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Serum corticosterone, interleukin-1 and tumour necrosis factor in rat experimental endotoxaemia: comparison between Lewis and Wistar strains

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1 Circulating corticosterone, interleukin-1 (IL-1) and tumour necrosis factor-α (TNFα) activities in serum of Lewis and Wistar rats were measured following injection of lipopolysaccharide (LPS). IL-1 was measured as 'lymphocyte activation factor' (LAF) activity following precipitation of inhibitory activity with polyethylene glycol. TNFα activity was measured as cytotoxic activity.

2 Compared to the Wistar, the Lewis rat had higher circulating LAF and TNF activities following LPS, and release of both cytokines was prolonged in this strain.

3 Corticosterone increases in response to LPS were less in the Lewis than in the Wistar rat following the initial peak at 1 h; basal corticosterone was lower in the Lewis rat.

4 Adrenalectomized Lewis rats had even greater amounts of circulating LAF and TNF activities following LPS than did intact animals; the effect of adrenalectomy was not however mimicked by acute treatment with the steroid receptor antagonist, RU486, suggesting that endogenous corticosteroids did not acutely control cytokine release.

5 Although *in vivo* administration of anti-murine IL-1α antiserum significantly lowered LAF activity of serum, circulating corticosterone in response to LPS was not affected. Similarly, treatment with anti-murine TNFα monoclonal antibody (mAb) abrogated TNF activity without affecting corticosterone, suggesting that other mediators may be responsible for corticosterone release following LPS.

6 This 'overproduction' of inflammatory cytokines together with lower circulating corticosterone may contribute to the susceptibility of the Lewis rat to diseases such as adjuvant arthritis or experimental allergic encephalomyelitis.

Keywords: Endotoxaemia; interleukin-1; tumour necrosis factor-α (TNFα); glucocorticosteroids

Introduction

Interleukin-1 (IL-1) and tumour necrosis factor-α (TNFα) are pluripotent cytokines with a central role in the onset and maintenance of chronic inflammatory conditions in both man and experimental animals (Miller & Dinarello, 1987; Arend & Dayer, 1990). Synthesis of these cytokines must be finely regulated and as with other inflammatory mediators, glucocorticoid hormones have the capacity to inhibit their generation, down-regulating transcription and translation of both IL-1 and TNFα (Beutler *et al.*, 1986; Knudsen *et al.*, 1987; Lee *et al.*, 1988). Moreover, IL-1 and TNFα are potent stimulators of the hypothalamo-pituitary-adrenal (HPA) axis leading to increased circulating corticosteroids (Besedovsky *et al.*, 1991). The importance of this immunoregulatory feedback is indicated by enhanced cytokine release in adrenalectomized (ADX) animals (Perretti *et al.*, 1989; Zuckerman *et al.*, 1989) which show exaggerated inflammatory responses (Flower *et al.*, 1986; Perretti *et al.*, 1991). The Lewis strain of rat is particularly susceptible to such chronic inflammatory models as cell-wall arthritis and experimental allergic encephalomyelitis (EAE): this susceptibility has been attributed to a defect in the HPA axis in this strain which results in lower corticosterone (CCS) responses following challenge with various stimuli including IL-1 (Sternberg *et al.*, 1989a; Mason, 1991). Additionally, under normal conditions, Lewis rats have lower basal CCS than other strains (Griffin & Whitacre, 1991; Villas *et al.*, 1991).

Amongst the many activities of IL-1 and TNFα, their roles as mediators of septic shock are well established, and cytokine levels are transiently elevated in animal models of shock and gram-negative sepsis (Dinarello, 1991). Lipopolysaccharide (LPS) is frequently used to induce release of IL-1 and TNFα, and this is associated with an increase in circulating corticosteroids. This latter effect partially explains the rapidly-induced tolerance to subsequent LPS challenge (Beutler *et al.*, 1986; Zuckerman *et al.*, 1991).

Most studies concerning LPS-induced cytokine release have been performed in the mouse. Here, using a recently-described method to remove inhibitory activity present in serum (Hopkins & Humphreys, 1990), we have been able to measure circulating lymphocyte activation factor (LAF) activity in the rat. Therefore, in the present study, we have compared the release of IL-1 and TNFα following LPS administration in Lewis and Wistar rats, and investigated the relationship between these cytokines and CCS. Furthermore, the effect of acute or prolonged ablation of corticosteroids by use of the steroid receptor antagonist, mifepristone (RU486) and adrenalectomy respectively were examined. A preliminary account of some of these results was presented to the British Pharmacological Society (Peers *et al.*, 1992).

