Structural determinants for the interaction of lipopolysaccharide binding protein with purified high density lipoproteins: role of apolipoprotein A-I

Tania Massamiri,* Peter S. Tobias,* and Linda K. Curtiss[1,*,†]

Department of Immunology* and Department of Vascular Biology,[†] The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Abstract The interaction of lipopolysaccharide binding protein (LBP) with apolipoprotein (apo)A-I on high density lipoproteins (HDL) was studied in solid phase ligand binding assays with a biotinylated LBP-specific antibody. The association was dependent on LBP concentration and enhanced in the presence of lipopolysaccharide (LPS). Maximal enhancement was measured at an LPS/LBP molar ratio of 6. To identify regions on apoA-I that participate directly or indirectly in the interaction between LBP and HDL, we attempted to inhibit LBP association with a panel of mapped apoA-I-specific monoclonal antibodies. Whereas some antibodies were effective inhibitors, others were not, even though they bound apoA-I. Furthermore, selected apoA-I synthetic peptides inhibited the antibody-mediated interference of the HDL/LBP interaction. Although no specific mechanism can be defined for the basis of the inhibitory effects of the antibodies on the association of LBP with HDL, we identified a role for three unique regions on apoA-I between residues 1–31, 95–164, and 178–200. These results suggested that apoA-I is a key component in the association of LBP with HDL and may play an important role in the biologic activity of LPS/LBP complexes.—**Massamiri, T., P. S. Tobias, and L. K. Curtiss.** Structural determinants for the interaction of lipopolysaccharide binding protein with purified high density lipoproteins: role of apolipoprotein A-I. *J. Lipid Res.* 1997. **38:** 516–525.

Supplementary key words septic shock • endotoxins • acute phase • epitopes

When endotoxins, lipopolysaccharides (LPS) associated with cell membranes of Gram-negative microorganisms, enter the blood stream they elicit inflammatory responses that lead to activation of various plasma proteins and cellular defense systems. These events can lead to endotoxic shock and frequently death (1–4). In whole blood, LPS-induced cellular responses are the net result of the interaction of LPS with various plasma components such as lipoproteins, soluble CD14, bactericidal/permeability increasing protein (BPI), and with specific membrane receptors (5–9). LPS binding protein (LBP) facilitates the interaction of LPS with lipoproteins and CD14 receptors, and can enhance the biologic consequences of BPI, LPS, or Gram-negative bacteria in vivo (5, 6, 9–12).

Protection against LPS endotoxicity by lipoproteins has been demonstrated using in vitro and in vivo models (13–17). Preferential association with high density lipoproteins (HDL) is reported (8, 18–22), albeit interactions with other lipoproteins also occur (14, 23). The mechanism by which LPS interacts with HDL is not well understood, although roles for phospholipids (20, 21), apoA-I (14, 16, 24, 25), LBP (24, 26), and factor H-related proteins (26) have been proposed.

Plasma HDL are heterogenous lipoproteins with respect to size, density, lipid, and protein composition (27–31). HDL represent a spectrum of lipoprotein particles that range from larger, less dense HDL species, to smaller, denser HDL particles (30). Genetic as well as exogenous factors influence the phenotype of this spectrum. Furthermore, the balance between the different HDL subpopulations is shifted by alimentation and disease states (32–35). ApoA-I is present on all HDL (29). It represents 75% of the HDL protein and plays an important role in lipid transport and metabolism (36). ApoA-I is a 243 amino acid protein composed of multiple, 11-residue homologous sequences that assemble into a series of amphipathic alpha helices (37) which mediates its association with lipids. In addition to its structural role on HDL, apoA-I modulates the activity

Abbreviations: LPS, lipopolysaccharide; LBP, lipopolysaccharide binding protein; HDL, high density lipoprotein; apoA-I, apolipoprotein A-I; BPI, bactericidal/permeability increasing protein; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; ELISA, enzyme-linked immunosorbent assay; CHO, Chinese hamster ovary.

[1]To whom correspondence should be addressed.

of selected enzymes that are found on HDL including the cholesterol esterifying enzyme, lecithin:cholesterol acyltransferase (LCAT), and a plasma neutral lipid exchange protein, cholesteryl ester transfer protein (CETP). Because both CETP and LBP interact with HDL, share sequence homology, and have a similar distribution of charged amino acids (38), the association of LBP with HDL and/or apoA-I may share similar functional and binding characteristics with the association of CETP with HDL.

Central to understanding how HDL neutralizes endotoxins is knowledge of the biochemical interactions between specific structural elements on HDL and LPS and/or LPS/LBP complexes. Therefore, we sought to clarify the role of LBP in modulating the binding of LPS to HDL and to identify structural elements of apoA-I that participate in this interaction. We have used an immunochemical approach to study the interaction between LBP and apoA-I on HDL. Studies of the conformation of apoA-I on HDL indicate that apoA-I epitopes are immunochemically heterogenous on HDL and do not necessarily bind antibody in a stoichiometric manner (27, 28, 39). Nevertheless we report here that some apoA-I-specific monoclonal antibodies inhibited the interaction of LBP with HDL and could be used to identify functional domains of apoA-I important for this interaction. Our results imply that apoA-I may play a role in the biologic activity of LPS/LBP complexes in plasma.

