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Biochimica et Biophysica Acta 1344 (1997) 210–220

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

LPS and cytokines regulate extra hepatic mRNA levels of apolipoproteins during the acute phase response in Syrian hamsters

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Received 12 June 1996; accepted 12 September 1996

Abstract

Altered hepatic expression of apolipoproteins occurs during the acute phase response. Here we examined whether the acute phase response alters extra hepatic expression of apolipoproteins. Syrian hamsters were injected with endotoxin (LPS), tumor necrosis factor (TNF), interleukin (IL)-1, or the combination of TNF + IL-1 and mRNAs for serum amyloid A (apoSAA), apolipoprotein (apo) J, apo E, apo A-I, and apo D, were analyzed. LPS increased mRNA levels for apoSAA in all tissues examined. LPS and TNF + IL-1 increased mRNA levels for apo J in kidney, heart, stomach, intestine, and muscle. Individually, TNF and IL-1 were less potent than the combination of the two cytokines. LPS decreased mRNA levels for apo E in all tissues, except for mid and distal intestine. TNF and IL-1 were less effective than LPS. LPS, TNF + IL-1 and TNF decreased mRNA levels for apo A-I in duodenum. mRNA for apo D decreased in heart, were unchanged in brain and increased in muscle, following LPS. The widespread extra hepatic regulation of the apolipoproteins during the acute phase response may be important for the alterations in lipid metabolism that occur during infection and inflammation as well as the immune response.

Keywords: Apolipoprotein; Acute phase response; Endotoxin; Cytokine; (Syrian hamster)

1. Introduction

Infection and inflammation induce dramatic changes in serum levels of certain proteins, the acute phase proteins, which are believed to play an important protective role in host defense [1]. Some acute phase proteins are increased, the positive acute phase proteins, whereas others are decreased, the negative acute phase proteins. Alterations in liver synthesis of these proteins are believed to be responsible for the changes in their serum levels. Although the liver is thought to be the source for most of the acute phase proteins in the vascular compartment, it has recently been recognized that several of these proteins are synthesized in extra hepatic tissues [2]. That acute

Abbreviations: Apo, apolipoprotein; apoSAA, serum amyloid A; CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; IL, interleukin; LCAT, lecithin:cholesterol acyltransferase; LPS, endotoxin; S.E.M., standard error of the mean; TNF, tumor necrosis factor; VLDL, very low density lipoprotein.

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PII S0005-2760(96)00143-9

phase proteins are produced in extra hepatic tissues suggests a broader role for these proteins in the host response to infection and injury than previously appreciated.

Some acute phase proteins are regulated in a similar manner in both hepatic and extra hepatic tissues. For example, serum amyloid A (apoSAA) and lipopolysaccharide binding protein, are increased in hepatic and extra hepatic tissues after administration of endotoxin (LPS) or casein [3–6]. In contrast, the synthesis of other acute phase proteins, such as α_1-acid glycoprotein, transferrin, and vitronectin, is only induced in the liver but not in most extra hepatic tissues [5,7]. Conversely, other acute phase proteins, like complement C2, are only induced in extra hepatic tissues (kidney and lung) but not in liver [8].

The host response to infection and inflammation is accompanied by profound alterations in lipid metabolism (reviewed in [9]). These alterations are thought to be part of the acute phase response [10] and to have potential beneficial effects [11]. In particular, the increase in serum lipid and lipoprotein levels may be beneficial as lipoproteins bind LPS and protect animals from its toxic effects [12–15]. Stimulation of hepatic lipogenesis and very low density lipoprotein (VLDL) production contribute to the increase in serum lipid and lipoprotein levels. In addition, changes in extra hepatic tissues such as decreased lipoprotein lipase activity in muscle and increased lipolysis and decreased lipoprotein lipase activity in adipose tissue participate in altering lipid metabolism during infection and inflammation [9,10].

Infection and inflammation also lead to alterations in apolipoproteins (apo) that may contribute to the changes in lipid metabolism. ApoSAA, which associates with high density lipoprotein (HDL), is a positive acute phase protein [3,16,17]. In addition, we have recently demonstrated that apo J is a positive acute phase protein, as both hepatic mRNA levels and serum concentrations of apo J increase following administration of LPS or cytokines [18]. In contrast, hepatic mRNA levels for apo E and apo A-I are either decreased or not changed dunng infection or inflammation [19–22].

Previous studies have focused on the effects of infection and inflammation on apolipoprotein expression in the liver and little information is available on their regulation in extra hepatic tissues. ApoSAA is induced in extra hepatic tissues during infection and inflammation [4,23,24] and preliminary data indicate that apo J is regulated in extra hepatic tissues by LPS and tumor necrosis factor (TNF) [18]. Acute phase regulation of other apolipoproteins synthesized in extra hepatic tissues has not been reported. In the present study we have determined the effects of LPS and cytokines on mRNA levels for apoSAA, apo J, apo E, apo A-I, and apo D during the acute phase response in hamsters. We used Syrian hamsters to investigate the effects of LPS and cytokines on lipid and lipoprotein metabolism because, in contrast to other rodents, cholesterol and lipoprotein metabolism in Syrian hamsters resembles that in humans. For example, the effects of dietary cholesterol and fatty acids on serum cholesterol levels and LDL metabolism are similar in Syrian hamsters and humans [25,26]. Additionally, as found in humans, hepatic VLDL from Syrian hamsters contains no apo B-48 and hamsters have cholesteryl ester transfer protein activity in plasma [27,28]. Furthermore, previous studies have shown that LPS and cytokines regulate triglyceride and cholesterol levels in Syrian hamsters [18–20,29].

