Role for Monokines in the Metabolic Effects of Endotoxin

Interferon-γ Restores Responsiveness of C3H/HeJ Mice In Vivo

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Abstract

To examine the role of cytokines in mediating the lipogenic effects of endotoxin (LPS), we studied the effects of LPS and cytokines on hepatic fatty acid synthesis in LPS-sensitive C3H/OuJ mice and in LPS-resistant C3H/HeJ mice, whose macrophages are defective in the ability to produce tumor necrosis factor (TNF) and IL-1 in response to LPS. HeJ mice were 16-fold less sensitive than OuJ mice to the lipogenic effect of LPS. In OuJ mice, 10 μg of LPS caused a maximal increase in hepatic lipogenesis (3.86±0.41-fold), whereas in HeJ mice the maximal increase was only 1.79±0.32-fold after 100 μg of LPS. This lipogenic response paralleled the decreased ability of LPS to increase hepatic and splenic levels of mRNAs for TNF and IL-1 and serum levels of TNF in HeJ mice. In contrast, the maximal effect of TNF on lipogenesis was greater and the sensitivity to TNF was increased 2.4-fold in HeJ mice compared to OuJ mice. Administration of IFN-γ before LPS in HeJ mice had no effect on IL-1 mRNA, but partially restored the LPS-induced increase in hepatic and splenic mRNA for TNF and serum TNF levels, which may account for the partial restoration of sensitivity to the lipogenic effect of LPS after IFN-γ treatment. These results indicate that cytokines produced by mononuclear leukocytes mediate the lipogenic effects of LPS. (*J. Clin. Invest.* 1992. 89:1603–1609.) Key words: cytokines • fatty acid synthesis • interleukin • macrophages • tumor necrosis factor

Introduction

In addition to destroying infectious agents and developing immunity to subsequent challenge by these agents, the host responds to infection with multiple changes in intermediary metabolism (1). Prominent among these changes is hypertriglyceridemia due to the accumulation of very low density lipoprotein (VLDL), which is seen both in response to endotoxin (LPS) and during infection (2–7). Infection or LPS may increase serum triglycerides by at least two mechanisms: (*a*) a decrease in triglyceride clearance due to reductions in lipoprotein lipase activity (5); (*b*) an increase in VLDL production that may be due to increased de novo synthesis of fatty acids (6) or increased reesterification of fatty acids derived from the periphery (7).

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The host response to infection is coordinated by a variety of cytokines including the tumor necrosis factors (TNF),[1] the interleukins (IL), and the interferons (IFN) (8–10). It is likely that these cytokines also mediate the metabolic disturbances that occur during infection. In vivo, TNF causes hypertriglyceridemia by increasing hepatic VLDL production through a combination of increased fatty acid synthesis in the liver and reesterification of peripherally derived fatty acids, rather than by decreasing lipoprotein lipase and triglyceride clearance (11–16). IL-1, IL-6, and IFN-α have also been shown to increase hepatic fatty acid synthesis (17, 18). TNF and IL-1 increase hepatic fatty acid synthesis at the same doses that induce fever, suggesting that the metabolic changes in the liver are closely linked to the response to infection (17).

Although it appears logical that cytokines would also mediate the metabolic disturbances of infection, their role has not been directly tested in vivo. Therefore, we have studied the response to LPS in an LPS-resistant mouse strain (C3H/HeJ) and an LPS-sensitive mouse strain (C3H/OuJ). C3H/HeJ mice show decreased responses to LPS, including a decrease in the ability of LPS to induce production of cytokines such as TNF and IL-1 by their macrophages (19–25). However, treatment of cultured macrophages from C3H/HeJ mice with IFN-γ in vitro restores the ability of LPS to increase levels of TNF and IL-1 mRNA and TNF protein in response to LPS in vitro (26–28). Recent data suggest that adherent monocytes or macrophages studied in vitro may show different regulation of both cytokine gene expression and cytokine protein production than is seen in vivo (10, 29).

Therefore, we have studied intact C3H/HeJ mice and demonstrate that, in parallel to their decreased ability to produce cytokine mRNA and protein in response to LPS in vivo, C3H/HeJ mice show a dramatically reduced ability to increase hepatic fatty acid synthesis after endotoxin challenge. However, treatment of C3H/HeJ mice in vivo with murine IFN-γ partially restores both the ability of these mice to produce TNF in response to LPS in vivo and the ability of LPS to stimulate hepatic fatty acid synthesis.

Methods

Materials. ^3H_2O (1 Ci/g) was purchased from ICN Radiochemicals (Cleveland, OH). [26-^{14}C]cholesterol (0.5 mCi/0.33), and [^{14}C]oleic acid (40–60 mCi/mol) were obtained from New England Nuclear (Boston, MA). CytoScint scintillation fluid was purchased from ICN Biomedicals, Inc. (Irvine, CA). *Escherichia coli,* strain 055:B5 endotoxin 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). Murine TNF-α with a specific activity of 2.9×10^7 U/mg and murine IFN-γ with a specific activity of 5×10^6 U/mg were kindly provided by Genentech, Inc. (South San

1. *Abbreviation used in this paper:* TNF, tumor necrosis factor.

Francisco, CA). Recombinant human IL-1β (112–269) with a specific activity of 5×10^7 U/mg was produced as described previously (30). WEHI 164 clone 13 cells were kindly provided by Dr. M. Palladino of Genentech, Inc. Multiprime DNA labeling system was obtained from Amersham Corp., (Arlington Heights, IL). The cDNA for murine TNF-α (31) was kindly provided by Dr. Bruce Beutler of the University of Texas, Southwestern Medical Center. The cDNA for murine IL-1β was kindly provided by Dr. Arjun Singh of Genentech, Inc. (32). The cDNA for actin was kindly provided by Dr. Peter Gunning of Stanford University.

Animals. Male mice, 4–5 wk old (C3H/OuJ and C3H/HeJ) were purchased from Jackson Laboratories Inc. (Bar Harbor, ME). C3H/OuJ is a substrain that was separated from the C3H/HeJ strain before the origin of the defective LPS response in C3H/HeJ; C3H/OuJ is sensitive to LPS and has many of the characteristics of the original CeH/HeJ strain (data on file, Jackson Laboratories, Inc.). Animals were maintained on a normal 12-h light cycle and were fed Purina Mouse Chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. On the morning of the study, after removal of food, animals were divided into groups of 5 and injected with the specified doses of LPS and/or cytokine or with the appropriate vehicle alone (controls). LPS was administered i.p. in 0.9% saline solution. Murine TNF-α, IL-1β, and IFN-γ were administered i.m. in 0.1% human serum albumin, a diluent which has previously been shown to have no effect on hepatic lipogenesis (17).