Methods

Animals

Male Wistar and Lewis rats (200–250 g) were obtained from Harlan-Olac, (Bicester, Oxon) and kept in the Animal Unit for at least 7 days before use. Animals received food and water *ad libitum*, and lighting was maintained on a 12 h cycle. ADX rats were obtained from the same source, and received saline drinking water. Adrenalectomy was confirmed

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by measurement of CCS. To minimise undue stress, animals were maintained in established groups, handled repeatedly and moved to the experimental room at least 16 h before the start of the experiment.

Experimental protocol

LPS (*E. coli* 055:B5) was administered i.p. in 1 ml sterile saline between 09 h 00 min and 11 h 00 min. After the indicated interval, animals were anaesthetized with CO_2, and a blood sample taken by cardiac puncture. Previous experiments indicated that this minimized stress to the animals and allowed measurement of basal CCS. Blood was allowed to clot at 4° for 2–3 h and centrifuged (600 *g*, 4°C, 30 min) to obtain sera which was stored in aliquots at − 20°C before assay for CCS or TNF activity, or at − 70°C before assay for LAF activity. All assays were performed within 4 weeks of serum collection. Dexamethasone (as phosphate) was administered s.c. and RU486 (mifepristone) was administered orally at the indicated time before LPS. Antiserum or antibodies were injected s.c. 16 h before LPS administration (Perretti *et al.*, 1992).

TNF bioassay

Serum TNF was assayed as cytotoxic activity against murine L-929 fibroblasts (Meager *et al.*, 1989). Briefly, L-M cells were seeded at $1-2 \times 10^5$ per well in flat-bottomed 96-well plates in RPMI-1640 medium containing 5% foetal calf serum (FCS) and incubated for 16 h, after which non-adherent cells were removed by washing in warmed balanced salt solution. Sterile filtered sera were diluted in RPMI-1640 (+ 5% FCS) containing 3 $\mu g\ ml^{-1}$ actinomycin D and serial five-fold dilutions added (volume 0.2 ml). After 20 h incubation, supernatants were discarded, the monolayer washed and adherent cells stained with crystal violet solution (0.5% in 20% methanol) for 10 min. Plates were rinsed with tap water and allowed to dry, after which the stained cells were lysed with 33% acetic acid, and optical density read at 570 nm. Standard curves were constructed using human recombinant TNFα; the limit of detection was approximately 10 $pg\ ml^{-1}$. Data are shown as TNF 'units', being 1/serum dilution giving 50% cytotoxicity (equivalent of approximately 100 $pg\ ml^{-1}$ human TNFα). Data are shown as values for individual sera, unless otherwise stated.

In experiments to confirm cytotoxic activity as being TNFα, filtered serum samples were preincubated for 2 h at 4°C with a monoclonal antibody (mAb) against murine TNFα at twice the final concentration before addition to cells as described above. In other experiments, sera were heated to 56°C for 30 min, centrifuged to remove precipitate then filtered and used as described above.

IL-1 bioassay

Sera were thawed and extensively dialysed ($1:1000 \times 3$) against phosphate buffered saline (PBS) and sterile filtered. IL-1 was assessed as LAF activity using the murine thymocyte co-stimulation assay as previously described (Perretti *et al.*, 1989) following incubation of dialysed, filtered sera with 12% polyethylene glycol (PEG; Hopkins & Humphreys, 1990): equal volumes of sera and 24% PEG were incubated for 30 min on ice then centrifuged and supernatants diluted with RPMI-1640 medium to obtain a starting dilution of 1/40. Incubations were performed in 96-well plates under the following conditions: 0.6×10^6 thymocytes from C3/HeJ mice aged 6–8 weeks (Harlan-Olac) were incubated in RPMI-1640 medium containing 5% FCS with 1.5 $\mu g\ ml^{-1}$ phytohaemagglutinin (PHA), 0.6% PEG and five fold serial dilutions of sera (prepared as above) for 72 h before pulsing with 1 μCi per well [^{3}H]-thymidine. After a further 16 h, the cells were harvested, and filters counted by liquid scintillation. Standard curves were run using recombinant human IL-1α. LAF activity is presented as ng ml^{-1} IL-1 activity calculated from the standard curve for a dilution of serum which gave approximately 50% maximum incorporation. The limit of detection was 5–10 $pg\ ml^{-1}$. All data shown are representative of at least two separate experiments conducted in triplicate with sera pooled from 2–4 rats.

