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Sites of action of IL-1 in the development of fever and cytokine responses to tissue inflammation in the rat

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1 The objective of the present study was to determine the sites of action of the cytokine, interleukin-1 (IL-1), in the febrile response to local inflammation in the rat, by comparing the importance of IL-1 in the local tissues, the circulation and the brain. This was achieved by injecting lipopolysaccharide (LPS, 100 μg kg^{-1}) into a subcutaneous air pouch and testing the effects of blocking IL-1 action with the human recombinant interleukin-1 receptor antagonist (IL-1ra) injected either into the air pouch, intraperitoneally (1 mg kg^{-1}, 0+1 h, i.p.), or intracerebroventricularly (200 μg/rat, 0+1 h, i.c.v.).

2 To investigate the effect of IL-1ra on fever and the induction of local and circulating cytokines (IL-1 and IL-6), separate experiments were performed in which groups of animals were killed 1.5, 3 or 5 h after LPS injection. Plasma and pouch fluid samples were collected for bioassay of IL-1 and IL-6.

3 Injection of LPS into the air pouch significantly increased (1.5°C) body temperature, local (air pouch) concentrations of bioactive IL-1 and IL-6, and circulating bioactive IL-6, compared to saline-treated controls.

4 Injection of IL-1ra into the pouch significantly attenuated LPS fever ($P<0.001$). This decrease in body temperature was associated with significant inhibition of local IL-1 bioactivity 1.5 (96%), 3 (84%) and 5 h (72%), and in bioactive IL-6 in pouch lavage fluid 1.5 (45%) and 5 h (35%), after LPS injection. The concentration of bioactive IL-6 in the plasma was significantly reduced (39%) at 3 h, when temperature was approaching the maximal value.

5 Both systemic (i.p.) and central (i.c.v.) administration of IL-1ra significantly attentuated LPS fever ($P<0.05$). However, it had no effect on either local concentrations of bioactive IL-1 or IL-6, or circulating IL-6, at any of the sample points.

6 These data suggest that IL-1 is released locally, at the site of tissue inflammation and that it is an important mediator of the febrile response to local inflammation. The results also indicate that IL-1 produced locally may contribute to the production of IL-6 which is released into the circulation, and that IL-1 has important actions in the generation of fever at other sites, including the brain.

Keywords: Interleukin-1; interleukin-6; interleukin-1 receptor antagonist; cytokines; lipopolysaccharide; fever; inflammation; subcutaneous air pouch

Introduction

Fever induced by lipopolysaccharide (LPS) or other exogenous pyrogens has been ascribed to the peripheral release of endogenous pyrogen(s) into the circulation. The cytokine interleukin (IL)-1 meets several of the criteria for an endogenous pyrogen (Kluger, 1991), but most studies have failed to demonstrate an elevation of IL-1 in the plasma of febrile animals (Bristow *et al*., 1991; Cooper *et al*., 1991) or man (Cannon *et al*., 1990; Engel *et al*., 1994). Nevertheless, systemic treatment of animals with anti-IL-1β antiserum (Rothwell *et al*., 1989; Long *et al*., 1990) attenuates LPS fever, suggesting that IL-1 induction is important, but may act via the release of a secondary pyrogen into the circulation. Since IL-1 can induce IL-6 release *in vivo* (Sironi *et al*., 1989) and IL-6 is a potent pyrogen that is detected readily in the circulation of febrile animals (Nijsten *et al*., 1987; LeMay *et al*., 1990a, c) IL-6 may be this secondary circulating mediator. The hypothesis that the rise in plasma IL-6 is stimulated, at least in part, by IL-1 is supported by *in vivo* evidence that increased circulating IL-6 is significantly inhibited by passive immuno-neutralisation of IL-1β (LeMay *et al*., 1990b). Our more recent studies (Luheshi *et al*., 1996), demonstrated that injection of recombinant IL-1 receptor antagonist (IL-1ra) intraperitoneally (i.p.) suppressed LPS fever. This inhibition of fever was associated with a significant reduction of circulating IL-6 bioactivity. We also demonstrated that central (intracerebroventricular, i.c.v.) injection of IL-1ra caused significant inhibition of LPS fever, in the absence of any effect on circulating IL-6, suggesting that brain IL-1 is important in the induction of fever.

