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Control of Lipopolysaccharide–High-Density Lipoprotein Interactions by an Acute-Phase Reactant in Human Serum†

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We have recently described several phenomena involving the interactions of lipopolysaccharides (LPS) from *Salmonella minnesota* Re595 (Re595-LPS) with rabbit serum, which are different in and unique to acute-phase serum as compared with normal serum (P. S. Tobias and R. J. Ulevitch, J. Immunol. 131:1913–1916, 1983). To determine whether these phenomena could also be observed in acute-phase human serum (APHS), we used APHS obtained from volunteers injected with etiocholanolone. As observed in acute-phase rabbit serum, we found that (i) in APHS, Re595-LPS forms a protein complex with a density of 1.3 g/cm^3 which does not form in normal human serum (NHS), (ii) in APHS, the $t_{1/2}$ for LPS–high-density lipoprotein (HDL) complexation is at least a factor of 10 slower than the $t_{1/2}$ for LPS–HDL complexation in NHS, (iii) when Re595-LPS serum mixtures are dialyzed against a low salt buffer, Re595-LPS precipitates in less soluble form from APHS than from NHS, and (iv) the precipitate from Re595-LPS-APHS mixtures includes a protein with a molecular weight of approximately 60,000 which does not precipitate from Re595-LPS-NHS mixtures or from NHS or APHS alone. These indications of an altered status of LPS in NHS and APHS suggest that one or more acute-phase reactants interact with Re595-LPS to modify its rate of binding to HDL.

When lipopolysaccharides (LPS) of gram-negative bacteria enter the bloodstream and initiate endotoxemia, their first interactions are with the humoral and cellular elements of the blood. A series of investigations from this and other laboratories (2, 5, 8, 10) of the interactions of LPS in blood have shown that LPS partitions between the tissues and plasma lipoproteins, binding specifically to high-density lipoprotein (HDL). We have previously presented data which suggested that acute-phase rabbit serum (APRS) modifies the interactions of LPS with HDL by slowing the rate of binding of LPS to HDL (9). Because we wished to determine whether our observations with APRS are relevant to humans, we extended our studies to acute-phase human serum (APHS). In this report, we demonstrate modification of the interaction of LPS with HDL by one or more acute-phase reactants found in APHS.

MATERIALS AND METHODS

LPS from *Salmonella minnesota* Re595 (Re595-LPS), either biosynthetically tritiated or unlabeled, was isolated as described previously (3). Biosynthetically tritiated Re595-LPS was isolated from bacteria grown in the presence of [^{3}H]acetate. The specific activity of the ^{3}H-Re595-LPS was 10^4 dpm/μg. The ^{3}H-Re595-LPS had the same bouyant density in CsCl as unlabeled Re595-LPS and interacted with HDL in normal rabbit serum (NRS) as described for ^{125}I-LPS (10). Etiocholanolone was used to induce an acute-phase response (6) in three human volunteers given an intramuscular injection of 0.3 mg of etiocholanolone per kg of body weight. Serum was collected at various times from 24 h before etiocholanolone injection to 120 h after injection. Sera were stored frozen for up to 1 week before testing. Observation and quantitation of Re595-LPS–protein and Re595-LPS–HDL complexes were performed by CsCl isopycnic density-gradient ultracentrifugation of mixtures of 1 ml of serum containing 20 mM EDTA and 10 μg of ^{3}H-Re595-LPS as previously described (9). Preparation of a euglobulin precipitate from Re595-LPS serum reaction mixtures was accomplished by permitting the reaction to proceed for 2 min at 37°C, stopping the reaction by rapid chilling in an ice bath, dialyzing the mixture overnight at 4°C against 2.5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)–15 mM NaCl (pH 7.65), collecting the precipitate by centrifugation, washing the precipitate two times with 10 mM HEPES–140 mM NaCl (pH 7.4), and finally suspending the product in distilled water for ease of handling (9). Re595-LPS in the supernatant, washes, and euglobulin precipitate was quantitated by measurement of ^{3}H. Liquid scintillation counting was done by using Scint A cocktail (Packard Instrument Co., Inc., Rockville, Md.) with quenching monitored by the external standard method. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done with the recipes of Laemmli (4). Measurement of serum C-reactive protein (CRP) levels was done in the clinical laboratory of the Scripps Clinic and Research Foundation.