In experiments to confirm the identity of LAF activity as IL-1, sera were dialysed and then incubated with specific antisera or pre-immune serum overnight at 4°C before incubation with PEG as described above, with antiserum at a final dilution of 1:720. In experiments using the IL-1 receptor antagonist protein (IL-1ra; McIntyre *et al.*, 1991), thymocytes were preincubated for 1 h with the protein at double the final concentration before addition of serum samples and incubation as above.

Assay of serum CCS

Serum CCS was assayed with a commercially available radioimmunoassay according to the manufacturer's instructions (ICN-Flow).

Materials

Mifepristone (RU486) was the kind gift of Roussel-Uclaf, Roumainville, France; recombinant human IL-1α was from National Institute for Biological Standards Control, South Mimms, Herts., batch 86.632 (sp. act. 5×10^6 u ml^{-1}); recombinant human TNFα and anti-murine TNFα mAb the kind gifts of Celltech, Slough, Berks.; anti-murine IL-1β antiserum the gift of Dr R.C. Newton, DuPont-Merck, Wilmington, DE, U.S.A. IL1-ra was the generous gift of Upjohn, Kalamazoo, MI, U.S.A. Anti-murine IL-1α antiserum was from Genzyme, Maidstone, Kent. L-M cells, balanced salts solutions and media were from ICN-Flow, High Wycombe, Bucks.; heat-treated FCS was from Difco, East Molesley, Surrey. [^{3}H]-thymidine (sp. act. 5 Ci $mmol^{-1}$) was from Amersham International, Aylesbury, Bucks. Dexamethasone phosphate was from Evans, Greenford, UK; PHA was from Wellcome Diagnostics, Beckenham, Kent; all other reagents were from Sigma, Poole, Dorset.

Statistics

Statistical differences were calculated by using Student's unpaired *t* test, regarding $P < 0.05$ as significant.

Results

Validation of bioassays

LAF activity was confirmed as predominantly IL-1 by the use of IL-1ra. Pre-incubation of the thymocytes for 1 h with IL-1ra before addition of samples significantly inhibited LAF activity of authentic IL-1α (0.5–50 ng ml^{-1} inhibited essentially by 100% by 5 $\mu g\ ml^{-1}$ IL-1ra) and of serum samples (Figure 1a). The precise nature of circulating LAF activity was determined using anti-murine IL-1α and anti-murine IL-1β antisera which significantly reduced LAF activity of serum samples (Figure 1a), and suggested that the majority of this activity in serum following LPS consists of IL-1α. With all these procedures, a maximal inhibition of approximately 75% was achieved.

Cytotoxic activity in serum was stable to heating to 56°C for 30 min (Figure 1b), indicating that complement did not account for significant amounts of TNF-like activity. Preincubation of serum samples (2 h, 4°C) with a mAb against murine TNFα completely removed cytotoxic activity, confirming its identity as TNFα (Figure 1b).

Figure 1 shows data with serum from Wistar rats: essentially the same results were obtained with Lewis serum i.e. maximum 75% reduction of LAF activity with IL-1ra and complete inhibition of cytotoxic activity with anti-TNFα.

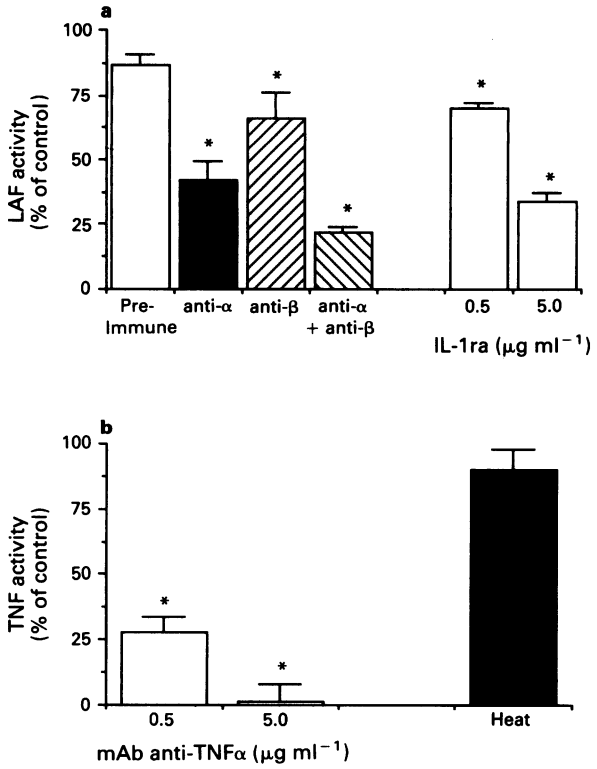

Figure 1 Validation of bioassays: (a) Effect of incubation with pre-immune serum of specific anti-sera raised against murine interleukin-1α (IL-1α) or IL-1β or IL-1ra upon lymphocyte activation factor (LAF) activity; (b) effect of preincubation with anti-murine tumour necrosis factor α-monoclonal antibody (TNFα mAb) or heating to 56°C for 30 min ('heat') upon cytotoxic activity. Data are shown as percentage of control, mean ± s.e.mean for triplicate measurements from serum pooled from 3 Wistar rats (a) 3 h and (b) 1 h following 1 mg kg^{-1} LPS i.p. *$P<0.05$ vs control response.

