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Endotoxin regulates the maturation of sterol regulatory element binding protein-1 through the induction of cytokines

Abstract

Endotoxin (LPS), by raising the levels of cytokines, markedly influences lipid metabolism. To clarify the molecular mechanism of this effect, we examined the action of endotoxin in vitro and in vivo on the regulation of sterol regulatory element binding protein-1 (SREBP-1). In HepG2 cells stimulated with LPS, a dose-dependent increase in the level of the mature form of SREBP-1 was observed. For

in vivo studies, endotoxin was administered intraperitoneally to CD1 mice fed with a standard or a cholesterol-enriched diet to increase the basal levels of circulating and liver cholesterol. Endotoxin raised cholesterol levels and stimulated the maturation of hepatic SREBP-1 in both normal and cholesterol-fed mice, indicating that the lipogenic effect of LPS was independent of endogenous sterol levels. To assess whether the lipogenic effect of endotoxin was linked to cytokine production, we administered LPS to C57Bl/6J endotoxin-sensitive and to C3H/HeJ endotoxin-resistant mice, which do not produce tumor necrosis factor in response to LPS. Significant induction of cholesterol levels and SREBP-1 activation was observed only in C57Bl/6J mice, indicating that cytokine production is crucial for the regulation of SREBP-1, and that the transcriptional activation of cholesterol biosynthesis may be part of the acute-phase response.

Full text

INTRODUCTION

Infection and inflammatory conditions are usually accompanied by multiple metabolic disturbances, including hypertriglyceridemia and increased lipogenesis [1-3]. Rates of *de novo* hepatic lipid synthesis are raised because of an increase in hepatic fatty acids, cholesterol synthesis and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity [4, 5]. The alterations of lipid homeostasis during inflammation have been ascribed to cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-1beta, and their effects. The cytokines significantly raise triglyceride and cholesterol serum levels and hepatic cholesterol synthesis [4, 5, 7]. Moreover, because anti-TNF antibodies block the effect of LPS on lipid metabolism, it was suggested that TNF might be the mediator of the endotoxin's metabolic action [4].

The molecular mechanism by which cytokines affect lipid metabolism is not completely known and no data are available on how they affect the control of genes expressing the enzymes involved in cholesterol and fatty acid biosynthesis. The transcription factors, sterol regulatory element binding protein (SREBP)-1 and SREBP-2 are particularly important because they promote the activation of genes encoding for key enzymes of cholesterol biosynthesis, such as HMG-CoA synthetase, HMG-CoA reductase, farnesyl diphosphate synthethase and squalene synthethase [9-11]. SREBP-1 and SREBP-2 are

anchored to the endoplasmic reticulum membrane and their activity is regulated by sterol levels [12-15]. When cells are deprived of sterols, two-step proteolytic cleavage releases a 68-kDa transcriptionally active fragment which migrates into the nucleus. When sterols accumulate in cells, this cleavage is prevented, so the transcription of target genes declines [12-15].

In vitro, SREBP-1 can be activated by TNF-alpha and IL-6 in cells of hepatic origin, whereas interferon-gamma reduced SREBP-1 expression in 3T3-L1 adipocytes, suggesting that, besides sterols, this transcription factor can also be regulated by pro-inflammatory cytokines through an original, sterol-independent, proteolytic pathway [16-18].

The aim of this study was to clarify whether endotoxin activates SREBP-1 maturation, *in vitro* and *in vivo*, and whether this effect is involved in the alteration of cholesterol homeostasis observed during inflammation. For the *in vitro* studies we exposed HepG2 cells to LPS. *In vivo*, we applied the LPS acute model of inflammation to CD1 mice fed a standard or a cholesterol-rich diet, to increase basal hepatic cholesterol levels. Then, to better elucidate the cytokines' role in SREBP-1 activation, we gave LPS to C57Bl/6J and C3H/HeN mice, sensitive to the action of the endotoxin, and C3H/HeJ mice, which are resistant because their macrophages do not respond by producing TNF-alpha [19].

MATERIALS AND METHODS

Materials

Bacterial lipopolysaccharide (LPS, from *Escherichia coli* 055:B5) was obtained from Difco (Detroit, MI, USA). Actinomycin D, leupeptin, aprotinin, phenylmethylsulfonyl fluoride, N-acetyl-leucine-leucine-norleucinal and horseradish peroxidase-labeled donkey anti-rabbit IgG antibody were from Sigma (St. Louis, MO, USA). Human SREBP-1 polyclonal antibody and rabbit beta-actin polyclonal antibody were from Santa Cruz Biotechnology (Santa Monica, CA, USA).

