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Role of Interleukin-1 in the Depression of Liver Drug Metabolism by Endotoxin

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Endotoxin-resistant C3H/HeJ mice were used to test the hypothesis that a macrophage product, possibly interleukin-1, might mediate the depression of liver cytochrome P-450-dependent drug metabolism in endotoxin-treated mice. Depression of liver drug metabolism by endotoxin was observed in normal mice (C3H/HeN) but not in C3H/HeJ mice. Serum transfer experiments demonstrated that a serum factor was responsible for the depression of liver drug metabolism. Experiments of passive transfer of peritoneal macrophages showed that this endotoxin-induced factor might be a macrophage product. In vitro experiments showed that endotoxin-stimulated monocytes produced a factor that depressed cytochrome P-450-dependent metabolism in cultured hepatocytes. Homogeneous human monocyte and recombinant interleukin-1 also depressed liver drug metabolism both in vivo and in vitro, suggesting that this macrophage product might be involved in the regulation of liver function by the immune system.

Bacterial endotoxins (lipopolysaccharides [LPS]) exert multiple effects on the liver, such as inhibition of glucocorticoid-mediated induction of tryptophan oxygenase and phosphoenolpyruvate carboxykinase (17), induction of acute-phase proteins (25), and depression of microsomal cytochrome P-450 and related drug-metabolizing enzymes (2, 12). Most of the in vivo effects of LPS, including depression of plasma iron and zinc (16), acute-phase response (26), glucocorticoid antagonism (17), antitumor effect (23), and fever (7), were reported to be mediated by macrophage products. Several of these mediators, including endogenous pyrogen, leukocytic endogenous mediator, and lymphocyte-activating factor, are now considered properties of the interleukin-1 (IL-1) family of polypeptides (7). The aim of this work was to investigate the possibility that the depression of liver drug metabolism by LPS might be also mediated via macrophage products, possibly IL-1. The experimental model used to test this hypothesis was the LPS-resistant C3H/HeJ mouse strain. Previous work showed that a normal endotoxemic response, with respect to fever and acute-phase response, can be elicited in C3H/HeJ mice by injection of IL-1 (16), and this strain of mice has been used successfully in studying the origin and nature of LPS-induced glucocorticoid-antagonizing factor (17).

MATERIALS AND METHODS

Animals and treatments. Adult male C3H/HeN mice (Charles River, Calco, Italy) or C3H/HeJ mice (Jackson Laboratories, Bar Harbor, Maine), 7 to 8 weeks of age, were used. Mice were treated with LPS from *Escherichia coli* serotype O55:B5 (Sigma Chemical Co., St. Louis, Mo.) at a dose of 2.5 μg per mouse, intravenously (i.v.), in 0.2 ml of sterile, pyrogen-free saline.

Twenty-four hours after treatment, mice were killed by cervical dislocation, and liver cytochrome P-450-dependent drug metabolism was assessed as ethoxycoumarin deethylase (ED) activity. ED was determined in liver homogenates by the fluorimetric method of Greenlee and Poland (13), and results are expressed as nanomoles per minute per gram of liver.

For serum transfer experiments, C3H/HeJ mice were given i.v. injections (0.2 ml) of serum from C3H/HeN mice bled 90 min after i.v. injection of 2.5 μg of LPS or saline alone.

Serum obtained from C3H/HeN mice treated with LPS or saline is referred to as LPS serum and control serum, respectively. C3H/HeJ mice were killed 24 h after serum injection, and ED activity was determined as described above.

PEC. Peritoneal exudate cells (PEC) were obtained from C3H/HeN mice treated 7 days earlier with casein (1 ml of a 6% solution, intraperitoneally [i.p.]). PEC consisted of macrophages (60 to 70%) and lymphocytes (30 to 40%). Neutrophils were less than 1%. PEC were harvested in phosphate-buffered saline, collected by centrifugation (400 × *g*, 10 min), and finally suspended at 10^7 cells per ml in Hanks balanced salt solution with or without LPS (10 μg/ml). A 0.5-ml amount of this suspension was injected i.p. into C3H/HeJ mice. Control mice received 0.5 ml of Hanks balanced salt solution alone or with LPS. Liver ED was determined 24 h after treatment.