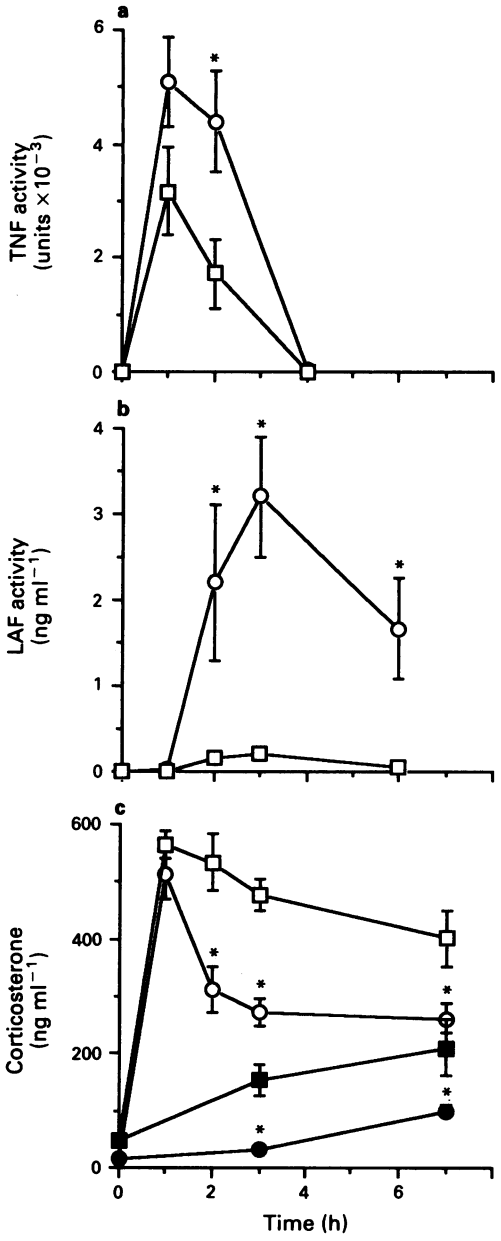

Figure 2 Time course of release of (a) tumour necrosis factor (TNF) activity (units), (b) lymphocyte activation factor (LAF) activity (ng ml^{-1}) and (c) corticosterone (CCS, ng ml^{-1}) following 1 mg kg^{-1} lipopolysaccharide (LPS) i.p. at T = 0 in (○) Lewis and (□) Wistar rats. CCS following saline injection is shown as solid symbols (●, ■). Data are shown as mean ± s.e.mean for at least 4 rats (a and c) or for 2 separate experiments performed in triplicate (b). *$P<0.05$, Lewis vs Wistar strain.

Effect of LPS upon serum IL-1, TNFα and CCS

No LAF or TNF activity was detectable in sera from untreated or saline-treated animals from either strain.

Administration of LPS 1 mg kg^{-1} i.p. caused the sequential appearance of TNFα and IL-1 in serum (Figure 2), accompanied by an increase in circulating CCS. TNF activity was detectable in sera from both strains 1 h after LPS, declining thereafter in the Wistar, but maintained at similar level in the Lewis for 2 h such that at this time there was significantly more TNF activity in Lewis serum (Figure 2a). TNF activity was no longer detectable in serum from either strain 4 h after LPS. In Wistar rats, LAF activity was detectable 2 h following LPS (0.17 ± 0.01 ng ml^{-1}), peaked at 3 h (0.22 ± 0.04 ng ml^{-1}), and then slowly declined (Figure 2b); no IL-1 activity was detectable 24 h after LPS (data not shown). Serum from Lewis rats consistently (3 experiments) contained significantly more IL-1 activity (5–10 fold) than did that from Wistar; the time-course of release was similar in both strains.