2. Materials and methods

2.1. Materials

[^{32}P]dCTP (3000 Ci/mmol, 10 mCi/ml) was purchased from New England Nuclear (Boston, MA). LPS (*E. coli* 55:B5) was purchased from Difco Laboratories (Detroit, Ml) and was freshly diluted to desired concentrations in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Irvine, CA). Human TNF-α with a specific activity of 5×10^7 U/mg was kindly provided by Genentech (South San Francisco, CA). Recombinant human interleukin (IL)-1β with a specific activity of 1×10^9 U/mg was generously provided by Immunex (Seattle, WA). MuItiprime DNA labeling system was purchased from Amersham International (Arlington Heights, IL). Mini spin columns were purchased from Worthington Biochemical (Freehold, NJ). Oligo (dt)-cellulose, type 77F was purchased from Pharmacia LKB (Uppsala, Sweden). Nitrocellulose was purchased from Schleicher and Schuell (Keene, NH). Kodak XAR5 film

was used for autoradiography. The cDNA for Apo J was purchased from the American Type Culture Collection (Rockville, MD): clone name HHCD19, ATCC No. 37909 [30]. Murine apolipoprotein E and A-I cDNA were kindly provided by Dr. John M. Taylor of the Gladstone Foundation, San Francisco [31]. The human SAA cDNA used (pA1a) corresponds to residues −10 to 55 of preSAA1 and cross-reacts with SAA_2 and SAA_3 [32,33]. pA1a also cross-hybridizes with hamster SAA mRNA [34]. The human apolipoprotein D cDNA [35] was used to probe for apo D.

2.2. Animals

Male Syrian hamsters (approx. 100–150 g) were purchased from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a room with lights on from 6 a.m. to 6 p.m. and were provided with rodent chow and water ad Iibitum. Animals were injected intraperitoneally (i.p.) with LPS, TNF + IL-1, TNF, or 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. The dose of LPS used (100 μg/100 g body weight (bw)) is far below the doses required to cause death in rodents in our laboratory ($LD_{50} \sim 5$ mg/100 g bw) but increases serum triglyceride and cholesterol levels and produces a maximal decrease in LCAT activity in Syrian hamsters [19,29]. The doses of TNF and IL-1 used (17 μg/100 g bw and 1 μg/100 g bw, respectively) increase serum triglycerides (TNF by 18% and IL-1 by 64% (unpublished observations)), increase serum cholesterol levels and decrease HDL cholesterol levels in Syrian hamsters [20]. In addition TNF and IL-1 at these doses reproduce many of the effects of LPS on lipid metabolism in Syrian hamsters [18,20]. The LPS content of these cytokine preparations is significantly lower than the amount of LPS that regulates lipid metabolism in vivo. Animals were studied 16 h after LPS or cytokine administration. The tissues were removed and stomach and intestine washed with saline, before freezing in liquid nitrogen. Three 5 cm segments of the intestine were isolated from the part just distal to the stomach, the middle part, and the part just proximal to the large intestines, corresponding to the duodenum, the jejunum, and the ileum, respectively.

2.3. Isolation of RNA and Northern blotting

Total RNA was isolated by a variation of the Guanidinium thiocyanate method [36]. Poly A RNA was isolated using oligo dT cellulose and quantified by measuring absorption at 260 nm. Equal amounts of poly A RNA (10 μg) were loaded on 1% agarose-formaldehyde gels and electrophoresed. The uniformity of sample applications was checked by ultraviolet visualization of the ethidium bromide stained gel before transfer to nitrocellulose membranes. Two different sets of Northern blots were prepared for most of the tissues. We and others have found that LPS increases mRNA levels of actin in liver [3,19] and adipose tissue (unpublished observations by this laboratory). LPS also increases hepatic mRNA levels for cyclophilin (unpublished observations by this laboratory). The mRNA levels of actin and cyclophilin, which are widely used for normalizing data, can therefore not be used in studies of LPS induction of the acute phase response. However, the direction of the change in mRNA levels following LPS or cytokine administration (increased for some apolipoproteins, decreased for others); the magnitude of the alterations (up to 35 fold increase and 80% decrease); and the relatively small standard error of the mean make it unlikely that the changes observed are due to unequal loading of mRNA. RNA probe hybridization was performed in 0.75 M sodium chloride, 0.075 M sodium citrate, 2% SDS, 10% dextran sulfate, 2 × Denhardt's solution and 100 μg/ml sheared salmon sperm DNA at 65°C overnight. Blots were either washed at 65°C in 0.03 M sodium chloride, 0.003 M sodium citrate, and 0.1% SDS or at 42°C in 0.3 M sodium chloride, 0.03 M sodium citrate, and 0.1% SDS. Blots were exposed to X-ray film and bands were quantified by densitometry. Duration of film exposure was varied to allow measurements using the linear portion of the curve — i.e., the exposure chosen was such that the absorbance of both the control and cytokine bands showed intensities that were linearly proportional to exposure time. The fold increase in the intensity of the bands between the control and the treated groups was then determined by densitometry. Because of the

huge increase in mRNA for apo J in the stomach, it was difficult to obtain an exposure of the Northern blot in which the bands for the control and the treated group were within the linear portion of the curve. We therefore can only determine the approximate increase in mRNA levels for apo J in the stomach.

2.4. Statistics

The results are expressed as means ± standard error of the mean (S.E.M.). Statistical significance was determined using a two-tailed Student's *t*-test.