Measurement of lipogenesis. ^3H_2O (10 mCi) was injected i.p. 4 h after LPS or 1 h after TNF or IL-1 administration. 1 h later the animals were weighed and a blood specimen was obtained. The animals were euthanized and incorporation of ^3H_2O into fatty acids in the liver was determined as described previously (17). The validity of our methodology for measuring lipid synthesis has been demonstrated in earlier publications (33, 34).

TNF assay. TNF activity in serum was measured using the TNF-sensitive cell line, WEHI 164 clone 13, in a cytotoxicity assay which was developed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium blue (35). Concentrations were calculated by comparison with a recombinant murine TNF-α standard.

Northern blot analysis. Total RNA was isolated using the guanidine isothiocyanate phenol-chloroform extraction method (36) and batch absorbed to oligo (dT)-cellulose; polyadenylated mRNA was then batch eluted (37). Identical amounts of RNA were fractionated in 1% agarose gels containing 2.2 M formaldehyde, then were transferred to nylon membranes electrophoretically. Membranes were prehybridized at 65°C for 1 h in 5× SSC, 2× Denhardt's, 2% SDS, and 10% dextran sulfate containing 100 μg/ml sheared salmon sperm DNA. cDNA probes were labeled by the random priming technique (37) using the Multiprime DNA labeling system. Hybridization with [^{32}P]cDNA probes was conducted at 65°C overnight in the same buffer. Blots were washed with 0.2× SSC, 0.1% SDS at room temperature for 30 min, then at 65°C for 1 h. Blots were exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) at −70°C using Cronex intensifying screens for the time indicated in the figure legends. Autoradiograph intensity was quantified by densitometry.

Statistics. Values are presented as mean±SEM. ANOVA was used for multiple comparisons. The independent t test was used to compare the same dose between the two mouse strains.

Results

Effect of LPS on hepatic fatty acid synthesis. The dose-response curves for LPS-stimulated hepatic fatty acid synthesis in OuJ and HeJ mice, measured at 4–5 h after LPS administration, are shown in Fig. 1. In OuJ mice, the half-maximal dose for increasing hepatic fatty acid synthesis is ~ 0.18 μg of LPS, whereas in HeJ mice, the half-maximal dose is ~ 2.6 μg of LPS. Hepatic fatty acid synthesis is stimulated 3.86±0.41-fold ($P < 0.005$ vs. saline control) at 10 μg of LPS in OuJ mice. In

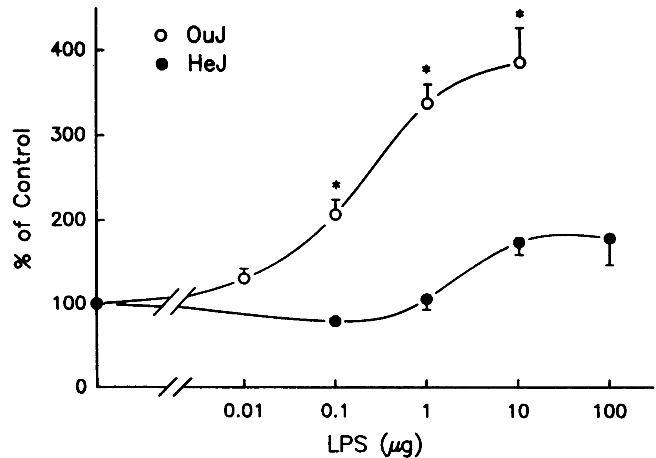

Figure 1. Effect of LPS on hepatic fatty acid synthesis in LPS-sensitive (C3H/OuJ) and LPS-resistant (C3H/HeJ) mice. Mice were injected with normal saline or the indicated concentration of LPS i.p. 4 h later the animals were injected with 10 mCi ^3H_2O and hepatic fatty acid synthesis measured as described under methods. (●) C3H/HeJ mice; (○) C3H/OuJ mice. n = 10–15 for each condition. *$P < 0.002$ vs. HeJ.

contrast, in HeJ mice, 100 μg of LPS stimulated hepatic fatty acid synthesis by only 1.79±0.32-fold ($P < 0.05$ vs. saline control). The ability of LPS to stimulate hepatic fatty acid synthesis in HeJ mice at 0.1–10 μg was significantly less than that seen with OuJ mice ($P < 0.002$).

Effect of LPS on cytokine mRNA. mRNA was prepared from mouse liver and spleen 2 h after LPS or saline administration. Northern blot analysis was performed using full-length cDNA probes for murine TNF and IL-1β (Fig. 2). TNF mRNA was not detected in the liver of saline-treated animals (Fig. 2). However, in OuJ mice, a small dose of LPS (0.01 μg) was able to induce significant levels of TNF mRNA. Levels of TNF mRNA increased with higher doses of LPS, and the effect began to taper between 1 and 10 μg of LPS. In contrast, HeJ mice were resistant to the ability of LPS to increase mRNA for TNF (Fig. 2, *right*); significant levels of TNF mRNA were detected after administration of 10 μg of LPS. Levels of TNF mRNA induced by 100 μg of LPS in HeJ mice are comparable to those

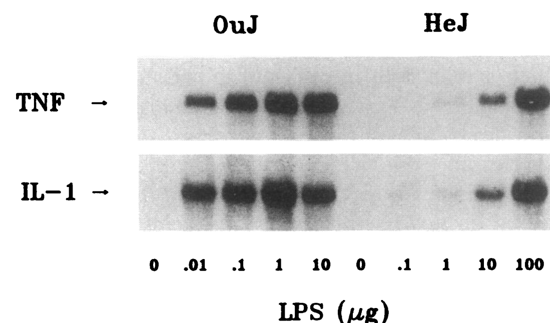

Figure 2. Effect of LPS on hepatic cytokine mRNA. C3H/OuJ or C3H/HeJ mice were injected with normal saline or the concentrations of LPS indicated. 2 h later livers were removed, RNA was extracted, and polyadenylated mRNA was isolated as described under methods. mRNA was subjected to gel electrophoresis, electrophoretically transferred, and probed with random primed [^{32}P]cDNA probes for TNF and IL-1 as indicated. Blots were exposed to film for 1 d for TNF and 8 h for IL-1.

