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The role of TNF-α in fever: opposing actions of human and murine TNF-α and interactions with IL-β in the rat

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1 The role of tumour necrosis factor-α (TNF-α) in fever is controversial. Some studies have indicated that TNF-α acts as a cryogen to inhibit fever, while others suggest that TNF-α is an endogenous pyrogen which mediates fever. The majority of studies in experimental animals supporting a cryogenic action have been conducted using human (h)TNF-α, which has been shown to bind only to one (p55) of the two TNF-α receptors in rodents.

2 The aim of the present investigation was to study the role of TNF-α in fever by comparing effects of hTNF-α, which binds only to the p55 receptor, with those of murine (m)TNF-α, which binds to both p55 and p75 TNF-α receptors, and to investigate the relationship between TNF-α and interleukin-1 (IL-1), an important endogenous pyrogen.

3 Injection of hTNF-α (0.3–10 μg kg^{-1}, i.p.) had no effect on core temperature in conscious rats (measured by remote radiotelemetry), whereas mTNF-α (3 μg kg^{-1}) induced fever which was maximal 1 h after the injection (38.2±0.2°C compared to 37.3±0.1°C in controls). Intracerebroventricular (i.c.v.) administration of either form of TNF-α elicited dose-dependent fever at doses higher than 0.12 μg kg^{-1}.

4 Peripheral injection of hIL-1β (1 μg kg^{-1}) resulted in fever (38.3±0.2°C compared to 37.2±0.1°C in controls at 2 h), which was significantly attenuated ($P<0.01$) by co-administration of a sub-pyrogenic dose of hTNF-α (1 μg kg^{-1}), but was unaffected by co-administration of mTNF-α (0.1 or 0.3 μg kg^{-1}, i.p.). In contrast, intracerebroventricular (i.c.v.) co-administration of a sub-pyrogenic dose (0.12 μg kg^{-1}) of hTNF-α did not attenuate fever induced by intraperitoneal (i.p.) injection of IL-1β, and sub-pyrogenic dose (0.12 μg kg^{-1}, i.c.v.) of mTNF-α significantly prolonged the febrile response to IL-1β. Pretreatment of animals with anti-TNF-α antiserum (i.c.v.) did not affect the febrile response to systemic IL-1β.

5 Animals injected i.p. with a pyrogenic dose of mTNF-α developed fever (38.2±0.2°C compared to 37.3±0.1°C in controls 2 h after the injection) that was completely abolished by peripheral administration of IL-1ra (2 mg kg^{-1}, $P<0.001$), while i.c.v. administration of IL-1ra (400 μg/rat) did not affect mTNF-α-induced fever.

6 These data indicate that endogenous TNF-α is probably a pyrogen and that previous results suggesting cryogenic actions of TNF-α resulted from the use of a heterologous protein in the rat. The markedly contrasting effects of mTNF-α and hTNF-α could result from different interactions with the two TNF-α receptor subtypes. The data also suggest that fever induced by exogenous TNF-α is mediated via release of IL-1β in peripheral tissues, but not in the brain.

Keywords: Fever; murine TNF-α; human TNF-α; IL-1; IL-1ra

Introduction

Injury, infection and inflammation elicit an array of local and systemic responses in mammals. These include immune activation, metabolic, endocrine and behavioural changes and the development of fever. The cytokines interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α play an important part in mediating both local and systemic responses, and have been implicated in the regulation of fever (see Kluger, 1991; Rothwell & Hopkins, 1995). There is considerable evidence supporting the function of IL-1 and IL-6 as endogenous pyrogens (Rothwell, 1990; LeMay *et al.*, 1990). However, the role of TNF-α in the regulation of fever is still controversial and its interactions with other pyrogens is not fully understood.

