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J Clin Invest. 1994;94(3):1304-1309. https://doi.org/10.1172/JCI117449.

Research Article

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Endotoxin and Cytokines Increase Hepatic Messenger RNA Levels and Serum Concentrations of Apolipoprotein J (Clusterin) in Syrian Hamsters

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Abstract

Infection and inflammation induce alterations in hepatic synthesis and plasma concentrations of the acute phase proteins. Our results show that apolipoprotein (apo) J is a positive acute phase protein. Endotoxin (LPS), tumor necrosis factor (TNF), and interleukin (IL)-1 increased hepatic mRNA and serum protein levels of apo J in Syrian hamsters. Hepatic apo J mRNA levels increased 10- to 15-fold with doses of LPS from 0.1 to 100 μg/100 g body weight within 4 h and were elevated for ≥ 24 h. Serum apo J concentrations were significantly increased by 16 h and further elevated to 3.3 times that of control, 24 h after LPS administration. Serum apo J was associated with high density lipoprotein and increased fivefold in this fraction, after LPS administration. Hepatic apo J mRNA levels increased 3.5- and 4.6-fold, with TNF and IL-1, respectively, and 8.2-fold with a combination of TNF and IL-1. Serum apo J concentrations were increased 2.3-fold by TNF, 79% by IL-1, and 2.9-fold with a combination of TNF and IL-1. These results demonstrate that apo J is a positive acute phase protein. (*J. Clin. Invest.* 1994. 94:1304–1309.) Key words: lipoproteins • HDL • acute phase reaction • lipopolysaccharides • immunologic factors

Introduction

Apolipoprotein (apo) J is a 70–80-kD glycoprotein consisting of two disulfide-linked subunits designated apo Jα (34–36 kD) and apo Jβ (36–39 kD) (1, 2). Apo J is also known as human serum protein-40,40, complement lysis inhibitor, clusterin, sulfated glycoprotein 2, testosterone-repressed protein message 2 (TRPM-2),[1] canine glycoprotein 80, and bovine glycoprotein III.

Apo J is found in normal human plasma (1) where it associates with a high density lipoprotein (HDL) particle (3) that may also contain apo A-I (3–5) and cholesterol ester transfer protein (5). It is expressed in many tissues, especially liver, brain, ovary, and testis (2, 6). The relative high abundance of apo J mRNA levels in the liver combined with the large size of the liver predict that the liver, rather than other organs is the major source of the circulating apo J (7).

Infection and inflammation induce changes in hepatic synthesis of the acute phase proteins (8). A common characteristic of many of these proteins is their involvement in defense mechanisms against tissue damage and infections. For example, fibrinogens participate in blood clotting and wound repair and complement C3 and C-reactive protein opsonize bacteria, parasites, and other foreign particles facilitating their clearance from the circulation by phagocytic cells. Furthermore, many acute phase proteins are proteinase inhibitors, such as α_2-macroglobulin and α_1-antitrypsin, that may serve to limit proteolysis to the site of infection or inflammation (8).

The host response to infection and inflammation is also accompanied by profound alterations in lipid metabolism, including increases in serum lipid and lipoprotein levels achieved by stimulation of hepatic lipogenesis and very low density lipoprotein (VLDL) production. These changes are thought to be part of the acute phase response (9) and to have potential beneficial effects (10), as lipoproteins have been shown to bind to endotoxin (LPS), thus protecting animals from the toxic effects of LPS (11). Lipoproteins also bind a variety of viruses blocking their cytopathic effects (12–16) and induce the lysis of the parasite, *Trypanosoma bruceii* (17, 18).

Among the physiological functions of apo J are several that may be associated with inflammation and the acute phase response. For example, apo J is increased in tissue injury (19, 20), can regulate complement function (21, 22), and may be involved in reverse lipid transport (3, 5, 23). Furthermore, tumor necrosis factor (TNF) increases apo J expression in L929 tumor cells (24) and infection with oncogenic retroviruses enhances apo J mRNA levels in quail embryo fibroblasts (25).

Regulation of hepatic apo J mRNA expression during the acute phase response has not been studied. The purpose of this study was to determine whether models for infection and inflammation induce changes in hepatic expression and serum concentrations of apo J.