EXPERIMENTAL PROCEDURE

Lipoproteins

Human HDL (d 1.063–1.21 g/ml) was isolated from pooled normal human plasma by standard ultracentrifugation techniques in the presence of protease inhibitors and antioxidants as described (40). After isolation, HDL was dialyzed extensively against 0.15 M NaCl containing 0.3 M EDTA and 1 mM probucol, filter sterilized, and stored at 4°C for up to 30 days. The apoprotein composition of the HDL was identified by polyacrylamide gel electrophoresis as described (27). Endotoxin contamination was prevented during lipoprotein isolation by using pyrogen-free sterile water for all reagents. All glassware was washed with 1% E-Toxa-Clean in sterile water, rinsed with sterile water, and baked at 185°C for 5 h. Endotoxin contamination of reagents and lipoproteins was monitored with the chromogenic LAL assay (Bio-Whittaker). HDL was iodinated to a specific activity of 9×10^5 cpm/μg using the iodine monochloride labeling method (41). All purified HDL preparations used contained no detectable LBP. This was assessed with an LBP immonoassay in which HDL was captured on plates coated with an anti-LBP monoclonal antibody (1E8, (42)). In this assay the minimal detectable level of LBP was in the range of 10 ng/ml.

Lipopolysaccharide binding protein

Human LBP was isolated from the medium of CHO cells transfected with a h-LBP cDNA and purified according to Theofan et al. (43). ^{35}S labeled LBP was purified from cells grown in [L−^{35}S]methionine according to Gegner, Ulevitch, and Tobias (42). The specific activity of [^{35}S]LBP was 652 cpm/ng and was quantified with an ELISA that was standardized against a known amount of LBP purified from human serum.

Monoclonal antibodies

All monoclonal antibodies to apoA-I have been described (40, 44, 45). They were obtained from multiple fusions of spleens from immunized Balb/c mice with P3Ag8.653.1 myeloma cells using standard fusion protocols (45). Immunogens included purified human HDL and isolated apoA-I. Purification of the antibodies was carried out by fast protein liquid chromatography on a Mono-Q HR 15/10 (100 mm × 16 mmID) anion exchange column (Pharmacia) (40). Antibodies were iodinated using the Iodobead method (40) and separated from free iodine by overnight dialysis.

Synthetic peptides

ApoA-I peptides were synthesized from the sequence published by Brewer and co-workers (37). Peptide synthesis was carried out using the solidphase method of Merrifield (46) on an Applied Biosystem ABI 430A automated peptide synthesizer using HOBt/DCC activation as described (45). The resultant peptide-resins were treated with 10% anisole/hydrogen fluoride at −4°C for 1 h and 10-μg samples were analyzed by high performance liquid chromatography using a Vydac C 18 column. The starting buffer contained 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B). The run consisted of a 20–70% gradient increase in solvent B over 20 min at 40°C. Preparative purification of the peptides used chromatography on a Water Auto 500 preparative high performance liquid chromatography (50 × 250 mm Vydec C 18 column, 15–20 μm) under the same conditions as described for analytical chromatography. Amino acid compositions of all peptides were measured after hydrolysis with a Beckman 6300 high performance

analyzer with internal standards. All peptides were lyophilized and stored under vacuum.

Cesium chloride density gradients

The interaction of LBP with purified human HDL was examined by isopycnic CsCl density gradient ultracentrifugation (47). Samples composed of purified human HDL and [^{35}S]LBP were incubated for 30 min at 37°C in PBS/EDTA (0.02 M), pH 7, and mixed with 3.8 ml of ice-cold 2.81 M CsCl, 0.15 M NaCl. The gradient was spun to equilibrium (16 h) at 45,000 rpm in a VTR 865 (Dupont/Sorvall) rotor at 4°C. The gradients were fractionated (9 drops per tube) and counted. HDL was consistently recovered from the top of the gradient as determined by the recovery of ^{125}I-labeled HDL.

HDL binding to LBP

Purified human HDL was immobilized for 2 h at 37°C on 96-well Microtiter Immunoassay plates (Immulon 4, Dynatech Labs, Inc.) at a concentration of 10 μg/ml in PBS (final volume 0.1 ml). After post-coating for 1 h at 37°C with 1% Bovuminar-protease free powder (Intergen Co.), the plates were incubated for 1 h at 37°C with 0.1 ml of either human LBP or preformed LBP/LPS complexes. The preformed complexes were prepared by incubating LBP (1 μg/ml) with LPS (the Rb form (List Biological Labs Inc.)) for 30 min at 37°C. A molecular weight of 5000 for Rb LPS (48) was used to calculate the LPS/LBP molar ratios. For competitive assays, HDL-coated plates were incubated for 1 h at 37°C with the competitor (HDL, apoA-I monoclonal antibodies, or apoA-I synthetic peptides in the presence of apoA-I antibodies) before addition of LBP or LPS/LBP complexes. After washing, LBP binding to the immobilized HDL was detected with a biotinylated, LBP-specific monoclonal antibody (18G4; 1 h at 37°C) followed by another incubation for 1 h at 37°C with a 1:500 dilution of biotin/streptavidin/peroxidase (Zymed). The substrate for the final colorimetric reaction, *o*-phenyldiamine (OPD, Zymed), was allowed to develop (5–10 min) at room temperature before the reaction was stopped with a solution of 4 N sulfuric acid. Plates were read at 590 nm.