In human subjects, administration of human (h)TNF-α at doses which mimic circulating concentrations after injection of bacterial lipopolysaccharide (LPS), causes fever comparable to that induced by LPS (Michie *et al.*, 1988), which supports the hypothesis that TNF-α mediates LPS-induced fever. Furthermore, a transient rise in both plasma and CSF concentrations of TNF-α has been observed after systemic injection of LPS or IL-1 in rodents (LeMay *et al.*, 1990; Klir *et al.*, 1993; Zeisberger & Roth, 1993). Although circulating concentrations of TNF-α do not correlate closely with fever, this may reflect involvement of other pyrogens or sites of action of TNF-α outside the circulation. A large body of evidence shows that peripheral administration of hTNF-α causes fever in mice (Dinarello *et al.*, 1986), rabbits (Nakamura *et al.*, 1988; Morimoto *et al.*, 1989; Hashimoto *et al.*, 1994), rats (Dinarello *et al.*, 1986; Coombes *et al.*, 1987) and man (Michie *et al.*, 1988; Jakubowski *et al.*, 1989). Furthermore, intracerebroventricular (i.c.v.) injection of hTNF-α causes fever in rats (Plata-Salamán *et al.*, 1988; Rothwell, 1988) and rabbits (Morimoto *et al.*, 1989). In contrast, some experimental data suggest a cryogenic (antipyretic) role for TNF-α. Stress-induced hyperthermia (Long *et al.*, 1990c) and fever caused by LPS injection (Long *et al.*, 1990a,b) are enhanced when endogenous TNF-α is neutralised by systemic injection of anti-TNF-α antiserum in rats,

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which suggests that endogenous TNF-α inhibits fever. However, these data are contradicted by studies on fever resulting from tissue injury caused by intramuscular injection of turpentine in rats, in which injection of the same neutralising TNF-α antiserum resulted in a reduced fever (Cooper *et al.*, 1994; Stefferl *et al.*, 1995a).

Further evidence for antipyretic effects of TNF-α is derived from the observation that administration of sub-pyrogenic doses of hTNF-α attenuates fever induced by either LPS or IL-1 in rats (Long *et al.*, 1992). This could be due, in part, to the existence of two distinct receptor types for TNF-α, namely p55 and p75, which have similar affinities for TNF-α, and are present on almost all cells (Tartaglia & Goeddel, 1992). Receptor function has not been fully established for the p55 and p75 receptors, but in the mouse and probably the rat, hTNF-α activates only the p55 receptor (Lewis *et al.*, 1991; Heller *et al.*, 1992), which provides an experimental tool to establish the function of each receptor type.

Although IL-1 has been identified as an endogenous pyrogen, its relationship to TNF-α is unclear. IL-1 has been implicated in responses to a variety of stimuli, including injection of TNF-α (Dinarello *et al.*, 1986); however, it has not been readily detected in the circulation of animals in which fever has been induced by LPS (Cannon *et al.*, 1990) or local inflammation (Cooper *et al.*, 1994).

The aim of this study was therefore two fold. Firstly to identify the difference between the action of human and murine (m)TNF-α in the rat, and secondly to determine the way in which two important pro-inflammatory cytokines, IL-1 and TNF-α, interact during the activation of the febrile response.

Methods

Male, Sprague-Dawley rats (Charles River, U.K.), weighing 200 to 300 g, were used in all experiments. Animals were housed singly on the day before the experiment, and were monitored under controlled environmental conditions (19–22 °C, 12 h light-dark cycle, light on at 08 h 00 min), with free access to food (CRM, Labsure) and water.

Recombinant human TNF-α (hTNF-α) and murine TNF-α (mTNF-α) were generous gifts from Knoll AG, Ludwigshafen, Germany and Professor W. Fiers, University of Gent, Belgium, respectively. Standard bioassay with WEHI 164 cells indicated that 1 iu of hTNF-α (87/650, NIBSC, South Mimms, UK, approximately 25 pg) was equivalent to approximately 19 pg of the hTNF-α preparation from Knoll AG, 5.8 pg mTNF-α from Professor W. Fiers and 6.5 pg of an interim murine TNF-α Standard from NIBSC (88/532). Recombinant human IL-1β was donated by Dr R. Newton (E.I. Dupont De Nemours & Co.) and had an activity of 2×10^8 iu mg^{-1} (rec.hIL-1β 86/680, NIBSC). All cytokine preparations were tested for endotoxin contamination by assessing pyrogenic activity following heat treatment, 90°C for 15 min. Human recombinant interleukin-1 receptor antagonist (IL-1ra) was a generous gift from Synergen, U.S.A. The rabbit anti-mouse TNF-α antiserum was a gift from Dr S. Kunkel, Michigan University, U.S.A. and has been used successfully in a number of studies to neutralize endogenous rat TNF-α *in vivo* (eg. Long *et al.*, 1990a;c; Cooper *et al.*, 1994; Stefferl *et al.*, 1995a). All dilutions were made in sterile, pyrogen-free saline (0.9% NaCl). Drugs were administered intraperitoneally (i.p.) in 0.2 ml sterile saline, or intracerebroventricularly (i.c.v.) via indwelling guide cannulae, in volumes ranging from 3 to 5 μl. In all experiments, control animals were injected with appropriate volumes of vehicle.

