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Interleukin-1 and tumour necrosis factor induce hepatic haem oxygenase

Feedback regulation by glucocorticoids

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During the acute-phase response to bacterial endotoxins [lipopolysaccharide (LPS)] in mice, the hepatic activity of haem oxygenase (HO) is increased. We investigated the effects of the potential humoral mediators of inflammation, interleukin-1 (IL-1) and tumour necrosis factor (TNF), on hepatic HO activity. In mice, IL-1 or TNF (5 μg) caused an elevation of HO activity comparable with that after LPS exposure (20 μg). The induction of HO by both cytokines was more pronounced in adrenalectomized mice. In the intact mice induction of HO activity by cytokines was observed earlier than depression of 7-ethoxycoumarin *O*-de-ethylase, a cytochrome *P*-450-dependent enzyme activity. Pretreatment with dexamethasone of the intact mice (3 mg/kg) or of the adrenalectomized mice (0.4 mg/kg) prevented the induction of HO activity caused by LPS and IL-1 respectively. These results suggest that: (1) HO activity is increased during an IL-1- or TNF-mediated acute-phase response, so haem metabolism might be a potential target of inflammation, and (2) HO induction by IL-1 and TNF does not require glucocorticoids, which in fact act as antagonists of this cytokine-induced effect.

INTRODUCTION

The acute-phase response plays an important role in the host reaction to infection or inflammation [1]. Whereas some of the changes associated with the acute-phase response might be important in the defence against pathogens, others serve as feedback mechanisms that inhibit the inflammatory response. This is the case of glucocorticoids released during inflammation or after injection of endotoxin [lipopolysaccharide (LPS)] [2]. Some of the acute-phase proteins are believed to have anti-inflammatory activities associated with their proteinase-inhibitory, antioxidant, immunosuppressive or cytokine-binding properties [1,3].

It is now established that cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF) are important mediators of the LPS-induced acute-phase response and of several of the actions of LPS *in vivo* [1,4]. In the liver, IL-1 or TNF induces a co-ordinated series of changes, including increased synthesis of acute-phase proteins, decreased albumin synthesis and depression of cytochrome *P*-450-dependent drug-metabolizing enzymes [1,5,6]. Haem oxygenase (HO) is the rate-limiting enzyme in the degradation of haem to bilirubin [7]. It cleaves the haem ring at the α-methene bridge to form biliverdin, which is further metabolized to bilirubin by biliverdin reductase. Induced HO is often associated with a decrease in cytochrome *P*-450 levels [8], and earlier studies reported that liver HO is induced *in vivo* in rats after LPS treatment [9,10].

Since previous work has indicated that IL-1 and TNF might mediate LPS-induced depression of cytochrome *P*-450 [11], we investigated the effect of these two cytokines on liver HO activity in mice *in vivo*. The effect of adrenalectomy or of exogenously administered glucocorticoids on the sensitivity of HO to IL-1 was also studied. Glucocorticoids interact with cytokines at different levels, acting as feedback inhibitors of their synthesis and action [2]; adrenalectomy sensitizes mice to the lethal action of IL-1 and increases its production in response to LPS [2,12]. On the other hand, some of the IL-1-induced acute-phase proteins require glucocorticoids in order to be induced, and their induction is inhibited in adrenalectomized mice [13].

In an attempt to investigate the role of IL-1 in the LPS-mediated induction of HO, we have used an IL-1 receptor antagonist [14–17] previously shown to inhibit several effects of IL-1 *in vivo* [14,17].

MATERIALS AND METHODS

TNF (recombinant human TNF-α) was kindly donated by BASF/Knoll, Ludwighafen, Germany, IL-1 (recombinant human IL-1-β) was a gift from Sclavo, Siena, Italy. LPS (Westphal preparation from *Escherichia coli* O55:B5) was from Sigma, St. Louis, MO, U.S.A. IL-1 receptor antagonist was kindly provided by Dr. P. Ralph (Cetus Corp., Emeryville, CA, U.S.A.), and was the intracellular form of the human IL-1 receptor antagonist (icIL-1ra) originally described by Haskill *et al.* [15], expressed in *E. coli*. The protein was 96% pure and contained less than 0.1 ng of LPS/mg of protein. Dexamethasone phosphate (DEX) was obtained from Laboratorio Farmacologico Milanese, Milan, Italy.