CCS was significantly elevated by LPS 1 h after injection in both strains (Figure 2c) and remained significantly elevated compared with saline-treated animals for at least 24 h, although by this time levels were only slightly higher than basal levels (90 ± 33 and 148 ± 12 ng ml^{-1} for Lewis and Wistar respectively). In the Wistar rats, the initial peak level was maintained for at least 3 h and declined slowly; in the Lewis rats, levels were equal to those in the Wistar rats at 1 h, but thereafter dropped, although still remaining significantly higher than basal CCS in this strain. It is noticeable that the Wistar rats had higher basal CCS, so that at 7 h post injection, CCS in LPS-treated Lewis rats was similar to that in saline-treated Wistar rats at the same time, although approximately double the Lewis basal levels. CCS levels in saline-treated Wistar rats were significantly higher than in Lewis rats as the rise in circadian CCS occurred during the afternoon (i.e. 3–7 h post injection).

The effect of LPS was dose-dependent (0.01–1 mg kg^{-1}, Figure 3). TNF data (Figure 3a) are shown 1 h following LPS when activity peaked in both strains; similarly LAF activity is given at 3 h post LPS (Figure 3b). In both cases, activity was detectable following 0.1 mg kg^{-1}, with significant difference in IL-1 between Lewis and Wistar rats at 1 mg kg^{-1}. There is no significant difference between strains in TNF release, although a trend is apparent; it is probable that at 2 h post-LPS, a significantly greater response would be seen in Lewis rats (see Figure 2). CCS data are shown at 3 h (Figure 3c), when levels in the Lewis rats are significantly

Table 1 Effect of pretreatment with dexamethasone (Dex, 0.5 mg kg^{-1}, s.c.) or RU486 (RU, 20 mg kg^{-1}, p.o.) upon serum lymphocyte activation factor (LAF) activity and tumour necrosis factor-α (TNFα) activity at the indicated time following 1 mg kg^{-1} lipopolysaccharide (LPS) in Lewis and Wistar rats

	TNF activity		*LAF activity*	
	Lewis	Wistar	Lewis	Wistar
Control (1 h)	4483 ± 1202	772 ± 122	ND	ND
+ Dex	912 ± 24*	264 ± 110*	ND	ND
+ RU	4154 ± 1291	1552 ± 668	ND	ND
Control (3 h)	42 ± 19	30 ± 6	3.97 ± 0.89	0.87 ± 0.04
+ Dex	NT	NT	0.29 ± 0.05*	0.14 ± 0.06*
+ RU	34 ± 10	18 ± 3	2.87 ± 1.58	1.10 ± 0.15

LAF activity is shown as ng ml^{-1} interleukin-1α (IL-1α) equivalents, mean ± s.e.mean for replicates from one experiment using serum pooled from 3 rats and TNF activity as units ml^{-1}, mean ± s.e.mean for 4–5 rats. ND: not detectable; NT: not tested. *$P<0.05$ vs appropriate controls.