Methods

Materials. [^{32}P]dCTP (3,000 Ci/mmol, 10 mCi/ml) was purchased from New England Nuclear (Boston, MA). LPS (*Escherichia coli* 55:B5) was purchased from Difco Laboratories (Detroit, MI) and was freshly diluted to desired concentrations in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Inc., Irvine, CA). Human TNF-α with a specific activity of 5×10^7 U/mg was kindly provided by Genentech, Inc. (South San Francisco, CA). Recombinant human interleukin (IL)-1β with a specific activity of 1×10^9 U/mg was generously provided

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Received for publication 4 October 1993 and in revised form 11 March 1994.

1. *Abbreviations used in this paper:* bw, body weight; LPS, endotoxin; SAA, serum amyloid A; TRPM-2, testosterone-repressed prostate message 2.

The Journal of Clinical Investigation, Inc.
Volume 94, September 1994, 1304–1309

by Immunex (Seattle, WA). Multiprime DNA-labeling system was purchased from Amersham International (Amersham, United Kingdom). Mini-spin columns were purchased from Worthington Biochemical Corporation (Freehold, NJ). Oligo (dt)-cellulose, type 77F, was purchased from Pharmacia LKB Biotechnology AB (Alameda, CA). Western Light chemiluminescent detection system was purchased from Tropix, Inc. (Bedford, MA). Nitrocellulose was purchased from Schleicher and Schuell (Keene, NH). Kodak XAR5 film was used for autoradiography. Rabbit anti-human apo J antisera was generated by injection into rabbits of synthetic peptides (ERKTUSNLEEAKKKKEDAL and KLRREL-DESLQAERLTRKY), which correspond to internal sequences of the Jα and Jβ chains, respectively. The DNA for apo J was purchased from the American Type Culture Collection (Rockville, MD): clone named HHCD19 (ATCC No. 37909).

Animals. Male Syrian hamsters (~ 100–120 g) were purchased from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a reverse light cycle room (3 a.m.–3 p.m. dark, 3 p.m.–3 a.m. light) and were provided with rodent chow and water ad lib. Animals were injected intraperitoneally with LPS, TNF, IL-1, or TNF + IL-1 at the indicated doses in 0.5 ml 0.9% saline or with saline alone. Subsequently, food was withdrawn from both control and treated animals because LPS and cytokines can induce anorexia. Animals were studied between 90 min and 24 h after LPS administration as indicated in Results.

Isolation of RNA and Northern blotting. Total RNA was isolated by a variation of the guanidinium thiocyanate method (26) as described previously (27). Northern blotting was performed as described previously (27). Blots were exposed to x-ray film and bands were quantified by densitometry. Duration of film exposure was varied to allow measurements on the linear portion of the curve.

Western blotting. For the determination of apo J protein, SDS-polyacrylamide gel electrophoresis was performed as described by Clarke et al. (28) with modifications described previously (27). Plasma, gel permeation chromatography fractions, and ultracentrifugation density gradient samples were analyzed in the reduced state using 10% polyacrylamide gel with a 4% stacking gel. Protein was transferred to nitrocellulose (0.2 mm pore size). Apo J protein was detected with rabbit antisera generated against the synthetic peptides described previously, using the Western Light chemiluminescent detection system (Tropix, Bedford, MA). Separate antisera against either the a or the b subunit of apo J reacted with bands running with apparent molecular mass of ~ 35 kD. After x-ray film development, band density was measured using an EC Apparatus Densitometer (EC Apparatus Corp., St. Petersburg, FL).

Gel permeation chromatography. Hamster plasma was separated by gel permeation chromatography using a 10 × 300 mm superose-12 column (Pharmacia). Plasma (100 μl) was eluted with 150 mM NaCl, 5 mM Tris, pH 7.4, 0.05% azide, and 0.04% EDTA at a flow rate of 0.4 ml/min and 80 200-μl fractions were collected. Cholesterol and triglyceride concentrations in the fractions were measured (diagnostic kits no. 351 and 337, respectively; Sigma Chemical Co., St. Louis, MO) and apo J was detected using the Western Light chemiluminescent detection system (Tropix).

Density gradient ultracentrifugation. Lipoproteins were separated by density gradient ultracentrifugation (29) modified as described previously (27). Fractions (0.5 ml) were collected, and cholesterol and triglyceride levels determined (diagnostic kits no. 351 and 337, respectively; Sigma Chemical Co.). Fractions with densities between 1.063 and 1.21 g/ml were combined as HDL.