Core body temperature was measured by remote radiotelemetry, in animals previously implanted intraperitoneally with temperature-sensitive radiotransmitters (Data Sciences, U.S.A.) under halothane/NO_2 anaesthesia. Some animals were also implanted with indwelling guide cannulae in the lateral ventricle of the brain (under sodium pentobarbitone anaesthesia 60 mg kg^{-1}) for i.c.v. injections. Animals were allowed to recover from surgery for at least seven days before the start of experiments.

All data are presented as mean ± s.e.mean. Statistical analysis was performed by multiple analysis of variance (MANOVA) for the timecourse of each experiment.

Results

All animals showed a transient rise in body temperature at the beginning of the experiments probably due to handling stress, but this response did not differ significantly between groups. Injection of heat-treated cytokines failed to affect body temperature (data not shown), indicating negligible levels of endotoxin contamination.

Effects of peripheral administration of hTNF-α or mTNF-α on fever induced by IL-1β

In order to establish the pyrogenic potential of hTNF-α or mTNF-α, dose-response experiments were performed. None of the doses of hTNF-α tested (0.3–3 μg kg^{-1}, i.p.) elicited significant fever up to 5 h post injection (Figure 1a) and hTNF-α even failed to elicit a pyrogenic response when administered at a dose of 10 μg kg^{-1} (Figure 1a, $P=0.38$, $n=6$). In further experiments, a dose of 1 μg kg^{-1} hTNF-α was used. In contrast, i.p. injection of 1 or 3 μg kg^{-1} mTNF-α caused an increase in core temperature (Figure 1b), which was maximal 1 h