Most of the studies described above have used systemic injection of LPS to investigate fever in rodents. However, this procedure does not permit investigation of local (tissue) cytokine induction or sites of action, since LPS can act at numerous tissue sites. We have demonstrated recently that injection of LPS into a subcutaneous air pouch induces increases in local concentrations of bioactive IL-1, IL-6 and tumour necrosis factor (TNF) and circulating IL-6 (Miller *et al*., 1996). The resultant fever develops in the absence of a significant increase in circulating bioactive IL-1 or TNF. The aim of the present study was to test the hypothesis that IL-1 acts locally at the sites of tissue damage and in the brain, by studying the effect of inhibiting IL-1 action at each of these sites on fever and the local and circulating IL-6 production induced by injection of LPS into a subcutaneous air pouch in the rat.

Methods

All experiments were performed on young adult, male Sprague-Dawley rats (Charles River, Kent, U.K.) of 250–350 g body weight. The animals were housed individually in a termperature-controlled room (19–22°C), artificially lit from 08 h 00 min to 20 h 00 min and were provided with food (CRM Labsure, U.K.) and water *ad libitum*.

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Core body temperature was monitored in free-moving animals by remote biotelemetry via small battery-operated, temperature-sensitive, radio-transmitters (Data Sciences, St Paul, U.S.A.) which were implanted, under halothane (3% in oxygen) anaesthesia, into the abdominal cavity, and were allowed six days recovery before experimentation. The output frequency (Hz) was monitored by antennae mounted in a receiver board situated beneath the individually caged animals and converted to degrees centigrade (°C).

Subcutaneous air pouches were formed under halothane anaesthesia, according to the method described by Edwards *et al*. (1981). Briefly, 20 ml of air, sterilised by passing through a 0.2 μm filter (Acrodisc, Gelman Sciences U.S.A.), was injected into the subcutaneous tissue of the dorsal midline, caudal to the scapulae. On day 3, pouches were re-inflated with 10 ml of sterile air, to maintain an open cavity. On day 6, LPS or saline (0.9%) was injected directly into the pouch.

The role of peripheral IL-1 in regulating the cytokine and febrile response to inflammation was investigated by injecting LPS (100 μg kg^{-1}, optimal dose determined by preliminary studies) intrapouch (i.po.), and recombinant human IL-1ra (1 mg kg^{-1}) (Amgen, U.S.A.) or saline (1 ml kg^{-1}) either i.po. or i.p. at 0+1 h. Control animals were injected with saline (1 ml kg^{-1}, 0+1 h either i.po. or i.p.). Due to a limited supply of IL-1ra, only one IL-1ra control group was used (IL-1ra saline 1 ml kg^{-1}, 0+1 h i.po., $n=4$, 5 h sample point). Separate experiments were performed in which groups of animals ($n=3-9$) were killed either 1.5, 3 or 5 h after injection of LPS or saline. Blood was collected by cardiac puncture, under terminal halothane anaesthesia, and plasma prepared. Sampling of the inflammatory exudate within the pouch was achieved by lavaging the pouch with 1 ml of saline. The lavage fluid was quickly aspirated and centrifuged for 5 min at 3000 *g*. Plasma and pouch lavage fluids were stored at -70°C until bioassay.

To investigate the involvement of brain IL-1 in the responses to local injection of LPS, IL-1ra (2×200 μg/rat, i.c.v.) or saline (2 μl, i.c.v.) was injected 0+1 h after a single injection of LPS (100 μg kg^{-1}, i.po). Separate experiments were performed in which groups of animals were killed 1.5, 3 or 5 h ($n=3-6$) after LPS or saline, and plasma and pouch fluid samples collected. I.c.v. injections were administered via indwelling guide cannulae, stereotaxically implanted in the right lateral ventricle of the brain under general halothane anaesthesia, six days before the start of the experiment.

Measurements of cytokine concentrations

Interleukin-6 bioactivity in plasma and pouch fluid samples was measured with the B9 cell line as described previously (Luheshi *et al*., 1996). Bioactive IL-1 in pouch fluid was measured with the D10(N4)M cell line (Hopkins & Humphreys, 1989). Units are referenced to the first international standards for human (h) IL-1β (86/680) and hIL-6 (89/548), each supplied by the National Institute for Biological Standards Control (South Mimms, U.K.). Results were calculated by use of the ELIPS programme (Bowman & Co. Ltd., Boreham Wood, U.K.). The assay detection limits, after allowing for dilution of samples into the assays, were 1 i.u. ml^{-1} for IL-1 in pouch fluid and 3 i.u. ml^{-1} for IL-6 in plasma and pouch fluid.

