Brief Definitive Report

Interleukin (IL)-6 Gene Expression in the Central Nervous System Is Necessary for Fever Response to Lipopolysaccharide or IL-1β: A Study on IL-6–deficient Mice

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Summary

Interleukin (IL)-6, IL-1β, and tumor necrosis factor α (TNF-α) are considered to act as endogenous pyrogens. Because of the complex pattern of cross-inductions between these cytokines, the relative role of the central and peripheral production of these cytokines in eliciting the fever response has not yet been clarified. The purpose of this study was to determine the role of IL-6 in the fever response by making use of mice carrying a null mutation in the IL-6 gene. The intraperitoneal injections of lipopolysaccharide (LPS) (50 μg/kg) and recombinant murine (rm) IL-1β (10 μg/kg), respectively, failed to evoke fever response in IL-6–deficient mice, whereas the same doses of LPS and rmIL-1β caused fever response in wild-type mice. The fever response could be induced in the IL-6–deficient mice by intracerebroventricular injection of recombinant human (rh) IL-6 (500 ng/mouse), whereas intracerebroventricular injection of rmIL-1β (100 ng/mouse) failed to produce fever response in the IL-6–deficient mice. These results suggest that central IL-6 is a necessary component of the fever response to both endogenous (IL-1β) and exogenous (LPS) pyrogens in mice and that IL-6 acts downstream from both peripheral and central IL-1β.

Fever is a systemic response that can be elicited by a multitude of exogenous stimuli (1–4). Work in the past decade has led to identification of several of the endogenous mediators of fever. IL-1α and IL-1β (5, 6), TNF-α (7), and IL-6 (6, 8–13) have been identified as putative endogenous pyrogens, based on experiments that showed that systemic or central intracerebroventricular application of these cytokines evokes fever response. In addition, it has been demonstrated that the mRNAs encoding these cytokines as well as their gene products are increased in the periphery (14, 15) and, more importantly, in some brain regions, including the hypothalamus, during fever caused by injection of bacterial LPS (16–19). Receptors for these cytokines are also found in the thermoregulatory regions of the brain, such as the preoptic area of the hypothalamus (4, 20–22).

IL-6, a proinflammatory cytokine (8), acts as an endogenous pyrogen (6, 8–13) in addition to its multiple effects on the immune system and in particular on hematopoiesis (8). The major inducers of IL-6 synthesis are IL-1 and TNF-α, which also act as inducers of each other's biosynthesis (7, 15, 16, 23–25). The study of the complex cascade of IL-1, TNF-α, and IL-6 effects is difficult because no reliable antagonists exist for TNF-α and IL-6, respectively; some TNF-α antagonists (26) as well as some antagonists of human IL-6 (27) but not of murine IL-6 action have been described.

Recently, we generated a strain of mice carrying a null mutation in the IL-6 gene, and therefore defective in IL-6 production (28). These mice (28, 29) represent a powerful tool to characterize unambigously the role of IL-6 in several physiological and pathological phenomena.

IL-6–deficient mice are unable to mount a normal inflammatory response to localized tissue damage generated by turpentine injection (30) but show no altered metabolic and lethality responses to TNF-α (31) and LPS (30).

In this article, we examine the fever response to the exogenous pyrogen LPS and the endogenous pyrogen IL-1β in IL-6–deficient mice and in wild-type littermates to define the role played by IL-6 in the pyrogenic response. To elucidate the role of peripheral and central pools of IL-6, which does not readily cross the blood–brain barrier, we have used both systemic (intraperitoneal) and intracerebroventricular injection of IL-6 to restore fever response in IL-6–deficient mice.