Cells

Human HepG2 cells were obtained from Istituto Zooprofilattico Sperimentale (Brescia, Italy) and were cultured as a monolayer in DMEM medium (Labtek, Eurobio, France) supplemented with 10% (vol/vol) fetal bovine serum (FBS, Labtek) and 20 U/ml penicillin/streptomycin (Labtek), in a humidified 5% CO_2 atmosphere. At about 80% confluence, cells were transferred to flat-bottomed, six-well plates (Falcon, Cockeysville, MD, USA) (1 **x** 10^6/ml/well) and pre-incubated for 24 hours in complete fresh DMEM supplemented with 10% FBS. Cells were then exposed to LPS (2-10 mug/10^6 cells/ml) for different times (45-210 min) at 37° C. At the end of incubation, supernatants were harvested, cells were washed twice with saline, detached with trypsin and kept at - 80° C until use.

Animals

Male CD1, C57Bl/6J, C3H/HeN and C3H/HEJ mice, 18-20 g body weight (Charles River Italy, Calco, Como, Italy) were used. Animals were housed at a constant temperature (22 ± 2° C) and relative humidity (60 ± 5%). CD1 mice were fed *ad libitum* for 17 days a standard diet or a diet supplemented with 0.6% cholesterol (Mucedola, Milano, Italy) [20]. Inflammation was induced by intraperitoneal injection of 2.5 mug/mouse of LPS. Controls received saline alone. At different times after treatment (from 30 min up to 36 hours) animals were anesthetized and the blood was collected from the retroorbital plexus. They were then killed by cervical dislocation and the liver was excised and immediately frozen at - 80° C.

Procedures involving animals and their care were conducted in conformity with national and international laws and policies (EEC Council Directive 86609, OJ L358, 1, 12 December 1987; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana n. 10, 18 February 1992; Guide for the Care and Use of Laboratory Animals,US National Research Council, 1996).

TNF-alpha

Serum TNF-alpha levels were measured according to Aggarwal *et al.* [21] by a standard cytotoxicity assay using L929 cells (Istituto Zooprofilattico Sperimentale, Brescia, Italy) in the presence of 1.0 mug/ml of actinomycin D.

Cholesterol

Lipids were extracted from liver homogenates according to Folch *et al.* [22]. Serum and liver cholesterol were assayed using an enzymatic assay kit (Boehringer Mannheim, Germany). Protein concentration was determined on cellular and liver homogenates using the Bio-rad protein assay (Bio-rad, Germany). Serum cholesterol was expressed as mg/dl. To avoid errors due to changes in liver weight, the hepatic cholesterol content was expressed as mug/mg protein.

Immunoblot analysis

Nuclear and membrane extracts were prepared from cellular and liver homogenates according to Sheng *et al*. [23]. Samples (25-50 mug proteins) of membrane and nuclear extract fractions were analysed by 8% SDS-PAGE, transferred to a nitrocellulose filter and incubated with a rabbit SREBP-1 polyclonal antibody or a rabbit beta-actin polyclonal antibody. The membrane-bound antibodies were visualized with the Enhanced Chemiluminescence Western Blotting Detection System Kit (ECL, Amersham Pharmacia Biotech, UK), using a horseradish peroxidase-labeled donkey anti-rabbit IgG antibody. Filters were exposed to Kodak film from 15 sec to 2 min. Quantitative data were obtained by scanning blots using a computerized image analyzer, and the precursor (125-kDa) and mature (68-kDa) levels were expressed as optical density. In our experimental conditions, the intensity of the signals from ECL was proportional to the amount of protein on the gel membrane (data not shown).

Statistical analysis

Data are expressed as the mean ± standard deviation. Statistical significance was determined by analysis of variance and Student's t test.

RESULTS AND DISCUSSION

Altered cholesterol homeostasis, associated with infection or inflammation, is believed to be part of the acute phase response [1-3]. Cytokines released during the inflammatory process activate hepatic cholesterol synthesis by raising the level of mRNA and the activity of key enzymes such as HMG-CoA reductase and synthethase [4-8]. Transcription of the enzymes required for cholesterol biosynthesis and transport is regulated by two transcription factors, SREBP-1

and SREBP-2 [10], whose activation is controlled by endogenous sterol levels [13-15].

This study aimed to elucidate the effect of endotoxin on the transcriptional control of cholesterol biosynthesis. We exposed HepG2 cells to LPS for different times and examined the levels of the mature and precursor form of SREBP-1. As shown in Figure 1, starting from 45 min of exposure, both endotoxin doses stimulated SREBP-1 maturation, with an increase of the 68-kDa mature form accompanied by the disappearance of the 125-kDa precursor form. The effect was maximal 90 min after LPS and declined by 210 min.

