Effect of interleukin-18 on mouse core body temperature

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Gatti, Silvia, Jennifer Beck, Giamilia Fantuzzi, Tamás Bartfai, and Charles A. Dinarello. Effect of interleukin-18 on mouse core body temperature. Am J Physiol Regulatory Integrative Comp Physiol 282: R702–R709, 2002; 10.1152/ajpregu.00393.2001.—We have studied, using a telemetry system, the pyrogenic properties of recombinant murine interleukin-18 (rmIL-18) injected into the peritoneum of C57BL/6 mice. The effect of IL-18 was compared with the febrile response induced by human IL-1β, lipopolysaccharide (LPS), and recombinant murine interferon-γ (rmIFN-γ). Both IL-1β and LPS induced a febrile response within the first hour after the intraperitoneal injection, whereas rmIL-18 (10–200 μg/kg) and rmIFN-γ (10–150 μg/kg) did not cause significant changes in the core body temperature of mice. Surprisingly, increasing doses of IL-18, injected intraperitoneally 30 min before IL-1β, significantly reduced the IL-1β-induced fever response. In contrast, the same pretreatment with IL-18 did not modify the febrile response induced by LPS. IFN-γ does not seem to play a role in the IL-18-mediated attenuation of IL-1β-induced fever. In fact, there was no elevation of IFN-γ in the serum of mice treated with IL-18, and a pretreatment with IFN-γ did not modify the fever response induced by IL-1β. We conclude that IL-18 is not pyrogenic when injected intraperitoneally in C57BL/6 mice. Furthermore, a pretreatment with IL-18, 30 min before IL-1β, attenuates the febrile response induced by IL-1β.

interleukin-1β; interferon-γ

INTERLEUKIN-18 (IL-18), initially characterized as interferon-γ (IFN-γ)-inducing factor, is mainly produced by activated macrophages and participates in the T helper cell type 1 (Th1) response during immunorecognition (33). IL-18 acts as a cofactor, inducing the production of IFN-γ usually in the presence of another stimulus [for instance, lipopolysaccharide (LPS) or IL-12], and potentiates Th1- and natural killer cell-induced cytotoxicity by increasing the expression of Fas ligand (6, 33).

The proinflammatory role of IL-18 has been extensively characterized in vitro, showing that IL-18 promotes the innate immune response, mainly inducing the production of tumor necrosis factor (TNF)-α, IL-1α/β, IL-6, IL-8, macrophage inflammatory protein-1α, and monocyte-chemoattractant protein-1 (12, 29, 34, 37) with no direct effect on the production of prostaglandins (PGs; Ref. 38). In vitro studies have also confirmed that mature IL-18 is produced by various cell types in response to endotoxins (LPS), and in vivo studies showed that IL-18 could be partially responsible for the LPS-induced lethality in mice (20, 39). During sepsis, circulating IL-18 levels are increased in humans (16).

The similarities between IL-18 and IL-1α/β, both in structural and in functional terms, have been highlighted by several studies. Both IL-1 and IL-18 gene expression are induced by LPS. However, differently from all other proinflammatory cytokines, 1) IL-18 gene expression is constitutively high, 2) IL-18 mRNA and IL-18 precursor protein (24 kDa) are broadly expressed in several tissues and cell types (4, 25, 30, 35, 36), and 3) IL-18 actions may be regulated by changes in concentration of IL-18 binding protein. Neuroendocrine cells in adrenal glands and pancreas (21) also express pro-IL-18 mRNA, possibly in response to stress events. By RT-PCR it was possible to demonstrate the constitutive expression of the mature transcript coding for IL-18 precursor in rat brain (42), probably mainly in astrocytes and microglia (4).

Similarly to IL-1β precursor, the pro-IL-18 is cleaved to the mature active form mainly by IL-1β-converting enzyme, and in the case of IL-18 the enzymatic cleavage is probably the main mechanism of control on the production of bioactive IL-18 (11, 14).