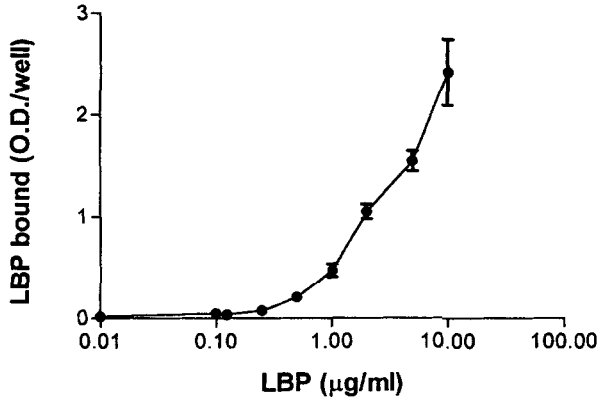

Fig. 1. Binding of LBP to immobilized purified human HDL. Plates were coated with 0.1 ml of HDL at 10 μg/ml for 2 h at 37 °C. Association of LBP with HDL (1 h at 37 °C) was detected with the biotinylated LBP-specific monoclonal antibody, 18G4. Results which are representative of 3–5 experiments were plotted as optical density per well (mean of triplicates ± SEM) .

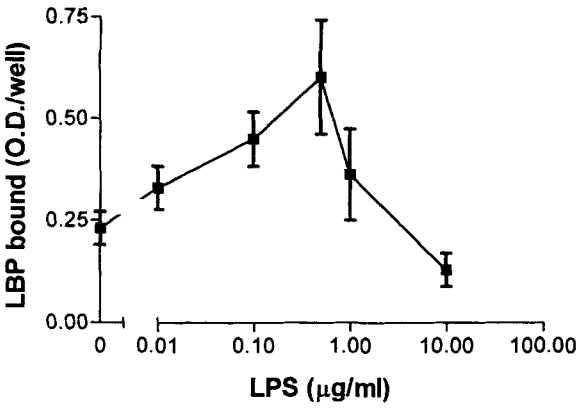

Fig. 2. Influence of LPS on the interaction of LBP with immobilized purified HDL. LBP was used at 1 μg/ml. The LBP/LPS complexes were allowed to preform for 30 min at 37°C before they were added to the HDL-coated plates. The association of LBP with HDL was detected as described with the biotinylated monoclonal antibody, 18G4. LPS concentrations of 0.01, 0.1, 0.5, 1.0, and 10 are equivalent to LPS/LBP ratios of 0.12, 1.2, 6, 12, and 120, respectively. Results are plotted as optical density per well (mean of triplicates ± SEM).

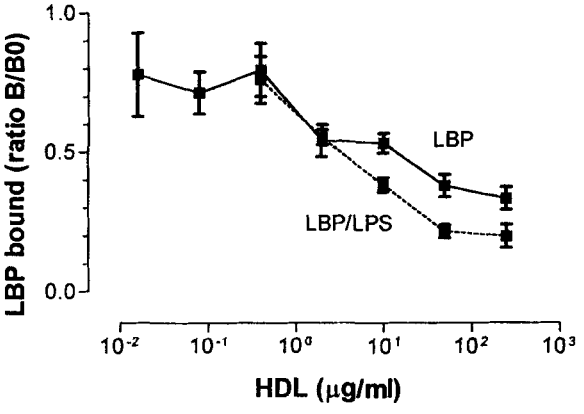

Fig. 3. Inhibition of the binding of LBP or LBP/LPS complexes to immobilized HDL by soluble HDL. LBP was used at 1 μg/ml and the LBP/LPS complexes (1 μg/ml and 0.1 μg/ml, respectively) were preformed (30 min at 37 °C) before they were added to the plate. The association of LBP with HDL was detected with biotinylated 18G4. Results are plotted as B/BO (mean ± SEM), where B = mean optical density in the presence of the competitor (HDL) and B0 = mean optical density in the absence of the competitor.