Statistics. The results are expressed as means±SEM. Statistical significance was determined using a two-tailed Student's *t* test.

Results

The effect of the administration of low dose [0.1 μg/100 g body weight (bw)] or high dose (100 μg/100 g bw) LPS on hepatic apo J mRNA levels in Syrian hamsters is shown in Fig. 1. At the earliest time point, 90 min, the lower dose of LPS had no effect on apo J mRNA levels but the higher dose increased apo J mRNA levels by 58%. 4 h after LPS administration, the lower dose of LPS increased apo J mRNA levels 9.5-fold and the higher dose of LPS increased them to 16-fold over that of control (Northern blot analysis is shown in Fig. 1, *bottom*). Elevated hepatic apo J mRNA levels persisted for ≥ 24 h after LPS administration (Fig. 1).

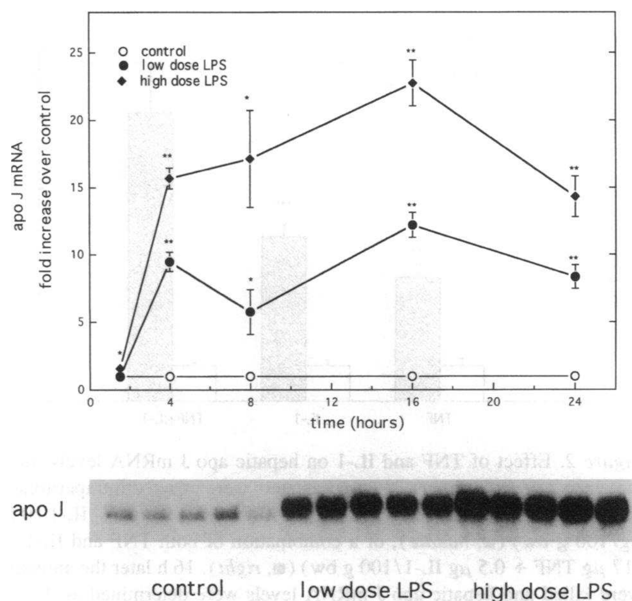

Figure 1. Top: Time course of the effect of LPS on hepatic apo J mRNA levels. Syrian hamsters were injected intraperitoneally with either saline (○), 0.1 μg/100 g bw LPS (●) or 100 μg/100 g bw LPS (◆). At the times indicated, the animals were killed and hepatic apo J mRNA levels were determined as described in Methods. Data are presented as means ±SEM ($n = 5$). *$P < 0.05$. **$P < 0.001$ vs. control. *Bottom:* Northern blot analysis of apo J mRNA from hamster liver 4 h after LPS administration.

A number of studies have shown that many of the immune and metabolic effects of LPS are mediated by TNF and IL-1. We therefore injected the hamsters with these cytokines to determine their effect on hepatic apo J mRNA levels. TNF (17 μg/100 g bw) and IL-1 (0.5 μg/100 g bw) increased hepatic apo J mRNA levels 3.5- and 4.7-fold, respectively (Fig. 2). Similar doses of TNF and IL-1 have been shown to induce fever (30) and changes in lipid metabolism in rodents (31). When TNF and IL-1 were administered simultaneously their effects were additive, increasing hepatic apo J mRNA levels by 8.2-fold over that of control (Fig. 2).

We next measured the levels of apo J circulating in the serum, using Western blotting. After gel electrophoresis of plasma, separate antisera against the a and the b subunits of apo J reacted with bands running with apparent molecular mass of ~ 35 kD (reduced). At 4 and 8 h after LPS administration, there was a decrease in serum apo J levels compared with that of controls (Fig. 3). However, 16 h after LPS administration serum apo J levels were significantly increased by 73% and 49%, with the lower dose and the higher of dose LPS, respectively. 24 h after LPS administration serum apo J levels were further increased to 3.3-fold over that of control with either dose of LPS (Fig. 3).