All agents were injected intraperitoneally in 0.2 ml of sterile pyrogen-free saline (0.9% NaCl). Male adult CD1 mice (22–24 g) were obtained from Charles River, Calco, Como, Italy. Adrenalectomy was performed under ether anaesthesia. Sham-operated mice were used as controls in these experiments. Adrenalectomized mice were maintained with 0.9% NaCl in drinking water, and were used 1 week after surgery. 7-Ethoxycoumarin *O*-de-ethylase (7-ECD) activity was measured according to Greenlee & Poland [18]. Microsomal cytochrome *P*-450 content was measured using the method of Omura & Sato [19]. HO activity was measured in a post-mitochondrial supernatant according to De Matteis & Gibbs [20], except that the

Abbreviations used: LPS, lipopolysaccharide; IL-1, interleukin-1; TNF, tumour necrosis factor; HO, haem oxygenase; DEX, dexamethasone; 7-ECD, 7-ethoxycoumarin *O*-de-ethylase; icIL-1ra, intracellular IL-1 receptor antagonist.

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Table 1. Induction of hepatic HO and depression of 7-ECD activity by IL-1 and TNF

IL-1 (5 μg/mouse) or TNF (5 μg/mouse) were given intraperitoneally. For each experiment, control mice received saline alone. Mice were kept fasted after treatment, and HO and 7-ECD activities were measured 6 or 24 h later, as indicated. Data are means $\pm$ S.E.M. of six mice per group. Numbers in parentheses are percentages of respective control values. All experiments were repeated at least twice, and data from a representative experiment are reported. $^{*}P < 0.05$, $^{**}P < 0.01$ versus respective control (Student's t test).

	HO (pmol/min per mg of protein)		7-ECD (pmol/min per mg of protein)	
Treatment	Control	Treated	Control	Treated
IL-1 (6 h)	9.1 $\pm$ 0.4	19.0 $\pm$ 1.1* (208)	246.2 $\pm$ 17.0	183.5 $\pm$ 25.0 (74)
IL-1 (24 h)	11.4 $\pm$ 0.1	18.1 $\pm$ 1.2** (158)	289.2 $\pm$ 20.1	132.4 $\pm$ 13.1** (46)
TNF (6 h)	10.1 $\pm$ 0.8	22.5 $\pm$ 0.8* (220)	330.4 $\pm$ 25.7	322.0 $\pm$ 19.1 (97)
TNF (24 h)	11.4 $\pm$ 0.7	18.8 $\pm$ 0.8** (165)	280.2 $\pm$ 12.9	209.3 $\pm$ 5.2** (75)

9000 g supernatant obtained from the 10% (w/v) liver homogenate was treated with a suspension of cellulose phosphate P11 cation exchanger (Whatman Chemical Separations Ltd., Maidstone, Kent, U.K.) to remove haemoglobin [21]. Proteins were determined by the method of Lowry *et al.* [22].

Table 2. Effect of DEX on hepatic HO induction by LPS

DEX was administered at a dose of 75 μg/mouse, 30 min before LPS treatment (20 μg/mouse). Mice were fasted until they were killed 24 h after treatment. Data are means $\pm$ S.E.M. of six mice per group. Numbers in parentheses are percentages of control values. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with saline group (Duncan's test); $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ compared with DEX/saline group (Duncan's test); $\ddagger P < 0.01$ compared with LPS group (Duncan's test).