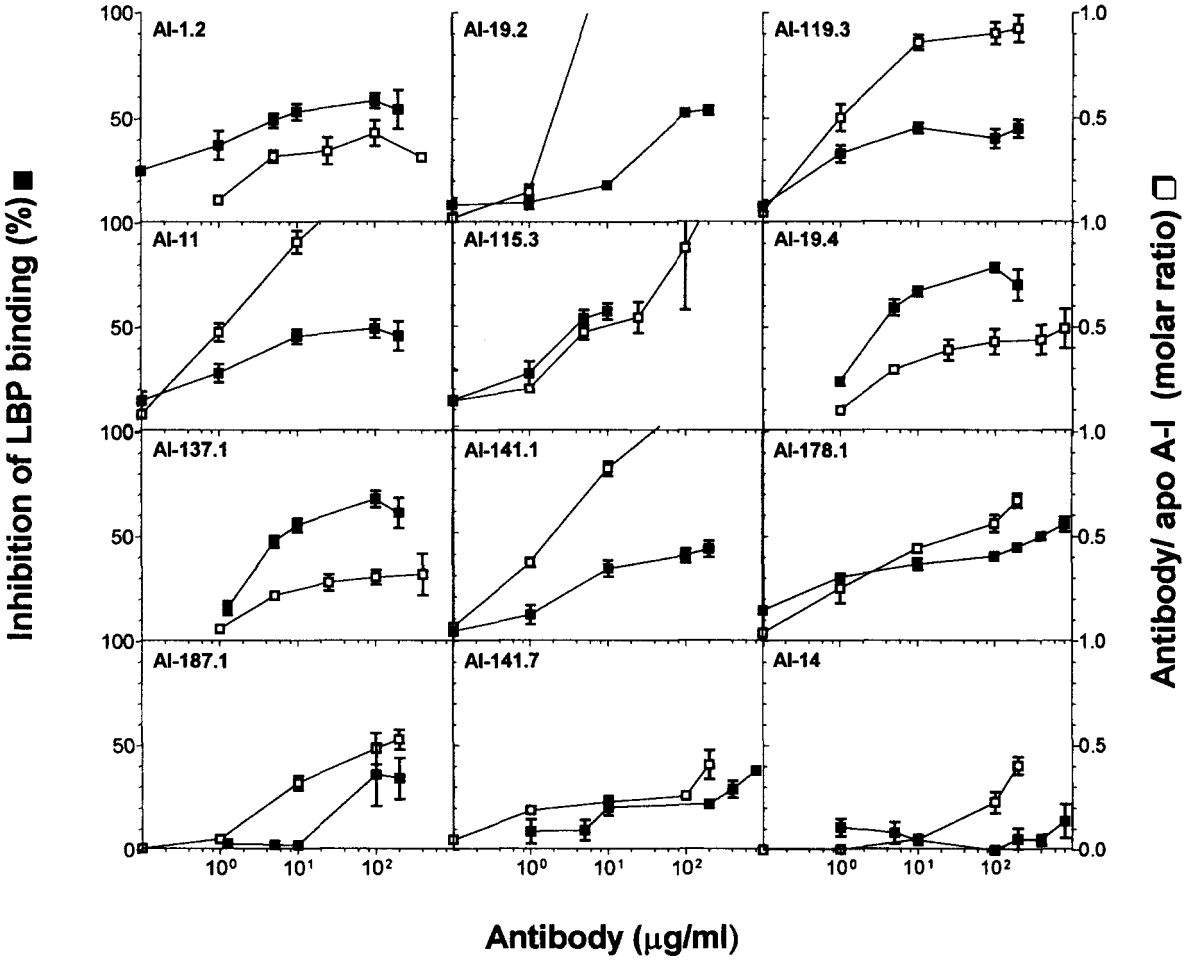

Fig. 4. Comparison of the binding capacity of each apoA-I antibody and its ability to inhibit the association of LBP with HDL. The % inhibition of LBP (1 μg/ml) association with immobilized HDL by increasing amounts of each apoA-I monoclonal antibody was plotted on the left axis (■), and the independent estimate of the amount (molar ratio) of antibody bound per apoA-I was plotted on the right axis (□). The amount of HDL-associated apoA-I bound per plate was estimated to be 57 ng/well. To facilitate the comparisons, only molar ratios between 0 and 1 are shown. The higher ratios seen with antibodies AI-19.2, AI-11, and AI-141.1 represent independent aggregation of the antibodies at the higher antibody concentrations.

ApoA-I antibody binding to HDL

To measure the ability of apoA-I monoclonal antibodies to bind to HDL, individual purified antibodies were radioiodinated and incubated with HDL-coated plates under the same assay conditions as those described for LBP binding to HDL. Binding of antibodies to HDL was quantified by measurement of ^{125}I cpm per well after subtraction of background, which was measured as the amount of binding to albumin-coated wells. Data were expressed as molar ratio of antibody per apoA-I. The amount of apoA-I protein bound per well was 57 ng. This was independently determined by measuring binding of ^{125}I-labeled HDL to wells (76 ng/well) and estimating that 75% of the labeled proteins on HDL were apoA-I.

RESULTS

Interaction of LBP and LPS with human HDL

Ulevitch and Johnston (47) and Ulevitch, Johnston, and Weinstein (8, 49) demonstrated an interaction between LPS and HDL using cesium chloride (CsCl) fractionation of LPS/serum mixtures. To examine the binding of LBP to HDL we first attempted to observe

an association of LBP with HDL by following a decrease in the buoyant density of [^{35}]LBP in CsCl gradients. In the presence of 1 mg/ml HDL, only 7.5% of the LBP could be recovered from the top of the gradient in the density range of HDL. This result is in accord with previous observations that LBP was not observed to be bound to HDL in the presence of high concentrations of (CsCl) (12). However, we were able to demonstrate an LBP/HDL association in saline with a solid phase assay. In this assay, HDL was immobilized on microtiter plates and LBP association with HDL was detected with a biotinylated LBP-specific monoclonal antibody. An LBP interaction with HDL was observed after incubation of LBP with immobilized HDL for 1 h at 37°C (**Fig. 1**). To examine the influence of LPS on this association of LBP with HDL, a nonsaturating amount of LBP (1 μg/ml) was pre-incubated with LPS for 30 min at 37°C before it was added to the immobilized HDL (**Fig. 2**). The effect of LPS was biphasic; low concentrations of LPS enhanced the association of LBP with HDL, whereas higher LPS concentrations diminished the association. Optimum binding was observed at a LPS/LBP molar ratio of 6. Direct evidence for specificity of the interaction between LBP and LBP/LPS complexes with immobilized HDL was demonstrated in competition assays. As shown in **Fig. 3**, soluble HDL inhibited the interaction of both LBP and LBP/LPS complexes with immobilized HDL.