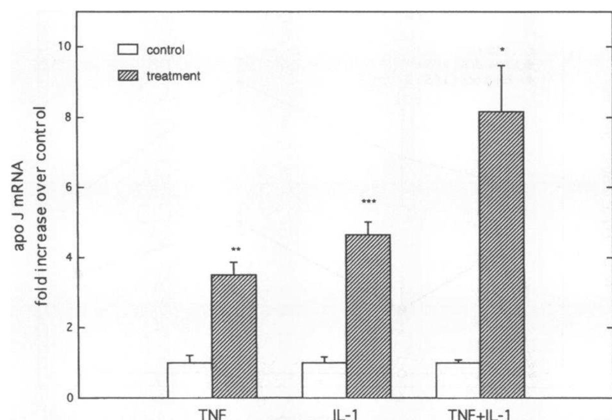

Figure 2. Effect of TNF and IL-1 on hepatic apo J mRNA levels. In three separate experiments, Syrian hamsters were injected intraperitoneally with either saline (□), TNF (17 μg/100 g bw) (■, *left*), IL-1 (0.5 μg/100 g bw) (■, *middle*), or a combination of both TNF and IL-1 (17 μg TNF + 0.5 μg IL-1/100 g bw) (■, *right*). 16 h later the animals were killed and hepatic apo J mRNA levels were determined as described in Methods. Data are presented as means±SEM (n = 4 or 5). $*P < 0.01$. $**P < 0.005$. $***P < 0.001$ vs. control.

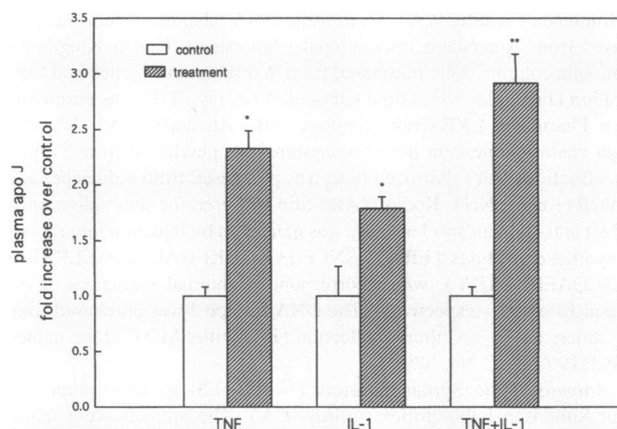

Figure 4. Effect of TNF and IL-1 on serum apo J levels. Syrian hamsters were injected intraperitoneally with either saline (□), TNF (17 μg/100 g bw) (■, *left*), IL-1 (0.5 μg/100 g bw) (■, *middle*), or a combination of both TNF and IL-1 (17 μg TNF + 0.5 μg IL-1/100 g bw) (■, *right*). 16 h later, the animals were killed and serum apo J levels determined as described in Methods. Data are presented as means±SEM (n = 5). $*P < 0.002$. $**P < 0.005$ vs. control.

TNF or IL-1 administration also increased serum apo J levels. 16 h after administration, serum apo J levels were increased by 2.3-fold with TNF and by 79% with IL-1 (Fig. 4). A combination of TNF and IL-1 increased serum apo J to 2.9-fold over that of control (Fig. 4).

To determine whether tissues other than the liver could contribute to the increase in serum apo J levels after endotoxin or cytokine administration, we next determined apo J mRNA levels in selected extrahepatic tissues. We chose to determine apo J in those tissues that others have shown apo J to be present in, i.e., testis, brain, stomach, kidney, heart, and spleen. In addition we, determined apo J mRNA levels in the intestine, because the intestine has been shown to express other apolipoproteins. High dose LPS decreased apo J mRNA levels in brain and testis by 36%, had no effect on mRNA levels in the intestine, and increased apo J mRNA levels in spleen, heart, kidney, and stomach (Table I). In contrast to the effect of LPS, TNF administration did not affect apo J mRNA levels in either stomach or kidney and only increased apo J mRNA levels in spleen and heart by twofold (Table I).

