the concentration of Soyacal to very high doses (10 mg/ml) had no further effect. Likewise, 10 mg/ml of Soyacal inhibited LTA activation by only 17% (Fig. 2C).

Because of the relative ineffectiveness of lipoproteins in inhibiting LTA activation of macrophages, we next determined whether lipoprotein-depleted plasma (LPDP) could enhance the ability of lipoproteins to neutralize LTA. Preincubation of LTA with LPDP had no effect on the ability of LTA to stimulate macrophage secretion of TNF (**Fig. 3A**). As expected, total plasma lipoproteins (d = 1.21 g/ml) also had no effect on LTA-induced TNF secretion (Fig. 3A). However, when LTA was incubated with both lipoproteins and 10% LPDP, the ability of LTA to stimulate macrophage secretion of TNF was dramatically curtailed (Fig. 3A). The concentration dependence for LPDP in promoting lipoprotein neutralization of LTA was next tested. Greater than 95% inhibition was seen at 10% LPDP and half maximal inhibition was seen at approximately 1% LPDP (Fig. 3B). Thus, pre-incubation of LTA with the combination of lipoproteins and LPDP resulted in a marked inhibition in the ability of LTA to activate macrophages.

We next determined the ability of specific lipoproteins in combination with LPDP to inhibit LTA activation of macrophages. When LTA was incubated with LPDP, the half maximal concentration of HDL required to inhibit the LTA-induced increase in TNF secretion by macrophages was between 10 and 20 μg/ml (**Fig. 4A**). In contrast, the same dose of HDL in the absence of LPDP had no effect on LTA-induced macrophage activation (Fig. 2A). Similarly, when LTA was incubated with LPDP and LDL, the ability of LTA to increase TNF secretion by macrophages was markedly inhibited (Fig. 4B). In contrast, LDL alone had virtually no effect on the ability of LTA to activate macrophages (Fig. 2B). Lastly, the combination of Soyacal and LPDP also dramatically inhibited LTA stimulation of TNF by macrophages (Fig. 4C), whereas Soyacal alone had almost no effect (Fig. 2C). Thus, in combination with LPDP, lipoproteins inhibit the ability of LTA to activate macrophages.

We next sought to determine which components of LPDP might enhance the ability of lipoproteins and lipid particles to neutralize LTA. Lipopolysaccharide-binding protein (LBP) is a plasma protein with homology to cholesteryl ester transfer protein and phospholipid transfer protein (53). LBP is known to both enhance the activation of macrophages by LPS in the absence of lipoproteins