RESULTS AND DISCUSSION

Etiocholanolone is a naturally occurring steroid metabolite experimentally useful for inducing inflammatory reactions and fever in humans. These responses typically begin within 8 to 20 h after injection and last for 2 to 6 h (6, 11). Etiocholanolone also induces typical plasma acute-phase reactant responses, for example, CRP and serum amyloid A, within 24 to 48 h after injection (6). Samples of human serum collected at various times before and after etiocholanolone injection were examined for their ability to form an Re595-LPS–protein complex with a density of 1.3 g/cm^3 (C1.3) by allowing the LPS-serum mixture to react for 10 min before equilibrium density-gradient centrifugation with CsCl. Over-

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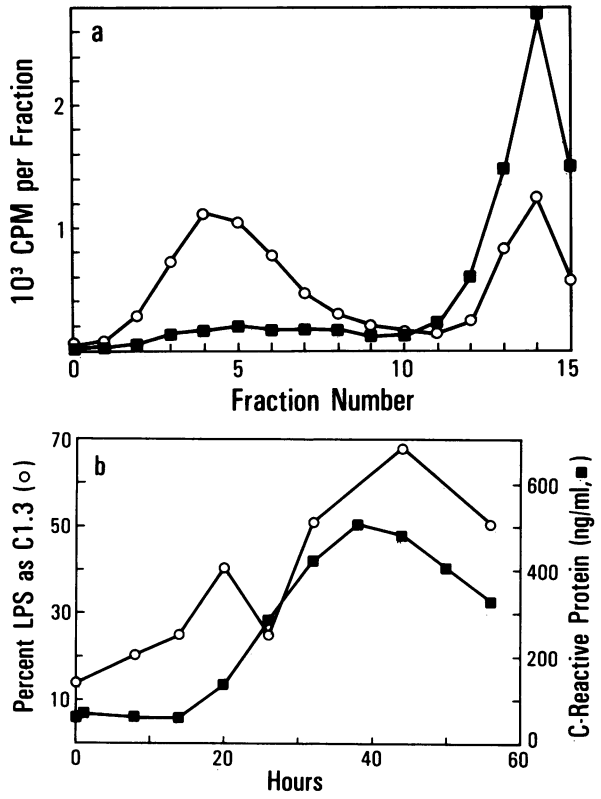

FIG. 1. Observation and quantitation of C1.3 in human serum collected before and after acute-phase induction. (a) Individual CsCl density gradients showing the inability and ability of normal (■) and acute (○) human sera to form C1.3, respectively. (b) The ability of human serum to form C1.3 (○) as a function of time after acute-phase induction with etiocholanolone and the CRP concentration (■) in human serum as a function of time after acute-phase induction with etiocholanolone.

all recovery of ^{3}H-Re595-LPS from these gradients was typically 65 to 75%. The 10-min reaction time was chosen from preliminary kinetic experiments as a reaction time which would allow most of the Re595-LPS to complex with HDL in normal serum but which would trap C1.3 before its LPS transferred to HDL. Results of this survey are shown in Fig. 1. Figure 1a shows a CsCl density gradient with sera collected before and 32 h after etiocholanolone injection; the appearance of a form of Re595-LPS in the bottom third of the gradient when APHS was used is evident. The density of this form of Re595-LPS was found to be 1.30 g/cm^3 by measurement of the refractive index. The amount of Re595-LPS at a density of 1.3 g/cm^3 as a function of time before or after etiocholanolone injection is shown in Fig. 1b. Figure 1b also shows the CRP concentrations of the same samples surveyed for C1.3. The ability of serum to form C1.3 follows a time course similar to the acute-phase CRP response which etiocholanolone induces (6).