To determine the *in vivo* effect of endotoxin on the transcriptional control of cholesterol, we applied the LPS acute model of inflammation to normal CD1 mice and to mice whose endogenous cholesterol levels had been raised by a cholesterol-enriched diet [20]. As shown in Figure 2A, there was a significant (p ¾ 0.01, Student's t test) increase in the levels of serum (128.4 ± 15 mg/dl *versus* 142.7 ± 10 mg/dl, mean ± standard deviation of 8 separate animals) and hepatic (99.6 ± 10 mug/mg protein *versus* 183.7 ± 27 mug/mg protein, mean ± standard deviation of 8 separate animals) cholesterol in mice fed with cholesterol-enriched diet. LPS caused significant increases in serum (1.6-fold) and hepatic (1.4-fold) cholesterol levels after 24 and 16 hours, respectively (Figure 2A). Similar effects were observed in mice fed with the cholesterol-enriched diet and treated with LPS, with an 1.6-fold increase in serum and a 1.5-fold increase in hepatic cholesterol at 24 and 16 hours (Figure 2A), indicating that the lipogenic effect of endotoxin was independent of the endogenous cholesterol.

We then examined the mature and precursor form of SREBP-1 in hepatic tissues from normal and cholesterol-enriched CD1 mice before and after LPS. Figure 2B reports the representative immunoblot analysis of the 125-kDa precursor and the 68-kDa mature form of SREBP-1, and of beta -actin used as a loading control. Quantitative data were obtained by densitometric analysis of immunoblots from liver homogenates of at least five animals. As shown in Figure 2B and C, endotoxin stimulated hepatic SREBP-1 maturation in CD1 mice at 6 hours and 16 hours, with an increase of the 68-kDa mature form and a disappearance of the 125-kDa precursor. This effect paralleled the changes in liver cholesterol content, suggesting that the activation of this transcription factor rapidly results in the synthesis of new cholesterol. The increase in cholesterol levels caused, *via* a negative feedback mechanism, a decrease in SREBP-1 maturation.

In fact, 24 hours after endotoxin administration, the level of the 125-kDa precursor form was similar to that in untreated mice, wheares there was still induction of the 68-kDa form. Nevertheless, the hepatic cholesterol content decreased suggesting that, at this time point, not all the mature form was transcriptionally active.

In CD1 mice fed the cholesterol-enriched diet, the high cholesterol levels caused a reduction of its *de novo* synthesis by a negative feedback loop [9, 10, 20] mediated by repression of the basal amounts of SREBP-1 precursor (Figure 2B). As in CD1 mice, in mice fed with the cholesterol-enriched diet, LPS also caused a time-dependent increase in the hepatic maturation of SREBP-1 (Figure 2B and C). A decrease in the precursor form and an increase in the mature form were observed in their liver homogenates 6 hours after LPS (Figure 2B). This caused a drop in the precursor-to-mature form ratio, from 7.11 in untreated, to 0.31 (Figure 2C). The precursor form disappeared 16 hours after the endotoxin but it had reappeared by 24 hours, and induction of the 68-kDa mature form was still evident (Figure 2B). These data indicate that high hepatic cholesterol levels did not antagonize the ability of LPS to increase cholesterol or to activate SREBP-1.

Several lines of evidence suggest that the genetic background may regulate the inflammatory response to endotoxin [6, 7]. To evaluate if genetic differences modulate the lipogenic effect of LPS too, we considered two inbred strains of mice (C57Bl/6J and C3H) which exhibit a more homogeneous genetic identity in respect to the outbred CD1 mice. These strains of mice exhibited a different ability to produce cytokines in response to LPS [6, 7]. To investigate whether the effect of endotoxin on cholesterol biosynthesis and SREBP-1 maturation was linked to cytokine production, we tested LPS-sensitive C57Bl/6J and C3H/HeN mice and LPS-resistant C3H/HeJ mice [19]. These latter are unresponsive to the endotoxin because their macrophages are unable to produce pro-inflammatory cytokines [19]. LPS, 2.5 mug/mouse, were injected intraperitoneally and serum TNF-alpha levels were determined after 30 min. As expected, serum TNF-alpha significantly increased in C57Bl/6J mice (from < 50 pg/ml basal to 210.8 ± 20 pg/ml after LPS; p ¾ 0.01, Student's t test, n = 6) and C3H/HeN mice (from < 50 pg/ml basal to 260.5 ± 15 pg/ml after LPS; p ¾ 0.01, Student's t test, n = 6), but not in the C3H/HeJ strain (< 50 pg/ml in basal and LPS-treated mice, n = 6), confirming its unresponsiveness [19].

To assess whether the C3H/HeJ strain was insensitive to LPS-induced lipogenesis as well, we compared the circulating and hepatic cholesterol levels in C3H/HeJ, C3H/HeN and C57Bl/6J mice given LPS. Although serum and liver cholesterol levels were very different among the strains considered, similarly to what was observed in CD1 mice, endotoxin, after 16 hours, significantly increased serum cholesterol in C57Bl/6J (1.6-fold, Figure 3A) and C3H/HeN (2-fold, from 98.7 ± 3.1 mg/dl of untreated to 185.4 ± 5.3 mg/dl of LPS-treated mice), whereas no variations were observed in C3H/HeJ mice (Figure 3A). These data indicate that the lipogenic effect of LPS is not related to genetic components but only to cytokines levels.