Monocyte supernatants. IL-1-containing supernatants were prepared by culturing Percoll-purified human peripheral blood monocytes for 48 h at 2×10^6 cells per ml in RPMI 1640 medium supplemented with 1% fetal calf serum (endotoxin-free, <0.1 ng/ml by the *Limulus* amebocyte lysate assay), with or without LPS (25 μg/ml). Medium alone, with or without LPS, served as the control. The IL-1 activity of the monocyte-plus-LPS supernatant was 650 U/ml by the thymocyte proliferation assay (22).

Hepatocyte isolation and culture. Hepatocytes were prepared from the livers of Crl:CD(SD)BR rats and purified from nonparenchymal liver cells by the method of Seglen (24), and the cells (0.9×10^6) were seeded in 9.6-cm^2 petri dishes. Hepatocyte culture medium was a mixture of 75% minimum essential medium and 25% medium 199 supplemented with 5% fetal calf serum, 0.2% bovine serum albu-

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TABLE 1. Effect of sera from LPS-treated mice on liver ED activity in C3H/HeJ mice[a]

Treatment	Mean ED (nmol/min per g of liver) ± SE	
	C3H/HeN	C3H/HeJ
Saline	14.9 ± 0.9 (100)	19.6 ± 0.9 (100)
LPS	8.7 ± 0.6 (58)*	19.0 ± 1.7 (97)
Control serum	ND[b]	19.0 ± 1.3 (97)
Post-LPS serum	ND	14.8 ± 1.3 (75)*

[a] C3H/HeJ mice were treated with control serum or post-LPS serum obtained from C3H/HeN mice 90 min after LPS treatment. ED was measured 24 h later. Data are for four to five mice per group. Numbers in parentheses are percentage of control values. *, $P < 0.05$ versus control by Student's t test.

[b] ND, Not done.

min, 1 mg of insulin, and 7×10^{-5} M hydrocortisone acetate. Polymixin B (5 μg/ml) was also added to inactivate the LPS present in the monocyte supernatants. Three hours after hepatocyte seeding, the medium was renewed and monocyte supernatants were added (0.2 ml per 2 ml of hepatocyte culture medium). Homogeneous human monocyte IL-1 was tested at a dose of 20 U/ml. After 18 h of culture the cells were homogenized in 0.05 M phosphate buffer, pH 7.4, and ED was measured.

IL-1. Human IL-1 was purified from the supernatant of adherent human blood monocytes as described previously (8). Briefly, the supernatant was purified by immunoabsorption (9), gel filtration, and chromatofocusing. With [^{35}S]methionine and [^{3}H]leucine intrinsic labeling, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography revealed radioactive amino acids corresponding to the positions of methionine-136 and leucine-142, -145, and -147 of the pI 7 IL-1 sequence predicted by the cDNA (1). Human recombinant pI 7 IL-1 was prepared as described elsewhere (5).