Statistical analysis

Values are presented as means$\pm$s.e.mean. All temperature data were analysed with area under the curve (AUC), followed by analysis of variance (ANOVA) and a Scheffe *post-hoc* test, with a 5% significance level. Cytokine data were also analysed by ANOVA. However, IL-6 values were first log transformed to permit parametric testing. Significant differences between LPS-treated groups are represented by $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

Results

Effect of local (intrapouch) injection of IL-1ra on cytokine induction and fever in response to LPS

Intrapouch (i.po.) injection of LPS significantly increased body temperature in all animals (Figure 1) compared to saline-injected controls. A small, probably stress-induced, increase in temperature (0.5°C) was observed in some animals in the first

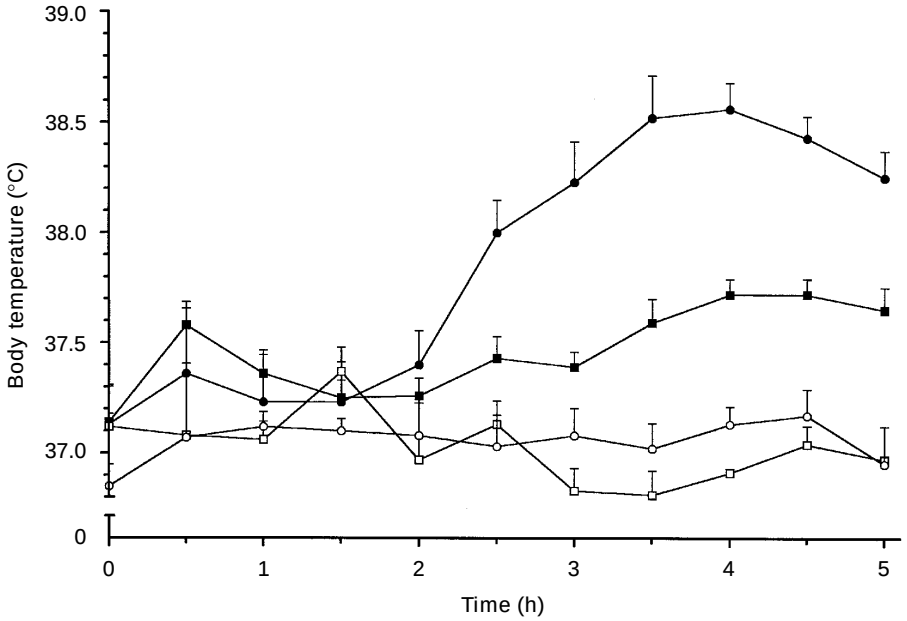

Figure 1 Thermal responses of rats injected intrapouch with LPS (100 μg kg^{-1}, 0 h) and IL-1ra (1 mg kg^{-1}, 0+1 h, i.po.) or saline (1 ml kg^{-1}, 0+1 h, i.po.). Symbols represent: saline (○), saline/IL-1ra (□), LPS/saline (●) and LPS/IL-1ra (■). Data are expressed as mean ($n=3-7$) and vertical lines show s.e.mean AUC; LPS/saline = 3.3 ± 0.5°C h^{-1} vs 0.9 ± 0.2°C h^{-1} for LPS/IL-1ra, ($P<0.001$).

hour, but thereafter, body temperature remained at basal levels in controls. Animals injected with LPS showed a significant increase in temperature by 2.5 h (LPS/Sal, 38.0 ± 0.2°C vs. 37.0 ± 0.2°C for saline-treated controls, $P<0.01$) and temperature was maximal 4 h after injection of LPS. Injection of IL-1ra (1 mg kg^{-1}, i.po., 0+1 h) delayed the onset of fever by 1 h (LPS/IL-1ra, 37.6 ± 0.1°C after 3.5 h vs 37.0 ± 0.1°C for controls, $P<0.05$), and significantly attenuated the magnitude of the peak fever (LPS/Sal, 38.6 ± 0.1°C after 4 h vs 37.7 ± 0.1°C for LPS/IL-1ra, $P<0.001$). Inhibition of the febrile response by IL-1ra was significant (AUC, $P<0.001$), though not at the 5 h sample point (LPS/Sal, 38.1 ± 0.5°C vs 37.6 ± 0.3°C for LPS/IL-1ra, $P<0.06$).