We therefore focused our attention on C57Bl/6J and C3H/HeJ mice because the lipogenic effect of cytokines was well characterized in these strains [5, 6, 19]. Figure 3A also shows that C3H/HeJ mice had significantly higher basal serum (86.5 ± 3.1 mg/dl, n = 8) and liver (64.0 ± 2.1 mug/mg protein, n = 8) cholesterol levels (p ¾ 0.01, Student's t test) than C57Bl/6J mice (51.1 ± 2.3 mg/dl and 40.5 ± 6.0 mug/mg protein, n = 8).

We then measured hepatic levels of the precursor and mature forms of SREBP-1. Unlike in C57Bl/6J mice, in the endotoxin-resistant strain, LPS did not modify the levels of either form of the transcription factor (Figure 3B). Of particular interest is the time-dependent induction of the mature form of SREBP-1 in C57Bl/6J mice after LPS. A maximal increase of the mature form was observed 16 h after the challenge (Figure 3B), accompained by a decrease of the precursor form level. This effect was paralleled by a decrease in the precursor-to-mature form ratio from 1.58 to 0.22 (Figure 3C). Twenty-four hours after LPS, the levels of both the mature and the precursor form were still higher than in untreated mice, whereas 36 hours after LPS, only the induction of the precursor form was detectable (Figure 3B).

Our findings indicate that, both *in vitro* and *in vivo*, LPS activates the maturation of SREBP-1, suggesting that this effect is at the basis of cytokine-induced lipogenesis. We found that high endogenous sterol levels, induced by a cholesterol-rich diet in mice, did not antagonize the ability of LPS to cause lipogenesis, reinforcing the idea that *in vivo* too, there is an original SREBP-1 regulatory mechanism encompassing endogenous cholesterol levels [16-18].

It is not clear why the host responds to infection or inflammation with increased cholesterol synthesis. This may be subordinate to cellular plasma membrane demands for cholesterol supplementation to preserve its integrity [24], or may reflect the increased need for molecules involved in the inflammatory reaction. For example, isoprenoids are important non-sterol intermediates in the cholesterol biosynthesis pathway which are also involved in the production of chemokines and cytokines [25-27].

Cholesterol is an important component of lipoproteins. It is known that the increase of serum and liver cholesterol induced by LPS is primarily due to an elevation of low density lipoprotein (LDL) cholesterol, whereas the high density lipoprotein (HDL) fraction declines [6, 7]. It is possible that the increased hepatic cholesterol synthesis induced by LPS allows for a higher production of lipoproteins, particularly HDL, which may be benefical for the body's host defence.

CONCLUSION

In conclusion, our data further suggest that activation of cholesterol biosynthesis is part of the acute-phase response and is rapid and insensitive to endogenous levels of sterols. This mechanism may therefore be vital for the host defenses.

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References

1. Alvarez C, Ramos A. 1986. Lipids, lipoproteins and apoproteins in serum during infections. *Clin. Chem.* 32: 142.

2. Cabana V G, Siegel J N, Sabesin S M. 1989. Effect of the acute phase response on the concentration and density distribution of plasma lipids and apoproteins. *J. Lipid Res.* 30: 39.

3. Kaufmann R L, Matson C F, Rowberg A H, Beisel W R. 1976. Defective lipid disposal mechanisms during bacterial infection in rhesus monkeys. *Metabolism.*

25: 615.

4. Memon R A, Grunfeld C, Moser A H, Feingold K R. 1993. Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism. *Endocrinology* 132: 2246.

5. Feingold K R, Soued M, Serio M K, Moser A H, Dinarello C A, Grunfeld C. 1989. Multiple cytokines stimulate hepatic lipid synthesis *in vivo. Endocrinology* 125: 267.

6. Feingold K R, Staprans I, Memon R, Moser A H, Shigenaga J K, Doerrler W, Dinarello C A, Grunfeld C. 1992. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J. Lipid Res.* 33: 1765.

7. Hardardottir I, Moser A H, Memon R, Grunfeld C, Feingold K R. 1994. Effects of TNF, IL-1 and the combination of both cytokines on cholesterol metabolism in Syrian hamsters. *Lymphokine Cytokine Res.* 13: 161.

8. Feingold K R, Pollock A S, Moser A H, Shigenaga J K, Grunfeld C. 1995. Discordant regulation of proteins of cholesterol metabolism during the acute phase response. *J. Lipid Res.* 36: 1474.