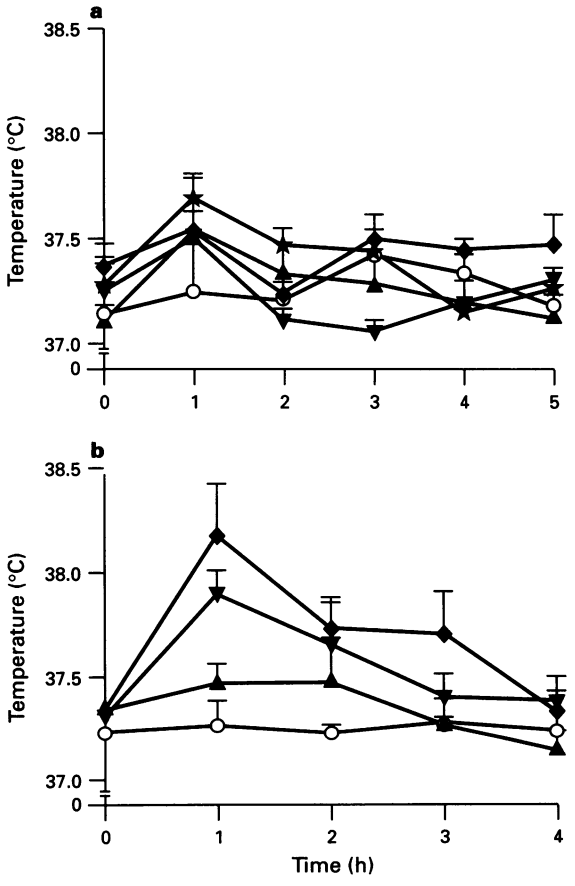

Figure 1 Effects of i.p. injection of mTNF-α or hTNF-α on body temperature: body temperature of animals injected with hTNF-α (a) did not rise significantly at any of the doses used (saline ○; 0.3 μg kg^{-1} ▲; 1 μg kg^{-1} ▼; 3 μg kg^{-1} ◆; 10 μg kg^{-1} ★; $P=0.38$ for 10 μg kg^{-1}). In contrast, animals injected with mTNF-α (b) developed fevers at 1 μg kg^{-1} ($P<0.05$) and 3 μg kg^{-1} ($P<0.05$) whereas injection of 0.3 μg kg^{-1} did not result in fever ($P=0.46$). Symbols used as for (a), $n=5-6$ in all groups.

after the injection and returned to baseline 4 h postinjection. Injection of 0.3 μg kg^{-1}, mTNF-α did not induce fever ($P=0.46$) up to 5 h postinjection. In further experiments, 0.3 μg kg^{-1} or 0.1 μg kg^{-1} mTNF-α were used as sub-pyrogenic doses.

A separate set of animals, injected with a maximal pyrogenic dose of IL-1β (1 μg kg^{-1}, i.p.) as determined from previous experiments (data not shown), developed fever which peaked after 2 h (38.3±0.2°C compared to control animals 37.2±0.1°C, $P<0.001$, Figure 2). Co-injection of hTNF-α (1 μg kg^{-1}, i.p.) significantly inhibited the febrile response to IL-1β over the time course (5 h) of the experiment, $P<0.01$. In contrast, 0.3 μg kg^{-1} mTNF-α did not affect the magnitude or duration of the febrile response to IL-1β.

Effects of central administration of hTNF-α or mTNF-α on fever induced by IL-1β

Sub-pyrogenic doses of hTNF-α or mTNF-α used for i.c.v. injections were determined in dose-response experiments. Although i.p. injection of 10 μg kg^{-1} hTNF-α failed to elicit fever, i.c.v. administration of 0.4 or 1.2 μg kg^{-1} hTNF-α or mTNF-α caused a significant increase in body temperature (Figure 3a,b) which peaked at 2 h and 3 h, respectively, and returned to baseline levels at 6–7 h postinjection. In subsequent experiments 0.12 μg kg^{-1} of either preparation was used as a sub-pyrogenic i.c.v. dose. I.c.v. injection of 0.12 μg kg^{-1} of either hTNF-α or mTNF-α had no effect on the maximum fever induced by systemic injection of IL-1β (1 μg kg^{-1}, i.p., Figure 4a,b). However, the response to IL-1β was prolonged significantly ($P<0.02$) for 2 h in the presence of mTNF-α.

Effects of i.c.v. anti-TNF-α antiserum on fever induced by systemic IL-1β

To investigate the role of endogenous brain TNF-α in the development of fever due to IL-1β, animals were pretreated with 3 μl anti-TNF-α antiserum i.c.v., 24 h prior to the start of the experiment, to allow distribution and penetration of brain tissue. Animals were then injected with IL-1β (1 μg kg^{-1}, i.p.) to induce fever. I.c.v. injections of antiserum or pre-immune serum had no effect on body temperature (data not shown). Injection of IL-1β (1 μg kg^{-1}, i.p.) in rats pretreated with preimmune serum resulted in a fever which was maximal 2 h later (38.2±0.2°C vs 37.2± 0.1°C in vehicle-treated animals, $P<0.001$). Pretreatment with anti-TNF-α antiserum (i.c.v.) did not affect the magnitude (38.1±0.2°C at 2 h) or duration (4.5 h) of febrile responses to systemic (i.p.) IL-1β (Figure 5).