In the above experiment animals were killed after 5 h and in two separate experiments, animals were killed 1.5 h or 3 h after the injection of LPS, to collect plasma and pouch fluid samples for cytokine bioassay. Fever induced by LPS was associated with significant increases in the concentrations of bioactive IL-1 and IL-6 in the pouch, and IL-6 in the plasma (Table 1). No such increases were observed in control animals. IL-1 bioactivity in the pouch fluid was significantly elevated at each sample point after LPS treatment compared to controls, and was maximal 3 h after injection ($P<0.001$). Injection of IL-1ra (1 mg kg^{-1}, 0+1 h) into the air pouch significantly attenuated the local IL-1 activity at 1.5 h (96%, $P<0.001$), 3 h (84%, $P<0.001$) and 5 h (72%, $P<0.01$) after administration of LPS. To determine whether the reduction observed in IL-1 bioactivity was due to the presence of injected IL-1ra remaining in the pouch fluid, a sample of pouch fluid from a 5 h LPS/Sal animal was mixed with an equal volume of pouch fluid from a 5 h Sal/IL-1ra animal. The IL-1 activity in the LPS/Sal pouch fluid sample (248 ± 17 i.u. ml^{-1}) was not affected by the addition of the Sal/IL-1ra pouch fluid (Sal/IL-1ra $\leqslant1\pm0$ i.u. ml^{-1}, LPS/Sal + Sal/IL-1ra 269 ± 26 i.u. ml^{-1}).

The local (air pouch) and circulating concentrations of bioactive IL-6 were also significantly increased at each sample point after injection of LPS into the pouch. Local IL-6 bioactivity was maximal 5 h after LPS injection, and increased by more than 100 fold in the plasma. Injection of IL-1ra into the pouch resulted in a 45% reduction in pouch fluid IL-6 bioactivity measured 1.5 h after LPS ($P<0.05$) and a 35% reduction after 5 h ($P<0.05$), but had no significant effect at 3 h ($P>0.4$). In contrast, the concentration of bioactive IL-6 in the plasma was significantly reduced after 3 h ($P<0.05$) and was not significantly affected after 1.5 h ($P>0.2$) or 5 h ($P>0.7$).

Effect of systemic injection of IL-1ra on cytokine induction and fever in response to LPS

To investigate the possibility that the effect of IL-1ra injected i.po. on fever and plasma IL-6 concentration was due to local rather than systemic effects resulting from 'seepage' from the pouch into the circulation, the same dose of IL-1ra used previously was injected directly into the periphery and tested against LPS i.po. Injection of LPS caused a significant rise in body temperature compared with the corresponding saline-treated group, which showed no significant change in temperature throughout the experiment (Figure 2). Animals injected with LPS first exhibited a significant increase in temperature after 2.5 h (LPS/Sal, 38.3 ± 0.3°C vs 37.1 ± 0.1°C for saline-treated controls, $P<0.05$) and this was maximal after 3.5 h ($P<0.001$). Intraperitoneal injection of IL-1ra (1 mg kg^{-1}, 0+1 h) significantly delayed the onset of LPS fever (LPS/IL-1ra after 3 h, 37.6 ± 0.2°C vs 36.8 ± 0.1°C for saline-treated controls, $P<0.05$), and reduced the maximal response (peak temperature for LPS/Sal, 38.5 ± 0.1°C after 3.5 h vs 38.0 ± 0.1°C for LPS/IL-1ra, $P<0.05$). The febrile response to LPS was significantly attenuated by i.p. injection of IL-1ra (AUC, $P<0.05$), but not at the 5 h sample point (LPS/Sal, 37.9 ± 0.1°C vs 37.7 ± 0.1°C for LPS/IL-1ra, $P>0.2$).