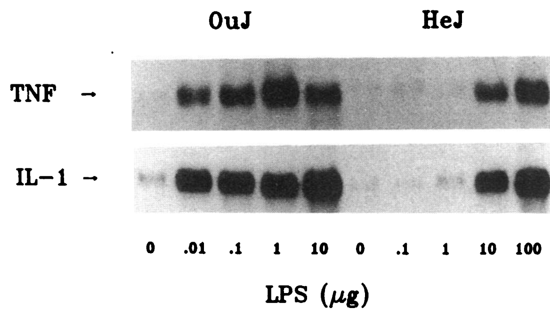

Figure 3. Effect of LPS on splenic cytokine mRNA in LPS-sensitive (C3H/OuJ) and LPS-resistant (C3H/HeJ) mice. The protocol used is identical to that in Fig. 2 except that spleens were used. Blots were exposed to film for 1 h for TNF and 2 h for IL-1.

induced by 1 μg of LPS in OuJ mice. A similar pattern was seen for LPS induction of the hepatic mRNA for IL-1 (Fig. 2).

In the spleen the same differential effect of LPS was seen on cytokine mRNAs (Fig. 3). Significant induction of TNF and IL-1 mRNAs could be seen at doses as low as 0.01 μg of LPS in OuJ mice. Maximal response was seen between 1 and 10 μg of LPS. In contrast, significant induction of the mRNA for TNF and IL-1 was not seen in the spleen of HeJ mice until LPS doses of 10 μg were used; 100 μg of LPS was needed in HeJ mice to reach mRNA levels for TNF and IL-1 that are comparable to those seen at much lower doses in OuJ mice (Fig. 3).

Effect of LPS on serum TNF levels. TNF was not detectable in the serum of mice 120 min after the administration of saline (Fig. 4). Administration of LPS caused a striking increase in serum TNF levels with a peak value of 10.1±2.5 ng/ml at 1 μg of LPS in OuJ mice (Fig. 4). In contrast, LPS is markedly less effective at inducing TNF in HeJ mice with a peak value of 0.18±0.03 ng/ml serum observed at 100 μg of LPS (Fig. 4, *inset*).

Effect of cytokines on lipid metabolism in OuJ and HeJ mice. In contrast to the decreased response to LPS, HeJ mice were significantly more sensitive to the effects of murine TNF

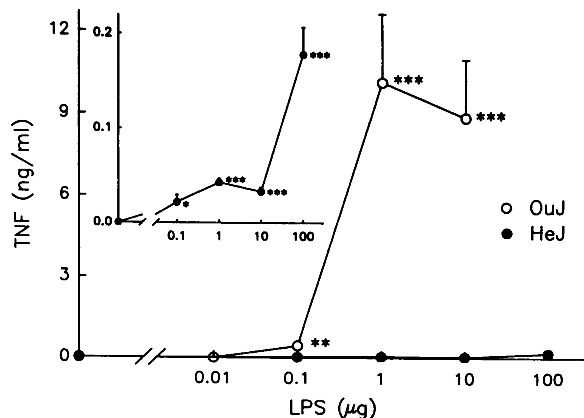

Figure 4. Effect of LPS on circulating TNF levels in LPS-sensitive (C3H/OuJ) and LPS-resistant (C3H/HeJ) mice. Mice were treated with normal saline or LPS at the concentrations indicated on the abscissa. 2 h later serum samples were drawn and TNF levels were measured by bioassay as described under Methods. (○) C3H/OuJ; (●) C3H/HeJ; *$P < 0.05$; **$P < 0.02$; ***$P < 0.005$ vs. control. $n = 5$ for each condition.

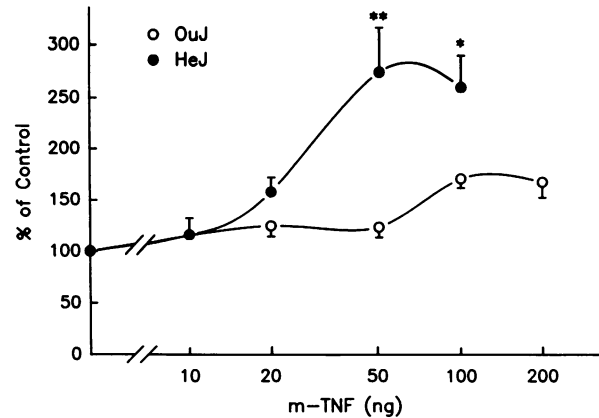

Figure 5. Effect of murine TNF on hepatic lipogenesis in LPS-sensitive (C3H/OuJ) mice and LPS-resistant (C3H/HeJ) mice. Mice were treated with normal saline or murine TNF at the concentrations indicated on the abscissa. 1 h later animals received 10 mCi of ^3H_2O and hepatic fatty acid synthesis was measured as described under Methods. (○) C3H/OuJ; (●) C3H/HeJ. *$P < 0.02$, **$P < 0.005$ vs. C3H/OuJ. $n = 10$ for each condition.

on lipid metabolism than were OuJ mice (Fig. 5). TNF increased hepatic fatty acid synthesis in both HeJ and OuJ mice; however, the ED_{50} for murine TNF was 26 ng in HeJ mice whereas in comparison the ED_{50} was 63 ng in OuJ mice. Maximal stimulation of hepatic de novo fatty acid synthesis was 2.7-fold at 50 ng in HeJ mice ($P < 0.001$ vs. saline control), whereas maximal stimulation was only 1.7-fold at 100 ng in OuJ mice ($P < 0.001$ vs. saline control). The effectiveness of the 50- and 100-ng doses of murine TNF was significantly greater in HeJ mice compared to OuJ mice (50 ng, $P < 0.005$; 100 ng, $P < 0.02$ for HeJ vs. OuJ). There was a trend for HeJ mice to be slightly more sensitive to the effects of IL-1 on hepatic lipogenesis, with an ED_{50} of 22 ng compared to an ED_{50} of > 50 ng in OuJ mice (Fig. 6). However, unlike the results found with TNF, maximal stimulation of lipogenesis by IL-1 was not greater in HeJ mice than in OuJ mice.