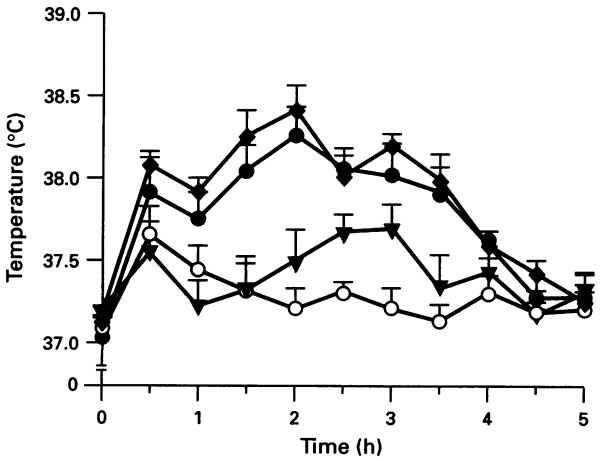

Figure 2 Effects of injection of non-pyrogenic doses of hTNF-α or mTNF-α on fever induced by IL-1β: rats ($n=4-8$) were injected with vehicle control (○) or IL-1β (1 μg kg^{-1}, i.p., ●) either alone or together with hTNF-α (1 μg kg^{-1}, i.p., ▼) or mTNF-α (0.3 μg kg^{-1}, i.p., ◆).

Effects of IL-1ra on fever induced by systemic mTNF-α

In this set of experiments, animals were injected with 3 μg kg^{-1} mTNF-α, which had elicited a maximal febrile response in dose-response studies (Figure 1). In one group of animals, the actions of endogenous IL-1 were investigated by co-injection of IL-1ra, either peripherally (i.p.) at a dose of 2 mg kg^{-1} or i.c.v. at a dose of 400 μg kg^{-1}, which is higher than a dose previously shown to inhibit IL-1 fever (Stefferl *et al.*, 1995a). Administration of mTNF-α (3 μg kg^{-1}, i.p.) resulted in fever ($P<0.001$) which reached a maximum of 38.2±0.2°C, 2 h after the injection (Figure 6), compared to 37.3±0.1°C in vehicle-treated animals at this time, and returned to baseline by 3.5 h. Co-injection of IL-1ra (2 mg kg^{-1}, i.p.) completely abolished the pyrogenic response to systemic injection of mTNF-α (37.4±0.1°C after 2 h, $P<0.001$, vs mTNF-α alone, Figure 6).

In experiments where IL-1ra (400 mg kg^{-1}) was injected i.c.v., mTNF-α (3 μg kg^{-1}, i.p.) resulted in fever ($P<0.01$) which was maximal (38.5±0.3°C) 2 h later. Injection of IL-1ra did not significantly modify fever induced by mTNF-α (3 μg kg^{-1}, i.p., data not shown). Injection of vehicle resulted in a body temperature of 37.2±0.1°C at this time-point.

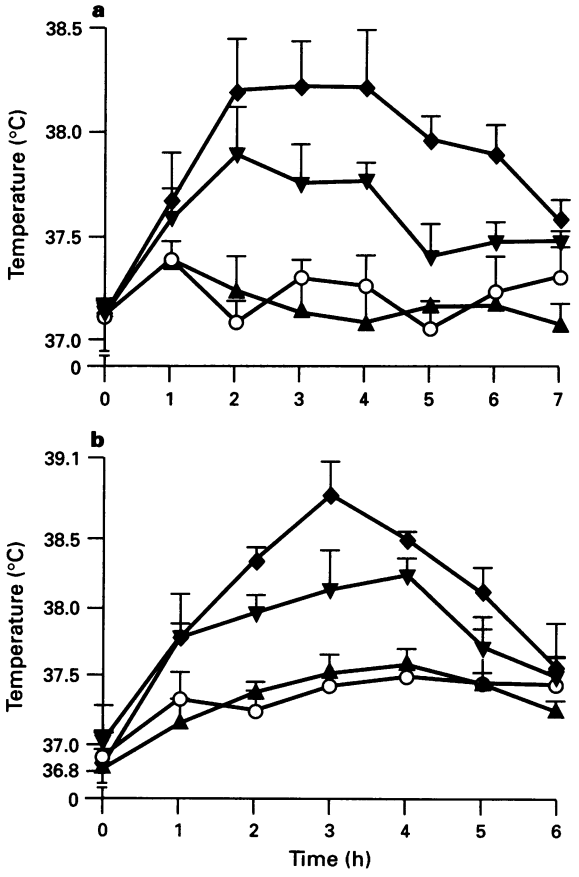

Figure 3 Effects of i.c.v. hTNF-α or mTNF-α on body temperature: animals ($n=5$) were injected i.c.v. with hTNF-α (a) or mTNF-α (b). The injection of 0.12 μg kg^{-1} (▲) of either preparation did not result in fever compared to vehicle controls (○), whereas at higher doses (0.4 μg kg^{-1} ▼; 1.2 μg kg^{-1} ◆) both hTNF-α and mTNF-α caused significant rises in body temperature ($P<0.01$ for 1.2 μg kg^{-1} of either preparation).