9. Goldstein J L, Brown M S. 1990. Regulation of the mevalonate pathway. (Review) *Nature* 343: 425.

10. Brown M S, Goldstein J L. 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. (Review) *Cell* 89: 331.

11. Jackson S M, Ericsson J, Metherall J E, Edwards P A. 1996. Role for sterol regulatory element binding protein in the regulation of farnesyl diphospate synthase and in the control of cellular levels of cholesterol and triglyceride: evidence from sterol regulation-defective cells. *J. Lipid Res.* 37: 1712.

12. Sakai J, Duncan E A, Rawson R B, Hua X, Brown M S, Goldstein J L. 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. *Cell* 85: 1037.

13. Sakai J, Nohturfft A, Cheng D, Ho Y K, Brown M S, Goldstein J L. 1997. Identification of complexes between the COOH-terminal domains of sterol regulatory element-binding proteins (SREBPs) and SREBP cleavage-activating protein. *J. Biol. Chem.* 272: 20213.

14. Sakai J, Nohturfft A, Goldstein J L, Brown M S. 1998. Cleavage of sterol regulatory element-binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. Evidence from *in vivo* competition studies. *J. Biol. Chem.* 273: 5785.

15. Wang X, Sato R, Brown M S, Hua X, Goldstein J L. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77: 53.

16. Lawler J F Jr, Yin M, Diehl A M, Roberts E, Chatterjee S. 1998. Tumor necrosis factor-alpha stimulates the maturation of sterol regulatory element binding protein-1 in human hepatocytes through the action of neutral sphingomyelinase. *J. Biol. Chem.* 273: 5053.

17. Gierens H, Nauck M, Roth M, Schinker R, Schurmann C, Scharnagl H, Neuhaus G, Wieland H, Marz W. 2000. Interleukin-6 stimulates LDL receptor gene expression *via* activation of sterol-responsive and Sp1 binding elements. *Arterioscler. Thromb. Vasc. Biol.* 20: 1777.

18. Waite K, Floyd Z E, Arbour-Reily P, Stephens J M. 2001. Interferon-gamma-induced regulation of peroxisome proliferator-activated receptor gamma and STATs in adipocytes. *J. Biol. Chem.* 276: 7062.

19. Rosenstreich D L, Vogel S N, Jacques A R, Wahl L M, Oppenheim J J. 1978. Macrophage sensitivity to endotoxin: genetic control by a single codominant gene. *J. Immunol.* 121: 1664.

20. Fruscella P, Romano M, Albani D, Bernasconi S, Sironi M, Luini W, Bruno A, Salmona M, Diomede L. 2000. Inhibition of HMG-CoA reductase activity by hypercholesterolemia reduces leukocyte recruitment and MCP-1 production in local inflammation. *Cytokine* 12: 1100.

21. Aggarwal B B, Kohr W J, Hass P E, Moffat B, Spencer S A, Henzel W J, Bringman T S, Nedwin G E, Goeddel D V, Harkins R N. 1985. Human tumor necrosis factor.

Production, purification and characterization. *J. Biol. Chem.* 260: 2345.

22. Folch J, Lees M, Sloane Stanley G H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497.

23. Sheng Z, Otani H, Brown M S, Goldstein J L. 1995. Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. (Review) *Proc. Natl. Acad. Sci. USA.* 92: 935.

24. Yeagle P L. 1985. Cholesterol and the cell membrane. (Review) *Biochim. Biophys. Acta.* 822: 267.

25. Terkeltaub R, Solan J, Barry M Jr, Santoro D, Bokoch G M. 1994. Role of the mevalonate pathway of isoprenoid synthesis in IL-8 generation by activated monocytic cells. *J. Leukoc. Biol.* 55: 749.

26. Kim S Y, Guijarro C, O'Donnell M P, Kasiske B L, Kim Y, Keane W F. 1995. Human mesangial cell production of monocyte chemoattractant protein-1: modulation by lovastatin. *Kidney Int.* 48: 363.

27. Romano M, Diomede L, Sironi M, Massimiliano L, Sottocorno M, Polentarutti N, Guglielmotti A, Albavi D, Bruno A, Fruscella P, Salmona M, Vecchi A, Pinza M, Mantovani M. 2000. Inhibition of monocyte chemotactic protein-1 synthesis by statins. *Lab. Invest.* 80: 1095.