Effect of IFN-γ on LPS responsiveness in HeJ Mice. Because treatment of macrophages from HeJ mice with IFN-γ in

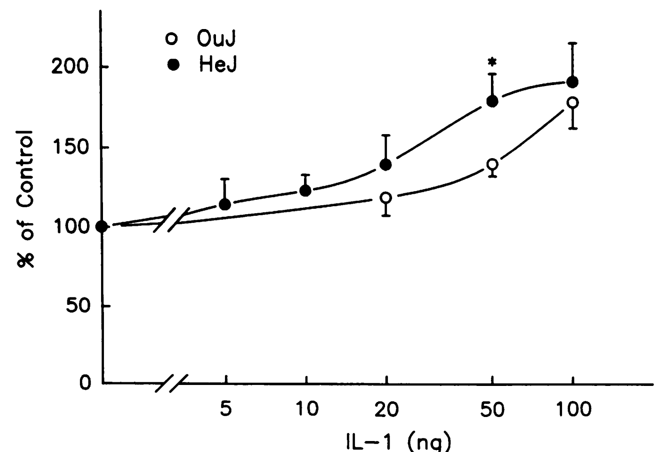

Figure 6. Effect of IL-1 on hepatic fatty acid synthesis in LPS-sensitive (C3H/OuJ) mice and LPS-resistant (C3H/HeJ) mice. Protocol is identical to that of Fig. 5 except that IL-1 was used. *$P < 0.05$ vs. C3H/OuJ. $n = 10$ for each condition.

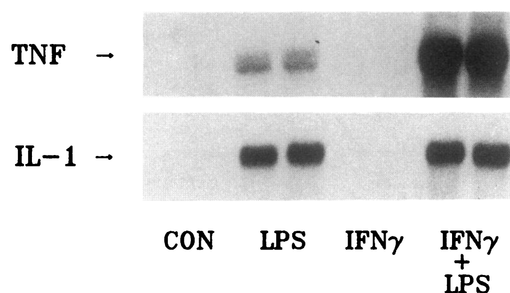

Figure 7. Effect of IFN-γ on LPS-induced hepatic cytokine mRNA in C3H/HeJ mice. Animals were treated with normal saline (*CON*), 5 μg of LPS (*LPS*), 50 μg of IFN-γ (*IFN-γ*) or the combination of 50 μg IFN-γ and 5 μg of LPS (*IFN-γ + LPS*). 2 h later livers were removed, mRNA was extracted and Northern blots were performed as described under Methods. Although the technique is identical to that in Fig. 2, the blots were exposed for 3 d for TNF and 24 h for IL-1.

vitro has been shown to restore their responsiveness to LPS (26–28), we determined whether treatment of intact HeJ mice with IFN-γ could restore the ability of LPS to induce cytokines and increase hepatic fatty acid synthesis in vivo. HeJ mice were treated with 50 μg of IFN-γ or saline i.m., followed by either saline or 5 μg of LPS i.p. 2 h later, livers and spleens were removed and poly A–rich RNA was prepared. Consistent with our earlier observations, 5 μg of LPS alone could induce a small amount of mRNA for TNF (Fig. 7). IFN-γ by itself had no effect on mRNA for TNF in liver. However, the combination of IFN-γ followed by LPS induced a ninefold increase in the mRNA for TNF in liver compared to endotoxin alone (also see Table I where data are expressed relative to the mRNA for actin).

As seen previously, there was negligible mRNA for IL-1 in the liver of saline-treated mice; however, 5 μg of LPS alone induced detectable amounts of mRNA for IL-1 in HeJ mice (Fig. 7, Table I). Administration of IFN-γ had minimal effect on IL-1 mRNA in liver. In contrast to the results with TNF, the combination of IFN-γ plus LPS induced little further increase in hepatic IL-1 mRNA compared to LPS alone.

Similar data were obtained when analyzing the mRNA for TNF and IL-1 in spleen (Fig. 8, Table I). LPS alone induced a small amount of mRNA for TNF; the combination of IFN-γ plus LPS resulted in a striking increase in the amount of TNF mRNA in spleen. LPS alone induced a significant increase in the mRNA for IL-1 in spleen. However, the combination of IFN-γ and LPS caused no further increase in splenic IL-1 and mRNA compared to LPS alone.

We then tested the effect of IFN-γ on serum TNF levels. Consistent with the data in Fig. 4, treatment of HeJ mice with 5 μg of LPS alone led to low but detectable levels of TNF in the serum (Table I). IFN-γ by itself was not able to induce detectable levels of TNF. However, the combination of 50 μg of IFN gamma and 5 μg of LPS produced a 17-fold increase in serum TNF levels compared to LPS alone (Table I). Thus, the effect of IFN-γ on the ability of LPS to induce TNF mRNA was paralleled by the effect of IFN-γ at enhancing the LPS-induced increases in serum TNF levels (Table I). Serum levels of IL-1 were not measured, owing to the difficulty in reliably interpreting circulating IL-1 levels given the presence of inhibitors in serum.

In parallel to its effects on TNF mRNA and circulating protein levels, IFN-γ was able to partially restore the ability of LPS to stimulate hepatic fatty acid synthesis in C3H/HeJ mice. In two separate experiments, HeJ mice were treated with two different doses of LPS that had no significant effect on hepatic fatty acid synthesis (Fig. 9). IFN-γ alone at 50 μg was also not able to stimulate hepatic lipogenesis significantly (Fig. 9). However, treatment of HeJ mice with 50 μg of IFN-γ just before treatment with LPS restored the ability of LPS to stimulate hepatic fatty acid synthesis at these doses (Fig. 9). The combination of 50 μg of IFN-γ and 5 μg of LPS gave a twofold stimulation of hepatic fatty acid synthesis over control levels.

Discussion

The host response to infectious and inflammatory stimuli results in changes in lipid metabolism such as increased plasma triglycerides, hepatic fatty acid synthesis, and VLDL production (1–7). The ability of cytokines to induce these same changes in lipid metabolism (11–18) suggested that cytokines mediate the metabolic response to infection. To examine this hypothesis, we studied the effects of LPS and cytokines on C3H/HeJ mice, a strain that is genetically resistant to the ef-

Table I. IFN-γ Enhances the Effects of LPS on Cytokines in HeJ Mice

	Control	LPS (5 μg)	IFN-γ (50 μg)	LPS plus IFN-γ
mRNA Levels (*arbitrary units relative to actin*)				
Liver				
TNF	0	2.8±0.032	0	25±4.2
IL-1	0	13±0.085	0.61±0.28	15±1.2
Spleen				
TNF	0	2.6±0.83	0.32±0.028	10±3.1
IL-1	0.36±0.054	11±5.7	0.93±0.27	8.2±1.7
Serum TNF Levels (*ng/ml*)	0	0.011±0.0029	0	0.182±0.059*

Cytokine mRNA levels were determined by analysis of Northern blots presented in Figs. 7 and 8 using densitometry and normalized to actin mRNA levels measured on the same blots; $n = 2$ for each condition and values are mean±SD. Serum TNF levels were determined by bioassay (35); $n = 5$ for each condition and values are mean±SE. * $P < 0.02$ vs. LPS alone.