Monoclonal antibodies to apoA-I interfered with the association of LBP with HDL

To examine the role of the major HDL apoprotein, apoA-I, multiple monoclonal antibodies specific for apoA-I were included individually in the HDL/LBP assays. The ability of each antibody to interfere with the association of LBP with HDL is illustrated in **Fig. 4.** Whereas none of the antibodies completely inhibited LBP binding, some of the antibodies (AI-19.4 or AI-137.1) inhibited up to 80%. Others (AI-1.2, AI-19.2, AI-11, AI-115.3, AI-119.3, AI-141, AI-178.1) inhibited the interaction by 50–60%. Interestingly, antibodies AI-14, AI-187.1, and AI-141.7 had little effect on the association of LBP with immobilized HDL.

Relationship between antibody binding capacity and inhibition of the LBP/HDL interaction

To verify that the antibodies, which had minimal effects on LBP/HDL interactions, bound immobilized HDL in the solid phase immunoassay, we independently examined the binding of each monoclonal antibody to HDL under conditions that duplicated the LBP/HDL association assays. Monoclonal antibody binding to immobilized HDL was determined with radioiodinated antibody and these data also are shown in Fig. 4. Although the antibody to apoA-I molar ratios were always less than 0.5, antibodies AI-1.2, AI-19.4, AI-137.1, and AI-178.1 inhibited LBP binding by 50% or more. In contrast, although AI-19.2, AI-11, AI-119.3, and AI-141.1 bound better to HDL, they too inhibited LBP binding by 50% or less. Furthermore, the antibodies that were poor functional inhibitors (such as AI-14, AI-141.7, and AI-187.1) did bind to the immobilized HDL. The relationship between the maximum binding capacity of the apoA-I monoclonal antibodies to HDL and their inhibition of the interaction of LBP with immobilized HDL is summarized in **Fig. 5**. The absence of any correlation (r = 0.011) between maximum antibody binding and inhibition of the LBP/HDL interaction indicated that LBP/HDL interactions were not just a reflection of the binding of any apoA-I antibody. Instead the antibody-mediated interference appeared to be a function of the epitope specificity of the antibodies.

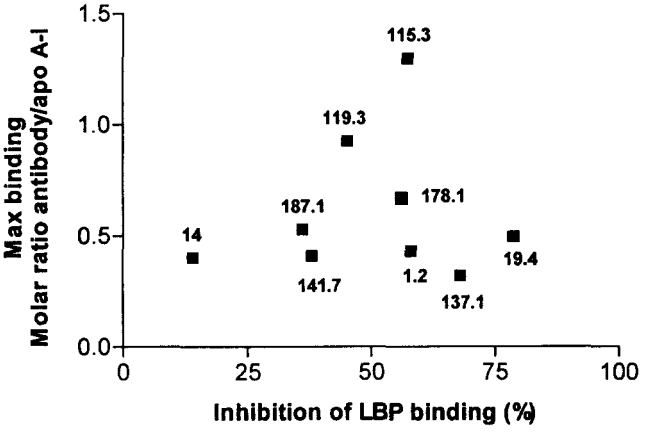

Fig. 5. Relationship between the maximum binding of each apoA-I-specific monoclonal antibody to immobilized HDL and its maximum inhibition of the LBP/HDL interaction. No correlation (r^2 = 0.011) was observed between the maximum binding capacity of an antibody to HDL (shown in Fig. 4) and its ability to inhibit the interaction of LBP with HDL (shown in Fig. 4).

ApoA-I synthetic peptides reversed the ability of an antibody to block LBP/HDL interactions

The epitope of each apoA-I monoclonal antibody is defined by its ability to bind a precise apoA-I synthetic peptide (44, 45). Therefore, further evidence for a specific interaction between LBP and apoA-I was demonstrated by examining the ability of an epitope-defining