3. Results

3.1. The effects of LPS and cytokines on mRNA levels for apoSAA in extra hepatic tissues

ApoSAA is a positive acute phase response protein that associates with HDL. LPS and inflammation increase mRNA levels for apoSAA in liver and extra hepatic tissues [3,4,23,24,37]. These previous studies were carried out in a variety of species and it should be noted that there are considerable species differences. Most species possess two main acute phase isoforms of hepatic origin apoSAA$_1$ and apoSAA$_2$, and a third form apoSAA$_3$ that is predominantly expressed in extra hepatic tissues [38]. In the present study we examined the effects of LPS on mRNA levels for apoSAA (including SAA$_1$, SAA$_2$ and SAA$_3$) in extra hepatic tissues in hamsters. Since induction of SAA during the acute phase response has been widely reported, although not with LPS in Syrian hamsters, these studies were performed to assure that our hamsters have the expected acute phase response and to allow comparison of expression of other apolipoproteins with apoSAA. As shown in Fig. 1, LPS administration induced mRNA levels for apoSAA in all tissues examined. As in other studies [3,4,23,24,37], mRNA for apoSAA was not detected in most tissues from control animals. However, the intestine was an exception. In the intestine low levels of mRNA for apoSAA were detected in the mid and distal part (jejunum and ileum) of control animals allowing quantification of the fold increase in mRNA levels in these parts of the intestine. LPS

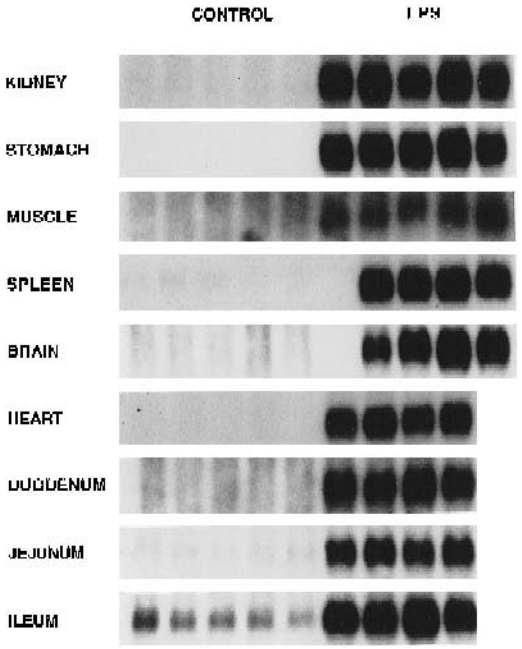

Fig. 1. The effects of LPS on mRNA levels for apoSAA in tissues. Syrian hamsters were injected intraperitoneally with either saline (control) or LPS (100 μg/100 g bw). Sixteen hours later the animals were killed, the respective organs were removed and mRNA for apoSAA was detected as described in Section 2. For kidney, stomach, and muscle $n = 5$; for the other tissues $n = 5$ for controls and $n = 4$ for LPS treated animals. Lane six is empty in spleen and brain.

increased mRNA levels for apoSAA in the jejunum by approx. 10-fold and in the ileum by 6- to 7-fold.

TNF and IL-1 mediate many of the immune and metabolic effects of LPS and they have been shown to increase hepatic mRNA levels and serum concentrations of apoSAA in hamsters [16,17]. In the present study, administration of these cytokines either in combination or by themselves induced apoSAA mRNA levels in most of the tissues examined (data not shown). The brain was the only tissue in which the combination of TNF + IL-1 was not effective in inducing mRNA levels for apoSAA. Similarly, as will be shown later, TNF and IL-1 did not affect mRNA levels for apo E in the brain, even though LPS did. The reason for the lack of effect of the cytokines on the brain may be an inability or decreased ability of these cytokines to cross the blood/brain barrier. The fold increase in mRNA

levels for SAA could only be determined in the jejunum and the ileum. The combination of TNF + IL-1 increased mRNA levels for apoSAA by 4- to 5-fold in the jejunum and by 2-fold in the ileum, but by themselves TNF and IL-1 did not increase mRNA levels for apoSAA in jejunum or ileum (data not shown).

Our results on the effects of LPS and cytokines on extra hepatic mRNA levels for SAA in hamsters are in accordance with results previously published on the effects of LPS and cytokines in other species and of cytokines in hamsters [4,23,24]. These results validate our model for studying the effects of endotoxin and cytokines on extra hepatic RNA levels for other apolipoproteins.

3.2. The effects of LPS and cytokines on mRNA levels for apo J in extra hepatic tissues

We have recently demonstrated that apo J is an acute phase protein [18]. Additionally, we observed that LPS increased mRNA levels for apo J in selected extra hepatic tissues [18]. We now confirm the results from our previous studies and, in addition, demonstrate that mRNA levels for apo J are increased in other extra hepatic tissues following LPS administration (Fig. 2). As seen previously [18], LPS significantly increased mRNA levels for apo J in kidney, heart, and stomach, with the increase in the stomach being most dramatic (approx. 35-fold). Similar to our previous findings, LPS did not affect mRNA levels for apo J in either spleen or brain. We previously reported a small increase in mRNA levels for apo J in the intestine, following LPS administration. In the present study we examined three different segments of the intestine, corresponding to the duodenum, the jejunum, and the ileum. Our results demonstrate that LPS significantly increases mRNA levels for apo J in all three segments of the intestine. The increase was greatest in the duodenum (11-fold) but less in the mid and distal intestine (jejunum and ileum) (2-fold). In situ hybridization has previously only demonstrated apo J in the duodenum [39]. Finally, in the present study, LPS also increased apo J mRNA levels in muscle (3-fold) (Fig. 2).