The cellular effects of IL-18 are a consequence of the specific interaction with the membrane-bound IL-18 receptor (IL-18R) complex (IL-18Rα/β). Signal transduction by IL-18 is highly analogous to that observed in the case of IL-1R type I (IL-1RI) (33). After binding to the IL-18Rα chain (24), IL-18/IL-18Rα complex recruits the IL-18 accessory protein (IL-18Rβ) (5, 23). This increases the affinity of the ligand for the receptor complex, and a signal is transduced. A soluble IL-18-binding protein binds and neutralizes IL-18 effects, reducing the amount of cytokine available for the interaction with IL-18R complex (38). In the periphery
the distribution of IL-18Rα is rather broad, and IL-18Rα mRNA can be detected mainly in lymphocytes and hematopoietic cells (32).

The intracellular, postsignal cascade of IL-18R kinases is nearly identical to that of IL-1R1. 1) IL-1RI and IL-18R signaling involves the recruitment of MyD88 and the activation of a common kinase, IL-1 receptor-associated kinase (IRAK; Refs. 17, 22). 2) Another kinase, known as TNF receptor (TNFR)-activating factor-6 (TRAP-6), contributes to the signal transduction system of both IL-1 and IL-18 receptors (27) and results in activation of nuclear factor (NF)-κB (26, 41). NF-κB translocation to the nucleus is associated with initiation of cyclooxygenase-2 (COX-2) gene expression.

The observed functional similarities between IL-18 and IL-1α/β suggest the presence of partially overlapping roles for these cytokines in the control of the acute phase response during infection. This is also suggested by the recent study of Kubota et al. (28), showing that IL-18 promotes sleep in rabbits and rats.

Considering that IL-1β is the main endogenous pyrogen and that IL-1β-induced fever is mediated by intrahypothalamic production of PGE2 (8), we studied the pyrogenic properties of IL-18 and compared the effect of IL-18 with the febrile response triggered by IL-1β and LPS, respectively.

IL-18 is not inducing PGE2 production in peripheral cells; this observation, however, is not per se predictive of a lack of pyrogenicity of this proinflammatory cytokine. IL-6, an endogenous pyrogen in humans and rabbits, does not induce COX-2 expression and PG production in monocytes or synovial fibroblasts. Moreover, IFN-γ, which is also pyrogenic in humans, suppresses LPS- and IL-1-induced PG production in monocytes. Therefore, to resolve this issue, we have tested the pyrogenicity of recombinant murine IL-18 in mice.

MATERIALS AND METHODS

Mice. C57BL/6J male mice (25–30 g, Biological Research Laboratories, Füllinsdorf) were used for this study. They were individually housed with free access to food and water at vivarium temperature of 25°C (30–40% humidity) for 7–10 days before the surgery (light from 7 AM to 7 PM). The animals were acclimated at 29.5°C after the surgery. All animals were kept on the same standard diet, Kliba no. 243 (12.6 MJ/kg).

Treatments. LPS was from Escherichia coli (serotype 026: B6, Sigma lot 107H4091, cell culture tested). Recombinant human (rh) IL-1β was from R&D Systems (Minneapolis, MN; helper T cells proliferation assays: ED50 = 5–10 pg/ml). Recombinant murine (rm) IFN-γ was from R&D Systems (antiviral test in L929; ED50 = 0.1–0.4 ng/ml). rmIL-18 was expressed and purified by Peprotech (Rocky Hill, NJ) (23). rmIL-12 was a kind gift of Genetics Institute (Andover, MA); the specific activity of IL-12 was 2.7 × 109 U/mg. LPS and cytokines were reconstituted in pyrogen-free saline solution. Aliquots were stored at −20°C, and each frozen aliquot was used only once. LPS or cytokines were injected intraperitoneally in pyrogen-free saline solution. Control mice were injected with pyrogen-free saline solution. All treatments were carried out between 9 AM and 11 AM. The volume of injection was calculated in proportion to the body weight, and it ranged between 0.1 and 0.2 ml. The total volume of liquids injected into the peritoneum, during multiple treatments, never exceeded 0.4 ml.