Discussion

Recent data have suggested that recombinant TNF-α from different species have differing affinities for the two known murine TNF receptors (p55 and p75) (Lewis *et al.*, 1991). These data, together with the discrepancy between pyrogenic and cryogenic activities shown for TNF-α, prompted the present study to compare the effects of hTNF-α and mTNF-α on body temperature in rats. Responses to peripheral injection of TNF-α indicate that the murine protein elicited fever at 1 $\mu g\ kg^{-1}$, while hTNF-α had no effect at doses up to 3 $\mu g\ kg^{-1}$. The mTNF-α was approximately 4 times more active than hTNF-α, in a standard bioassay, which may reflect a difference in receptor affinities for human and murine TNF-α, since the bioassay target cells (WEHI-164) are murine. To test the possibility that the discrepancy between the two different preparations in inducing fever was not a direct reflection of their activity *in vitro*, a dose of 10 $\mu g\ kg^{-1}$ hTNF-α was injected (i.p.), but this also failed to induce fever. Previous studies have shown that peripheral injection of doses of up to 50 $\mu g\ kg^{-1}$ of hTNF-α do not cause significant fever in rats (Long *et al.*, 1992), suggesting a genuine difference between human and murine TNF-α. More critical, and perhaps surprising, is the demonstration that murine and human TNF-α preparations had very similar effects on fever when injected i.c.v. The mechanisms underlying these varied responses to central and peripheral injections of hTNF-α are unclear, but some possibilities can be considered. Firstly, hTNF-α and mTNF-α may be cleared at different rates in peripheral tissues, but not in the brain. Another possibility is that the TNF-α receptors which mediate fever differ between the CNS and periphery. As yet there is no direct evidence to support this, because there is no consensus about the location or nature of TNF-α receptors expressed in the brain (Cunningham *et al.*, 1993; Boka *et al.*, 1994).

Further differences between murine and human preparations become apparent from the observed effects of TNF-α on fever induced by IL-1β. A subpyrogenic dose of hTNF-α significantly attenuated fever when co-administered peripherally with IL-1β, thus confirming the findings of Long *et al.* (1992). This observation led Long *et al.* (1992) to suggest that endogenous TNF-α is a cryogen, and that its main action is to limit the severity of febrile responses, an effect that may be mediated by vasopressin (Derijk & Berkenbosch, 1993). In contrast, we observed that peripheral injection of mTNF-α failed to attenuate febrile responses to IL-1β, which questions the hypothesis that TNF-α is an endogenous cryogen.

These results are critical to understanding the role of endogenous TNF-α in the regulation of fever, namely whether it acts as a cryogen, as indicated by the effects of hTNF-α, or as a pyrogen. Our results suggest that the cryogenic actions of hTNF-α are an experimental artefact, resulting from the use of species heterologous protein which has different receptor affinities from the homologous protein. To exclude the possibility that the cryogenic actions of hTNF-α was attributable to an effect that is induced only by sub-pyrogenic doses of TNF-α, a