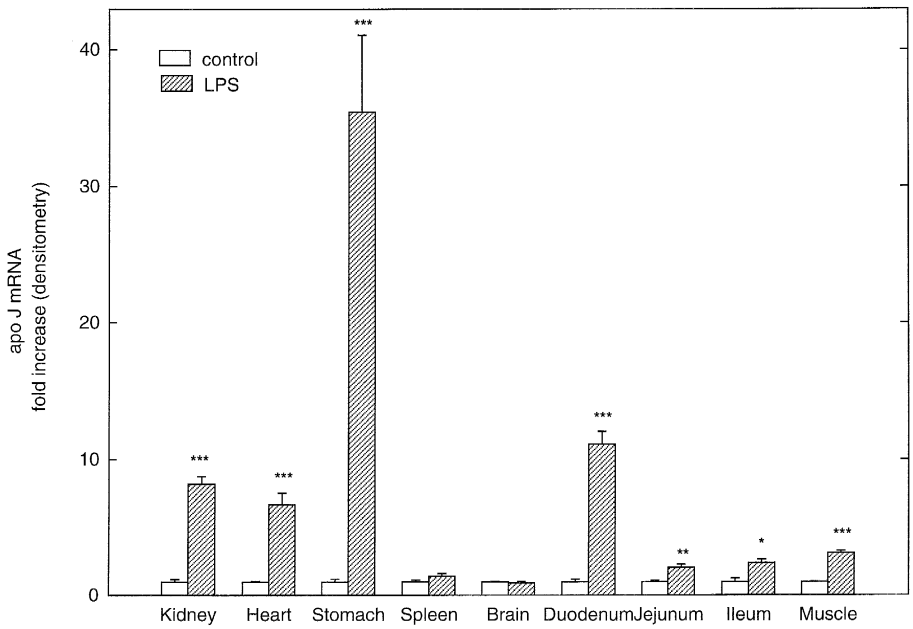

Fig. 2. The effects of LPS on mRNA levels for apo J in tissues. Syrian hamsters were injected intraperitoneally with either saline (control) (open bars) or LPS (100 μg/100 g bw) (striped bars). Sixteen hours later the animals were killed, the respective organs were removed and mRNA for apo J was detected as described in Section 2. Data are presented as means ± S.E.M. ($n = 4$–10). * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ vs. controls.

Table 1
The effects of TNF and IL-1 on mRNA levels for apo J in extra hepatic tissues

Tissues	TNF + IL-1 (17 μg + 1 μg/100 g bw)	TNF (17 μg /100 g bw)	IL-1 (1 μg /100 g bw)
	Fold increase over control		
Kidney	2.1 ± 0.44 *	1.5 ± 0.12	1.4 ± 0.18
Heart	2.4 ± 0.26 **	2.0 ± 0.23 *	1.3 ± 0.12
Stomach	21.6 ± 5.9 *	0.83 ± 0.10	7.9 ± 1.2 ***
Duodenum	3.0 ± 0.72 *	3.1 ± 0.38 **	2.5 ± 0.14 ***
Jejunum	1.7 ± 0.21 *	1.4 ± 0.39	1.0 ± 0.33
Ileum	1.9 ± 0.39	1.7 ± 0.23	1.9 ± 0.10 **
Muscle	2.2 ± 0.18 ***	1.3 ± 0.10	1.0 ± 0.11

Syrian hamsters were injected intraperitoneally with either saline (control), TNF + IL-1, TNF, or IL-1. Sixteen hours later the animals were killed, the respective organs were removed and mRNA for apo J was detected as described in Methods. Data are presented as means ± S.E.M. ($n = 5$–10). * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ vs. controls.

TNF + IL-1 in combination increased mRNA levels for apo J in all tissues in which they had been increased by LPS — i.e. kidney, heart, stomach, all three segments of the intestine, and muscle — demonstrating that these cytokines regulate apo J in a manner similar to that of LPS (Table 1). Administration of the two cytokines together was not as potent as LPS and therefore other factors may be needed to precisely mimic the effects of LPS. Individually, TNF and IL-1 increased apo J mRNA levels in some of the tissues but were for the most part less effective than the combination of the two (Table 1).

3.3. The effects of LPS and cytokines on mRNA levels for apo E in tissues

Apo E mediates the clearance of VLDL and chylomicron remnants and has been postulated to play a role in cholesterol efflux from peripheral cells. Apo E is synthesized in a number of tissues besides the liver [40]. Apo E may be a negative acute phase protein as LPS administration and turpentine-induced inflammation decrease hepatic mRNA levels for apo E in Syrian hamsters and rats [19,21]. In contrast, other studies have found that LPS administration and tur-

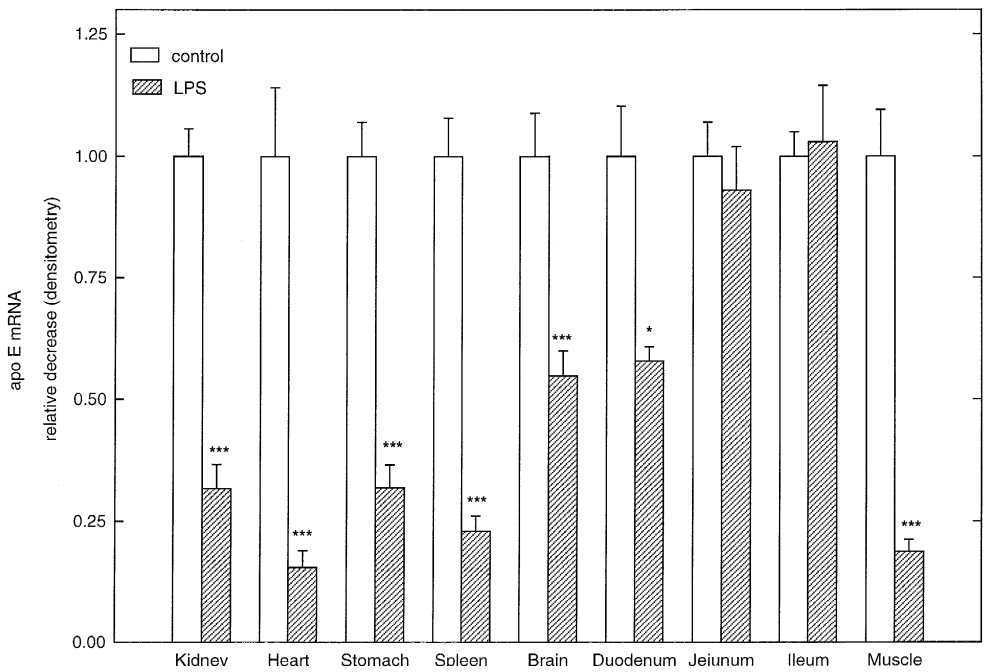

Fig. 3. The effects of LPS on mRNA levels for apo E in tissues. Syrian hamsters were injected intraperitoneally with either saline (control) (open bars) or LPS (100 μg/100 g bw) (striped bars). Sixteen hours later the animals were killed, the respective organs were removed and mRNA for apo E was detected as described in Section 2. Data are presented as means ± S.E.M. ($n = 4$–10). * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ vs. controls.