Materials and Methods

Materials. LPS from *Escherichia coli* (055:B5, purified by phenol extraction and gel filtration chromatography) was from Sigma

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/96/01/311/06 $2.00
Volume 183, January 1996 311–316

Chemical Co. (St. Louis, MO); the same lot of LPS was used in all experiments. Recombinant murine (rm) IL-1β was a gift from Dr. P. T. Lomedico (Hoffman-La Roche, Basel, Switzerland). Rompun was from Bayer AG (Leverkusen, Germany) and Ketalar was from Parke-Davis, S.A. (Barcelona, Spain). Recombinant human (rh) IL-6 was produced in *E. coli* at the Institute for Research in Molecular Biology ([IRBM] Rome, Italy) as described by Arcone et al. (32). The bioactivity (2×10^8 U/mg) was assayed using 7TD1 cell growth assay (33). All substances were diluted in pyrogen-free saline solution the day of the experiment.

Animals. 13–18-wk-old IL-$6^{-/-}$ male mice and IL-$6^{+/+}$ littermates were bred at IRBM. The generation of this strain of mice and its genetic background has been described by Poli et al. (28). The mice were housed one per cage in a temperature-controlled room, with food and water ad libitum and under a 12-h light/12 h dark diurnal cycle (light at 8.00 a.m.). The temperature in the room was kept at 30 ± 1°C. The animals were acclimated for at least 1 wk before starting any experimental procedure. The experiments started when the mice reached a body weight of 30 ± 2 g; no differences in body weight were observed between IL-6–deficient and wild-type mice.

Measurement of Body Temperature. A radiotransmitter was inserted in the mouse peritoneal cavity under anesthesia with ketamine (Ketalar, 50 mg/kg) and xylazin (Rompun, 10 mg/kg). All mice with a radiotransmitter inserted were indistinguishable (for their body weight and activity) from normal mice 4 d after the surgery. After implantation, the animals were allowed to recover for at least 5 d before starting any experimental procedure. The core body temperature and activity (data not shown) of the animals were measured using battery-operated biotelemetry devices (model XM-FH; Mini-Mitter Co., Inc., Sunriver, OR) as described by Kozak et al. (34). Body temperature values were recorded at 10 min intervals beginning at least 24 h before the injection of LPS, rmIL-1β, or rhIL-6 and continued for at least 48 h after the injections. All treatments began between 10:00 and 11.30 a.m., during the light period.

Intracerebroventricular injection of 10 μl was carried out under ether anesthesia, at the site described by Haley and McCormick (35). The needle was lowered 3.5 mm after penetration.

Statistical Analysis. All data were reported as mean + SE. Analysis of variance followed by Fisher's protected least significant difference test was used to analyze statistical differences. The average group size was five. Animals with consecutive missing temperature recordings because of failure of the telemetry system were excluded from the statistical analysis.