Implant of telemetry probes. Telemetry probes (Vitalview 4000, MiniMitter, Sunriver, OR) were inserted into the mouse peritoneum under systemic anesthesia with diazepam (5 mg/kg ip) and ketamine (100 mg/kg ip). For the postoperative analgesic treatment, buprenorphine (0.05 mg/kg sc) was used twice a day for 1 day after the surgery. Animals were then acclimatized for 5–7 days at 29.5 ± 0.5°C of ambient temperature (30–40% humidity) before any further treatment.

Measurement of core body temperature in mice. Core body temperature and horizontal motor activity values were recorded every minute in freely moving animals housed in single cages, starting from the evening before the day of the experiment and until 18 h after the injections. This study received the approval of the Cantonal Veterinary office of the city of Basel, Switzerland.

Measurement of IFN-γ levels in the serum of mice treated with IL-18. Mice (8 wk old) were treated for 4 days with IL-18 or murine IL-12 (400 ng/mouse ip). The serum was taken from the retroorbital plexus 2 h after the last injection. IFN-γ levels were measured with an enzyme-linked immunosorbent assay kit from Endogen (Wobur, MA).

Statistical analysis. Core body temperature was recorded every minute, and data were averaged every 10 min. The results were analyzed using ANOVA followed by post hoc tests (UNISTAT software package).

RESULTS

Effect of IL-18, IL-1β, and LPS on core body temperature of C57BL/6J mice. IL-1β (10 μg/kg ip) causes fever in mice acclimated in thermoneutral environment (29.5 ± 0.5°C) (Fig. 1A). During the fever response, the core body temperature increases about 1–1.5°C, with a maximal rise within 2 h after the intraperitoneal injection. The core body temperature of mice injected with IL-1β is different from that of saline-injected animals for ~6 h after the treatment. The core body temperature of treated mice was not significantly different from that of mice injected with saline solution (~24 h after the treatment (data not shown).

Under the same experimental conditions, LPS (50 μg/kg ip) triggers a similar febrile response within 1 h after the injection, and the fever reaches peak elevation within the second hour after the injection (data not shown). The LPS used during this study is highly purified cell culture-tested LPS from E. coli with <1% protein content. The doses of the two pyrogens IL-1β and LPS were chosen as the minimal doses causing a reproducible febrile response in this strain of mice (data not shown).

Animals consistently exhibited a similar stress reaction immediately after the intraperitoneal injection, with a sudden hyperthermia lasting ~45 min after the injection (Fig. 1). This reaction was longer (~60 min) in the case of animals injected twice (Fig. 2).

Under our experimental conditions, neither rmIL-18 (10–50 μg/kg) nor rmIFN-γ (10–150 μg/kg) (Fig. 1, B and C, respectively) induced a sustained increase of the core body temperature within 5–6 h after the treatment. No changes in the circadian rhythm of the core
body temperature were observed during the 24 h after the treatment (data not shown).

No signs of sickness or changes in horizontal locomotor activity were observed in mice treated with IL-18.

The doses of rmIL-18 used in this study (10–50 μg/kg; 0.25–1.25 μg/mouse) are in the range of IL-18 doses active in suppressing the growth of MetA tumor cell ascites and increasing histidine decarboxylase activity in mouse tissues (31, 43).

At a dose of 200 μg/kg of IL-18, no sustained effect on core body temperature was observed (data not shown).

Effect of a pretreatment with IL-18 or IFN-γ on IL-1β- and LPS-induced fever. As described above, mice pretreated (30 min) with IL-18 (10–200 μg/kg ip) developed a reduced febrile response to IL-1β (10 μg/kg ip) (Fig. 2). This reduction in IL-1β-induced fever by IL-18 was dose dependent. In fact, the pretreatment with IL-18 at 10 μg/kg resulted in a slight reduction of IL-1β-induced fever, whereas the injection of 50 μg/kg of IL-18 30 min before IL-1β resulted in a significant shortening of the febrile period in each of the IL-18-treated animals. The highest IL-18 dose tested in this study, 200 μg/kg, completely blocked IL-1β-induced
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Fever response. In contrast, similar attenuation of LPS-induced fever (50 μg/kg) was not observed with a pretreatment by IL-18 (50 μg/kg) (Fig. 3). Thus, in our experimental conditions, the suppression of fever by pretreatment with IL-18 (50 μg/kg ip) appears to be effective for IL-1β and not for LPS.