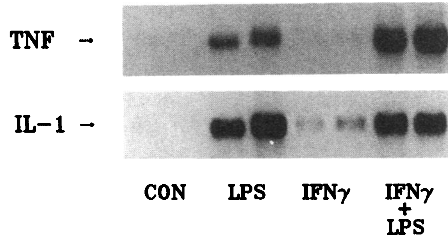

Figure 8. Effect of IFN-γ on LPS-induced splenic cytokine mRNA in C3H/HeJ mice. The protocol is identical to that in Fig. 7 except that spleens were removed for extraction and the exposure was 1 h for TNF and 2 h for IL-1.

fects of LPS (19–25). Previous studies have shown that macrophages from C3H/HeJ mice do not produce TNF or IL-1 mRNA or protein in response to LPS in vitro (24, 25). Beutler et al. (24) have proposed that the defect in the HeJ mouse occurs at two levels: LPS is less able to induce the mRNA for TNF and there is a block in translation of the mRNA with decreased production of TNF in C3H/HeJ macrophages studied in vitro. The resistance of C3H/HeJ mice to endotoxin is genetic (19–25), but this block is not absolute. Treatment of cultured macrophages from C3H/HeJ mice with IFN-γ in vitro restores their ability to respond to LPS with an increase in TNF and IL-1 mRNA and secretion of TNF in vitro (26–28); the secretion of IL-1 was not specifically studied in those experiments (27). However, recent data raise questions as to whether cytokine induction in vitro is different from that seen in vivo, perhaps owing to the process of adherence of cells to plastic or to low level contamination with LPS (10, 29).

In this article we show that genetic resistance to the ability of LPS to induce cytokines is paralleled by resistance to one of the metabolic effects of LPS, the ability to stimulate hepatic lipogenesis. We have shown that C3H/HeJ mice are resistant to the ability of LPS to induce the mRNA for TNF and IL-1 in liver and spleen in vivo when compared to the LPS-sensitive strain C3H/OuJ. Furthermore, this study demonstrates that LPS was less effective at increasing circulating TNF levels in C3H/HeJ mice. Previous data have suggested that the major source of TNF in the circulation is the splanchnic bed (38), and it is therefore likely that both liver and spleen contribute to circulating TNF levels. To our knowledge the resistance of C3H/HeJ mice to LPS induction of TNF and IL-1 has not previously been demonstrated in vivo. With regards to other effects of LPS in vivo, several laboratories have shown that LPS is less capable of inducing circulating granulocyte/macrophage colony-stimulating factor and interferon activity in C3H/HeJ mice (39–41).

Consistent with the proposal of Beutler et al. (24) based on in vitro studies, we find evidence in vivo for a defect in the post-transcriptional regulation of TNF protein in addition to the defect in induction of TNF mRNA. The maximal dose of LPS tested in C3H/HeJ mice (100 μg) induces similar levels of TNF mRNA in liver and spleen compared to much lower doses in C3H/OuJ mice. Yet the absolute levels of TNF protein found in the circulation of C3H/HeJ mice under these conditions of LPS stimulation remain strikingly lower than the level of TNF seen in the circulation of C3H/OuJ mice having similar mRNA levels. It remains to be determined whether these post-transcriptional changes occur at the level of translation of TNF mRNA, within the secretory pathway for TNF protein or at the level of degradation of protein. Thus, although in some in vitro macrophage/monocyte systems cytokine mRNA may be increased without production of cytokine protein as a result of the conditions of incubation in vitro (29), the C3H/HeJ mouse shows defects in both expression of TNF mRNA and the appearance of TNF protein in vivo, similar to what is seen with their macrophages in vitro.

In parallel to the resistance of C3H/HeJ mice to cytokine induction by LPS, these mice were resistant to the ability of LPS to stimulate hepatic fatty acid synthesis, showing both a decrease in the sensitivity to LPS and a decrease in the maximal responsiveness to LPS. This finding indicates that products of LPS-activated mononuclear leukocytes mediate the effect of LPS on hepatic fatty acid synthesis.

We also studied the relative ability of cytokines to directly stimulate hepatic fatty acid synthesis in these two mice strains. In contrast to the resistance to LPS, C3H/HeJ mice were significantly more responsive to the ability of TNF to stimulate hepatic fatty acid synthesis than C3H/OuJ mice, both in terms of maximal response and sensitivity. It is likely that C3H/HeJ mice are not exposed to significant amounts of circulating TNF during life, owing to their defect in the ability to induce TNF. Therefore these mice may have an upregulated response to TNF.

IFN-γ treatment of macrophages from C3H/HeJ mice in vitro partially restores their ability to respond to LPS by increasing TNF mRNA and protein levels in vitro (26–28). We

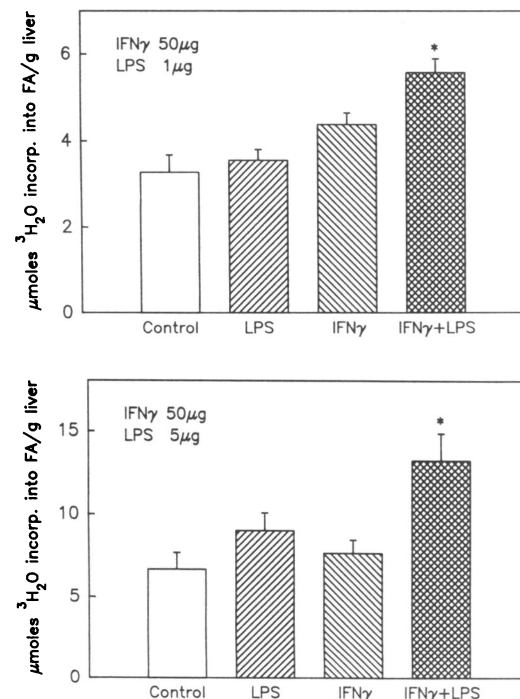

Figure 9. Effect of IFN-γ on LPS-stimulated hepatic fatty acid synthesis in C3H/HeJ mice. Mice were administered IFN-γ (50 μg) or saline i.m. followed by LPS at 1 μg (*upper panel*) or 5 μg (*lower panel*) or saline i.p. where indicated. Four hours later animals received 10 mCi ^3H_2O and hepatic fatty acid synthesis was measured as described under Methods. n = 5 for each condition in the upper panel and n = 10 in the lower panel. *$P < 0.025$ compared to LPS alone, IFN-γ, or control.