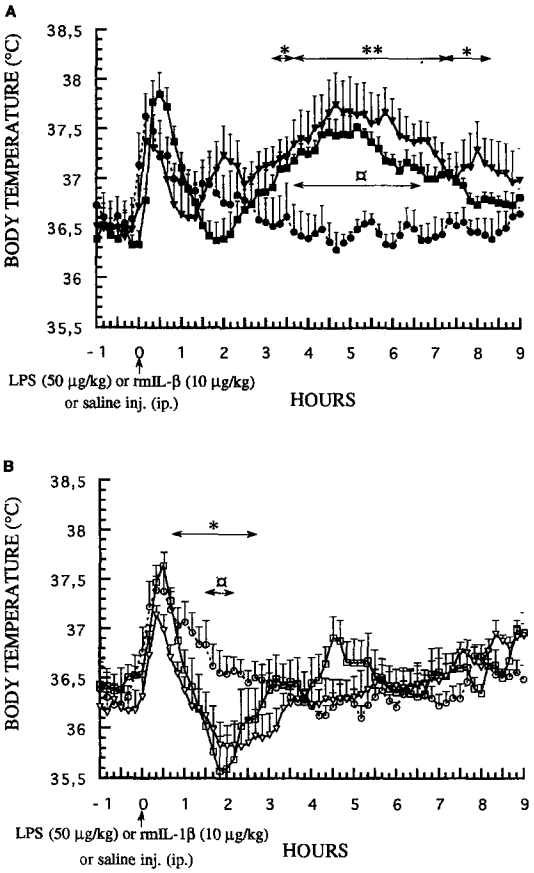

Figure 1. Effect of LPS (50 μg/kg i.p.) or rmIL-1β (10 μg/kg i.p.) injection on the core body temperature of IL-6–deficient and wild-type mice measured at 30 ± 1°C of ambient temperature. Data are mean + SE. ** $P < 0.01$, saline- versus LPS-injected mice; * $P < 0.05$, saline- versus LPS-injected mice; ¤ $P < 0.05$, saline- versus rmIL-1β–injected mice. (*A*) LPS (50 μg/kg i.p.) or rmIL-1β (10 μg/kg i.p.) effects on body temperature in wild-type mice. Saline- injected mice (●; $n = 6$); LPS-injected mice (▼; $n = 5$); and rmIL-1β–injected mice (■; $n = 5$). (*B*) LPS (50 μg/kg i.p.) or rmIL-1β (10 μg/kg i.p.) effects on body temperature in IL-6–deficient mice. Saline-injected mice (○; $n = 5$); LPS-injected mice (▽; $n = 5$); and rmIL-1β–injected mice (□; $n = 4$).

Results

We have studied the circadian variations of body temperature in wild-type and IL-6–deficient mice, respectively. No difference was observed in the daily rhythm of temperature changes between these two groups of mice when kept at 30 ± 1°C (data not shown), suggesting that normal regulation of body temperature is not affected by the absence of IL-6 expression in the mice with null mutation of the IL-6 gene.

LPS injection (50 μg/kg i.p.) caused a reproducible fever response in the wild-type mice (Fig. 1 *A*) (maximal change in body temperature ~1.4°C), starting 1.5 h after the LPS injection and reaching maximum after 4.5 h. No fever was observed in the IL-6–deficient mice (Fig. 1 *B*) upon the same LPS treatment. The handling stress caused temperature elevation and was present and had the same magnitude in the IL-6–deficient mice as in wild-type littermates. The injection of LPS (50 μg/kg i.p.) induced in all the IL-6–deficient mice (and not in the wild-type mice) a significant reduction in body temperature (~0.5°C) during the first 1–2.5 h after LPS injection (36).

The intraperitoneal injection of an endogenous pyrogen, rmIL-1β (10 μg/kg i.p.) into wild-type mice (Fig. 1 *A*) caused fever for at least 3 h starting ~3 h after the rmIL-1β injection, whereas the same dose of rmIL-1β did not cause a fever response when injected into the IL-6–deficient mice (Fig. 1 *B*). Raising the dose of rmIL-1β to 25 μg/kg i.p. caused hypothermia in the wild-type mice (data not shown), indicating that this is a toxic dose, yet not even this larger dose of rmIL-1β evoked any fever response in the IL-6–deficient mice (data not shown).

Injection of rhIL-6 (250 μg/kg i.p.) alone caused no fe-

ver response in either the wild-type or the IL-6–deficient mice (Fig. 2, *A* and *B*), although a transient hyperthermic period was observed upon injection of the IL-6–deficient mice with this dose of rhIL-6 (Fig. 2 *B*).

To examine whether this peripheral injection of rhIL-6 could restore the fever response to LPS in IL-6–deficient mice, we combined rhIL-6 and LPS and injected these agents intraperitoneally. Fig. 2 *A* shows that this combination of rhIL-6 (250 μg/kg i.p.) and LPS (50 μg/kg i.p.) caused a large (maximal change 1.8°C) and sustained fever response in wild-type mice, which was faster in onset than the fever response caused by LPS alone (see Fig. 1 *A*) but which otherwise closely resembles the LPS-induced fever (see Fig. 1 *A*). In the IL-6–deficient mice, the fever response (maximal change 1°C) to combined intraperitoneal injection of rhIL-6 and LPS was atypical in kinetics, without a clear peak, and it was significantly smaller in magnitude; furthermore, the response was also delayed (Fig. 2 *B*). The duration of this fever hyperthermia was also much longer (8 h) than fever caused by the same treatment in the wild-type mice (6 h) (Fig. 2 *A*).