Apo J has been shown to be primarily associated with HDL in the basal state (5). We next determined whether LPS administration increased apo J in the HDL fraction and whether other lipoprotein fractions acquired apo J after LPS administration. Apo J has been reported to disassociate from HDL during ultracentrifugation (5) and in the present study apo J could not be detected in the HDL fraction after density gradient ultracentrifugation of hamster plasma (data not shown). Fractionation of plasma by gel permeation chromatography was therefore employed to separate HDL from VLDL and low density lipoprotein (LDL). The triglyceride and cholesterol concentrations of the

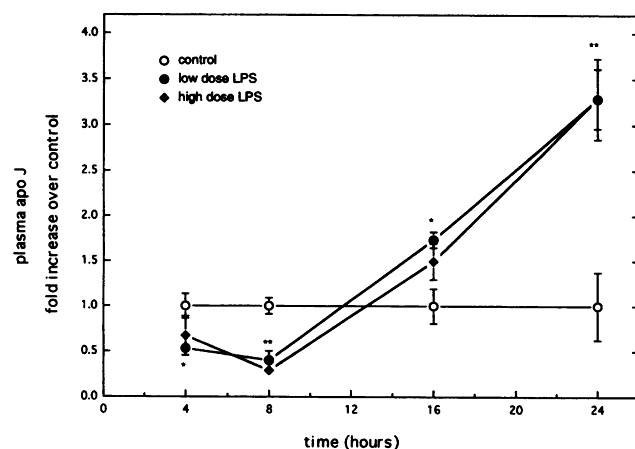

Figure 3. Time course of the effect of LPS on serum apo J levels. Syrian hamsters were injected intraperitoneally with either saline (○), 0.1 μg/100 g bw LPS (●) or 100 μg/100 g bw LPS (♦). At the times indicated, the animals were killed and serum apo J levels determined as described in Methods. Data are presented as means±SEM (n = 5). $*P < 0.05$. $**P < 0.02$ vs. control.

Table I. The effect of LPS and TNF on apo J mRNA Levels in Selected Extrahepatic Tissues

Tissue	LPS (100 μg/100 g bw)	TNF (17 μg/100 g bw)
	Fold increase over control	
Brain	0.64 ± 0.08	ND*
Testis	0.64 ± 0.09	ND
Intestine	1.13 ± 0.16	ND
Spleen	1.97 ± 0.24‡	1.97 ± 0.09§
Heart	7.81 ± 1.21‡	1.98 ± 0.23‡
Kidney	8.72 ± 0.95§	1.47 ± 0.11
Stomach	>42.91 ± 4.56§	1.24 ± 0.42

Syrian hamsters were injected intraperitoneally with either saline (control), LPS, or TNF. 16 h later the animals were killed, the respective organs were removed, and apo J mRNA levels were determined as described in Methods. Data are presented as means ± SEM (n = 4–5). *ND, not determined. ‡$P < 0.05$. §$P < 0.001$.

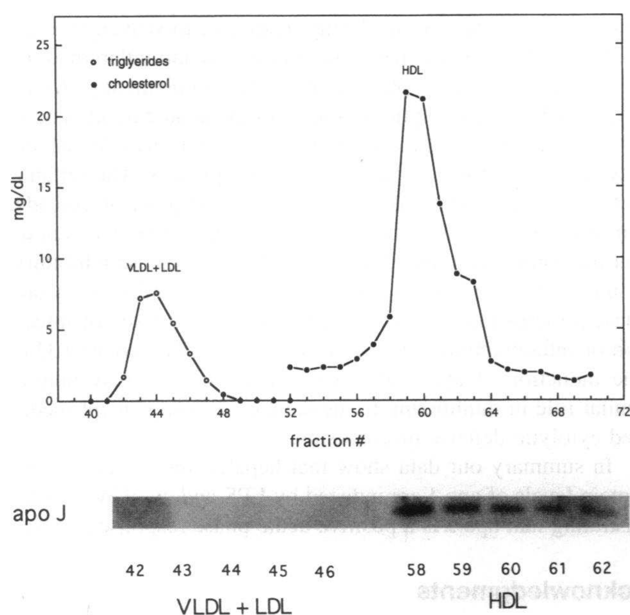

Figure 5. Top: Triglyceride and cholesterol concentrations in plasma fractionated by gel permeation chromatography. Plasma from male Syrian hamsters was fractionated by gel permeation chromatography, and triglyceride and cholesterol concentrations were determined as described in Methods. *Bottom:* Apo J in VLDL + LDL and HDL size fractions from gel permeation chromatography. The presence of apo J was noted in HDL as determined by Western blotting as described in Methods.

fractions were measured to determine the location of the VLDL + LDL and the HDL peaks (Fig. 5, *top*). In control plasma apo J was detected in the HDL fraction but not in the VLDL + LDL fraction (Fig. 5, *bottom*) nor in the small molecular weight fractions that follow HDL (data not shown). After LPS administration (16 h) apo J in the HDL fraction increased fivefold over that of control (Fig. 6). As in controls, apo J was not detected in other fractions after LPS administration (data not shown).