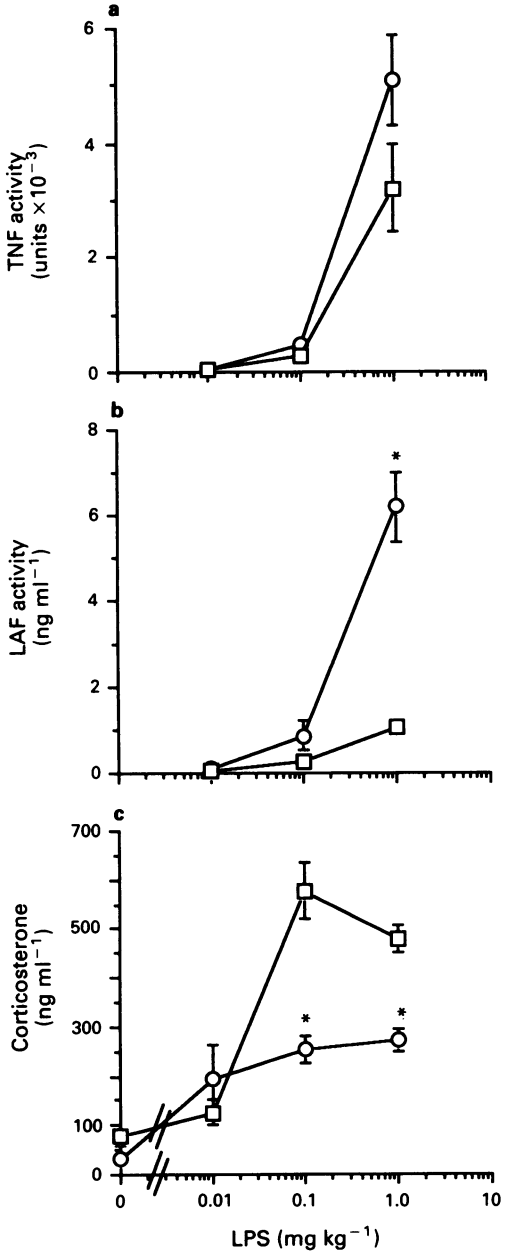

Figure 3 Dose-response to lipopolysaccharide (LPS): (a) tumour necrosis factor (TNF) activity (units), (b) lymphocyte activation factor (LAF) activity (ng ml^{-1}) and (c) corticosterone (CCS, ng ml^{-1}) in response to i.p. LPS in (○) Lewis and (□) Wistar rats. Data are shown as mean ± s.e.mean for at least 4 rats (a and c) or for 3 separate experiments performed in triplicate (b). *$P<0.05$ Lewis vs Wistar strain.

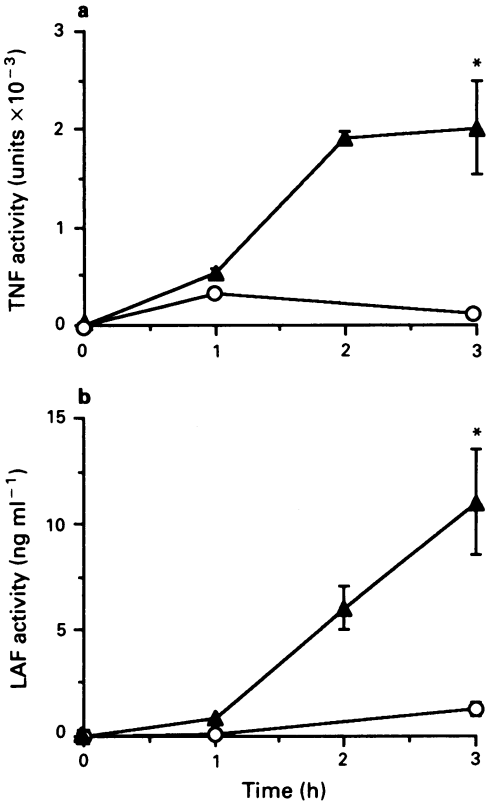

Figure 4 Effect of adrenalectomy upon release of (a) tumour necrosis factor (TNF) activity (units) and (b) lymphocyte activation factor (LAF) activity (ng ml^{-1}) in control (○) and adrenalectomized (ADX) (▲) Lewis rats following 1 mg kg^{-1} lipopolysaccharide (LPS) i.p. Data are shown as mean ± s.e.mean for 4 rats. *$P<0.05$ ADX vs control rats.

lower than those in the Wistar rats; 1 h following LPS however there is no significant difference in CCS at any of the doses used (data not shown) indicating the importance of the time of measurement. An increase in CCS was seen at all doses of LPS tested.

Modulation of cytokine release by exogenous and endogenous corticosteroids

Dexamethasone (0.5 mg kg^{-1}, s.c.) 1 h before injection of LPS significantly inhibited release of both IL-1 and TNF in both strains (Table 1). To discover whether endogenous CCS chronically affected cytokine release, ADX Lewis rats were injected with 1 mg kg^{-1} LPS. These animals appeared markedly ill following LPS injection, and were destroyed at 3 h since it was believed that they would not survive to 6 h.

Table 2 Effect of *in vivo* pretreatment with anti-murine tumour necrosis factor-α monoclonal antibody (TNFα mAb) or anti-murine interleukin-1α (IL-1α) antiserum upon corticosterone (CCS), TNF and lymphocyte activation factor (LAF) activity

Treatment	*CCS*	*TNF activity*	*LAF activity*
Control 1 h	385 ± 20	2338 ± 1092	ND
+ anti-TNFα	329 ± 16	< 10	ND
Control 3 h	222 ± 28	ND	0.95 ± 0.18
+ anti-TNFα	200 ± 26	ND	0.57 ± 0.14
+ anti-IL-α	223 ± 63	ND	0.46 ± 0.17*

Lewis rats were pretreated with mAb (20 mg kg^{-1}) or antiserum (20 mg kg^{-1}) s.c. 16 h before i.p. injection of 1 mg kg^{-1} LPS. CCS is shown as ng ml^{-1}, TNF activity as units and LAF activity as ng ml^{-1} IL-1α, mean ± s.e.mean for at least 4 rats. ND: not detectable.
*$P < 0.05$ vs appropriate controls.