In the above experiment, animals were killed after 5 h and in two separate experiments, cytokines were measured in the air pouch and plasma 1.5 h or 3 h after injection of LPS. In animals injected with LPS/saline, the concentration of bioactive IL-1 in air pouch lavage fluid was significantly elevated at each sample point compared to controls (Table 2). Intraperitoneal treatment with IL-1ra had no significant effect on local (air pouch) IL-1 bioactivity measured at any time point ($P>0.4$). The concentrations of bioactive IL-6 in the pouch and plasma were also significantly elevated after injection of LPS compared to the corresponding control group. IL-1ra injected i.p. had no significant effect on the concentration of bioactive IL-6 in the pouch or in plasma at any of the time points.

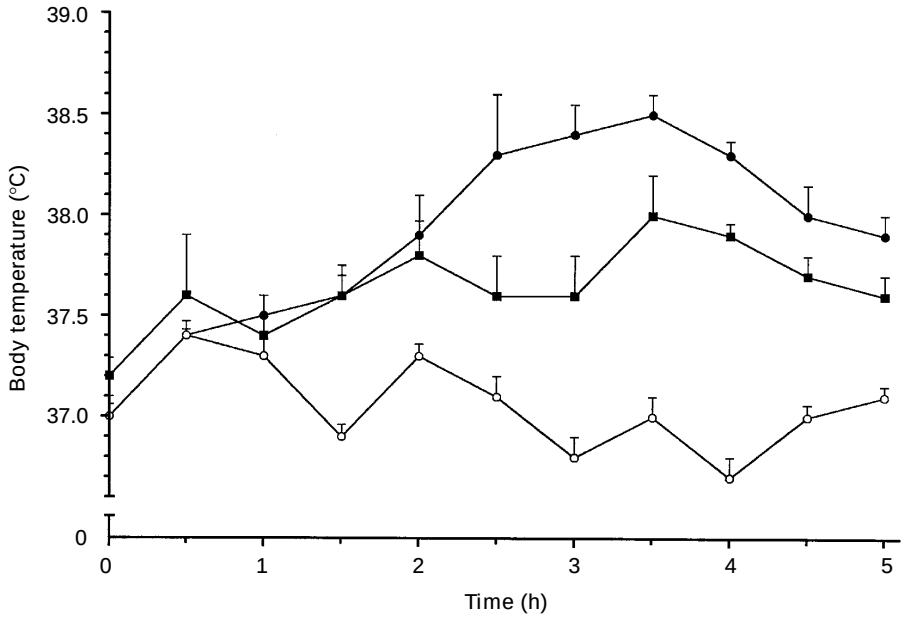

Figure 2 Thermal responses of rats injected intrapouch with LPS (100 μg kg^{-1}, 0 h) and IL-1ra (1 mg kg^{-1}, 0+1 h, i.p.) or saline (1 ml kg^{-1}, 0+1 h, i.p.). Symbols represent: saline (○), LPS/saline (●) and LPS/IL-1ra (■). Data are expressed as mean ($n=3-6$) and vertical lines show s.e.mean AUC; LPS/saline $=2.3\pm0.3$°C h^{-1} vs 1.0 ± 0.3°C h^{-1} for LPS/IL-1ra ($P<0.05$).

Effect of intracerebroventricular (i.c.v) injection of IL-1ra on cytokine induction and the pyrogenic response to LPS

Animals injected with LPS/saline exhibited a significant increase in body temperature 2 h after LPS injection (LPS/Sal, 37.8 ± 0.2°C vs 37.1 ± 0.1°C for saline-treated controls, $P < 0.05$) and was maximal after 3 h ($P < 0.01$) (Figure 3). I.c.v. administration of IL-1ra (200 μg/rat, 0 + 1 h) significantly inhibited LPS fever ($P < 0.05$), but had no significant effect on IL-1 or IL-6 bioactivity in pouch fluid or plasma at any of the time-points tested (Table 3).

Discussion

Our previous studies have shown that the cytokines IL-1 and IL-6 are induced locally at the site of inflammation in a se-

Table 1 Concentrations of bioactive IL-1 and IL-6 after injection of LPS (100 μg kg^{-1}, 0 h, i.po.) and IL-1ra (1 mg kg^{-1}, 0 + 1 h, i.po.) or saline (1 ml kg^{-1}, 0 + 1 h, i.po.)