Discussion

Infection and inflammation induce dramatic changes in the concentrations of many plasma proteins, designated the acute phase proteins. These changes are involved in the defense mechanism against tissue damage and infection and are believed to have potential beneficial effects (32). The changes in hepatic synthesis of the acute phase proteins, after inflammatory stimuli, are thought to be mediated by cytokines, particularly TNF, and the interleukins (32, 33). Administration of LPS has been used to mimic infection, and many of the effects of LPS have been shown to be mediated by cytokines.

The present study indicates that apo J is an acute phase protein positively regulated during infection and inflammation. LPS administration increased both hepatic mRNA levels and serum protein levels of apo J in the Syrian hamster. The mRNA levels were increased by 10- to 15-fold 4 h after LPS administration and elevated apo J mRNA levels persisted for $\geq$ 24 h. Serum apo J concentrations were transiently decreased 4 and 8 h after LPS administration. This decrease may be due to increased uptake and degradation of apo J or to protein leakage from the circulation after LPS administration. Serum apo J concentrations then increased and were elevated by 3.3-fold over that of control 24 h after LPS administration. The doses of LPS used in this study are small compared with doses used in previous studies. The lower dose of LPS used in this study (0.1 μg/100 g bw) is far below the doses required to cause death in rodents in our laboratory ($LD_{50} \sim 5$ mg/100 g bw) but produces close to a maximal increase in serum triglyceride levels (34). The induction of apo J is therefore among the most sensitive host responses to LPS. The cytokines, TNF and IL-1, which mediate many of the effects of LPS and the changes in hepatic acute phase protein synthesis, also increased hepatic apo J mRNA levels and serum apo J concentrations, supporting the concept of apo J as an acute phase response protein.

Although previous studies have convincingly shown that the liver is the dominant source for acute phase plasma proteins in the vascular compartment (35), we can not totally rule out that tissues other than the liver could contribute to serum apo J levels. In fact, LPS administration in addition to increasing apo J mRNA levels in the liver, markedly increased apo J mRNA levels in kidney, heart, and stomach and caused a twofold increase in the spleen. However, TNF administration, which increased serum apo J levels to a comparable degree as LPS, did not affect apo J mRNA levels in either stomach or kidney and only increased apo J mRNA levels modestly in spleen and heart (less than twofold). Considering that the liver is a much larger organ than either the spleen or heart; that apo J is much more abundant in the liver than in spleen or heart (2); and that TNF increases apo J mRNA levels in the liver by 3.5-fold, compared to only 2-fold in either heart or spleen, it is likely that the increase in serum apo J levels after TNF administration is primarily due to the increase in apo J in the liver rather than other tissues. The role of the increased apo J production in extrahepatic tissues remains to be elucidated.

The increase in apo J expression and protein levels during the acute phase response is specific since other apoproteins do not change in the same manner. Regulation of these apoproteins, as has been observed with other hepatic proteins, is species specific. Hepatic apo E mRNA levels *decrease* after LPS and TNF administration to Syrian hamsters (27, 36). In rats LPS does not affect hepatic apo E mRNA levels (37, 38). Apo E mRNA in freshly isolated rat Kupffer cells and macrophage secretion of apo E decrease after LPS administration (37, 39). Hepatic apo A-I mRNA levels are not affected by LPS, TNF, or IL-1 in the Syrian hamster (27, 36). In mice apo A-I mRNA

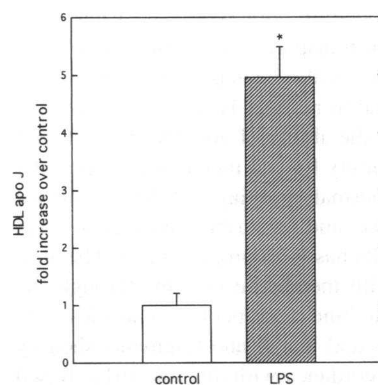

Figure 6. Effect of LPS on apo J concentrations in HDL. Syrian hamsters were injected intraperitoneally with either saline (□) or 100 μg/100 g bw LPS (■). 16 h later the animals were killed and plasma was fractionated by gel permeation chromatography. The fractions corresponding to HDL (fractions 58–64) were pooled and serum apo J levels determined as described in Methods. Data are presented as means±SEM (n = 5). *$P < 0.005$ vs. control.

levels in liver decrease after LPS administration (40). Apo CII serum concentrations decrease in mice after LPS administration (41). In rats apo B mRNA levels in liver extracts are not affected by TNF (38). Thus in contrast to apo J, apo A-I, B, CII, and E either decrease or are not affected by LPS or cytokines. This suggests that the increase in apo J during the acute phase response is a specific response and is not seen with most other apolipoproteins.