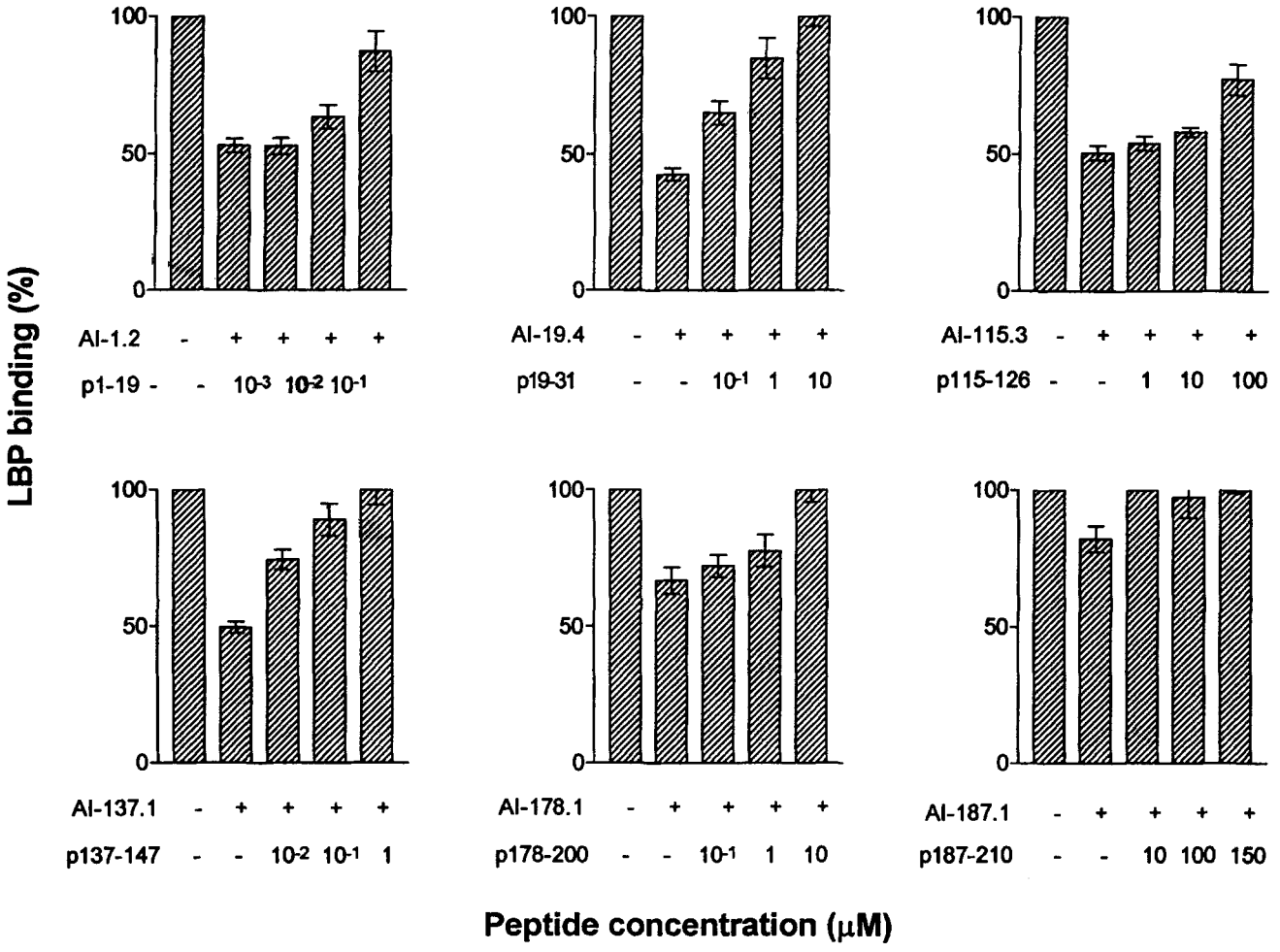

Fig. 6. Epitope-defining apoA-I synthetic peptides interfere with antibody-mediated inhibition of the LBP/HDL interaction. LBP was used at 1 μg/ml. The effect of selected monoclonal antibodies in the absence and presence of their respective epitope-defining peptides was plotted as the percent inhibition of LBP association with HDL (1 h at 37°C). The concentration of each antibody was chosen to achieve 50% inhibition of total LBP binding. These concentrations were: 5 μg/ml for antibody AI-1.2; 10 μg/ml for antibody AI-19.4; 10 μg/ml for antibody AI-115.3; 10 μg/ml for antibody AI-137.1; 10 μg/ml for antibody AI-178.1; and 120 μg/ml for antibody AI-187.1. p1-19, p-31, p-126, p-147, p-200, and p-210 are synthetic peptides representing apoA-I amino acid residues 1–19, 19–31, 115–126, 137–147, 178–200 and 187–210, respectively (22, 23).

synthetic peptide to interfere with the binding of an antibody to HDL and thereby block the LBP/HDL interaction (**Fig. 6**). Only high-affinity antibody/peptide pairs were examined. Antibody concentrations that reduced LBP/HDL interactions by 50% were used and antibody AI-187.1, which did not block LBP/HDL interactions, was included as a negative control. Despite differences in affinities among defining peptides and their respective antibodies, full reversal was observed in all cases. However, when these same concentrations of peptides were incubated with inappropriate antibodies, these antibodies bound HDL as expected (data not shown), indicating that the effect of the peptides was antigen specific.