To study the role of centrally produced IL-6 in the fever response to rmIL-1β and rhIL-6, we carried out intracerebroventricular injections of these two endogenous pyrogens. Fig. 3 *A* shows that both rmIL-1β (100 ng/mouse icv.) and rhIL-6 (500 ng/mouse icv.), respectively, caused fever in the wild-type mice. The maximum fever (1.2°C) was reached 5 h after the injections of either of the two endogenous pyrogens. Injection of rhIL-6 (500 ng/mouse

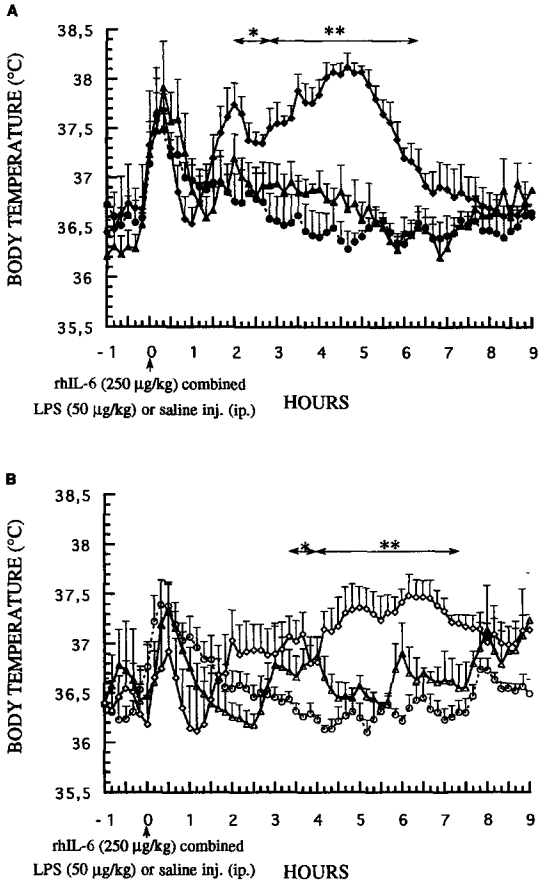

Figure 2. Effects of the systemic injection of rhIL-6 (250 μg/kg i.p.) and combined LPS (50 μg/kg i.p.) and rhIL-6 (250 μg/kg i.p.) on the core body temperature of IL-6–deficient and wild-type mice measured at 30 ± 1°C of ambient temperature. Data are mean + SE. ** $P < 0.01$ saline- versus rhIL-6–combined LPS–injected mice; * $P < 0.05$ saline- versus rhIL-6–combined LPS–injected mice. (*A*) The effects of the systemic injection of rhIL-6 (250 μg/kg i.p.) and combined LPS (50 μg/kg i.p.) and rhIL-6 (250 μg/kg i.p.) into wild-type mice. Saline-injected mice (●; $n = 6$); rhIL-6–combined saline-injected mice (▲; $n = 5$); and rhIL-6–combined LPS–injected mice (◆; $n = 4$). (*B*) The effects of the systemic injection of rhIL-6 (250 μg/kg i.p.) and combined LPS (50 μg/kg i.p.) and rhIL-6 (250 μg/kg i.p.) into IL-6–deficient mice. Saline-injected mice (○; $n = 5$); rhIL-6–combined saline–injected mice (△; $n = 4$); and rhIL-6–combined LPS–injected mice (◇; $n = 4$).