Figures

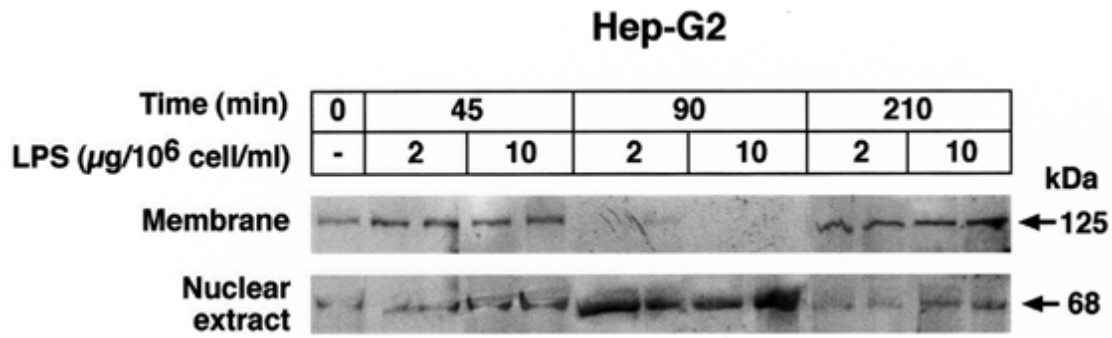


Figure 1
Effect of endotoxin on SREBP-1 activation in HepG2

cells. Western blot analysis of membrane and nuclear extracts of HepG2 cells stimulated with endotoxin (LPS). Cells were pre-incubated for 24 hours in complete fresh medium supplemented with 10% FBS and then exposed to LPS (2 and 10 mug/10^6 cells/ml) for up to 210 minutes. The membrane and nuclear extracts (25 mug protein per lane) were analysed by SDS-PAGE and transferred electrophoretically to a nitrocellulose filter. Immunoblot analysis was carried out with rabbit SREBP-1 polyclonal antibody and the bound antibodies were visualized as described in Methods. Arrows indicate the 125-kDa precursor and the 68-kDa mature form of SREBP-1. One representative filter of three independent experiments is shown.

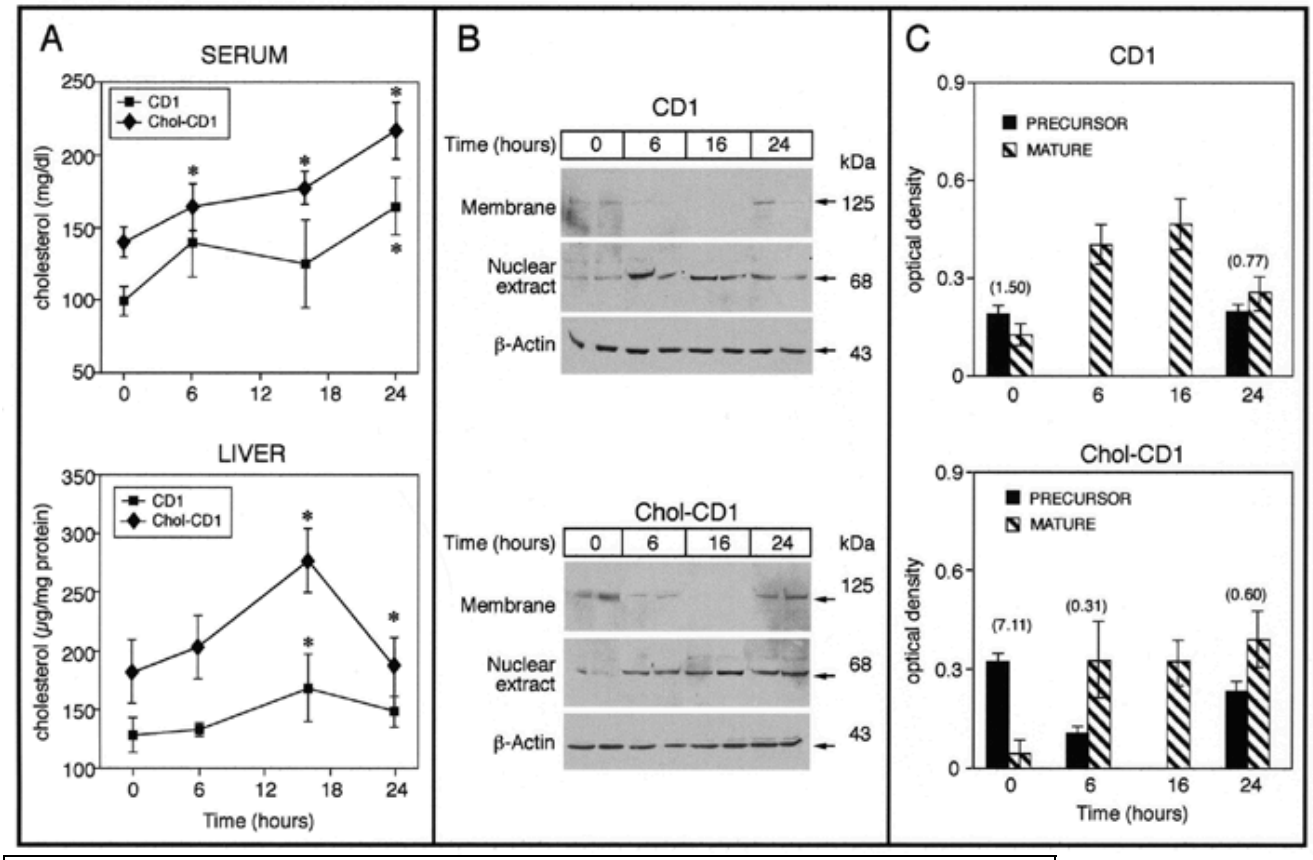


Figure 2

Effect of endotoxin on cholesterol levels and hepatic SREBP-1 activation in CD1 mice. LPS, 2.5 mug/mouse, was injected intraperitoneally to CD1 mice fed a standard