Figure 4 shows that ADX Lewis rats released significantly more TNF than did intact animals (Figure 4a): at 1 h, there was no significant difference between ADX and intact animals, but in ADX rats, TNF release was prolonged, with high levels of activity detectable at 3 h. Similarly, LAF activity was significantly more elevated in serum from ADX rats at 3 h after LPS injection (Figure 4b), and unlike intact animals, was just detectable 1 h post LPS.

In order to investigate the acute role of endogenous corticosteroids released as part of the response to LPS in control of cytokine release, animals were pretreated with the steroid receptor antagonist, RU486 (20 mg kg^{-1}, p.o.) 1 h before injection of LPS. This dose did not significantly affect serum TNF in either rat strain 1 h or 3 h following LPS (Table 1). RU486 pretreatment did not affect serum IL-1 measured at 3 h in either strain, and in one experiment, had no effect upon serum IL-1 measured 6 h after LPS in the Wistar rats (data not shown).

Interaction between cytokines and CCS release

The mechanism by which LPS stimulates the HPA axis remains unclear, although many cytokines including IL-1 and TNFα are able to cause corticosteroid release. In order to investigate the involvement of IL-1 and TNFα in the elevation of CCS, Lewis rats were injected with anti-murine TNFα mAb (20 mg kg^{-1}) or with anti-murine IL-1α antiserum (20 mg kg^{-1}) or with pre-immune serum s.c. Table 2 shows that although anti-TNFα mAb effectively neutralised serum cytotoxic activity at 1 h, there was no significant effect of the antibody upon CCS at this time, suggesting that TNFα was not vital for HPA axis stimulation. Three hours after LPS, although the anti-IL-1α antiserum significantly reduced LAF activity, there was no significant effect upon circulating CCS. Moreover, anti-TNFα mAb did not significantly affect either CCS or LAF activity measured at 3 h.

Discussion

These data confirm in two strains of rat the sequential release of TNF and IL-1 activity accompanied by increased CCS following LPS *in vivo* described in other species (Dinarello, 1991). The CCS response appears to be more sensitive to LPS than is cytokine release, as seen with responses to 0.01 mg kg^{-1} (Figure 3): this may be a genuine phenomenon, since for example, the CCS increase occurs at lower doses of LPS than does pyresis (Derijk *et al.*, 1991) rather than a lack of sensitivity of our cytokine assays. We have measured serum IL-1 activity using bioassay following the removal of inhibitory activity, a problem which has confounded such measurements until recently; we believe that this is the first account of serum IL-1 activity following LPS in the rat. Although specific inhibitory activity may be present in serum (Shaw, 1990) it has been suggested that PEG precipitation is likely to remove non-specific inhibitory activity (Hopkins & Humphreys, 1990) and the assay may therefore reflect biologically available IL-1. The identity of LAF activity as predominantly IL-1 has been confirmed by the use of IL-1ra and by specific antisera; the remaining LAF activity not neutralised by these procedures may be due to other cytokines released during endotoxaemia such as IL-2 or IL-6. Serum cytotoxic activity has been identified as entirely TNFα by use of specific mAb against murine TNFα.

One important observation reported here is the apparent 'over-production' of cytokines in the Lewis compared with the Wistar rat, seen predominantly as a sustained release of TNF, and as an increased and sustained release of LAF activity. The pattern of the CCS response is also different, with a transient peak in the Lewis compared to maintained elevation in the Wistar rat. It has been suggested that the Lewis strain has a defect in HPA axis responses to a variety of stimuli including IL-1 (MacPhee *et al.*, 1989; Sternberg *et al.*, 1989a,b), and this appears also to be the case for the maintained response to LPS, although the immediate response is identical in both strains. We also confirmed the observations of others (Griffin & Whitacre, 1991; Villas *et al.*, 1991) that circadian CCS is lower in the Lewis rat. Since IL-1 and TNF are down-regulated by corticosteroids (Beutler *et al.*, 1986; Knudsen *et al.*, 1987; Lee *et al.*, 1988) and ADX animals show increased cytokine release (Perretti *et al.*, 1989; Zuckerman *et al.*, 1989) the over-production of cytokines in the Lewis rat may be a consequence of lower circulating CCS in this strain.