Table 2
The effects of TNF and IL-1 on mRNA levels for apo E in extra hepatic tissues

Tissues	TNF + IL-1 (17 μg + 1 μg/100g bw)	TNF (17 μg /100 g bw)	IL-1 (1 μg /100 g bw)
	Fold increase over control		
Kidney	1.2 ± 0.12	0.84 ± 0.11	1.2 ± 0.06
Heart	0.35 ± 0.06 *	0.69 ± 0.04 *	0.59 ± 0.11 *
Stomach	0.70 ± 0.13	0.92 ± 0.12	1.2 ± 0.13
Spleen	0.39 ± 0.06 * * *	0.67 ± 0.07	0.95 ± 0.11
Brain	0.84 ± 0.07	0.99 ± 0.15	1.2 ± 0.10
Duodenum	0.53 ± 0.13 *	0.57 ± 0.16	0.40 ± 0.13
Jejunum	0.95 ± 0.22	1.5 ± 0.19	1.5 ± 0.18
Ileum	1.3 ± 0.10	1.2 ± 0.10	0.97 ± 0.15
Muscle	0.33 ± 0.03 * * *	0.39 ± 0.07 *	0.48 ± 0.10

Syrian hamsters were injected intraperitoneally with either saline (control), TNF + IL-1, TNF, or IL-1. Sixteen hours later the animals were killed, the respective organs were removed and mRNA for apo E was detected as described in Section 2. Data are presented as means ± S.E.M. ($n = 5–10$). * $P < 0.05$, * * $P < 0.005$, * * * $P < 0.0005$ vs. controls.

pentine-induced inflammation do not affect hepatic mRNA levels for apo E in rats [41,42]. LPS administration decreased mRNA levels of apo E in freshly isolated rat Kupffer cells [42] and in vitro incubation with LPS decreased macrophage synthesis and secretion of apo E in most [43,44] but not all studies [45]. In the present study we demonstrate that LPS decreases apo E mRNA levels in all extra hepatic tissues studied in the hamster, except for the mid and distal intestine (jejunum and ileum) (Fig. 3). The decrease in mRNA levels for apo E was greatest in kidney, heart, stomach, spleen, and muscle (70%–80%), but still of considerable magnitude in duodenum and the brain (40–50%) (Fig. 3).

Previous studies have shown that the combination of TNF + IL-1 decreases hepatic mRNA levels for apo E, although individually these cytokines have no effect [20,46]. In the present study the combination of TNF + IL-1 decreased mRNA levels for apo E in most of the tissues studied (Table 2). The decrease was significant in heart, spleen, duodenum and muscle (50–70%), but less in other tissues (Table 2). In contrast, TNF or IL-1 alone only decreased apo E mRNA levels in the heart. Others have demonstrated that TNF or IL-1 decrease apo E secretion by macrophages in vitro [47]. Macrophages may indeed be a major source of apo E as bone marrow transplantation to apo E deficient mice leads to greater or equivalent levels of mRNA for apo E in tissues of these mice than in normal mice [48].

3.4. The effects of LPS and cytokines on mRNA levels for apo A-I

Apo A-I, the major constituent of HDL, is only synthesized in intestine and liver. LPS administration and turpentine-induced inflammation have been shown not to affect hepatic mRNA levels for apo A-I in hamsters or rats [1,9,21]. In contrast, LPS decreased hepatic apo A-I mRNA levels in mice [22] and inflammation decreased serum apo A-I levels in rabbits [49]. We now demonstrate that LPS administration to hamsters decreases mRNA levels for apo A-I in the duodenum by 55%, but does not affect apo A-I mRNA levels in the jejunum or the ileum (Table 3). Apo A-I mRNA was not detected in other tissues.

Table 3
The effects of LPS, TNF, and IL-1 on mRNA levels for apo A-I in the intestine

Tissues	LPS (100 μg/100 g bw)	TNF + IL-1 (17 μg + 1 μg/100g bw)	TNF (17 μg/100 g bw)	IL-1 (1 μg/100 g bw)
	Fold increase over control			
Duodenum	0.45 ± 0.09 *	0.30 ± 0.06 * *	0.66 ± 0.03 *	0.86 ± 0.09
Jejunum	1.1 ± 0.08	1.31 ± 0.07 *	1.0 ± 0.20	0.78 ± 0.04
Ileum	1.1 ± 0.18	1.1 ± 0.13	1.2 ± 0.15	1.0 ± 0.08

Syrian hamsters were injected intraperitoneally with either saline (control), LPS, TNF + IL-1, TNF, or IL-1. Sixteen hours later the animals were killed, the intestines removed and washed with saline. Three five cm segments of the intestine, corresponding to the duodenum, jejunum, and the ileum, were isolated and mRNA for apo A-I was detected as described in Section 2. Data are presented as means ± S.E.M. ($n = 5–10$). * $P < 0.05$, * * $P < 0.005$ vs. controls.

In our previous study, the combination of TNF + IL-1 slightly decreased hepatic mRNA levels for apo A-I [20] and others have shown IL-1 administration to decrease circulating apo A-I levels in cynomolgus monkeys [50]. In vitro studies have also demonstrated decreased synthesis of apo A-I in mouse hepatocytes during acute inflammation [51] and decreased concentrations of apo A-I in the medium from HepG2 cells following incubation with TNF or IL-1 [52]. In the present study the combination of TNF + IL-1 as well as TNF by itself decreased apo A-I mRNA levels in the duodenum (Table 3).