RESULTS

LPS (2.5 μg per mouse, i.v.) caused a marked depression (42%) of liver cytochrome P-450-dependent metabolism, as measured by ED activity 24 h after treatment (Table 1). By contrast, LPS did not affect liver drug metabolism in C3H/HeJ mice. However, when C3H/HeJ mice were treated with 0.2 ml of serum collected from normally responsive C3H/HeN mice 90 min after an i.v. injection of LPS, a depression (25%) of liver drug metabolism was observed. The drug metabolism-depressing activity of post-LPS serum was present 90 min after LPS treatment but was not detected after 6 or 24 h (Table 2). The rapid release of this factor suggests a possible similarity with other known LPS-induced factors (IL-1, glucocorticoid-antagonizing factor, and tumor necrosis factor). Since these mediators are of macrophage origin, and a defective response of macrophages to LPS has been suggested to the mechanism of the LPS resistance of the C3H/HeJ mouse strain (18), we investigated the possible macrophage origin of the drug metabolism-depressing mediator(s). In the experiment reported in Table 3, peritoneal macrophages elicited with casein from C3H/HeN mice were injected i.p. into C3H/HeJ mice. Transfer of macrophages from normal mice into LPS-resistant mice rendered the latter responsive to LPS with respect to the depression of liver drug metabolism. Since most of the liver effects of LPS, particularly the induction of acute-phase proteins, have been shown to be mediated by IL-1, we used two other experimental models of IL-1-mediated acute-phase response, namely i.p. injection of casein- and silver nitrate ($AgNO_3$)-induced local inflammation (26). Both treatments caused a marked depression of liver drug metabolism (Table 4), and in this case no difference was observed between the two mouse strains, in agreement with previous reports indicating that while C3H/HeJ mice are hyporesponsive to LPS, they respond to non-LPS inflammatory agents (26). To test the hypothesis that IL-1 might have a role in this depression of liver drug metabolism, we studied the in vivo effect of homogeneous human monocyte IL-1 and recombinant human IL-1. A depression of liver ED activity was observed with all the IL-1 preparations (Table 5). A dose-response experiment of the in vivo effect of recombinant IL-1 on liver ED is reported in Table 6.

To further characterize the role of macrophage products, and possibly of IL-1, in the LPS-induced depression of liver metabolism, we studied the effect of the conditioned medium from human monocytes stimulated with LPS on isolated rat hepatocytes in vitro. Rat hepatocytes were used because of the higher stability of cytochrome P-450 in cultured rat hepatocytes than in mouse hepatocytes.

IL-1-containing supernatants (monocytes plus LPS) depressed P-450-dependent drug metabolism in rat hepatocytes in a 20-h culture (Table 7). Homogeneous human monocyte

TABLE 2. Time course of drug metabolism-depressing activity in post-LPS serum[a]

Time after LPS (h)	Mean ED (nmol/min per g of liver) ± SE	
	Control serum	Post-LPS serum
1.5	15.2 ± 0.8	11.5 ± 1.1 (76)*
6	15.1 ± 1.8	16.1 ± 1.3 (106)
24	14.9 ± 1.4	14.2 ± 0.8 (95)

[a] C3H/HeJ mice were treated with control serum or post-LPS serum obtained from C3H/HeN mice 90 min, 6 h, or 24 h after LPS treatment. ED was measured in recipient mice 24 h later. Data are for four to five mice per group. ED activity in saline-treated, C3H/HeJ mice was 14.5 ± 1.1 nmol/min per g of liver. Numbers in parentheses are the percentage of respective controls. *, $P < 0.05$ versus respective control by Student's t test.

TABLE 3. Induction of LPS responsiveness in C3H/HeJ mice after transfer of PEC from C3H/HeN mice[a]

Treatment	Mean ED (nmol/min per g of liver) ± SE
Saline	16.0 ± 1.4 (100)
LPS	15.1 ± 0.7 (94)
PEC	15.3 ± 2.1 (96)
PEC plus LPS	11.1 ± 0.7 (69)*

[a] C3H/HeJ mice received PEC from C3H/HeN mice as described in Materials and Methods. ED was measured 24 h later. Data are for four to five mice per group. Numbers in parentheses are percent of control values. *, $P < 0.05$ versus control by Student's t test.

TABLE 4. Depression of liver drug metabolism by casein- and silver nitrate-induced local inflammation in LPS-responsive and LPS-resistant mice[a]

Treatment	Mean ED (nmol/min per g of liver) ± SE	
	C3H/HeN	C3H/HeJ
Control	23.8 ± 1.5 (100)	21.1 ± 1.1 (100)
Casein	13.3 ± 0.8 (56)*	12.5 ± 1.7 (59)*
$AgNO_3$	12.4 ± 0.8 (52)*	11.0 ± 2.4 (52)*

[a] Mice were treated with casein (50 mg per mouse, i.p.) or $AgNO_3$ (10 mg per mouse, subcutaneously) 24 h before the mice were killed. Data are for five mice per group. Numbers in parentheses are percent of control values. *, $P < 0.05$ versus controls by Student's t test.