(CD1) or a cholesterol-enriched (Chol CD1) diet. Before (time 0) and 6, 16 and 24 hours after LPS, animals were killed and the blood and liver were collected. **A:** serum and hepatic cholesterol levels. Serum and hepatic cholesterol were measured as described in Methods. Each value is the mean ± standard deviation of at least five determinations on different animals. * p ¾ 0.01 according to Student's t test *versus* time 0. **B:** immunoblots of anti-SREBP-1 antibody. Liver homogenates were used to prepare the membrane and nuclear extract as described in Methods. Aliquots of the membrane and nuclear extracts, containing the same amount of proteins (50 mug protein per lane), were analyzed by SDS-PAGE, transferred electrophoretically to a nitrocellulose filter and immunoblot analysis was carried out using a rabbit SREBP-1 polyclonal antibody. A rabbit beta-actin polyclonal antibody was used to verify that the same amount of protein was loaded in each lane. Western blot analysis, representative of two of five livers, is shown. The arrows indicate the 125-kDa precursor and the 68-kDa mature form of SREBP-1 and the 43-kDa band of beta-actin. One representative filter of three independent experiments is shown. **C:** quantitative data of hepatic SREBP-1. Immunoreactivity of the Western blots from livers of CD1 mice given LPS was quantified by scanning densitometry, as reported in Methods. The time of exposure to LPS is plotted against the precursor and mature levels of SREBP-1, expressed as optical density. Each bar represents the mean ± standard deviation (three experiments) of the optical density of immunoreactivity of SREBP-1 antibody. The numbers above the bars indicate the precursor-to-mature form ratio.