3.5. The effects of LPS and cytokines on mRNA levels for apo D

Apo D associates with HDL and has been reported to be present in a number of tissues, including heart, kidney, spleen, brain, intestine, and liver, in humans, monkeys, rabbits, or rats [35,53–55]. The present study is the first to demonstrate apo D mRNA in tissues from hamsters. Apo D mRNA was detected by Northern blotting in heart, muscle, and brain but not in liver, stomach, kidney, spleen, or intestine. In contrast to our results with the other apolipoproteins, the effects of LPS and cytokines on apo D were not unidirectional. LPS decreased mRNA levels for apo D in the heart by around 60%; did not affect mRNA levels for apo D in the brain; and increased apo D in muscle by almost 5-fold (Table 4). LPS did not induce detectable levels of apo D mRNA in tissues where it was not detected in control animals. The combination of TNF + IL-1 decreased apo D mRNA levels in the heart by 15% but did not significantly increase apo D mRNA levels in muscle (Table 4).

Table 4
The effects of LPS, TNF, and IL-1 on mRNA levels for apo D in extra hepatic tissues

Tissues	LPS (100 μg/100g bw)	TNF + IL-1 (17 μg + 1 μg/100g bw)
	Fold increase over control	
Heart	0.41 ± 0.04 ***	0.75 ± 0.06 *
Brain	0.86 ± 0.08	n.d.
Muscle	4.8 ± 0.62 ***	1.2 ± 0.17

Syrian hamsters were injected intraperitoneally with either saline (control) or TNF + IL-1. Sixteen hours later the animals were killed, the respective organs were removed and mRNA for apo D was detected as described in Section 2. Data are presented as means ± S.E.M. ($n = 5$–10). * $P < 0.05$, *** $P < 0.0005$ vs. controls. n.d.: not determined.

4. Discussion

Our results demonstrate that mRNA levels for several apolipoproteins (apo SAA, apo J, apo E, apo A-I, and apo D) are regulated in extrahepatic tissues during the acute phase response. Although mRNA levels do not always correlate perfectly with expression of protein it is likely that secretion at least qualitatively parallels mRNA levels. The regulation of apoSAA, apo J, apo E, apo A-I, and apo D during the acute phase response suggests a potential role for these apolipoproteins in local and systemic host response to infection and inflammation. The alterations in these apolipoproteins may be important for the changes in lipid and cholesterol metabolism that occur during the acute phase response as well as in the immune response to infection and inflammation.

The profound alterations in lipid and lipoprotein metabolism that occur during infection and inflammation are thought to be beneficial to the host [9,10], as lipoproteins bind endotoxin, viruses and urate crystals and protect from their toxic and inflammatory effects [12,56–58]. Lipoproteins may also lyse parasites [59–61]. Regulation of apolipoprotein expression during the acute phase response may play a role in these alterations in lipid and lipoprotein metabolism. For example, decreased expression of apo E during infection and inflammation may contribute to increased serum lipoprotein levels, as apo E facilitates uptake of lipoproteins by the low density lipoprotein receptor and low density lipoprotein receptor related protein. The importance of apo E production in lipoprotein clearance has been demonstrated in apo E deficient mice, which are characterized by marked hyperlipidemia. When extra hepatic production of apo E was induced in apo E deficient mice by bone marrow transplantation, lipoprotein clearance was promoted and serum cholesterol levels were normalized [48].

Apo SAA promotes cellular uptake of cholesterol [62] and apoSAA rich HDL particles have higher affinity for macrophages and lower affinity for hepatocytes than apoSAA poor HDL [63,64]. Therefore,

apo SAA may be a signal to redirect HDL cholesterol away from the liver and to peripheral cells. Increased expression of apoSAA during the acute phase response may thus increase the availability of cholesterol for peripheral cells, which may have increased cholesterol requirements during infection and inflammation. Induction of apo J may play a similar role, as apo J has been shown to participate in mobilization, uptake and redistribution of lipids from damaged or lipid loaded cells [65]. Local induction of apo J during the acute phase response may also be beneficial in protecting membranes against potentially harmful oxidized fatty acids and toxic lipid byproducts formed during the response to infection and inflammation [66]. Apo D may play a similar role as it has been proposed to protect cells and tissues from oxidant damage [67].

In addition to playing a role in altering lipid metabolism, the changes in expression of apolipoproteins may be involved in other aspects of the immune response to infection and inflammation. For example, apo J is an inhibitor of the lytic terminal complement cascade [68]. The activation of the complement cascade during infection and inflammation plays a key role in the local host defense process. The inflammatory and destructive properties of the complement cascade can, however, lead to systemic complications, which makes it essential that the complement attack is confined. Apo J may confine the complement cascade; thus increased expression of apo J during infection and inflammation may be beneficial. Other examples of acute phase proteins limiting systemic toxicity by confining the acute phase response are the protease inhibitors, α_2-macroglobulin and α_1-antitrypsin, which limit proteolysis to the site of infection or inflammation [69].

ApoSAA and apo E may also be involved in the immune response to infection and inflammation. ApoSAA induces migration, adhesion and tissue infiltration of monocytes and polymorphonuclear cells to inflamed areas during the acute phase response [70] and apo E inhibits mitogen or antigen-driven lymphocyte proliferation and neutrophil function [71]. Induction of apoSAA and reduction of apo E in tissues during the acute phase response may thus be important for the cellular response to infection and inflammation.

In summary, we have shown that there is significant regulation of expression of the apolipoproteins, apoSAA, apo J, apo E, apo A-I, and apo D, during the acute phase response. The changes in these apolipoproteins may play a role in the alterations in lipid metabolism that occur in response to infection and inflammation as well as in the local immune response to infection and injury.

Acknowledgements

This work was supported by grants from the Research Service of the Department of Veterans Affairs and the NIH (DK40990).