Sample point (h)	*Treatment*	*Pouch IL-1* (i.u. ml^{-1})	*Pouch IL-6* (i.u. ml^{-1})	*Plasma IL-6* (i.u. ml^{-1})
1.5	Saline	< 1	21 ± 10	< 3
	LPS/Saline	68 ± 10	16,333 ± 2,682	269 ± 34
	LPS/IL-1ra	< 1***	9,009 ± 1,002*	238 ± 61
3	Saline	< 1	14 ± 8	< 3
	LPS/Saline	131 ± 21	32,805 ± 6,451	406 ± 51
	LPS/IL-1ra	21 ± 3***	26,300 ± 4,614	249 ± 30*
5	Saline	3 ± 1	20 ± 12	< 3
	Saline/IL-1ra	3 ± 1	8 ± 5	< 3
	LPS/Saline	126 ± 26	49,963 ± 4,567	250 ± 45
	LPS/IL-1ra	35 ± 14**	32,589 ± 3,468*	231 ± 22

Data are expressed as mean ± s.e.mean ($n = 3-9$). *$P < 0.05$, **$P < 0.001$ and ***$P < 0.001$.

Table 2 Concentrations of bioactive IL-1 and IL-6 after injection of LPS (100 μg kg^{-1}, 0 h, i.po.) and IL-1ra (1 mg kg^{-1}, 0 + 1 h, i.p.) or saline (1 ml kg^{-1}, 0 + 1 h, i.p.)

Sample point (h)	*Treatment*	*Pouch IL-1* (i.u. ml^{-1})	*Pouch IL-6* (i.u. ml^{-1})	*Plasma IL-6* (i.u. ml^{-1})
1.5	Saline	< 1	6 ± 5	< 3
	LPS/Saline	54 ± 25	4,563 ± 271	90 ± 14
	LPS/IL-1ra	58 ± 14	3,470 ± 767	86 ± 24
3	Saline	< 1	< 3	< 3
	LPS/Saline	55 ± 12	24,232 ± 6,531	311 ± 53
	LPS/IL-1ra	65 ± 8	21,019 ± 5,443	246 ± 48
5	Saline	3 ± 1	6 ± 3	< 3
	LPS/Saline	110 ± 26	15,709 ± 5,118	87 ± 18
	LPS/IL-1ra	86 ± 19	11,025 ± 5,606	98 ± 26

Data are expressed as mean ± s.e.mean ($n = 3-7$).

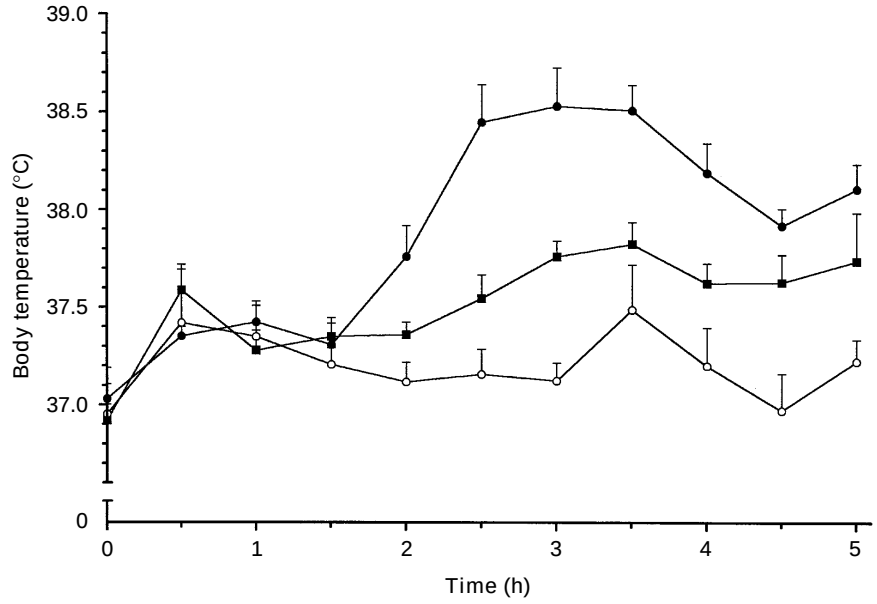

Figure 3 Thermal responses of rats injected intrapouch with LPS (100 μg kg^{-1}, 0 h) and IL-1ra (200 μg/rat, 0 + 1 h, i.c.v.) or saline (2 μl/rat, 0 + 1 h, i.c.v.). Symbols represent: saline (○), LPS/saline (●) and LPS/IL-1ra (■). Data are expressed as mean ($n = 3-6$) and vertical lines show s.e.mean AUC; LPS/saline = 3.0 ± 0.5°C h^{-1} vs 1.2 ± 0.4°C h^{-1} for LPS/IL-1ra ($P < 0.05$).