ApoA-I antibodies also inhibited the interaction of LPS/LBP complexes with HDL

Because we observed that LPS could enhance the association of LBP with HDL (Fig. 2), we examined the effect of each antibody on the association of LPS/LBP complexes (**Fig. 7**). LPS/LBP complexes were prepared by pre-incubating 1 μg/ml of LBP with 0.5 μg/ml of LPS for 30 min at 37°C and were studied under conditions identical to that illustrated in Fig. 4 for LBP alone. Although, as expected, the absolute amount of LBP bound to HDL was increased, a direct one to one relationship (r^2 = 0.939) was observed between antibody-mediated inhibition of LPS/LBP and the inhibi-

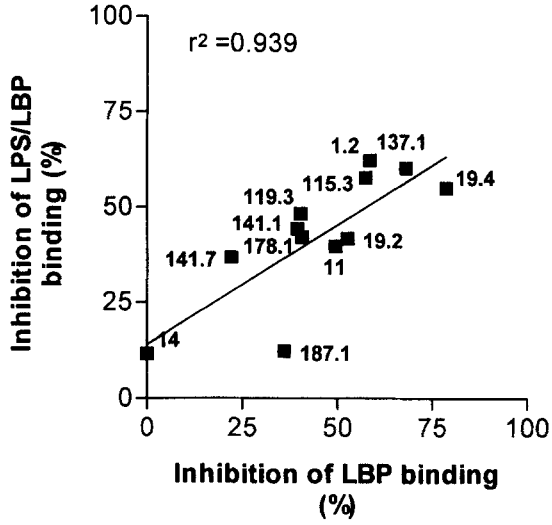

Fig. 7. Correlation between antibody-mediated percent inhibition of LBP and LBP/LPS interactions with HDL. The maximum percent inhibition for each antibody was chosen for this analysis. The inhibition by antibody of the association of LBP (1 μg/ml) with HDL was obtained from Fig. 4. The inhibition by antibody of the association of LBP/LPS complexes was obtained from identical assays performed with LBP/LPS complexes (1 μg/ml/0.5 μg/ml) preformed for 1 h at 37 °C.

tion of LBP association with HDL. This suggests that both LPS/LBP complexes and LBP interacted with the same apoA-I domains on HDL.

DISCUSSION

To address the functional contribution of specific apoA-I epitopes to the interaction of LBP and LPB/LPS complexes with HDL, we examined the effects of apoA-I monoclonal antibodies on this association in a purified in vitro system. Our data demonstrated that: *1*) LBP associated with purified HDL in the absence or presence of LPS (Figs. 1, 2); *2*) the association of LBP/LPS complexes with HDL was dependent on the LPS/LBP ratio (Fig. 3); and *3*) only some epitopes on apoA-I play a role in the association of LBP and LBP/LPS complexes with HDL (Figs. 4, 6, 7).

The critical effect of plasma HDL in reducing physiologic responses to LPS and clearing it from the circulation is well established (8, 18–22), yet the role of LBP in mediating the association of LPS with HDL is not. Our data confirm recent reports that LBP binds HDL (24, 26). Wurfel et al. (24) reported that reconstituted HDL (R-HDL) is not sufficient to neutralize the biological activity of LPS, but that LBP acts as a cofactor in the neutralization of LPS by R-HDL. Conversely, our data show that LPS can influence the association of LBP with HDL (Fig. 2). LPS increased the association of LBP with HDL within molar ratios of LPS to LBP between 0.125 to 6, but inhibited this interaction at ratios greater than 12. The loss of LBP binding at high concentrations of LPS could suggest that LPS completed with LBP for HDL, however, the observation that apoA-I antibodies did not distinguish between the association of LBP or LPB/LPS complexes (Fig. 7) implies that both LBP and LPS/LBP complexes can interact with the same or similar sites on HDL. A possible explanation for the observed increase in LBP association in the presence of LPS in our system is that LPS/LBP complexes can self-associate on HDL. Thus, when LPS is in large excess relative to LBP, LPS aggregates LBP and restricts its association with HDL. Whether such an interaction also occurs in vivo is difficult to predict. However, a similar phenomenon was observed for the binding of LPS and LBP on mCD14 (42).

The function of apoA-I in protecting against endotoxins has been observed both in vitro (14, 24, 25) and in vivo (21). Our studies provide evidence for a direct role for apoA-I in the association of LBP and LBP/LPS complexes with HDL. Furthermore, these results begin to define specific regions of apoA-I on HDL that contribute either directly or indirectly in the interaction between LBP and purified HDL. These regions are defined by the antibody epitopes (40, 44, 45), and these epitopes are displayed in **Fig. 8** on a linear model of apoA-I. Antibodies AI-1.2, AI-19.2, and AI-19.4 identify epitopes at the N terminus and are not part of any predicted alpha helical region. Antibodies AI-19.2 and AI-19.4 share overlapping epitopes. In contrast, epitopes for antibodies AI-14, AI-115.3, AI-119.3, AI-141.1, AI-178.1, and AI-187.1 are in regions of apoA-I that include both part of an amphipathic helix and a proline punctuated beta turn that interrupts the alpha helical segments. Among these antibodies, overlapping epitopes are shared between antibodies: AI-119.3, AI-115.3 and AI-137.1; AI-141.1, AI-137.1 and AI-119.3; and AI-178.1, AI-187.1 and AI-141.7. Antibody AI-141.7 identifies an epitope that is confined to helix 8. Because different antibodies with unique as well as overlapping epitopes inhibited the LBP/HDL interaction, at least three regions of apoA-I located between residues 1–31, 95–164, and 178–200 must participate either directly or indirectly in this association.