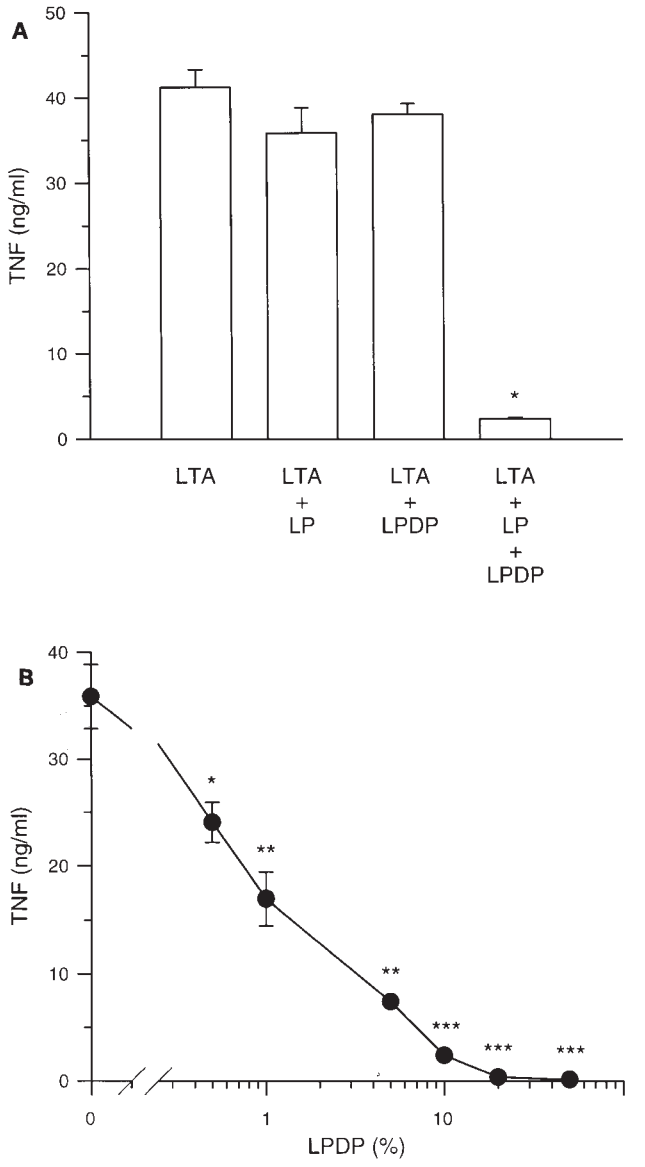

Fig. 3. Lipoprotein-depleted plasma (LPDP) enhances lipoprotein neutralization of LTA. Macrophages were incubated for 16 h in the presence of LTA (10 μg/ml) that had been pre-incubated as described under Methods in the presence or absence of total plasma lipoproteins (LP) prepared by centrifugation at d 1.21 gm/cm^2 (final concentration 0.2 mg/ml of cholesterol) and/or LPDP. TNF in the media was then assayed as described under Methods; n = 3 for each point. Values are mean ± SEM. A: The effect of LP, LPDP and LP + LPDP on LTA stimulation of TNF. *$P < 0.001$ compared to LTA alone, LTA + LPDP and LTA + LP using Student-Neuman-Keuls. B: Concentration dependence for LPDP. Where error bars are not visible, they are smaller than the symbol. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. no LPDP.

and to facilitate LPS binding to lipoproteins. LBP also binds LTA (57). **Figure 5** shows that when LBP is added to a concentration of HDL that alone is not able to inhibit LTA, LBP allows HDL to inactivate LTA. The dose–response curve for LBP gives an ID_{50} of 0.3 μg/ml, with 75% inhibition at 1 μg/ml of LBP (Fig. 5). The content of LBP in normal human plasma has been reported to be between 4 and 18 μg/ml (58–60). Thus, 10% LPDP would