Pretreatment/ treatment	HO (pmol/min per mg of protein)
Saline/saline	8.0 $\pm$ 0.5 (100)
Saline/LPS	18.5 $\pm$ 2.1**†† (231)
DEX/saline	8.2 $\pm$ 0.8 (102)
DEX/LPS	13.0 $\pm$ 0.9*†‡ (162)

Table 3. Effect of adrenalectomy on HO induction by IL-1 and TNF

Sham-operated or adrenalectomized (ADX) mice received IL-1 (5 μg/mouse), TNF (5 μg/mouse) or saline. Mice were fasted until they were killed 6 h after treatment. Data are means $\pm$ S.E.M. of six mice per group. Numbers in parentheses are percentages of respective sham/saline controls. Experiments were repeated at least twice and data from a representative experiment are shown. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with sham/saline group; $\dagger P < 0.01$ compared with sham/IL-1 or sham/TNF group; $\ddagger P < 0.01$ compared with ADX group (all by Duncan's test).

Pretreatment/ treatment	HO (pmol/min per mg of protein)
Sham/saline	9.1 $\pm$ 0.7 (100)
Sham/IL-1	16.0 $\pm$ 0.2* (175)
ADX/saline	12.3 $\pm$ 1.0 (135)
ADX/IL-1	25.6 $\pm$ 2.7**†‡ (281)
Sham/saline	10.0 $\pm$ 0.8 (100)
Sham/TNF	20.0 $\pm$ 1.1** (200)
ADX/saline	13.0 $\pm$ 0.7 (130)
ADX/TNF	39.0 $\pm$ 4.5**†‡ (390)

RESULTS

Effects of IL-1 and TNF on liver HO and 7-ECD activities

Hepatic HO activity was measured after treatment with LPS, IL-1 or TNF. LPS was utilized as a positive control, since it induces liver HO [9,10]; 24 h after LPS injection (20 μg/mouse, intraperitoneal), HO activity was 82% higher than in control mice (23.2 $\pm$ 0.2 pmol/min per mg of protein in the treated group versus 12.7 $\pm$ 0.7 pmol/min per mg of protein in the control group; $P < 0.01$ by Student's t test). IL-1 (5 μg/mouse) or TNF (5 μg/mouse) induced HO to a slightly lesser extent (58% and 65% respectively) (Table 1). However, when HO activity was measured 6 h after administration of the two cytokines, induction by IL-1 and TNF was greater than that by LPS (108% and 120% increases respectively), suggesting that the effect of the cytokines is more marked shortly after administration. With LPS, HO activity after 6 h was 71% higher than in control mice (24.0 $\pm$ 1.9 pmol/min per mg of protein in the treated group versus 14.6 $\pm$ 1.1 pmol/min per mg of protein in the saline group; $P < 0.01$ by Student's t test).

Table 1 compares the effect of cytokines on HO activity and 7-ECD activity, the latter taken as an index of the cytochrome P-450-dependent drug-metabolizing system. Unlike HO, 7-ECD activity was not significantly altered by IL-1 or TNF after 6 h, but it was decreased to 46% and 75% of control values 24 h after IL-1 and TNF respectively.

Modulation by glucocorticoid hormones of IL-1- and TNF-mediated HO induction

In a first set of experiments in intact mice, the synthetic glucocorticoid DEX [75 μg/mouse, i.e. 3 mg/kg intraperitoneally 30 min before LPS (20 μg/mouse); Table 2] significantly decreased HO induction by LPS measured 24 h after treatment. Therefore the influence of glucocorticoid hormones on HO induction in the acute-phase response was further investigated.

HO induction by IL-1 or TNF was assessed in adrenalectomized mice (Table 3). The effect in adrenalectomized mice of the same doses of IL-1 and TNF used in intact mice was studied at 6 h to avoid the previously reported toxicity of these cytokines observed at later times in adrenalectomized mice [12].