have now demonstrated that IFN-γ has similar properties in intact mice. Treatment of C3H/HeJ mice with IFN-γ partially restores the ability of LPS to increase levels of the mRNA for TNF in both liver and spleen. In parallel to the increase seen in TNF mRNA, we found that IFN-γ-treated C3H/HeJ mice produced higher circulating TNF levels in response to LPS. While IFN-γ treatment of C3H/HeJ mice restores the ability of lower-dose LPS (5 μg) to induce maximal levels of TNF mRNA, the levels of circulating TNF found under these conditions, while adequate to induce hepatic fatty acid synthesis, still remain significantly below that found in C3H/OuJ mice. This difference in TNF mRNA and circulating protein may be another example of the defect in post-transcriptional regulation found earlier in vitro (24). Thus, the effect of IFN-γ on TNF previously found using peritoneal macrophages in culture also occurs in tissue macrophages of the spleen and in Kupffer cells in the liver. In contrast to other in vitro data (27), we did not find that pretreatment with IFN-γ led to an increased ability of LPS to induce IL-1 mRNA; it is possible that higher doses or different times are necessary to see such an effect of IFN-γ on IL-1.

Likewise, treatment with IFN-γ partially restored the ability of C3H/HeJ mice to respond to LPS with an increase in hepatic fatty acid synthesis. This experiment provides further evidence that products of activated macrophages are responsible for the metabolic effects of LPS. However, activated macrophages produce a wide array of cytokines and other active substances; thus it remains possible that the effects of LPS are mediated in part by cytokines or products other than TNF (39–41).

The data presented here and elsewhere emphasize that there is a close linkage between the immune or inflammatory response and lipid metabolism, particularly in the liver. Multiple cytokines regulate hepatic and adipose tissue lipid metabolism (11, 16–18, 42–44). These changes in lipid metabolism occur early after administration of cytokines (11, 16–18) and were seen here within 4 h after the response to LPS. The doses of TNF and IL-1 required to stimulate hepatic fatty acid synthesis are similar to those that induce fever (17). Here we show that the dose of LPS required to stimulate hepatic fatty acid synthesis in the sensitive mouse strain, C3H/OuJ, is several orders of magnitude less than that which induces death in LPS sensitive mice (19–21). Thus, it is not surprising that treatment with IFN-γ, which begins to restore the ability of LPS-resistant C3H/HeJ mice to respond to LPS with TNF production also restores the ability of these mice to respond to LPS with an increase in hepatic fatty acid synthesis; C3H/HeJ mice are also more sensitive to TNF, requiring less TNF to increase hepatic lipogenesis compared to C3H/OuJ mice.

The resistance of C3H/HeJ mice to the effects of LPS includes resistance to LPS-induced lethality (19–21), which is also likely to be due to the defect in macrophage TNF and IL-1 secretion, inasmuch as both TNF and IL-1 have been implicated as mediators of LPS-induced shock (45–47). However, this defect in LPS responsiveness dramatically reduces the ability of C3H/HeJ mice to successfully survive infection (48–51), because such cytokines are critical for host defense. Pretreatment of C3H/HeJ mice with TNF and/or IL-1 restores their ability to survive gram negative infection (52, 53). Antibody blockade of TNF also induces susceptibility to infection (54, 55). Thus, the induction of cytokines such as TNF and IL-1 plays a significant role in host defense. In addition to modulating cells of the immune system, LPS, TNF, and IL-1 induce the acute-phase response, a change in the program of liver protein synthesis and secretion that also plays a role in host defense (56, 57). It is likely that the ability of these cytokines to increase hepatic fatty acid synthesis and VLDL secretion (11–18) is part of the acute-phase response. Recent evidence indicates that such changes in lipid metabolism may contribute to host defense. VLDL can bind LPS and prevent LPS lethality, a phenomenon shared by other lipoproteins (58, 59). In addition, VLDL in the circulation of normal humans contains sequestered LPS that has most likely been scavenged during the course of physiological low grade endotoxinemia (59). Lipoproteins also inactivate viruses and parasites (60–65). It is therefore possible that the role of the tight linkage between the immune response and lipid metabolism is to increase circulating lipoprotein levels during infection or to at least maintain lipoproteins at the normal level of circulation in the face of LPS or infection-induced anorexia.

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References

1. Beisel, W. R. 1975. Metabolic response to infection. *Annu. Rev. Med.* 26:9–20.

2. Gallin, J. I., D. Kaye, and W. M. O'Leary. 1969. Serum lipids in infection. *N. Engl. J. Med.* 281:1081–1086.

3. Lees, R. S., R. H. Fiser, Jr., W. R. Beisel, and P. J. Bartelloni. 1972. Effects of an experimental viral infection on plasma lipid and lipoprotein metabolism. *Metab. Clin. Exp.* 21:825–833.

4. Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1972. Infection with *Diplococcus pneumoniae* and *Salmonella typhimurium* in monkeys: changes in plasma lipids and lipoproteins. *J. Infect. Dis.* 125:54–60.

5. Kaufmann, R. L., C. F. Matson, and W. R. Beisel. 1976. Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanisms. *J. Infect. Dis.* 133:548–555.

6. Guckian, J. D. 1973. Role of metabolism in pathogenesis of bacteremia due to *Diplococcus pneumoniae* in rabbits. *J. Infect. Dis.* 127:1–8.

7. Wolfe, R. R., J. H. F. Shaw, and M. J. Durkot. 1985. Effect of sepsis on VLDL kinetics: responses in basal state and during glucose infusion. *Am. J. Physiol.* 248:E732–E740.

8. Baron, S., F. Dianzani, G. J. Stanton, editors. 1981. The interferon system: a review to 1982. *Tex. Rep. Biol. Med.* Parts I and II:1–715.