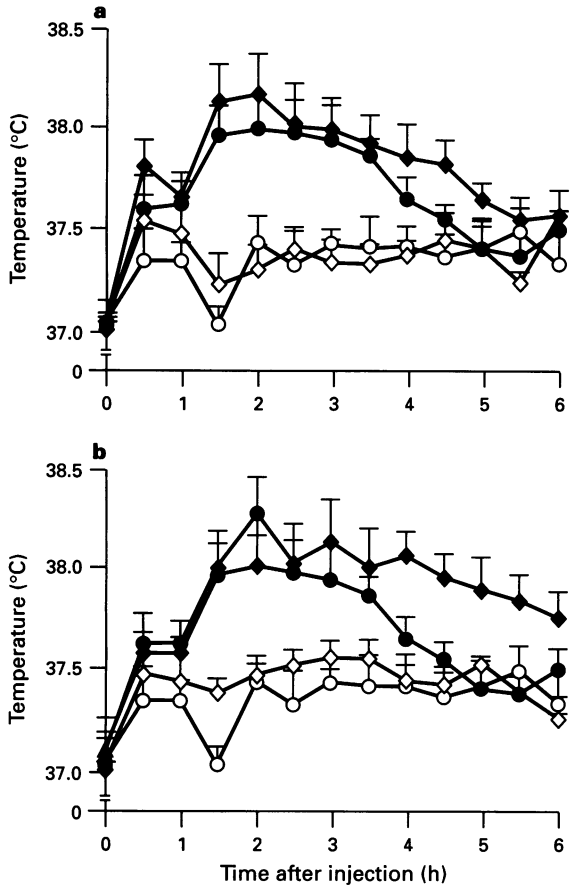

Figure 4 Effects of injection of subpyrogenic doses of hTNF-α and mTNF-α on fever elicited by IL-1β: rats ($n=6-10$) were injected i.p. with saline (○) or 1 $\mu g\ kg^{-1}$ IL-1β i.p. (●). The effect of 0.12 $\mu g\ kg^{-1}$, i.c.v. hTNF-α (a) or mTNF-α (b) on the saline (◇) or IL-1β (◆) responses is also shown.

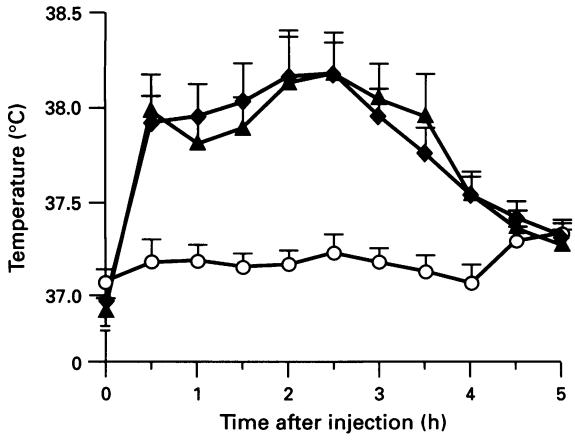

Figure 5 The effect of anti-TNF-antiserum on IL-1β induced fever: rats ($n=12$) injected with IL-1β (1 $\mu g\ kg^{-1}$, i.p., ●) exhibited a highly significant febrile response when compared with saline (○)-treated animals ($P<0.001$). Treatment with anti-TNF-antiserum (3 μl/rat i.c.v., ▲) had no effect on the febrile response to IL-1β.

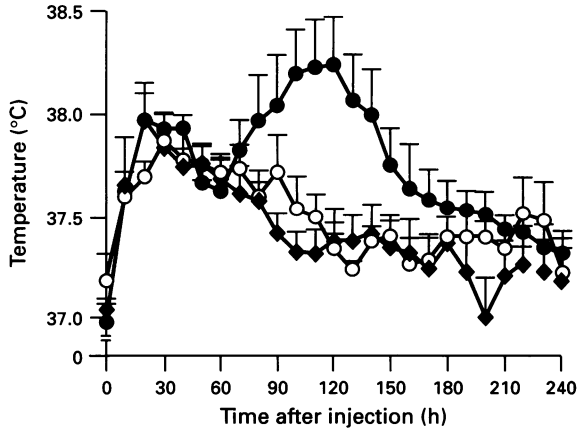

Figure 6 The effect of injection of IL-1ra on fever induced by mTNF-α: injection of 3 $\mu g\ kg^{-1}$ mTNF-α i.p., alone (●) or co-injected with 2 $mg\ kg^{-1}$ IL-1ra, i.p. (◆) is compared to vehicle control (○), ($n=5$). The reduction in fever induced by IL-1ra was significant at $P<0.001$.

lower dose of mTNF-α (0.1 μg kg^{-1}, i.p.) was used, but this also failed to inhibit the febrile responses to IL-1β (data not shown). Hence, it appears that hTNF-α and mTNF-α have genuinely different effects on IL-1-induced fever in the rat.