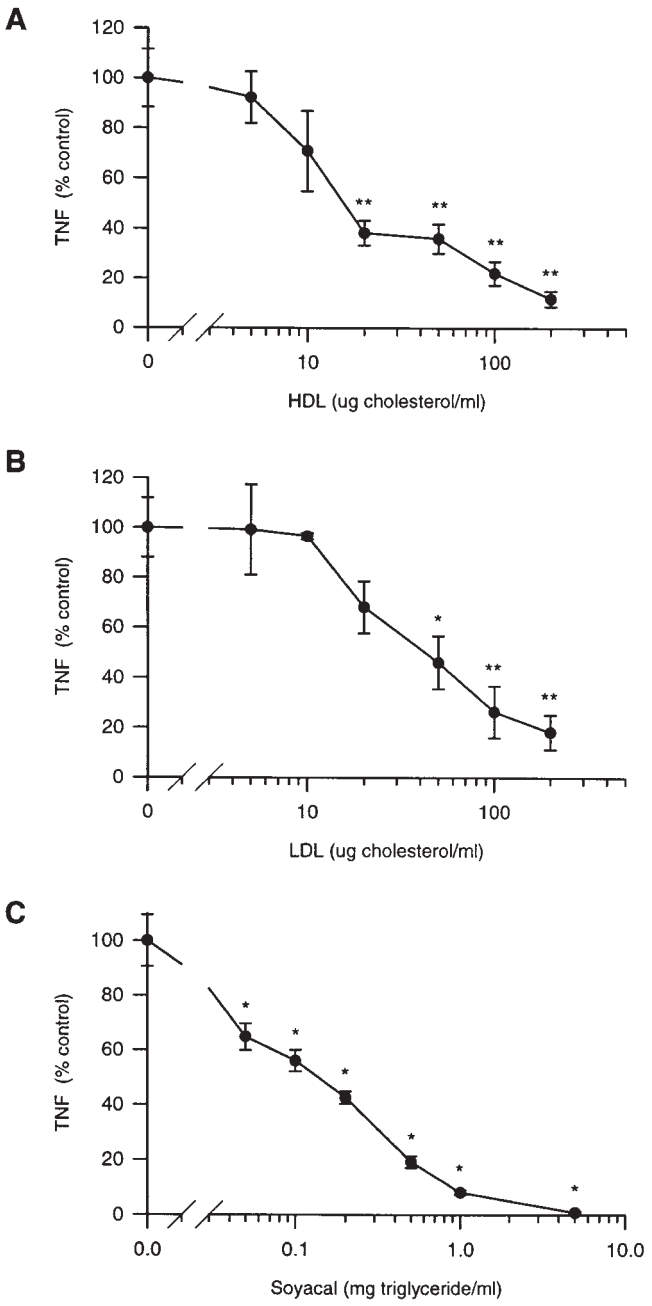

Fig. 4. Effect of lipoprotein fractions on LTA-stimulated TNF secretion. LTA (10 μg/ml) was pre-incubated in the presence of 10% LPDP plus varying concentrations of lipoprotein lipid as indicated on the abscissa as described under Methods (A: HDL, B: LDL, C: Soyacal). The mixture was then added to freshly washed macrophages and incubated for 16 h. Media were removed and assayed for TNF by ELISA; n = 3–6 for each point. Values are mean ± SEM. Where error bars are not visible, they are smaller than the symbol. *$P < 0.01$, **$P < 0.001$ vs. no lipid.

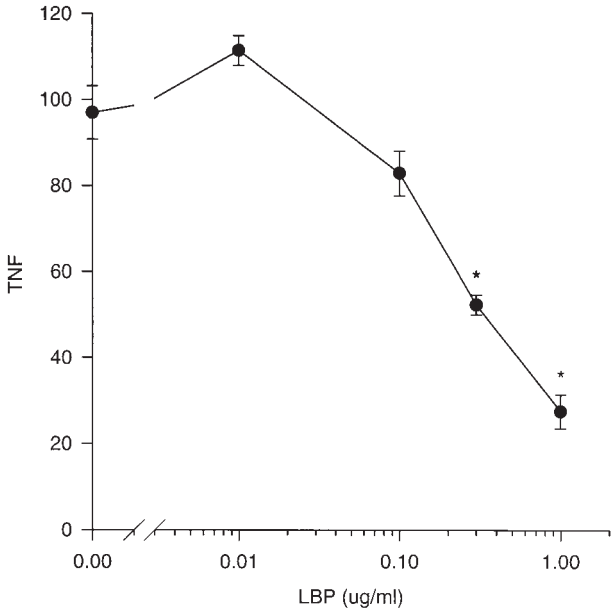

Fig. 5. Effects of LBP on HDL neutralization of LTA. LTA (10 μg/ml) and HDL (100 μg/ml cholesterol) were pre-incubated with the concentrations of LBP indicated on the abscissa as described under Methods. The mixture was then added to freshly washed macrophages and incubated for 16 h. Media were removed and assayed for TNF by ELISA; n = 5 for each point. Values are mean ± SEM. *$P < 0.001$ vs. no LBP.

contain approximately 0.4–1.8 μg of LBP; these data demonstrate that 1 μg of LBP is as effective as 10% LPDP in inhibiting LTA.

As a consequence, we next tested whether a neutralizing antibody to LBP could block the ability of the combination of LPDP and HDL to inhibit LTA. As shown in the experiment presented in **Fig. 6**, anti-LBP reduced the ability of LPDP to facilitate HDL inhibition of LTA (53 ± 8.3% average of 3 similar experiments).

DISCUSSION

The close linkage of the changes in lipid metabolism that occur during infection and inflammation to the host immune response has led to the proposal that changes in lipoprotein levels and metabolism are a component of the acute phase response (3, 61). The acute phase response plays a role in host defense as part of innate, non-adaptive immunity (3). Increases in the circulating levels of many acute phase proteins are beneficial to the host. For example, complement 3 and C reactive protein help in the opsonization of bacteria, immune complexes, and foreign particles (3, 62). C reactive protein is associated with VLDL and LDL (63, 64). Alpha 2 macroglobulin, alpha 1 proteinase inhibitor, and several other acute phase proteins are inhibitors of leukocyte proteases and thereby may serve to limit proteolysis to the site of inflammation (3). It is likely that the changes in lipoprotein levels and metabolism during the acute phase response may also have beneficial effects.