TABLE 5. Depression of liver drug metabolism by IL-1 in LPS-responsive and LPS-resistant mice[a]

Treatment	Mean ED (nmol/min per g of liver) ± SE	
	C3H/HeN	C3H/HeJ
Saline	26.1 ± 2.1 (100)	17.5 ± 2.1 (100)
Human monocyte IL-1	18.2 ± 1.5 (70)*	12.5 ± 1.4 (71)*
Human recombinant IL-1	21.0 ± 1.5 (80)*	13.7 ± 1.1 (78)*

[a] IL-1 was given i.v. at 40 U per mouse, 24 h before the mice were killed. Data are for four mice per group. Numbers in parentheses are percent of control values. *, $P < 0.05$ by Student's t test.

IL-1 was also active in depressing ED activity in this experimental model.

DISCUSSION

The cytochrome P-450-dependent drug-metabolizing system of the liver is a metabolic system involved in the detoxification of foreign compounds, including drugs, and in the metabolic activation of chemical carcinogens and anticancer drugs. The depression of this system by LPS has been reported (2, 12). To investigate the possible role of macrophages in the depression of liver drug metabolism by LPS, we took advantage of the experimental model used by Moore and colleagues in the characterization of glucocorticoid-antagonizing factor (17). Serum transfer experiments demonstrated the presence of a serum factor that affected liver drug metabolism in LPS-resistant mice. This demonstrated that a second mediator, rather than LPS per se, was responsible for the depression of liver drug metabolism. In fact, this mediator was present shortly after treatment with LPS (i.e., at 90 min and not at 24 h; see Table 2), further supporting the hypothesis that this mediator might be responsible for the depression of liver drug metabolism observed at 24 h but not at 90 min (data not shown) and is not just secondary to this "hepatotoxic" effect of LPS. Since LPS is a known interferon inducer (15) and interferon has been reported to depress liver cytochrome P-450-dependent drug metabolism (11, 19, 21), we considered the possibility that interferon might account for the drug metabolism-depressing activity of post-LPS serum. However, the interferon titer of this serum was low (400 U/ml), and injection of 0.2 ml of serum resulted in a dose of interferon equal to 80 U per mouse. Since higher doses of interferon (10^4 to 10^5 U per mouse) are required to significantly depress liver drug metabolism (19), we could rule out that the serum mediator under investigation was interferon. The observation that transfer of peritoneal macrophages can render C3H/HeJ mice responsive to drug metabolism depression by LPS, together with the ability of LPS-stimulated human monocytes to release a factor(s) that depresses drug-metabolizing enzymes in cultured rat hepatocytes, strongly supported the hypothesis that the mediator involved was a non-species-specific macrophage product such as IL-1 (interferon, unlike IL-1, is strictly species specific). The ability of purified IL-1, including recombinant IL-1, to depress liver drug-metabolizing enzymes in vivo and in vitro suggests that this mediator, known to mediate other nonimmunologic effects of LPS, might have a role in the effect of LPS on liver drug metabolism. This work provides an experimental system for studying the regulation of liver drug metabolism by the immune system through a soluble mediator of macrophage origin and might be important in defining the mechanism of depression of liver drug metabolism observed in pregnancy (6) and various pathological conditions including traumatic injury (14) and burn (3, 4).

TABLE 6. Depression of liver drug metabolism by different doses of recombinant IL-1[a]

IL-1 dose (μg)	Mean ED (nmol/min per g of liver) ± SE
0 (saline control)	31.69 ± 2.54 (100)
0.15	24.35 ± 1.71 (77)
0.30	24.35 ± 1.66 (77)
0.60	22.37 ± 2.97 (71)
2.50	21.45 ± 0.69 (68)*
5.00	19.90 ± 2.00 (63)*

[a] Recombinant IL-1 was given i.v. at the dose indicated 24 h before mice were killed; 1 μg of recombinant IL-1 corresponded to 40 U. Data are for four mice per group. Numbers in parentheses are percent of control values. *, $P < 0.05$ by Student's t test.