Examination of the sequences upstream of the 5′ end of the coding sequences for homologues of apo J reveals several sequence elements that have been linked to regulation of the acute phase protein genes. The upstream control region of the rat TRPM-2 gene contains the acute phase response element CTGGGA (42, 43). Acute phase response factor and IL-6 regulatory element binding protein bind to the acute phase response element and their binding activity is stimulated by both IL-6 and leukemia-inhibitory factor (43). An AP-1 binding motif is present on two rat genomic clones of clusterin (44), on the rat TRPM-2 gene (42), and on the human apo J gene (45). The AP-1 binding element is thought to confer responsiveness to TNF-α, IL-1 and IL-6 (46, 47), and the acute phase response induced by Freund's adjuvant increased binding activity by AP-1 (47). The TRPM-2 gene furthermore contains clustered motifs for CCAAT-binding transcription factor and CCAAT recognition factor (42). Apo J expression may thus be controlled by several of the regulatory elements that have been shown to control many of the acute phase proteins, further supporting it being an acute phase response protein.

In contrast to these conventional apolipoproteins, serum amyloid A (SAA), which behaves as an apolipoprotein (48), is also positively regulated during the acute phase response. Hepatic SAA mRNA levels in mice increase after LPS administration (49), and both hepatic mRNA levels and serum concentrations of SAA are elevated in hamsters after TNF and IL-1 administration (50, 51). SAA is associated with HDL (52), and becomes the major HDL apoprotein after LPS administration to mice (53) and injection of croton oil to rabbits (54). Apo J, like SAA, is associated with HDL in plasma (3, 5) and in the present study apo J was detected in HDL in the basal state and increased fivefold in the HDL fraction, after administration of LPS. Another acute phase protein, C-reactive protein, interacts with triglyceride-rich and apo B–containing particles (55, 56) but apo J was not detected in the VLDL + LDL fraction, in the present study, in either the basal state or after LPS administration.

Many physiological roles and functions have been proposed for apo J. Based on its association with HDL, apo J may play a role in reverse cholesterol transport (5). A role for SAA in reverse cholesterol transport has also been proposed, suggesting that it may represent a signal to redirect HDL to sites of tissue destruction (57). Based on the ability of apo J to interact with cell surfaces (58) and its affinity for cell membranes, especially those that are damaged, abnormal, or dying (59, 60), a role for apo J in mobilization, uptake, and redistribution of lipids from damaged or lipid-loaded cells has been proposed (3). The ability of apo J to associate with membrane proteins through hydrophobic interactions and to bind hydrophobic molecules, e.g., potentially toxic fatty acids and membrane fragments shed by degenerative cells, are in accordance with this role (61). Based on this, induction of apo J, during the acute phase response, may be beneficial and provide a mechanism to rid the host of potentially harmful oxidized fatty acids and to scavenge toxic lipid byproducts synthesized during infection and inflammation.

Apo J, furthermore, plays a role in the complement pathway as an inhibitor of the lytic terminal complement cascade (62). LPS administration activates the complement cascade, which plays a key role in the local host defense process. The inflammatory and destructive properties of the complement cascade can lead to systemic complications, which makes it essential that the complement attack is confined. The protease inhibitors α_2-macroglobulin and α_1-antitrypsin are examples of acute phase proteins that, by limiting proteolysis to the site of infection or inflammation, serve to decrease systemic toxicity (32). The induction of apo J after LPS administration may play a similar role in minimizing tissue damage in complement-mediated cytolytic defense mechanisms.

In summary our data show that hepatic mRNA and serum protein levels of apo J are induced by LPS and cytokines, demonstrating that apo J is a positive acute phase response protein.

Acknowledgments

This work was supported by a grant from the Research Service of the Department of Veterans Affairs and National Institutes of Health grant DK-40990.

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