The time interval of pretreatment seems to be crucial for the IL-18 effect in suppressing IL-1β fever in mice. In fact, mice pretreated with the highest dose of IL-18 used in this study (200 μg/kg) mounted a normal IL-1β-induced febrile response when IL-18 was injected 1 h before IL-1β (Fig. 4) compared with 30-min pretreatment (Fig. 2).

Because IL-18 is an inducer of IFN-γ, particularly with IL-12 or other T-cell activators, we examined the possible effect of IFN-γ on IL-1β-induced fever. A pretreatment (30 min) with IFN-γ (50 μg/kg ip) did not reduce IL-1β-induced fever under these experimental conditions (Fig. 5).

Serum levels of IFN-γ. IFN-γ was increased slightly in the circulation of mice injected each day, for 4 days, with IL-18 (16 μg/kg ip). However, the serum levels were not statistically different from those observed in control mice injected with saline (Table 1). In contrast, mice injected with IL-12 exhibited high levels of IFN-γ.

DISCUSSION

Fever is a stereotypic response to endogenous and exogenous pyrogens, associated with increased serum levels of proinflammatory cytokines (IL-1α/β, TNF-α, and IL-6) and hypothalamic production of PGs (mainly PGE2), that trigger fever interacting with EP3 receptors (13). Exogenous pyrogens, like LPS, trigger fever via the interaction with Toll-like receptors (TLR) expressed in macrophagic cells, endothelial cells, or perivascular cells of the organum vasculosum lamina terminalis, with either direct release of PGE2 or the subsequent release of endogenous pyrogens (i.e., cytokines) (13).

In the case of intraperitoneal injection of IL-1β (10 μg/kg) into mice, the febrile response is only partially due to the circulating proinflammatory cytokine (18). In fact, the effect of the intraperitoneal injection of IL-1β is amplified by the local production of IL-1α/β, TNF-α, and IL-6 by peritoneal macrophages, and fever is also triggered via the activation of the sensory afferent part of the vagal nerve. In rats a surgical cut of the sensory part of this nerve in the subdiaphragmatic region completely prevents the development of IL-1β-induced fever after intraperitoneal injection of IL-1β.
Moreover, the synthesis of IL-1β can be detected in the vagal nerve and in the nodose ganglion soon after the intraperitoneal injection of LPS (15). Therefore, we hypothesized that intraperitoneal injection of IL-18 would also cause fever by similar mechanisms. However, intraperitoneal injection of IL-18 did not produce fever.

Endogenous or exogenous pyrogens can cause fever in all endotherms, because they trigger the shift upward of the hypothalamic core temperature set point, with a subsequent increase in metabolic rate and facultative thermogenesis. In the case of small rodents like mice and rats, it is necessary to acclimate the animals in thermoneutral conditions (29–30°C ambient temperature for mice) before the treatment, to allow them to mount a proper febrile response (16). C57BL/6 mice are an inbred strain commonly used for studies of in vivo effects of cytokines and of T-cell-mediated immune responses associated with the production of IFN-γ (1, 3).

In the experimental conditions used in the present study both IL-1β (10 μg/kg ip) and LPS (50 μg/kg ip) cause a prolonged fever response in C57BL/6 mice. LPS- and IL-1β-induced fevers in mice are very similar both in terms of time course and of magnitude of the effect (data not shown) despite the fact that LPS fever was sustained in our experimental conditions by the induction of several endogenous pyrogens (TNF-α, for instance). In fact, LPS-induced fever is still present in IL-1α/β knockout. Moreover, LPS-induced fever, in rabbits and in humans, is not affected by the coinfusion of IL-1 receptor antagonist (IL-1ra; Ref. 13).