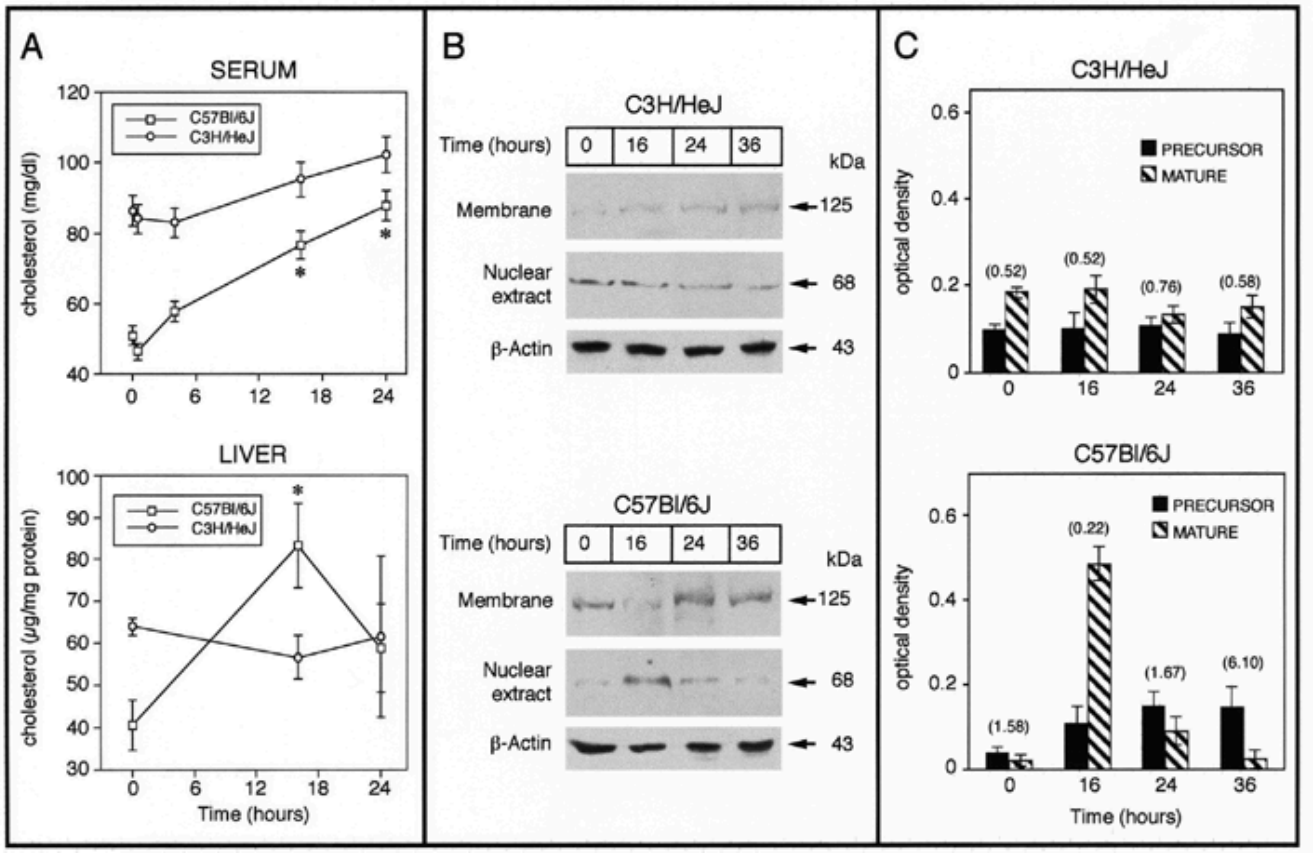


Figure 3

Effect of endotoxin on cholesterol levels and hepatic SREBP-1 activation in C57Bl/6J and C3H/HeJ mice. LPS, 2.5 mug/mouse, were injected intraperitoneally to C57Bl/6J endotoxin-sensitive and C3H/HEJ endotoxin-resistant mice. Before (time 0) and 16, 24 and 36 hours after LPS, animals were killed and the blood and liver were collected. **A:** serum and hepatic cholesterol levels. Serum and hepatic cholesterol were measured as described in Methods. Each value is the mean ± standard deviation of at least five determinations on different animals. *p ¾ 0.01 according to Student's t test *versus* time 0. **B:** immunoblots of anti-SREBP-1 antibody. Liver homogenates were used to prepare the membrane and nuclear extract as described in Methods. Aliquots of the membrane and nuclear extracts containing the same amount of proteins (50 mug protein per lane) were analysed by SDS-PAGE and transferred electrophoretically to a nitrocellulose filter. Immunoblot analysis was carried out with rabbit SREBP-1 polyclonal

antibody. We used a rabbit beta-actin polyclonal antibody to verify that the same amount of protein was loaded in each lane. One representative Western blot analysis of three independent experiments is shown. Arrows indicate the 125-kDa precursor and the 68-kDa mature form of SREBP-1 and the 43-kDa band of beta-actin. **C:** quantitative data of hepatic SREBP-1. Immunoreactivity of the Western blots from at least five livers of C57Bl/6J and C3H/HeJ mice given LPS was quantified by scanning densitometry, as reported in Methods. The time of exposure to LPS is plotted against the precursor and mature levels of SREBP-1, expressed as optical density. The numbers above the bars indicate the precursor-to-mature form ratio. Each bar represents the mean of at least three determinations calculated from on three experiments.

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SREBP-1, LPS, HepG2, liver, mice.

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Ulrich Böcker, Tobias Manigold, Joanna M. Watson, Manfred V. Singer, Siegbert Rossol
human, cytokine, bacterial antigen, CD14, signal transduction.

Interleukine-6 (IL-6) synthesis and gp130 expression by human pericardium

Emmanuelle Menet, Pierre Corbi, Cécile Ancey, Franck Morel, Adriana Delwail, Martine Garcia, Anne-Marie Osta, John Wijdenes, Daniel Potreau, Jean-Claude Lecron
IL-6, gp130, pericardium.

Plasmodium falciparum liver-stage antigen-1 peptide-specific interferon-gamma responses are not suppressed during uncomplicated malaria in African children

Adrian J.F. Luty, Martina Bongartz, Philipp Rezbach, Jean-Francois Faucher, Michael R. Hollingdale, Peter G. Kremsner
malaria, cytokine, interferon-gamma, liver.

Immunoexpressions of p21, Rb, mcl-1 and bad gene products in normal, hyperplastic and carcinomatous human prostates

Mar Royuela, María Isabel Arenas, Fermín R. Bethencourt, Manuel Sánchez-Chapado, Benito Fraile, Ricardo Paniagua
p21, Rb, mcl-1, bad, human prostate, benign prostatic hyperplasia, prostatic carcinoma.

Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic

myelodepression in patients with malignant tumors I. Phase I-II clinical trials of recombinant human interleukin-1 beta as a leukopoiesis stimulator in cancer patients receiving combination chemotherapy

M.L. Gershanovich, L.V. Filatova, S.A. Ketlinsky, A.S. Simbirtsev
human recombinant interleukin-1 beta, myelopoiesis, myelodepression, cancer chemotherapy.

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human recombinant interleukin-1 beta, leukopoiesis, myelodepression, cancer chemotherapy.

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