Adrenalectomy *per se* did not significantly increase HO activity, but it potentiated HO induction by IL-1 (5 μg/mouse) or TNF (5 μg/mouse) 6 h after their injection. When adrenalectomized mice were treated with DEX (10 μg/mouse, i.e. 0.4 mg/kg, 30 min before IL-1; Table 4), induction of HO was completely blocked. However, under these experimental conditions the cytochrome P-450 content did not change significantly after treatment with IL-1 or IL-1 plus DEX. It should be noted that

Table 4. Effect of DEX on hepatic HO induction and cytochrome *P*-450 content in adrenalectomized mice treated with IL-1

DEX was administered at a dose of 10 μg/mouse, 30 min before IL-1 (5 μg/mouse). Mice were fasted after treatment and killed 6 h later. Data are means ± S.E.M. of six mice per group. Numbers in parentheses indicate percentages of respective saline controls. Experiments were repeated at least twice and data from a representative experiment are shown. *$P < 0.01$ compared with saline group; †$P < 0.01$ compared with DEX/saline group; ‡$P < 0.01$ compared with DEX/IL-1 group (all by Duncan's test).

Pretreatment/ treatment	HO (pmol/min per mg of protein)	*P*-450 (nmol/mg of protein)
Saline/saline	14.7 ± 0.7 (100)	0.89 ± 0.03 (100)
Saline/IL-1	45.3 ± 2.6*†‡ (208)	0.79 ± 0.07 (89)
DEX/saline	10.7 ± 0.7 (72)	0.81 ± 0.09 (91)
DEX/IL-1	13.0 ± 1.0 (121)	0.72 ± 0.06 (81)

Table 5. Effect of icIL-1ra on HO induction by LPS

icIL-1ra was administered at a dose of 0.18 mg/mouse three times: simultaneously with, and 2.5 h and 6 h after LPS administration (20 μg/mouse). Mice were fasted until they were killed, 24 h after treatment. Data are means ± S.E.M. of six mice per group, except for the icIL-1ra/saline group (three mice). Numbers in parentheses are percentages of respective saline controls. *$P < 0.01$ compared with saline/saline group; †$P < 0.01$ compared with icIL-1ra/saline group (Duncan's test).

Treatment	HO (pmol/min per mg of protein)
Saline/saline	8.9 ± 1.1 (100)
Saline/LPS	28.3 ± 4.1*† (320)
icIL-1ra/saline	12.2 ± 2.8 (138)
icIL-1ra/LPS	22.6 ± 4.0* (255)

DEX alone did not alter HO activity or cytochrome *P*-450 content. Administration of DEX *per se* to intact mice at the doses used in these experiments did not significantly affect either cytochrome *P*-450 content or HO activity 24 h after treatment. HO activity was 13.0 ± 1.2, 14.7 ± 0.8 and 15.0 ± 1.0 pmol/min per mg of protein respectively for the control, DEX (10 μg/mouse) and DEX (75 μg/mouse) groups, while in the same groups the cytochrome *P*-450 content was respectively 0.96 ± 0.03, 0.92 ± 0.07 and 0.75 ± 0.05 nmol/mg of protein.

Effects of IL-1 receptor antagonist on HO induction by LPS

To quantify the role of IL-1 in LPS induction of HO, we tested a specific IL-1 receptor antagonist (icIL-1ra). For this purpose, icIL-1ra was administered three times (simultaneously, and 2.5 h and 6 h after LPS) at a dose of 0.18 mg/mouse, in association with LPS (20 μg/mouse). As shown in Table 5, icIL-1ra partially inhibited the induction of HO by LPS (155 % versus 220 % in the presence and absence respectively of antagonist).

DISCUSSION

The present paper shows that HO is induced by administration *in vivo* of the cytokines IL-1 and TNF, which are considered to be important mediators of the inflammatory response [1,4]. This suggests that haem catabolism might be altered during the acute-phase response and that HO might behave like an acute-phase protein.