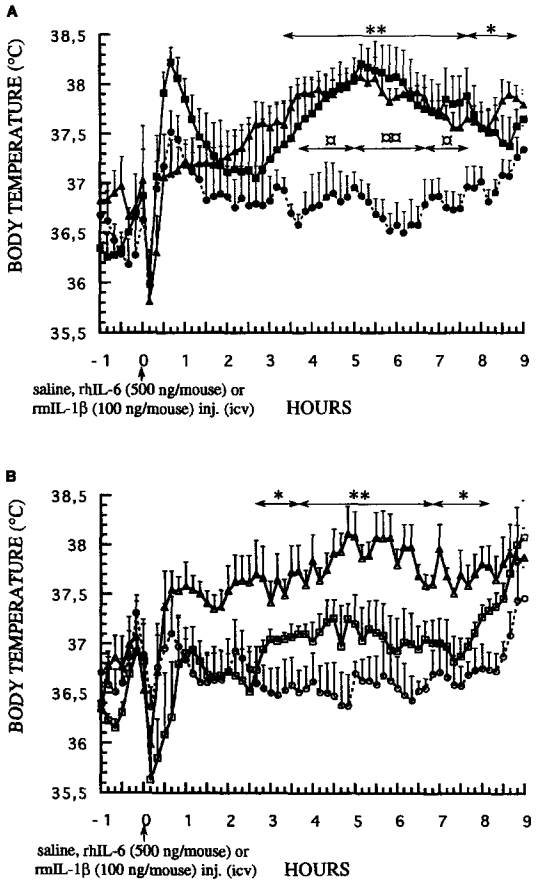

Figure 3. Effects of intracerebroventricular injection of rhIL-6 (500 ng/mouse, in 10 μl) and rmIL-1β (100 ng/mouse, in 10 μl), respectively, on the core body temperature of IL-6–deficient and wild-type mice measured at 30 ± 1°C of ambient temperature. Data are mean + SE. ** $P < 0.01$ saline- versus rhIL-6–injected mice; * $P < 0.05$ saline- versus rhIL-6–injected mice; ¤¤ $P < 0.01$ saline- versus rmIL-1β–injected mice; ¤ $P < 0.05$ saline- versus rmIL-1β–injected mice. (*A*) The effects of the intracerebroventricular injection of rhIL-6 (500 ng/mouse, in 10 μl) and rmIL-1β (100 ng/mouse, in 10 μl), respectively, into wild-type mice. Saline-injected mice (●; $n = 6$); rhIL-6–injected mice (▲; $n = 5$); and rmIL-1β–injected mice (■; $n = 4$). (*B*) The effects of the intracerebroventricular injection of rhIL-6 (500 ng/kg, in 10 μl) and rmIL-1β (100 ng/kg, in 10 μl), respectively, into IL-6–deficient mice. Saline-injected mice (○; $n = 6$); rhIL-6 injected mice (△; $n = 5$); and rmIL-1β injected mice (□; $n = 4$).

icv.) but not of rmIL-1β (100 ng/mouse icv.) caused fever in the IL-6–deficient mice (Fig. 3 *B*). Fever caused by rhIL-6 lasted at least 9 h and reached a maximum temperature elevation of 1.6°C. The fever induced by rhIL-6 continued into the "dark period" (when the core body temperature of rodents is higher), so that the exact time when the fever peak subsided is hard to determine, but on the next morning all of these animals had normal temperature. The large initial hypothermic response seen in both wild-type and IL-6–deficient mice is probably attributable to the ether anaesthesia applied in these intracerebroventricular injection experiments.

Discussion

Fever is one of the systemic inflammatory responses (1–4) that can be evoked by bacterial infection on the periphery or centrally. The ability of peripherally injected bacterial LPS to evoke distinct and long-lasting fever peak (34) is attributed to its ability to induce peripheral as well as central production of endogenous pyrogens such as IL-1β, TNF-α, and IL-6 (7, 15–19) and prostaglandins (9).

This study clearly shows that the LPS-mediated fever response in mice is dependent on expression of IL-6, as the IL-6–deficient mice could not mount a fever response (Fig. 1 *B*), whereas their wild-type litter mates responded with fever to LPS (Fig. 1 *A*). Earlier reports on IL-6–deficient mice indicated that, when injected with LPS, these mice produce a normal corticosterone response and a threefold enlarged serum TNF-α response (29). Obviously, the elevated serum TNF-α response to LPS is not sufficient to evoke a fever in these IL-6–deficient mice.