Endogenous corticosteroids have important anti-inflammatory roles even in the Lewis strain however (MacPhee *et al.*, 1989), and adrenalectomized Lewis rats show significantly increased levels of both IL-1 and TNF activity following LPS, confirming previous observations in adrenalectomized rats and mice (Perretti *et al.*, 1989; Zuckerman *et al.*, 1989; Parant *et al.*, 1991). Interestingly, the steroid antagonist, RU486, failed to affect cytokine release (confirming the observations of Hawes *et al.* (1992) upon TNFα) suggesting that it is the preceding level of corticosteroids that may determine the size of acute response to LPS; CCS released as part of the response may have a role in the 'switching off' of the gene following the response (Evans & Zuckerman, 1991), although this was not apparent over the short-term. Others have shown that RU486 reduces survival over several days in models of septic shock (Hawes *et al.*, 1992; Lazar *et al.*, 1992). It seems unlikely that break-through of CCS occurred in our experiments; the dose of RU486 used effectively blocks the anti-inflammatory actions of dexamethasone (Peers *et al.*, 1988; and unpublished observations), and in addition, circulating CCS were elevated in these animals (unpublished observations), indicating effective block of CCS negative feedback on the HPA axis. It should be noted that dexamethasone administered 1 h before LPS (albeit at a moderate-to-high anti-inflammatory dose) significantly inhibited TNF and LAF activities at 1 and 3 h respectively, suggesting that the 'lag phase' for inhibition of synthesis is not necessarily long, and a response to endogenous CCS could have been observed. *In vitro* evidence indicates that glucocorticoids have significant actions upon cytokine gene activation when applied before, but are much less effective when applied after the stimulus (Lee *et al.*, 1988). Taken together, these observations suggest that endogenous corticosteroids released in response to LPS may help to protect against the effects of released cytokines, rather than affecting their release in the short-term: glucocorticoids protect ADX animals against IL-1 as well as adjuvant-induced death (Perretti *et al.*, 1991). Corticosteroids are involved in the development of tolerance to LPS (Beutler *et al.*, 1986; Evans & Zuckerman, 1991), although LPS-tolerant mice release reduced TNFα but normal amounts of IL-1 in response to LPS, again suggesting that corticosteroid control of TNFα

and IL-1 *in vivo* may differ. Another possibility for the discrepancy between ADX animals and those treated acutely with RU486 is the possible involvement of adrenaline in control of cytokine release (Severn *et al.*, 1992). Clearly ADX animals will not show increases in circulating adrenaline following LPS, although RU486 treatment should not affect this response in intact animals. It would be interesting to investigate combined treatment with RU486 and β-adrenoceptor blockers upon cytokine release following LPS.

The mechanism by which inflammatory stimuli activate the HPS axis is a subject of much debate, with IL-1 a major candidate (Rivier *et al.*, 1989) and other cytokines including TNFα and IL-6 are able to increase CCS (Besedovsky *et al.*, 1991). Given the time-course of cytokine release, it is tempting to speculate that the initial rise in CCS following LPS may be due to TNFα, with the subsequent plateau phase due to IL-1 and possibly IL-6. Our observations with anti-TNFα and anti-IL1 do not support this suggestion however. The anti-TNFα mAb completely blocked *in vitro* cytotoxic activity, yet did not affect CCS levels at 1 h. LAF activity measured at 3 h was not significantly reduced by anti-TNFα suggesting that TNFα alone is not responsible for IL-1 release by LPS (Dinarello *et al.*, 1986; Zuckerman *et al.*, 1991). Similarly, the polyclonal anti-murine IL1α significantly reduced serum LAF activity without affecting CCS measured at 3 h; it is possible that sufficient IL-1 remained (either remaining IL-1α or IL-1β which is also present, Figure 1) to stimulate the CCS response. From our data, neither TNFα nor IL-1α appear fully to account for HPA axis activation by LPS. Alternative mechanisms by which LPS activates the HPS axis have been suggested, and the ability of pyrogenic doses of LPS to bypass the macrophage, a likely source of cytokines, has been observed (Derijk *et al.*, 1991). Interestingly, LPS has been shown to increase CCS in rats with lesions of the paraventricular nuclei (Elenkov *et al.*, 1992), suggesting a direct effect on the pituitary. Given that the lesion in the Lewis rat is believed to be globally-defective CRF release (Calogero *et al.*, 1992), such a mechanism may explain the pattern of CCS response in the Lewis strain.

In conclusion, our data show that while release of the cytokines IL-1 and TNF in response to LPS is tonically controlled by endogenous corticosteroids, corticosteroids released during the response do not acutely control cytokine generation. Moreover, TNFα and possible also IL-1 are not responsible for HPA axis activation during the early part of the response. Overproduction of cytokines in the Lewis rat may have implications concerning their susceptibility to models of experimental diseases such as adjuvant arthritis or EAE, where these cytokines appear to be involved (Jacobs *et al.*, 1991; Bromberg *et al.*, 1992): it has been suggested that imbalances in the cytokine network may contribute to immune dysregulation in the certain strains of mouse (Levine *et al.*, 1991).

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