Human IFN-γ causes fever when injected subcutaneously in humans or intraperitoneally in rabbits. In mice, however, rmIFN-γ does not cause fever when injected in doses ranging from 10 to 150 μg/kg ip (2). In the same experimental model, murine IL-18 (10–200 μg/kg ip) does not cause a significant and sustained increase of the core body temperature (Fig. 1). These results confirmed our earlier unpublished observations using human IL-18 in mice.

Recently, Reznikov et al. (38) reported a significant biological difference between the two proinflammatory cytokines: IL-1β and IL-18. In vitro, IL-18 does not trigger PGE2 production in human peripheral blood mononuclear cells and, when coincubated with IL-1β, even causes a marked reduction of PGE2 production (IFN-γ mediated). Therefore, in vivo studies are required to understand if IL-18 is not causing fever because of a lack of production of PGE2 in the hypothalamus. However, the evidence that IL-18 is not pyrogenic when injected intraperitoneally in mice sug-

Fig. 4. Effect of a high dose of IL-18 on IL-1β-induced fever in mice when IL-18 is injected 1 h before IL-1β. IL-18 (200 μg/kg, n = 3) was injected 1 h before IL-1β (10 μg/kg) intraperitoneally. Control animals were injected with saline solution 1 h before IL-1β injection (n = 2) or saline injection (n = 2).

Fig. 5. Effect of a pretreatment with IFN-γ on IL-1β-induced fever in mice. Recombinant murine IFN-γ (50 μg/kg ip, n = 3) was injected 30 min before IL-1β (10 μg/kg ip). Control animals were injected with saline solution 30 min before IL-1β injection (n = 7) or saline injection (n = 10).
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Table 1. IFN-γ levels in the serum of mice treated with IL-18 and IL-12

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IFN-γ, ng/ml</th>
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<tbody>
<tr>
<td>Saline</td>
<td>0.14 ± 0.07</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.58 ± 0.32</td>
</tr>
<tr>
<td>IL-12</td>
<td>18.66 ± 9.29</td>
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Data are means ± SE; n = 4. Mice were treated for 4 days with saline, interleukin (IL-18 (16 μg/kg), or IL-12 (16 μg/kg), and the serum was taken 2 h after the last injection. IFN-γ, interferon-γ.

suggests a main difference in the neurological effects of IL-1β and IL-18.

Experimental antipyretic strategies usually block the effect of endogenous pyrogens (IL-1β, IL-6, and TNF-α) via the inhibition of PGE2 formation by inhibiting COX enzymes (COX-1 and/or COX-2). In each case, the antipyretic agent is injected 30 min before the pyrogen. Using a classical protocol for the study of antipyretic agents, we show in the present study that IL-18 reduces the fever response induced by rhIL-1β but not LPS in a dose-dependent manner.

It is unlikely that IFN-γ is responsible for the observed effect of IL-18 on IL-1β fever. In fact, rmIL-18 (16 μg/kg) injected intraperitoneally in mice for 4 days does not increase IFN-γ levels in the serum, and, more importantly, a pretreatment with IFN-γ (50 μg/kg ip) does not modify IL-1β-induced fever (Fig. 5).

A central production of IFN-γ at the level of periventricular organ or hypothalamus could, however, play a role in the control of the pyrogenic response induced by IL-1β. Similarly to what was observed by Reznikov et al. (38) in blood mononuclear cells, central IL-1β-induced PG production could be reduced by a cotreatment with IL-18 because of the local induction of IFN-γ.

Several peripheral or central events could as well account, at least partially, for the observed pharmacological IL-18 antagonism on IL-1β-induced fever. IL-18 could, for instance, act on endogenous antipyretic/anti-inflammatory mechanisms such as IL-4 secretion, the epoxygenase pathway, or the central release of arginine vasopressin or IL-1ra, for instance (13).