IL-1β is known to be a most efficient endogenous pyrogen whether injected intraperitoneally or intracerebroventricularly (5). In wild-type mice intraperitoneal injection of rmIL-1β caused a typical fever response (Fig. 1 *A*), whereas such response was absent in the IL-6–deficient mice (Fig. 1 *B*). Thus, it appears that fever response to an endogenous pyrogen such as IL-1β also requires IL-6 expression. Both IL-1β and TNF-α are strong inducers of IL-6 expression (12, 14–16) in wild-type animals, and thus it is plausible that IL-6 acts downstream from these cytokines in the fever response.

It is noteworthy that not even the centrally applied rmIL-1β (Fig. 3 *B*) caused fever in the IL-6–deficient mice, whereas the same dose caused rapid and long lasting fever in the wild-type mice (Fig. 3 *A*), suggesting that brain expression and presence of large doses of IL-1β are insufficient for evoking the fever response in the absence of IL-6; thus, IL-6 must act downstream from the central IL-1β.

To examine whether it is the central or peripheral pool of IL-6 that is required in the fever response, we injected mice with IL-6 intraperitoneally (Fig. 2) and intracerebroventricularly (Fig. 3). It is known that IL-6 is an endogenous pyrogen that acts centrally (6, 9, 10, 16, 18). Our data showing that peripheral injection of large doses of IL-6 is unable to evoke fever response in wild-type (Fig. 2 *A*) as well as in IL-6–deficient mice (Fig. 2 *B*) are in line with these reports (9–13).

In the IL-6–deficient mice, the combination of intraperitoneal injection of LPS and rhIL-6 produced some small, atypical fever response (Fig. 2 *B*), with slow onset, which may reflect some leakage of IL-6 from the peripheral to the central compartment. Cytokine transport through the blood–brain barrier has earlier been suggested by Banks et al. (37), and it is possible that some IL-6 transport (38) into the brain occurs; in addition, clearance of IL-6 may also be changed in the presence of LPS. Nevertheless, the lack of effect of intraperitoneally injected IL-6, alone and together with LPS, on the fever response clearly demonstrates that interpretations of results of intracerebroventricular injection of pyrogens that hold that these agents cause fever after having leaked from the brain to the periphery or induced a large response in the periphery first cannot hold for IL-6.

The key finding of this study using IL-6–deficient mice is that centrally applied IL-6 causes a rapid and long-lasting fever response not only in wild-type (Fig. 3 *A*) but also in IL-6–deficient mice (Fig. 3 *B*). These data confirm that IL-6 is a centrally acting pyrogen as described earlier (6, 9, 16, 18), and demonstrate that, in IL-6–deficient mice, the IL-6 receptor–gp 130 complex was intact and capable of signaling. It should be mentioned that gp 130 is used by several other IL-6–related cytokines such as ciliary neurotrophic factor (CNTF), leukemia-inhibitory factor, oncostatin M, and IL-11, which have overlapping inflammatory activities (39) and of which CNTF and leukemia-inhibitory factor also have been shown to occur in the central nervous system (40). CNTF was even proposed to act as an endogenous pyrogen in rabbits (41), yet none of these IL-6–related cytokines seems to compensate for lack of expression of IL-6 in the fever response.

In summary, using mice with null mutation in the IL-6 gene, we could demonstrate that central expression of IL-6 is a necessary component of the febrile response to both LPS and IL-1β. Furthermore, it appears that neither TNF-α nor IL-1β can bypass central IL-6 in production of the fever response to LPS or IL-1β in mice.

This study was supported by funds from the Swedish Medical Research Council and by TRION FOU AB. S. Gatti was the recipient of a fellowship from the Italian National Institute of Public Health: Italian national program for health workers on HIV infections. Z. Chai was the recipient of an exchange fellowship between Peking University and Stockholm University.

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Received for publication 31 July 1995 and in revised form 5 September 1995.

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