The effect of discrete apoA-I antibodies on the association of LBP with HDL can result from: *1*) direct interference with a specific LBP recognition site on apoA-I in HDL, *2*) bulk steric hinderance that would affect only a subpopulation of HDL, or *3*) a conformational change in apoA-I and/or HDL that indirectly restricts the LBP/HDL interaction. At this time no distinction can be made between these potential mechanisms.

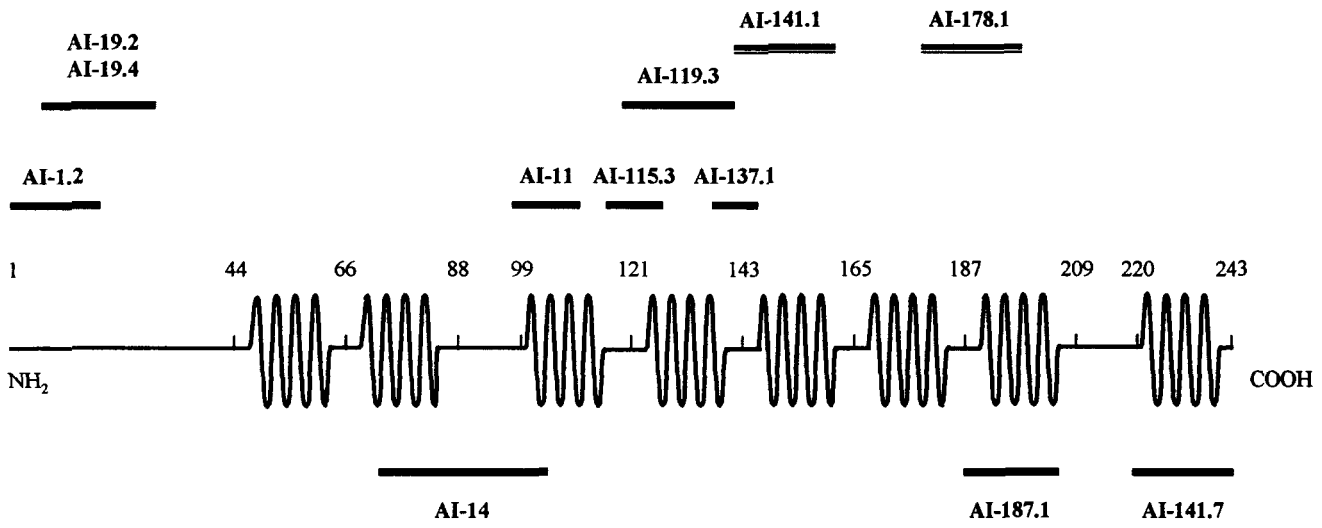

Fig. 8. Schematic representation of apoA-I antibody epitopes on a linear model of apoA-I. The predicted amphipathic alpha helices are represented by the wave structures. The epitope for each monoclonal antibody is represented by a bold line. Antibodies shown above the linear model interfered with the association of HDL and LBP, and those represented by the solid line were better inhibitors of LBP binding than those antibodies represented with a double line. Antibodies shown below the linear model were poor inhibitors of the interaction of LBP with HDL.

Studies using homogenous preparations of HDL subpopulations or structural apoA-I mutants should help elucidate the mechanism involved in mediating the inhibitory effect of specific apoA-I antibodies. Furthermore, it must be emphasized that the regions identified need not necessarily be directly involved in protein–protein interaction as would be suggested by a specific LBP site on apoA-I in HDL. The regions (epitopes) that we identified could be important for conferring a particular conformation of apoA-I on HDL that is required for interaction with LBP. Interestingly, monoclonal antibodies have been used to identify the region including residues 95–164 to be important for both LCAT activation and cellular cholesterol efflux. Antibodies to epitopes 95–121 (40) and 99–186 (50) impede the interaction between LCAT and apoA-I and epitopes spanning this central region also are involved in cellular cholesterol efflux (51, 52).

We did not observe complete inhibition of the association of LBP with HDL by any single antibody. Previous studies with these monoclonal antibodies led to the conclusion that there is intrinsic heterogeneity in the expression of intramolecular loci representing the epitopes of these antibodies. This inability of any antibody to inhibit 100% of LBP to HDL suggests that the interaction of LBP with apoA-I must be with a larger area of the apoA-I surface than the interaction of any single antibody. Studies are underway to determine which specific HDL subpopulations are involved in LBP and LBP/LPS interactions.

Neutralization of LPS by HDL is important in the detoxification of endotoxins. Our results have shown that in the absence of other serum factors, the plasma protein, LBP, interacted with apoA-I on HDL and that this association was enhanced in the presence of LPS. This extends the role of LBP in mediating physiologic responses to LPS to encompass its ability to interact with apoA-I on HDL. Furthermore, these studies have demonstrated that specific structural elements of apoA-I are key components of the interaction of LBP and LPS/LBP complexes with HDL.

We thank Kathi Richards, Yili Guo, Virginia Patch, David Bonnet, and Katrin Soldau for their expert technical assistance. This work was supported by NIH grants HL-43815 (LKC) and AI-32021 (PST). TM was supported by NIH training grant HL-

07195. This is manuscript #10069-IMM from The Scripps Research Institute.

Manuscript received 23 September 1996 and in revised form 23 December 1996.

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