Table 3 Concentrations of bioactive IL-1 and IL-6 after injection of LPS ($100\,\mu g\,kg^{-1}$, 0 h, i.po.) and IL-1ra ($1\,mg\,kg^{-1}$, 0 + 1 h, i.c.v.) or saline ($2\,\mu l$, 0 + 1 h, i.c.v.)

Sample point (h)	*Treatment*	*Pouch IL-1* (i.u. ml^{-1})	*Pouch IL-6* (i.u. ml^{-1})	*Plasma IL-6* (i.u. ml^{-1})
1.5	Saline	<1	18 ± 8	7 ± 4
	LPS/Saline	76 ± 14	$13{,}339\pm1{,}996$	166 ± 20
	LPS/IL-1ra	61 ± 5	$18{,}325\pm2{,}907$	128 ± 27
3	Saline	<1	12 ± 5	<3
	LPS/Saline	93 ± 15	$37{,}156\pm1{,}285$	423 ± 47
	LPS/IL-1ra	103 ± 28	$32{,}992\pm7{,}528$	438 ± 55
5	Saline	2 ± 1	9 ± 5	<3
	LPS/Saline	185 ± 20	$26{,}350\pm8{,}610$	176 ± 39
	LPS/IL-1ra	148 ± 41	$12{,}180\pm4{,}860$	179 ± 70

Data are expressed as mean ± s.e.mean ($n = 3-6$).

quential manner, in response to injection of LPS into a subcutaneous air pouch in the rat (Miller *et al*., 1996). The objective of the present study was to investigate the role of IL-1 in the febrile response to localized inflammation, and specifically to test the hypothesis that IL-1 acts locally at the inflammatory site and in the brain to modulate fever. These proposals were tested by studying the effects of IL-1ra, a naturally occurring receptor antagonist which blocks IL-1 binding to its Type I receptor (Dripps *et al*., 1991) and has previously been used to inhibit the action of IL-1 in a number of physiological responses (Ohlsson *et al*., 1990; McIntyre *et al*., 1991; Opp & Krueger, 1991; Bluthé *et al*., 1992), including fever (Smith & Kluger, 1992; Luheshi *et al*., 1996).

The results of the present study confirm earlier observations that fever induced by injection of LPS into a subcutaneous air pouch is accompanied by significant increases in IL-1 and IL-6 bioactivity in the air pouch and IL-6, but not IL-1, in the circulation (Miller *et al*., 1996). The production of these cytokines precedes the rise in temperature, which suggests that they could mediate the development of fever in experimental animals.

Administration of IL-1ra into the site of inflammation (intrapouch) significantly attentuated LPS-induced fever (AUC, $P<0.001$) and reduced the local concentration of bioactive IL-1 at each of the time points tested. We initially considered that the reduction in bioactivity could be due to the presence of IL-1ra that had been injected into the pouch. However, no inhibition of IL-1 activity was observed when pouch fluid from an LPS/Sal animal was mixed 1:1 with pouch fluid from a control animal injected with Sal/IL-1ra. Since samples from pouches injected with Sal/IL-1ra were only available for the 5 h time point it is certainly likely that sufficient IL-1ra was present to inhibit IL-1 at the earlier time points.

Injection of IL-1ra i.po. also resulted in a significant decrease in production of IL-6 in the air pouch, both before the onset of fever (1.5 h, $P<0.05$) and after the peak fever (5 h, $P<0.05$). In contrast, plasma IL-6 bioactivity was reduced significantly only at 3 h, when the fever was approaching the maximal value. Our previous study (Miller *et al*., 1996), which investigated the kinetics of local and circulating cytokine induction in response to i.po. injection of LPS, has shown a delay of approximately 1 h between detection of the first significant increases in local (0.5 h) and the plasma (1.5 h) IL-6. This observation may explain the failure to detect a significant reduction in plasma IL-6 at 1.5 h and the subsequent attenuation observed at 3 h. However, the possibility that plasma IL-6 is released independently of local IL-1 cannot be excluded.