However, the lack of effect of IL-18 pretreatment on LPS-induced fever suggests the presence of cellular events specific for IL-1RI-mediated signal transduction. The observed attenuation of IL-1β fever could be, for instance, the result of mechanisms of sequestration at the receptor accessory protein level or at the level of intracellular signaling proteins, like MyD88 or IRAK. In vitro studies are necessary to further address this hypothesis. However, when considering this hypothesis, we should remember that LPS also mobilizes IRAK1/2-TRAF6. Thus possible functional interactions between IL-18 and IL-1 receptor signaling must be upstream from IRAK1/2-TRAF6 in the TLR-signaling pathway.

The effect of IL-18 on IL-1β fever is dependent on the time protocol of the pretreatment. IL-18 (200 μg/kg) is able to inhibit completely IL-1β-induced fever when injected 30 min before IL-1β (Fig. 2), whereas the effect is no longer present if the same dose of IL-18 is injected 1 h before IL-1β (Fig. 4).

It is rather difficult to explain the time dependence of the antipyretic effect of the IL-18 pretreatment with the present, rather scanty, knowledge of in vivo IL-18 effects. Our experimental observation could be consistent with the hypothesis of cross-talk between fast intracellular events associated with the activation of IL-1R and/or IL-18 receptor complexes.

A similar time-dependent effect could be observed while studying the antipyretic effect of IL-1ra pretreatment: in this case, the blockade of IL-1 receptors with IL-1ra is effective on IL-1β-induced fever response when IL-1ra is injected 15 min before IL-1β (7, 13) or after IL-1β because of the short plasma half-life of IL-1ra. Pharmacokinetic reasons cannot be ruled out also in the case of the observed time dependence of the IL-18 effect.

Moreover, because of the tight time dependence of the IL-18 effect on IL-1β, we cannot rule out completely that the observed lack of effect of IL-18 on LPS-induced fever is due to the protocol of treatment we used. We are further exploring this possibility.

This is the first study addressing the effect of a systemic treatment with IL-18 on core body temperature. We conclude that IL-18 is not causing fever per se when injected intraperitoneally in C57BL/6 mice. These results are in agreement with the in vitro observation that IL-18 is not able to trigger the production of PGE2 in macrophage-like cells (38) and with the study of Kubota et al. (28) showing no changes in brain temperature in rats treated with intraperitoneal IL-18. Moreover, a recent study carried out by Stuyt RJJ, Netea MG, Kullberg BJ, and van der Meer JWM, (unpublished data) has further confirmed that recombinant human IL-18 (1 μg/kg iv) is not pyrogenic in rabbits.

IL-18 (10–200 μg/kg ip) exhibits an in vivo effect when tested in a protocol of pretreatment on IL-1β-induced fever. In fact, we could observe a significant reduction of IL-1β-induced fever when IL-18 is injected 30 min before the pyrogen. Considering that this effect is specific for IL-1β-induced fever and that it is not observed when IL-18 is injected 1 h before IL-1β, we suggest the presence of cross-talk at the level of early IL-1R-mediated intracellular events.

Perspectives

New ligands belonging to the structural IL-1 family have been recently discovered together with a large series of TLR receptors. All the known ligands of the IL-1R structural superfamily exhibit high selectivity for the different receptors, raising a lot of interesting, still unanswered, questions about the structural requirements of ligand-receptor interaction for agonist activity and about the common or different intracellular complexes involved in signal transduction. The picture is getting broader and more detailed, mostly thanks to in vitro studies.
In vivo studies on the neurological effects of cytokines of the IL-1 structural superfamily, like that by Kubota et al. (28) or this study, could also contribute to the effort of understanding the biology of the system. If IL-18 is a modifier of IL-1β response, this effect could be important, especially considering that IL-1α/β are the most potent among the pyrogenic, anorectic, and somnogenic substances we know.

Fever, in particular, is a stereotyped and phylogenetically very ancient reaction to infection. The knowledge concerning other proinflammatory cytokines of the IL-1 structural superfamily and their effect on IL-1-induced fever is also of importance for our understanding of the molecular mechanisms inducing and sustaining the pyrogenic response both in the periphery and at the hypothalamic level.

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