If the cryogenic effects of hTNF-α are due to the use of species heterologous protein in the rat, two possible hypotheses can be considered. Firstly, hTNF-α could exert an antagonistic action at one of the rat receptor(s), and therefore inhibit the effects of endogenous TNF-α. If endogenous TNF-α acts as a pyrogen via interaction with both receptors in the periphery, then inhibition of its actions at one (e.g. p75) could lead to a reduction of fever. Recent results with mice deficient in the p75 receptor suggest that both receptors are required for some actions of TNF (Erickson *et al.*, 1994). Furthermore, selective activation of p55 receptors could mimic the effects of an endogenous cryogen if, for example, p55 induces responses which inhibit fever, such as hypothalamic-pituitary adrenal (HPA)-activation. A second possibility is that p55 and p75 receptor subtypes may be involved in cryogenic and pyrogenic effects of TNF-α respectively.

In agreement with previous findings (Long *et al.*, 1992), we observed that i.c.v. injection of hTNF-α failed to inhibit fever induced by systemic injection of IL-1β. This contrasts with effects of systemic injections of hTNF-α on fever induced by IL-1β (see above), and could reflect the involvement of different receptors in the brain. Similarly, mTNF-α failed to inhibit the febrile response to IL-1β, but instead prolonged the response (Figure 4b). The fact that central administration of anti-TNF-α antiserum did not influence fever induced by IL-1β, indicates that endogenous brain TNF-α does not participate in the fever induced by peripheral IL-1β. It is unlikely that the lack of effect observed in this experiment was due to poor penetration of the antiserum in brain tissue, thus preventing it from reaching its putative site(s) of action since previous studies, using the same antiserum injected −24 h i.c.v., have shown an inhibition of fever induced by localized peripheral inflammation (Stefferl *et al.*, 1995a).

One of the main questions addressed in this study was whether TNF-α and IL-1β interact in the periphery to induce fever. IL-1 can induce the release of TNF-α in the mouse (Vogels *et al.*, 1994), but the hypothesis that IL-1β is directly involved in pyrogenic responses to TNF-α has not been tested. To investigate this, the effects of IL-1ra on febrile responses to TNF-α were tested. Peripheral injection of IL-1ra inhibits fever due to tissue injury (Luheshi *et al.*, 1994) or LPS (Bate *et al.*, 1994) and, in this study, completely abolished fever induced by systemic injection of mTNF-α. This suggests that TNF-α induces IL-1 *in vivo*, and that IL-1 is an important mediator of fever induced by TNF-α. Hashimoto *et al.* (1994) recently reported that injection of anti-IL-1β antiserum in rabbits fails to inhibit pyrogenic responses to TNF-α. However, although this antiserum neutralised IL-1β *in vitro*, it only partially inhibited the action of exogenous IL-1β *in vivo*. The effects of systemic IL-1ra on fever induced by TNF-α suggest a sequential induction of TNF-α then IL-1 in the generation of fever. In contrast, central injection of IL-1ra (400 μg kg^{-1}) had no effects on fever. Although it has been reported that actions of IL-1β in the brain are not inhibited by IL-1ra (Kent *et al.*, 1992), subsequent experiments performed in this laboratory have shown that IL-1ra does inhibit pyrogenic effects of IL-1 in the brain, measured by radio-telemetry in free-moving rats (Stefferl *et al.*, 1995b).

In summary, the data presented in this study suggest that mTNF-α is a pyrogen in the rat, and that endogenous TNF-α acts as a pyrogen following injection of IL-1β. The peripheral pyrogenic actions of mTNF-α are not mimicked by hTNF-α, which may act as an antagonist or exert differential effects at each of the two receptors in the periphery. Although hTNF-α does not induce fever in the rat when administered peripherally, it does do so when administered centrally. This indicates differences in the nature or function of the receptors involved in peripheral and central pyrogenic activity of TNF-α. Finally, pyrogenic responses to peripheral TNF-α appear to be mediated via IL-1, which is not involved in the central mediation of fever induced by TNF-α.

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