The above data suggest that IL-1 acts locally at the sites of inflammation to activate fever indirectly through the release of secondary mediator(s) such as IL-6. However, it is possible that IL-1ra injected into the air pouch may 'leak' into the circulation and act at other sites. To test this possibility, an additional experiment was performed in which IL-1ra was injected i.p. at the same dose as that used in the air pouch and its effect on the local and circulating cytokine bioactivity and fever was investigated. Systemic injection of this dose of IL-1ra caused inhibition of fever (56%, $P<0.05$) which was considerably less than that produced by injection of IL-1ra into the pouch (73%, $P<0.001$). Furthermore, inhibition of LPS fever by systemic injection of IL-1ra was achieved in the absence of any significant reduction in the local IL-1 bioactivity at any of the time points tested. This observation indicates that IL-1ra injected i.p. acts outside the air pouch. IL-1 produced in the air pouch could be released in small amounts into the circulation, though this seems unlikely in view of the fact that no increase in circulating IL-1 was detected by the sensitive bioassay.

A further possibility is that IL-1ra injected i.p. acts in the brain. This is supported by the finding that i.p. injection of IL-1ra failed to inhibit increases in local or circulating IL-6, even though IL-1 is an important inducer of this cytokine. The observation that administration of IL-1ra i.po. inhibited fever and IL-6, indicates that IL-1 mediates fever through release of IL-6 into the circulation. However, the finding that systemically administered IL-1ra failed to inhibit bioactive levels of plasma IL-6, but not fever suggests that the IL-1ra could be acting at different sites, which are not dependent on the induction of IL-6 in the circulation. One possibility is that IL-1ra injected systemically acts directly on areas of the brain responsible for triggering fever, either by crossing the blood-brain barrier or at sites lacking a blood-brain barrier (Blatteis, 1992). Active transport mechanisms from blood to brain have also been described for various cytokines including IL-1 (Banks *et al*., 1991), IL-6 (Banks *et al*., 1994), and IL-1ra (Gutierrez *et al*., 1994). Another possibility is that systemic IL-1 activates central febrile pathways via activation of neural afferents such as the vagus (Bluthé *et al*., 1994; Layé *et al*., 1995).

The importance of brain IL-1, following local (i.po.) injection of LPS was investigated directly in this study. IL-1ra (200 μg/rat, 0 + 1 h) injected i.c.v. inhibited fever (60%, $P<0.05$) induced by i.po. LPS, but was not as effective as when injected at the site of inflammation ($P<0.001$). I.c.v. IL-1ra also failed to influence the elevation in local IL-1 and IL-6, or circulating IL-6, at any time point. These data are consistent with previous studies showing induction of IL-1 in the brain after peripheral LPS (Layé *et al*., 1994), and are in agreement with our previous studies demonstrating that i.c.v. injection of IL-1ra inhibits the febrile response to i.p. LPS (Luheshi *et al*., 1996). Inhibition of fever by IL-1ra in the present study was independent of either local or ciculating cytokines and conflicts with studies showing that brain IL-1 induced IL-6 in the perpiphery (De Simoni *et al*., 1990). It is unlikely that the dose of IL-1ra injected i.c.v. has systemic actions since similar doses were previously injected systemically with no effect on fever or cytokine bioactivity (G. Luheshi, unpublished data).

Recent studies have shown that IL-6 and tumour necrosis factor-α (TNF-α), but not IL-1, are produced in brain areas responsible for thermoregulation (Klir *et al*., 1993) and that

the appearance of these cytokines is consistent with the development of the fever after i.p. LPS. Furthermore, the production of brain IL-6 in particular is inhibited by the introduction of an antibody to IL-1, suggesting that IL-1 is probably produced in areas of the brain peripheral to the posterior hypothalamus and that it acts via the release of IL-6 in the brain to induce fever (Klir *et al*., 1994).

In conclusion, the results presented in this study indicate that the cytokines IL-1 and IL-6 are produced locally by an inflammatory stimulus. The importance of IL-1 in the subsequent febrile response is supported by the observation that IL-1ra administered locally, systemically or into the brain inhibits LPS fever. These data suggest that IL-1 acts locally to induce the release of IL-6, which may represent an important circulating pyrogen. However, these data also suggest that brain IL-1 has an important role in the development of the febrile response and that this effect is not mediated via regulation of peripheral cytokine (IL-6) production.

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