A possible role for HO in inflammation has been suggested by other evidence: the gene for rat HO contains a heat-shock consensus element [23], and in the mouse the synthesis of a protein (p32) with strong sequence similarity to rat HO is induced after heat shock [24]. The synthesis of heat-shock proteins can be induced not only by heat, which is a major clinical sign of inflammation, but also by other forms of cellular injury such as oxidative stress, and it has been suggested that heat-shock proteins have antioxidant properties [25]. In human skin fibroblasts u.v. irradiation and treatment with H_2O_2, both conditions that cause an oxidant stress in the cell, result in induction of HO mRNA [26]. Bilirubin, the end-product of the HO-catalysed reaction, has been suggested as a physiological antioxidant [27].

As regards the possibility that IL-1 and TNF might be the mediators involved in the induction of HO after LPS administration, it should be noted that LPS induces the formation of various cytokines [28], and administration of cytokines themselves often induces other cytokines [29–32]. Furthermore, it cannot be ruled out that HO might also be induced directly by LPS. However, the HO-inductive response to IL-1 and TNF appeared to be a rather rapid process, fully evident in a few hours. If one considers that after LPS treatment the maximum TNF and IL-1 levels are observed at 1 and 3 h respectively [4,32] and that LPS administration induces HO activity by 6 h, this is consistent with the idea that these two cytokines have a role in HO induction by LPS. Furthermore, by using an IL-1 receptor antagonist, we obtained partial inhibition of LPS induction of HO, which suggests some involvement of IL-1 in this process.

Induction of HO is often accompanied by perturbations of haem metabolism such as depression of cytochrome *P*-450 and the related drug-metabolizing system [8,33]. It has been suggested that haem mediates HO induction by LPS [8]. However, the source of haem that becomes available and affects HO activity after LPS exposure is still not clear, since the time course of HO induction did not correlate with the *P*-450 decrease [8]. Furthermore, LPS induces HO activity in Kupffer cells *in vivo* and in erythrophagocytic macrophages *in vitro*; both of these cell types contain very low intrinsic cytochrome *P*-450 concentrations [9].

IL-1 and TNF have been reported to affect cytochrome *P*-450 levels as well as cytochrome *P*-450-dependent drug-metabolizing activity [5,6,34,35]; IL-1 was also suggested to be the mediator responsible for loss of *P*-450 after LPS administration [6,35]. However, no significant decrease in cytochrome *P*-450 content has been shown previously before 24 h after administration of the cytokines, except in the case of repeated doses of IL-1 [36]. Our data show that IL-1- and TNF-mediated induction of HO is apparent well before any detectable decrease in a cytochrome *P*-450-dependent reaction. This suggests that at least the metabolic capacity of cytochrome *P*-450 might be maintained in the first few hours after cytokine administration. In adrenalectomized mice, which are in general more susceptible to cytokines than intact mice, the decrease in cytochrome *P*-450 in the IL-1 group 6 h after administration did not reach statistical significance.

Thus these data do not permit us to conclude that HO induction by IL-1 or TNF is a result of haem liberated from cytochrome *P*-450. In fact, IL-1 significantly suppresses the mRNA content of one of the two major constitutive cytochromes *P*-450 in rat liver [36]; thus, after IL-1 administration, free haem not utilized for cytochrome *P*-450 synthesis might contribute to HO induction.

We also observed that glucocorticoids acted as antagonists of HO induction by IL-1. HO induction by IL-1 was more pronounced in adrenalectomized mice, indicating that induction does not require glucocorticoids, and administration of DEX to

the mice treated with IL-1 lowered HO activity to values comparable to those in non-IL-1-treated mice. This latter effect was obtained in the absence of any evident toxicity which might unspecifically delay or decrease HO induction, and of changes in cytochrome *P*-450 levels in comparison with the group treated with IL-1 alone.

These effects of glucocorticoids agree with the postulated feedback system *in vivo*, involving the neuroendocrine system, which leads to corticosterone production after IL-1 or TNF administration and subsequent regulation of the activity of these cytokines [2]. They provide another indication of the potential role of glucocorticoids in the regulation of HO activity [37], as suggested by studies on the induction of HO in the liver *in vivo* by metals [38] and in macrophages *in vitro* during erythrophagocytosis [39].

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