Extensive studies have demonstrated that lipoproteins play a role in neutralizing LPS. HDL, LDL, and triglyceride-rich lipoproteins bind LPS and the ability of LPS to stimulate cytokine production by macrophages in vitro is inhibited (14–17). While the cytokines produced by macrophages coordinate the host's response to infection and

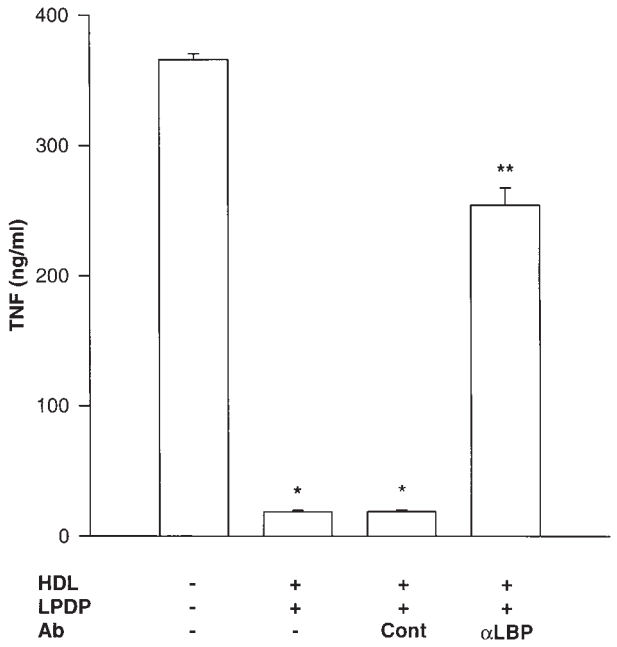

Fig. 6. Antibody to LBP inhibits the ability of LPDP to facilitate HDL neutralization of LTA. Using protocols similar to previous figures, LTA (10 μg/ml) was incubated in the presence of 10% LPDP, HDL (100 μg/ml), control IgG (2 mg/ml), and/or IgG prepared from antibody to LBP (2 mg/ml), a quantity more than sufficient to neutralize several μg of LBP. Media were removed and assayed for TNF by ELISA; n = 3 for each group. Values are mean ± SEM. *$P < 0.001$ vs. LTA alone, LTA plus LPD plus anti-LBP; **$P < 0.001$ vs. LTA alone, statistics were done by ANOVA using Student-Newman-Keuls Multiple Comparison Test.

inflammation, there is also abundant evidence demonstrating that many of the adverse effects of infection and inflammation are secondary to the overproduction of these cytokines. The toxic effects of LPS in vivo can be ameliorated by inhibiting cytokine production or action. Studies have shown that incubating LPS with all lipoprotein classes prior to administration to rodents reduces LPS toxicity (18, 19). The physiological significance of lipoproteins in protecting from LPS-induced lethality is further demonstrated in animals whose lipoprotein levels are modulated experimentally. For example, lipid lowering by drugs markedly increased the sensitivity to LPS-induced mortality (20). The increase in LPS-induced mortality in the hypolipidemic animals was associated with a greater increase in serum TNF levels than observed in animals with normal lipid levels. In addition, when animals whose endogenous lipid levels were reduced by drug therapy were administered exogenous lipoproteins, which increased levels of serum lipids into the physiological range, the increased mortality to LPS was reversed (20). Furthermore, increasing serum HDL levels by making transgenic mice that overexpressed human apoA-I also led to a reduced cytokine response to LPS treatment and improved survival (21). Finally, recent studies indicate that recombinant HDL can provide protection from the toxicity of experimental endotoxemia in humans (65, 66).