References

[1] Kushner, I. (1991) Eur. Cytokine Net. 2, 75–80.
[2] Colten, H.R. (1992) J. Appl. Physiol. 72, 1–7.
[3] Morrow, J.F., Stearman, R.S., Peltzman, C.G. and Potter, D.A. (1981) Proc. Natl. Acad. Sci. USA 78, 4718–4722.
[4] Ramadori, G., Sipe, J.D. and Colten, H.R. (1985) J. Immunol. 135, 3645–3647.
[5] Kalmovarin, N., Friedrichs, W.E., O'Brien, H.V., Linehan, L.A., Bowman, B.H. and Yang, F. (1991) Inflammation 15, 369–379.
[6] Su, G.L., Freeswick, P.D., Geller, D.A., Wang, Q., Shapiro, R.A., Wan, Y.-H., Billiar, T.R., Tweardy, D.J., Simmons, R.L. and Wang, S.C. (1994) J. Immunol. 153, 743–752.
[7] Seiffert, D., Crain, K., Wagner, N.V. and Loskutoff, D.J. (1994) J. Biol. Chem. 269, 19836–19842.
[8] Falus, A., Beuscher, H.U., Auerbach, H.S. and Colten, H.R. (1987) J. Immunol. 138, 856–860.
[9] Hardardóttir, I., Grunfeld, C. and Feingold, K.R. (1994) Curr. Opin. Lipidol. 5, 207–215.
[10] Grunfeld, C. and Feingold, K.R. (1991) Trends Endocrinol. Metab. 2, 213–219.
[11] Grunfeld, C. and Feingold, K.R. (1992) Proc. Soc. Exp. Biol. Med. 200, 224–227.
[12] Harris, H.W., Grunfeld, C., Feingold, K.R. and Rapp, J.H. (1990) J. Clin. Invest. 86, 696–702.
[13] Levine, D.M., Parker, T.S., Donnelly, T.M., Walsh, A. and Rubin, A.L. (1993) Proc. Natl. Acad. Sci. USA 90, 12040–12044.
[14] Ulevitch, R.J., Johnston, A.R. and Weinstein, D.B. (1981) J. Clin. Invest. 67, 827–837.
[15] Munford, R.S., Hall, C.L., Lipton, J.M. and Dietschy, J.M. (1982) J. Clin. Invest. 70, 877–888.
[16] Dowton, S.B., Peters, C.N. and Jestus, J.J. (1991) Inflammation 15, 391–397.
[17] Niewold, T.A., Gruys, E., Arakawa, T., Shirahama, T. and Kisilevsky, R. (1993) Scand. J. Immunol. 37, 29–32.