We next investigated the kinetics of Re595-LPS–HDL complexation in sera taken either before (NHS) or 32 h after (APHS) etiocholanolone injection. To obtain these data, Re595-LPS–serum mixtures were sampled at various times after mixing and examined with CsCl gradients. The formation of Re595-LPS–HDL complexes is plotted as for a first-order reaction in Fig. 2. Complexation of Re595-LPS with HDL in NHS has a $t_{1/2}$ of about 7 min, whereas in APHS the reaction has a $t_{1/2}$ of about 52 min. This difference

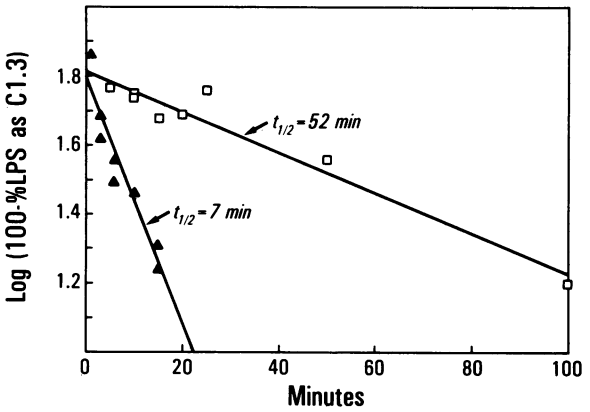

FIG. 2. Kinetics of Re595-LPS–HDL complex formation in NHS (▲) or APHS (□). NHS was collected just before etiocholanolone injection, and APHS was collected 32 h postinjection.

is virtually identical to that seen in rabbit serum in which the $t_{1/2}$s are 2 to 4 min and 40 to 80 min for NRS and APRS, respectively (9).

We also observed that the solubility properties of Re595-LPS were different in NHS and APHS. The euglobulin precipitate dialysis procedure resulted in Re595-LPS being distributed in the dialyzed supernatant, washes, and final precipitate as shown in Table 1. By using NHS or APHS taken from the same volunteer before and after etiocholanolone injection, some 52% of the input LPS was recovered. Of this recovered LPS, only 6.3% was recovered in the final precipitate when NHS was used, whereas 59% was recovered in the final precipitate when APHS was used. When the final euglobulin precipitates prepared from NHS and APHS in the absence and in the presence of Re595-LPS were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3), the precipitate from the LPS-APHS reaction mixture (Fig. 3, lane e) contained a unique protein with an apparent molecular weight of 64,500 that was not found in any of the other precipitates. Also shown in Fig. 3, lane a (containing rabbit LPS-APRS euglobulin precipitate) is gp60, the protein which precipitates from APRS only in the presence of C1.3. Although the human protein appeared to be slightly larger than rabbit gp60, this comparison may not be valid because the molecular weights of glycoproteins do not dependably correlate with their mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1). The other difference that we noted between the APRS and APHS was that the ability of APRS to form C1.3 was stable at 4°C for months, whereas the ability of APHS to form C1.3 was not stable, even when sera were stored at −20°C, for more than several weeks.

We conclude from these results, as we did previously for

TABLE 1. Percent recovery of ^{3}H-Re595-LPS from euglobulin precipitation[a]

LPS fraction	% ^{3}H-Re595-LPS recovered with:	
	NHS (SD)	APHS (SD)
Not precipitated	91.3 (4.7)	39.5 (6.5)
Recovered in washes	2.5 (0.25)	1.5 (0.1)
In precipitate	6.3 (4.4)	59.0 (7)

[a] Overall recovery of LPS from two experiments was 52%.