TABLE 7. Depression of ED activity in rat hepatocytes incubated in vitro with supernatants from human monocytes or purified IL-1[a]

Treatment	Mean ED (pmol/min per mg of protein) ± SE
Expt 1	
Medium (control)	71.4 ± 7.6 (100)
Medium plus LPS	70.7 ± 8.7 (99)
Monocytes	63.7 ± 4.1 (89)
Monocytes plus LPS	40.9 ± 3.2 (57)*
Expt 2	
Control	60.4 ± 2.4 (100)
Human IL-1	44.8 ± 2.0 (75)*

[a] Incubation conditions are described in Materials and Methods. Data are for four samples per group. Numbers in parentheses are percent of control values. *, $P < 0.05$ by Student's t test.

The data reported do not rule out the possibility that other LPS-induced mediators might also contribute to the depression of cytochrome P-450-dependent metabolism by LPS, and certainly interferon and possibly tumor necrosis factor might be important in this effect.

As far as the mechanism of this effect of IL-1 is concerned, our data do not distinguish whether the depression of liver cytochrome P-450 is due to increased degradation or decreased synthesis. While interferon-mediated depression of cytochrome P-450 was suggested to be mediated via increased degradation (10, 11), IL-1 might be effective by another mechanism. In fact, the general effect of IL-1 on the liver is a switch from the normal proteins to the acute-phase proteins, and decreased gene expression was reported for albumin (20). It is therefore tempting to hypothesize that IL-1 might have a regulatory action on cytochrome P-450 at the transcriptional level.

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LITERATURE CITED

1. **Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello.** 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. Proc. Natl. Acad. Sci. USA 81:7907–7911.
2. **Bissel, D. M., and L. E. Hammaker.** 1976. Cytochrome P-450 heme and the regulation of hepatic heme oxygenase activity.