While LPS is a component of gram-negative bacteria and is thought to be responsible for much of the toxicity of these microorganisms, LTA is a component of the wall and membrane of gram-positive bacteria (46, 47). Studies by several laboratories have shown that LTA stimulates cytokine production by macrophages and can induce toxicity when administered to animals (48–51). Moreover, studies by our laboratory have shown that LTA administration can induce changes in lipid and lipoprotein metabolism in rodents that are similar to that produced by LPS (52). In the present study we addressed whether lipoproteins were capable of neutralizing LTA stimulation of macrophages in vitro.

As reported previously by other laboratories, we found that LTA stimulates TNF production by macrophages. In contrast to LPS, lipoproteins alone do not inhibit TNF secretion by LTA-treated macrophages. Some, but not all, of the lack of efficiency of lipoproteins against LTA may be due to the larger mass of LTA needed to activate macrophages. However, the addition of small amounts (1–10%) of lipoprotein-depleted plasma (LPDP) facilitates the ability of lipoproteins to inhibit LTA activation of macrophages. HDL, LDL, and Soyacal (a triglyceride- and phospholipid-containing particle used for intravenous nutrition) were all capable of inhibiting the activation of macrophages by LTA when LPDP was present. The effective concentrations of HDL and LDL (ID_{50} = 14 and 40 mg/dl, respectively) are within the physiologic range. Thus, while lipoproteins alone are capable of inhibiting LPS activation of macrophages, in contrast, LTA neutralization by macrophages requires LPDP.

Based on the failure of Soyacal alone to inhibit LPS, we would speculate that circulating lipoproteins contain cofactors in sufficient quantity to facilitate LPS, but not LTA, binding to lipoproteins. Studies by other investigators have shown that apolipoprotein E and LBP, which are associated with lipoproteins, particularly HDL, facilitate LPS binding to lipoproteins (67, 68). In fact, we have shown that the combination of LPDP and Soyacal, but not either LPDP or Soyacal, alone, inhibits the ability of LPS to stimulate TNF secretion in macrophages (data not shown), suggesting that under some conditions LPDP might facilitate LPS binding with lipoproteins. In the case of LTA, the lipoproteins (HDL and LDL) probably do not contain sufficient quantities of these cofactors to allow for inhibition in the absence of serum.

Our studies have further demonstrated that LBP is an important serum factor in facilitating LTA neutralization by HDL. The addition of purified LBP in combination with HDL resulted in an inhibition of LTA-induced macrophage activation. The concentration needed to promote neutralization is consistent with the amount of LBP found in LPDP, which induces similar neutralization of macrophage activation when incubated with lipoproteins. More importantly, antibodies that neutralize LBP resulted in a significant decrease (53%) in the ability of LPDP to facilitate HDL inhibition of LTA activation of macrophages. These results suggest that LBP is an important serum factor that facilitates HDL inhibition of LTA activation of macrophages, but that serum factors in addition to LBP may also play a role. The molecular interactions of

LBP with lipoproteins and bacterial fragments is an important topic for future studies.

It is likely that lipoproteins play other important roles in protecting the host from injury. Alterations in lipoprotein levels, composition, and metabolism may result in the redistribution of nutrients to cells that are important in host defense (1, 27). Additionally, lipoproteins have been shown to compete with certain viruses for cellular receptors and to directly neutralize viruses by binding (26–30). HDL from humans and baboon lyse the parasite *Trypanosoma brucei brucei*; the human haptoglobin-related protein that resides on HDL has been shown to be responsible for lysing the parasite (31–33). Lastly, LDL binds to the parasite *Schistosoma mansoni* and helps target macrophages that kill the parasite (69, 70). Thus, by a variety of different mechanisms, lipoproteins may play a role in host defense. The ability of lipoproteins to neutralize LTA extends this beneficial function to gram-positive infections such as those caused by staphylococcus and streptococcus. Thus, the changes in lipoprotein levels and metabolism that occur during infection and inflammation may be part of the host's innate immune response. In this light, it is of interest that in long-term human cohort studies, low levels of serum cholesterol at entry predict an increased risk of death from infection at later time points (71). Moreover, very recent studies have demonstrated that low serum cholesterol levels are a marker of increased subsequent risk to infection with HIV and death from AIDS (72, 73). Thus, numerous different groups have provided evidence suggesting that lipoproteins play a role in the host's defense against infection.

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