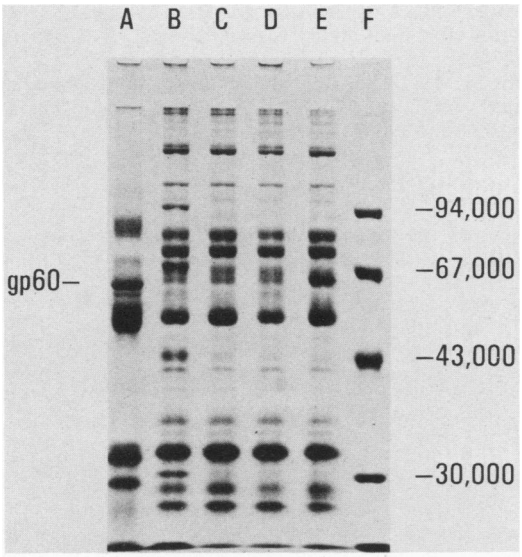

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel analysis. Each lane received approximately 3 μg of protein. Lanes: A, rabbit LPS-APRS euglobulin with gp60 marked; B, euglobulin from NHS; C, euglobulin from NHS with LPS; D, euglobulin from APHS; E, euglobulin from APHS with LPS; and F, molecular weight markers.

the rabbit, that component(s) of the acute-phase human response interact with Re595-LPS to reduce the rate of binding of LPS to HDL. The identity of the responsible acute-phase reactant is not currently known. In the rabbit system, gp60 is not an acute-phase reactant, although it associates with the euglobulin precipitate only in the presence of C1.3 (P. S. Tobias and R. J. Ulevitch, manuscript in preparation). Addition of rabbit CRP to NRS at levels characteristic of acute-phase serum does not reconstitute the observed phenomena. Additionally, reconstitution of ultracentrifugally delipoproteinated NRS with HDL from APRS does not reconstitute the observed phenomena. These observations argue that the prototypical acute-phase reactants CRP and serum amyloid A are not involved (P. S. Tobias and R. J. Ulevitch, in preparation). The remaining known acute-phase reactants do not undergo large concentration changes between the normal and acute states and are therefore, in our opinion, unlikely to cause the qualitative differences between normal and acute-phase serum we observed.

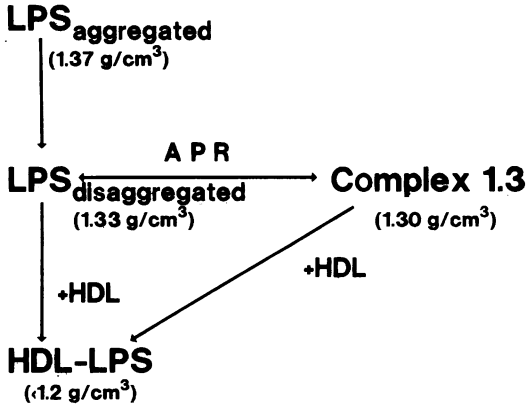

FIG. 4. Hypothetical progression of LPS from an aggregated to a disaggregated state and thence to an HDL complex with the formation of C1.3 if acute-phase reactants (APR) are present.

Our current mechanistic hypothesis to explain these results is summarized in Fig. 4. The progression of Re595-LPS from an aggregated to a disaggregated state and thence to an HDL complex is what has been observed in NRS (10). We simply elaborated on that progression by the addition of C1.3. There is no direct evidence to support our assumption of a disaggregated state of LPS as an intermediate between the LPS aggregates and C1.3, just as there is no evidence for or against direct transfer of LPS from C1.3 to HDL. These are simply our current hypotheses. We also assumed that C1.3 includes an acute-phase reactant and the human analog of rabbit gp60. Experiments are in progress to isolate the acute-phase reactant, to examine the endotoxic properties of C1.3, and to explore the mechanisms of LPS complexation to serum constituents.

Previous studies have shown that the mode of presentation of LPS, i.e., as a purified aggregated isolate or as an HDL complex, can significantly modify its endotoxic properties. Re595-LPS–HDL complexes have a significantly reduced capacity to produce fever, neutropenia, and complement activation compared with parent LPS, although they retain the ability to produce hypotensive shock and coagulopathy in experimental animals (5). We think it is likely that C1.3 will similarly display its own unique set of endotoxic properties. Thus, the acute-phase response may include a mechanism for dealing with potentially injurious LPS. It is noteworthy that tolerance to the toxic effects of endotoxin can be induced in rabbits and other experimental animals by repetitive administration of LPS (7) in a manner which would be expected to induce acute-phase reactants. Whether our results are relevant to understanding the phenomenon of tolerance to LPS remains to be determined.

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