9. Oppenheim, J. J., and S. Cohen, editors. 1983. Interleukins, lymphokines and cytokines. *In* Proceedings of the Third International Lymphokine Workshop. Academic Press, Inc., New York. 1–799.

10. Dinarello, C. A. 1991. Interleukin-1 and interleukin-1 antagonism. *Blood.* 77:1627–1652.

11. Feingold, K. R., and C. Grunfeld. 1987. Tumor necrosis factor alpha stimulates hepatic lipogenesis in the rat in vivo. *J. Clin. Invest.* 80:184–190.

12. Grunfeld, C., R. Gulli, A. H. Moser, L. A. Gavin, and K. R. Feingold. 1989. The effect of tumor necrosis factor administration in vivo on lipoprotein lipase activity in various tissues of the rat. *J. Lipid Res.* 30:579–585.

13. Feingold, K. R., M. Soued, I. Staprans, L. A. Gavin, M. E. Donahue, B. J. Huang, A. H. Moser, R. Gulli, and C. Grunfeld. 1989. The effect of TNF on lipid metabolism in the diabetic rat: evidence that inhibition of adipose tissue lipoprotein lipase activity is not required for TNF-induced hyperlipidemia. *J. Clin. Invest.* 83:1116–1121.

14. Chajek-Shaul, T., G. Friedman, O. Stein, E. Shiloni, J. Etienne, and Y. Stein. 1989. Mechanisms of the hypertriglyceridemia induced by tumor necrosis factor administration to rats. *Biochim. Biophys. Acta.* 1001:316–324.

15. Feingold, K. R., M. K. Serio, S. Adi, A. H. Moser, and C. Grunfeld. 1989.

Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. *Endocrinology.* 124:2336–2342.

16. Feingold, K. R., M. Soued, S. Adi, I. Staprans, R. Neese, J. Shigenaga, W. Doerrler, A. H. Moser, C. A. Dinarello, and C. Grunfeld. 1991. Effect of interleukin-1 on lipid metabolism in the rat: Similarities to and differences from tumor necrosis factor. *Arterioscler. Thromb.* 11:495–500.

17. Feingold, K. R., M. Soued, M. K. Serio, A. H. Moser, C. A. Dinarello, and C. Grunfeld. 1989. Multiple cytokines stimulate hepatic lipid synthesis in vivo. *Endocrinology.* 125:267–274.

18. Grunfeld, C., A. Adi, M. Soued, A. Moser, W. Fiers, and K. R. Feingold. 1990. The search for mediators of the lipogenic effects of tumor necrosis factor: potential role for interleukin-6. *Cancer Res.* 50:4233–4238.

19. Sultzer, B. M. 1968. Genetic control of leucocyte responses to endotoxin. *Nature (Lond.).* 219:1253–1254.

20. Sultzer, B. M. 1972. Genetic control of host responses to endotoxin. *Infect. Immun.* 5:107–113.

21. Chedid, L., M. Parant, C. Damais, F. Parant, D. Juy, and A. Galelli. 1976. Failure of endotoxin to increase nonspecific resistance to infection of lipopolysaccharide low-responder mice. *Infect. Immun.* 13:722–727.

22. Watson, J., and R. Riblet. 1974. Genetic control of responses to bacteria lipopolysaccharides in mice. I. Evidence for a single gene that influences mitogenic and immunogenic responses to lipopolysaccharides. *J. Exp. Med.* 140:1147–1161.

23. Watson, J., R. Riblet, and B. A. Taylor. 1977. The response of recombinant in-bred strains of mice to bacterial lipopolysaccharides. *J. Immunol.* 118:2088–2093.

24. Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science (Wash. DC).* 232:977–979.

25. Rosenstreich, D. L., S. N. Vogel, A. R. Jacques, L. M. Wahl, and J. J. Oppenheim. 1978. Macrophage sensitivity to endotoxin: genetic control by a single codominant gene. *J. Immunol.* 121:1664–1670.

26. Beutler, B., V. Tkacenko, I. Milsark, N. Krochin, and A. Cerami. 1986. Effect of gamma interferon on cachectin expression by mononuclear phagocytes: reversal of the lps-d (endotoxin resistance) phenotype. *J. Exp. Med.* 164:1791–1796.

27. Collart, M. A., D. Belin, J.-D. Vassali, S. De Kossodo, and P. Vassali. 1986. Gamma interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin-1 and urokinase genes, which are controlled by short-lived repressors. *J. Exp. Med.* 164:2113–2118.

28. Hogan, M. M., and S. N. Vogel. 1988. Production of tumor necrosis factor by rIFN-gamma-primed C3H/HeJ (Lps^d) macrophages requires the presence of lipid A-associated proteins. *J. Immunol.* 141:4196–4202.

29. Schindler, R., B. D. Clark, and C. A. Dinarello. 1990. Dissociation between interleukin-1b mRNA and protein synthesis in human peripheral blood mononuclear cells. *J. Biol. Chem.* 265:10232–10237.

30. Dinarello, C. A., J. A. Cannon, J. W. Mier, H. A. Burnheim, A. LoPreste, D. L. Lynn, R. N. Love, A. C. Webb, P. E. Auron, R. C. Reuben, et al. 1986. Multiple biological activities of human recombinant interleukin 1. *J. Clin. Invest.* 77:1734–1737.

31. Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shimer, and A. Cerami. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA.* 83:1670–1674.

32. Gray, P. W., D. Glaister, E. Chen, D. V. Goeddel, and D. Pennica. 1986. Two interleukin-1 genes in the mouse: cloning and expression of the cDNA for murine interleukin-1 beta. *J. Immunol.* 137:3644–3648.

33. Feingold, K. R., M. H. Wiley, A. MacRae, S. R. Lear, A. Zsigmond, and M. D. Siperstein. 1983. De novo sterologenesis in the intact rat. *Metab. Clin. Exp.* 32:75–81.

34. Feingold, K. R., M. H. Wiley, A. MacRae, A. H. Moser, S. R. Lear, and M. D. Siperstein. 1982. The effect of diabetes mellitus on sterol synthesis in the intact rat. *Diabetes.* 31:388–395.

35. Espevik, T., and J. Nissen-Meger. 1986. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J. Immunol. Methods.* 95:99–105.

36. Chomezinsky, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidiniun thiocianate-phenol-chloroform extraction. *Anal. Biochem.* 162:150–159.

37. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

38. Fong, Y., M. A. Marano, L. L. Moldawer, H. Wei, S. E. Calvano, J. S. Kenney, A. C. Allison, A. Cerami, G. T. Shires, and S. F. Lowry. 1990. The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. *J. Clin. Invest.* 85:1896–1904.

39. Apte, R. N., O. Ascher, and D. H. Pluznik. 1977. Genetic analysis of generation of serum interferon by bacterial lipopolysaccharide. *J. Immunol.* 119:1898–1902.

40. Apte, R. N., and D. H. Pluznik. 1976. Genetic control of lipopolysaccharide induced generation of serum colony stimulating factor and proliferation of splenic granulocyte/macrophage precursor cells. *J. Cell Physiol.* 89:313–324.

41. Vogel, S. N., R. N. Moore, J. D. Sipe, and D. L. Rosenstreich. 1980. BCG-induced enhancement of endotoxin sensitivity in C3H/HeJ mice. I. In vivo Studies. *J. Immunol.* 124:2004–2009.

42. Keay, S., and S. E. Grossberg. 1980. Interferon inhibits the conversion of 3T3-L1 mouse fibroblast into adipocytes. *Proc. Natl. Acad. Sci. USA.* 77:4099–4103.

43. Patton, J. S., H. M. Shepard, H. Wilking, G. Lewis, B. B. Aggarwal, T. E. Eessalu, L. A. Gavin, and C. Grunfeld. 1986. Interferons and tumor necrosis factors have similar catabolic effects on 3T3-L1 cells. *Proc. Natl. Acad. Sci. USA.* 83:8313–8317.

44. Beutler, B. A., and A. Cerami. 1985. Recombinant interleukin-1 suppresses lipoprotein lipase activity in 3T3-L1 cells. *J. Immunol.* 135:3969–3971.

45. Beutler, B., I. W. Milsark, and A. C. Cerami. 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science (Wash. DC).* 229:869–871.

46. Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and C. A. Cerami. 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature (Lond.).* 330:662–664.

47. Wakabayashi, G., J. A. Gelfand, J. F. Burke, R. C. Thompson, and C. A. Dinarello. 1991. A specific receptor antagonist for interleukin-1 prevents *Escherichia coli*-induced shock in rabbits. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 5:338–343.

48. Robson, H. G., and S. I. Vas. 1972. Resistance of in-bred mice to *Salmonella typhimurium. J. Infect. Dis.* 126:378–386.

49. von Jeney, N., E. Gunther, and K. Jann. 1977. Mitogenic stimulation of murine spleen cells: relation to susceptibility to Salmonella infection. *Infect. Immun.* 15:26–33.

50. O'Brien, A. D., D. L. Rosenstreich, I. Scher, G. Campbell, R. P. MacDermott, and S. B. Formal. 1980. Genetic control of susceptibility to *Salmonella typhimurium* in mice: role of the Lps gene. *J. Immunol.* 124:20–24.

51. Hagberg L., R. Hull, S. Hull, J. R. McGhee, S. M. Michalek and C. S. Eden. 1984. Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. *Infect. Immun.* 46:839–844.

52. Cross, A. S., J. C. Sadoff, N. Kelly, E. Bernton, and P. Gemski. 1989. Pretreatment with recombinant murine tumor necrosis factor alpha/cachectin and murine interleukin-1 alpha protects mice from lethal bacterial infection. *J. Exp. Med.* 169:2021–2027.

53. Czuprynski, C., and J. F. Brown. 1987. Recombinant murine interleukin-1 alpha enhancement of nonspecific antibacterial resistance. *Infect. Immun.* 55:2061–2065.

54. Nakane, A., T. Minagawa, and K. Kato. 1988. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against Listeria monocytogenes infection. *Infect. Immun.* 56:2563–2569.

55. Kindler, V., A. P. Sappino, G. E. Grau, P. F. Piguet, and P. Vassalli. 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell.* 56:731–740.

56. Perlmutter, D. H., C. A. Dinarello, P. I. Punsal, and H. R. Colten. 1986. Cachectin/tumor necrosis factor regulates hepatic acute phase gene expression. *J. Clin. Invest.* 78:1349–1354.

57. Darlington, G. J., D. R. Wilson, and L. B. Lachman. 1987. Monocyte conditioned medium, interleukin-1 and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. *J. Cell Biol.* 103:787–793.

58. Ulevitch, R. J., A. R. Johnston, and D. B. Weinstein. 1979. New function for high density lipoproteins: their participation in intravascular reactions of bacterial lipopolysaccharides. *J. Clin. Invest.* 64:1516–1524.

59. Harris, H. W., C. Grunfeld, K. R. Feingold, and J. H. Rapp. 1990. Human VLDL and chylomicrons can protect against endotoxin-induced death in mice. *J. Clin. Invest.* 86:696–702.

60. Shortridge, K. R., W. K. Ho, A. Oya, and M. Kobayashi. 1975. Studies on the inhibitory activities of human serum lipoproteins for Japanese encephalitis virus. *Southeast Asian J. Trop. Med. Public Health.* 6:461–466.

61. Leong, J. C., J. P. Kane, O. Oleszko, and J. A. Levy. 1977. Antigen specific nonimmunoglobulin factor that neutralizes xenotropic virus is associated with mouse serum lipoproteins. *Proc. Natl. Acad. Sci. USA.* 74:276–280.

62. Seganti, L., M. Grass, P. Matromarino, A. Pana, F. Superti, and N. Orsi. 1983. Activity of human serum lipoproteins on the infectivity of rhabdoviruses. *Microbiology.* 6:91–99.

63. Heumer, H. P., H. J. Menzel, D. Potratz, B. Brake, D. Falke, G. Utermann, and M. P. Dierich MP. 1988. Herpes simplex virus binds to human serum lipoproteins. *Intervirology.* 29:68–76.

64. Rifkin, M. R. 1978. Identification of the trypanocidal factor in normal human serum: high density lipoprotein. *Proc. Natl. Acad. Sci. USA.* 75:3450–3454.

65. Hajduk, S. L., D. R. Moore, J. Vasudevacharya, H. Siqueira, A. F. Torri, E. M. Tytler, and J. D. Esko. 1989. Lysis of trypanosoma bruceii by a toxic subspecies of human high density lipoprotein. *J. Biol. Chem.* 264:5210–5217.