[18] Hardardóttir, I., Kunitake, S.T., Moser, A.H., Doerrler, W.T., Rapp, J.H., Grunfeld, C. and Feingold, K.R. (1994) J. Clin. Invest. 94, 1304–1309.
[19] Feingold, K.R., Hardardóttir, I., Memon, R., Krul, E.J.T., Moser, A.H., Taylor, J.M. and Grunfeld, C. (1993) J. Lipid Res. 34, 2147–2158.
[20] Hardardóttir, I., Moser, A.H., Memon, R., Grünfeld, C. and Feingold, K.R. (1994) Lymphokine Cytokine Res. 13, 161–166.
[21] Tu, G.F., De Jong, F., Apostolopoulos, J., Nagashima, M., Fidge, N., Schreiber, G. and Howlett, G. (1987) Inflammation 11, 241–251.
[22] Lowell, C.A., Stearman, R.S. and Morrow, J.F. (1986) J. Biol. Chem. 261, 8453–8461.
[23] Meek, R.L. and Benditt, E.P. (1986) J. Exp. Med. 164, 2006–2017.
[24] Rygg, M., Husby, G. and Marhaug, G. (1993) Scand. J. Immunol. 38, 417–422.
[25] Spady, D.K. and Dietschy, J.M. (1988) J. Clin. Invest. 81, 300–309.
[26] Spady, D.K. and Dietschy, J.M. (1985) Proc. Natl. Acad. Sci. USA 82, 4526–4530.
[27] Arbeeny, C.M., Meyers, D.S., Bergquist, K.E. and Gregg, R.E. (1992) J. Lipid Res. 33, 843–851.
[28] Quig, D.W., Arbeeny, C.M. and Zilversmit, D.B. (1991) Biochim. Biophys. Acta 1083, 257–264.
[29] Ly, H., Francone, O.L., Fielding, C.J., Shigenaga, J.K., Moser, A.H., Grunfeld, C. and Feingold, K.R. (1995) J. Lipid Res. 36, 1254–1263.
[30] Adams, M.D., Kelley, J.M., Gocagne, J.D., Dobnick, M., Polymeropoulos, M.H., Xiao, H., Merril, C.R., Wu, H., Olden, B., Moreno, R.F., Kerlavage, A.R., et al. (1991) Science 252, 1651–1656.
[31] Elshourbagy, N.A., Liao, W.S., Mahley, R.W. and Taylor, J.M. (1985) Proc. Natl. Acad. Sci. USA 82, 203–207.
[32] Sipe, J.D., Woo, P., Goldberger, G., Cohen, A.S. and Whitehead, A.S. (1986) Characterization of two distinct serum amyloid A gene products defined by their complementaiy DNAs, in Amyloidosis (Glenner, G.G., Osserman, E.F., Benditt, E.P., Calkins, E., Cohen, A.S. and Zucker-Franklin, D., eds.), pp. 57–60, Plenum Press, New York.
[33] Sipe, J.D., Colten, H.R., Goldberger, G., Edge, M.D., Tack, B.F., Cohen, A.S. and Whitehead, A.S. (1985) Biochemistry 24, 2931–2936 (Erratum Biochemistry (1986) 25, 3736).
[34] De Beer, M.C., De Beer, F.C., Beach, C.M., Gonnerman, W.A., Carreras, I. and Sipe, J.D. (1993) J. lmmunol. 150, 5361–5370.
[35] Drayna, D., Fielding, C., McLean, J., Baer, B., Castro, G., Chen, E., Comstock, L., Henzel, W., Kohr, W., Rhee, L., Wion, K. and Lawn, R. (1986) J. Biol. Chem. 261, 16535–16539.
[36] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[37] Webb, C.F., Tucker, P.W. and Dowton, S.B. (1989) Biochemistry 28, 4785–4790.
[38] Husby, G., Marhaug, G., Dowton, B., Sletten, K. and Sipe, J.D. (1994) Int. J. Exp. Clin. Invest. 1, 119–137.
[39] Aronow, B.J., Lund, S.D., Brown, T.L. and Harmony, J.A.K. (1993) Proc. Natl. Acad. Sci. USA 90, 725–729.
[40] Driscoll, D.M. and Getz, G.S. (1984) J. Lipid Res. 25, 1368–1379.
[41] Schreiber, G., Aldred, A.R., Thomas, T., Birch, H.E., Dickson, P.W., Tu, G.F., Heinrich, P.C., Northemann, W., Howlett, G.J., De Jong, F.A. and Mitchell, A. (1986) Inflammation 10, 59–66.
[42] Dawson, P.A., Lukaszewski, L.M., ElIs, P.F., Malbon, C.C. and Williams, D.L. (1989) J. Lipid Res. 30, 403–413.
[43] Werb, Z. and Chin, J.R. (1983) J. Biol. Chem. 258, 10642–10648.
[44] Menju, M., Tajima, S. and Yamamoto, A. (1989) J. Biochem. 106, 505–510.
[45] Van Lenten, B.J., Fogelman, A.M., Seager, J., Ribi, E., Haberland, M.E. and Edwards, P.A. (1985) J. lmmunol. 134, 3718–3721.
[46] Delers, F., Mangeney, M., Raffa, D., Vallet-Colom, I., Daveau, M., Tran-Quang, N., Davrinches, C. and Chambaz, J. (1989) Biochem. Biophys. Res. Commun. 161, 81–88.
[47] Zuckerman, S.H., Evans, G.F. and O'Neal, L. (1992) Atherosclerosis 96, 203–214.
[48] Linton, M.F., Atkinson, J.B. and Fazio, S. (1995) Science 267, 1034–1037.
[49] Cabana, V.G., Siegel, J.N. and Sabesin, S.M. (1989) J. Lipid Res. 30, 39–49.
[50] Ettinger, W.H., Miller, L.A., Smith, T.K. and Parks, J.S. (1992) Biochim. Biophys. Acta 1128,186–192.
[51] Baumann, H., Jahreis, G.P. and Gaines, K.C. (1983) J. Cell Biol. 97, 866–876.
[52] Ettinger, W.H., Varma, V.K., Sorci-Thomas, M., Parks, J.S., Sigmon, R.C., Smith, T.K. and Verdery, R.B. (1994) Arterioscler. Thromb. 14, 8–13.
[53] Smith, K.M., Lawn, R.M. and Wilcox, J.N. (1990) J. Lipid Res. 31, 995–1004.
[54] Provost, P.R., Weech, P.K., Tremblay, N.M., Marcel, Y.L. and Rassart, E. (1990) J. Lipid Res. 31, 2057–2065.
[55] Boyles, J.K., Notterpek, L.M., Wardell, M.R. and Rall Jr., S.C. (1990) J. Lipid Res. 31, 2243–2256.
[56] Sernatinger, J., Hoffman, A., Hardman, D., Kane, J.P. and Levy, J.A. (1988) J. Gen. Virol. 69, 2657–2661.
[57] Superti, F., Seganti, L., Marchetti, M., Marziano, M.L. and Orsi, N. (1992) Med. Microbiol. Immunol. 181, 77–86.
[58] Terkeltaub, R.A., Dyer, C.A., Martin, J. and Curtiss, L.K. (1991) J. Clin. Invest. 87, 20–26.
[59] Xu, X., Remold, H.G. and Caulfield, J.P. (1993) Am. J. Pathol. 142, 685–689.
[60] Hajduk, S.L., Moore, D.R., Vasudevacharya, J., Siqueira, H., Torri, A.F., Tytler, E.M. and Esko, J.D. (1989) J. Biol. Chem. 264, 5210–5217.
[61] Smith, A.B., Esko, J.D. and Hajduk, S.L. (1995) Science 268, 284–286.
[62] Liang, J. and Sipe, J.D. (1995) J. Lipid Res. 36, 37–46.
[63] Kisilevsky, R. and Subrahmanyan, L. (1992) Lab. Invest. 66, 778–785.
[64] Banka, C.L., Yuan, T., De Beer, M.C., Kindy, M., Curtiss, L.K. and De Beer, F.C. (1995) J. Lipid Res. 36, 1058–1065.

[65] Jenne, D.E., Lowin, B., Peitsch, M.C., Böttcher, A., Schmitz, G. and Tschopp, J. (1991) J. Biol. Chem. 266, 11030–11036.
[66] Jordan-Starck, T.C., Witte, D.P., Aronow, B.J. and Harmony, J.A.K. (1992) Curr. Opin. Lipidol. 3, 75–85.
[67] Peitsch, M.C. and Boguski, M.S. (1990) The New Biologist 2, 197–206.
[68] Murphy, B.F., Saunders, J.R., O'Bryan, M.K., Kirszbaum, L., Walker, I.D. and d'Apice, A.J.F. (1989) Int. Immunol. I, 551–554.
[69] Richards, C., Gauldie, J. and Baumann, H. (1991) Eur. Cytokine Net. 2, 89–98.
[70] Badolato, R., Wang, J.M., Murphy, W.J., Lloyd, A.R., Michiel, D.F., Bausserman, L.L., Kelvin, D.J. and Oppenheim, J.J. (1994) J. Exp. Med. 180, 203–209.
[71] Mistry, M.J., Clay, M.A., Kelly, M.E., Steiner, M.A. and Harmony, J.A. (1995) Cell. Immunol. 160, 14–23.