Arch. Biochem. Biophys. **176**:91–102.
3. **Ciaccio, E. I., and R. J. Fruncillo.** 1979. Decreased aryl hydrocarbon hydroxylase after a 15% burn injury. Biochem. Pharmacol. **28**:3151–3152.
4. **Ciaccio, E. I., and R. J. Fruncillo.** 1979. Urinary excretion of D-glucaric acid by severely burned patients. Clin. Pharmacol. Ther. **25**:340–344.
5. **Dayer, J.-M., B. de Rochementeix, B. Burrus, S. Demczuk, and C. A. Dinarello.** 1986. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. J. Clin. Invest. **77**:645–648.
6. **Dean, M. E., and B. H. Stock.** 1975. Hepatic microsomal metabolism of drugs during pregnancy in the rat. Drug Metab. Dispos. **3**:325–331.
7. **Dinarello, C. A.** 1985. An update on human interleukin-1: from molecular biology to clinical relevance. J. Clin. Immunol. **5**:287–297.
8. **Dinarello, C. A., H. A. Bernheim, J. G. Cannon, G. LoPreste, S. J. C. Warner, A. C. Webb, and P. E. Auron.** 1985. Purified 35-S-methionine, 3-H-leucine labelled human monocyte interleukin-1 with endogenous pyrogen activity. Br. J. Rheumatol. **24**(Suppl.):59–64.
9. **Dinarello, C. A., L. Renfer, and S. M. Wolff.** 1977. Human leukocytic pyrogen: purification and development of a radioimmunoassay. Proc. Natl. Acad. Sci. USA **74**:4624–4627.
10. **El Azhary, R., K. W. Renton, and G. J. Mannering.** 1980. Effect of interferon inducing agents (polyriboinosinic acid · polyribocytidylic acid and tilorone) on the heme turnover of hepatic cytochrome P-450. Mol. Pharmacol. **17**:395–399.
11. **Ghezzi, P., M. Bianchi, L. Gianera, S. Landolfo, and M. Salmona.** 1985. Role of reactive oxygen intermediates in the interferon-mediated depression of hepatic drug metabolism and protective effect of N-acetylcysteine in mice. Cancer Res. **45**:3444–3447.
12. **Gorodischer, R., J. Krasner, J. J. McDevitt, J. P. Nolan, and S. J. Yaffe.** 1976. Hepatic microsomal drug metabolism after administration of endotoxin in rats. Biochem. Pharmacol. **25**:351–353.
13. **Greenlee, W. F., and A. Poland.** 1978. An improved assay of 7-ethoxycoumarin O-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Pharmacol. Exp. Ther. **205**:596–605.
14. **Griffeth, L. K., G. M. Rosen, C. Tschanz, and E. J. Raukman.** 1983. Effects of model traumatic injury on hepatic drug metabolism in the rat. I. In vivo antipyrine metabolism. Drug Metab. Dispos. **11**:517–525.
15. **Ho, M.** 1964. Interferon-like viral inhibitor in rabbits after intravenous administration of endotoxin. Science **146**:1472–1474.
16. **Kampschmidt, R. F., L. A. Pulliam, and H. F. Upchurch.** 1980. The activity of partially purified leukocytic endogenous mediator in endotoxin-resistant C3H/HeJ mice. J. Lab. Clin. Med. **95**:616–623.
17. **Moore, R. N., K. J. Goodrum, R. E. Couch, and L. J. Berry.** 1978. Elicitation of endotoxemic effects in C3H/HeJ mice with glucocorticoid-antagonizing factor and partial characterization of the factor. Infect. Immun. **19**:79–86.
18. **O'Brien, A. D., E. S. Metcalf, and D. L. Rosenstreich.** 1982. Defect in macrophage effector function confers Salmonella typhimurium susceptibility on C3H/HeJ mice. Cell. Immunol. **67**:325–333.
19. **Parkinson, A., J. Lasker, M. J. Kramer, M.-T. Huang, P. E. Thomas, D. E. Ryan, L. M. Reik, R. L. Norman, W. Levin, and A. H. Conney.** 1982. Effects of three recombinant human leukocyte interferons on drug metabolism in mice. Drug Metab. Dispos. **10**:579–585.
20. **Ramadori, G., J. D. Sipe, C. A. Dinarello, S. B. Mizel, and H. R. Colten.** 1985. Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. J. Exp. Med. **162**:930–942.
21. **Renton, K. W., and G. J. Mannering.** 1976. Depression of hepatic cytochrome P-450-dependent monooxygenase systems with administered interferon inducing agents. Biochem. Biophys. Res. Commun. **73**:343–348.
22. **Rossi, V., F. Breviario, P. Ghezzi, E. Dejana, and A. Mantovani.** 1985. Prostacyclin synthesis induced in vascular cells by interleukin-1. Science **229**:174–176.
23. **Ruff, M. A., and G. E. Gifford.** 1981. Tumor necrosis factor, p. 235–272. In E. Pick (ed.), Lymphokines, vol. 2. Academic Press, Inc., New York.
24. **Seglen, P. O.** 1976. Preparation of isolated rat liver cells, p. 29–83. In D. M. Prescott (ed.), Methods in cell biology, vol. 13. Academic Press, Inc., New York.
25. **Sipe, J. D., S. N. Vogel, J. L. Ryan, K. P. W. J. McAdam, and D. L. Rosenstreich.** 979. Detection of a mediator derived from endotoxin-stimulated macrophages that induces the acute phase serum amyloid a response in mice. J. Exp. Med. **150**:597–606.
26. **Sipe, J. D., S. N. Vogel, M. B. Sztein, M. Skinner, and A. S. Cohen.** 1982. The role of interleukin 1 in acute phase serum amyloid A (SAA) and serum amyloid P (SAP) biosynthesis. Ann. N.Y. Acad. Sci. **389**:137–150.