Impaired febrile responses to immune challenge in mice deficient in microsomal prostaglandin E synthase-1

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Fever is an adaptive response to infectious and inflammatory processes that is elicited and coordinated by the central nervous system. Fever, traditionally, has been thought to be initiated by pyrogenic cytokines acting on the preoptic area of brain (3, 6, 45), but because the blood-brain barrier is impermeable to cytokines, the mechanism by which these peripheral signals are transmitted into central nervous signals was long obscure. However, recent studies carried out in this and other laboratories have demonstrated that immune stimuli, such as peripheral administration of cytokines or LPS, as well as chronic inflammatory conditions such as adjuvant-induced arthritis, evoke the expression of the inducible PGE2 synthesizing enzymes cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) (24) in brain vascular cells (5, 8, 10, 12, 26, 56), indicating that the peripheral immune signals are transduced in these cells into a prostaglandin signal (1, 2). These observations, which are consistent with findings suggesting a critical role for PGE2 in the generation of fever (21, 47, 53), were further corroborated in studies of mPGES-1-deficient mice. In contrast to wild-type controls, these mice showed no fever and no central PGE2 synthesis after peripheral injection of LPS, but they displayed an intact and receptor-specific pyretic capacity in response to centrally administered PGE2 (14).

While these observations demonstrate that induced PGE2 production by mPGES-1 is necessary for LPS-induced fever, they raise the question as to whether this is a general mechanism for fever during immune challenge, i.e., whether it is elicited not only during endotoxemia, but also during cytokine-mediated immune responses. LPS is known to elicit a cascade of cytokine synthesis, including the formation of IL-1β, IL-6, and TNF-α (11, 23, 55), and receptors for these cytokines are expressed by the brain vasculature (15, 26, 37, 54). Hence, while it is possible that the LPS-induced fever is cytokine-mediated, LPS could also exert its effect on the brain directly by a Toll-like receptor 4 (TLR4), which is expressed in the leptomeninges, choroid plexus, and circumventricular organs, although not on brain endothelial cells (32), thereby bypassing the cytokine pathway. Thus it has been shown that inhibitory factor κBα, an index of NF-κB activity, and COX-2 transcripts are expressed in the endothelial cells of the brain vasculature after LPS challenge also in IL-1-deficient mice (31), and that TLR4-mutated mice are endotoxin resistant (41).

Therefore, in the present study, using mPGES-1-deficient mice, we examined the febrile response, as well as motor activity, in an animal model that is dependent on intact cytokine signaling, namely the aseptic inflammation induced by subcutaneous injection of turpentine (16). We also studied these responses in mPGES-1-deficient mice following intraperitoneal administration of IL-1β, and we examined the role of mPGES-1 in the circadian temperature variation and in stress-induced fever, phenomena that both have been suggested to be prostaglandin dependent (25, 36, 46, 49).

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Cage-exchange stress experiment. Mice were briefly anesthetized with isoflurane (4%) and subcutaneous injection of turpentine. Statistical differences in the cage-exchange experiment were also assessed by a four-way ANOVA, with genotype, treatment (cage exchange/control), and time as fixed factors and animals as random factor, followed by single degree of freedom test. Animals were nested within treatments and constituted a random factor, whereas all other factors were regarded as fixed factors. Treatments constituted either of four combinations: wild-type mice + saline, wild-type mice + turpentine, knockout mice + saline, or knockout mice + turpentine. Statistical differences in the responses to intraperitoneal injection of IL-1β were assessed by two-tailed t-test. A difference was considered to be significant if P < 0.05. When applicable, multiple comparisons were corrected for by the Bonferroni method.

RESULTS

Diurnal changes in core temperature and motor activity. The recordings of the diurnal core temperature changes of wild-type and mPGES-1 knockout mice displayed no significant differences between the two groups (Fig. 1). Also the motor activity displayed by these mice was nearly identical to those displayed by the temperature recordings (Fig. 2).

Intraperitoneal injection of IL-1β. The animals were briefly restrained and injected with 600 ng ip (≈30 μg/kg ip) of recombinant mouse IL-1β expressed in E. coli (R&D Systems; endotoxin content <0.1 ng/1 μg of IL-1β), diluted in 100 μl 0.9% NaCl. Mice in the control groups received an equal volume of saline. None of these mice had been subjected to any previous experiments.

Statistical analyses. The values are presented as means ± SE. Significant differences in the turbineptine experiment were assessed by a four-way ANOVA, with light/dark period, 24 h cycle, treatment, and animal as factors, followed by Tukey’s pairwise comparisons or single degree of freedom test. Animals were nested within treatments and constituted a random factor, whereas all other factors were regarded as fixed factors. Treatments constituted either of four combinations: wild-type mice + saline, wild-type mice + turpentine, knockout mice + saline, or knockout mice + turpentine. Statistical differences in the responses to intraperitoneal injection of IL-1β were assessed by two-tailed t-test. A difference was considered to be significant if P < 0.05. When applicable, multiple comparisons were corrected for by the Bonferroni method.

MATERIALS AND METHODS

Animals. Mice with a deletion of the Ptges gene, which encodes mPGES-1, were generated by breeding heterozygous littermates of the DBA/1lacJ strain, as previously reported (52). The animals were kept one per cage in a pathogen-free facility at an ambient temperature of 27 ± 1°C, with food and water available ad libitum. All experimental procedures were approved by the Animal Care and Use Committee at the University of Linköping.

Telemetric recordings. At least 1 wk before the experiments, the mice were briefly anesthetized with isoflurane (4%) and implanted in the peritoneal cavity with a transmitter that records core temperature and motor activity (Data Science International, St. Paul, MN). A receiver, which transmits the signals on line to the connected computer, was placed beneath each cage. The animal’s motor activity was qualitatively assessed from the change in position of the transmitter in relation to the receiver and the speed with which movement occurred. The recordings were started at least 1 h before injection, and data were obtained every 2 min throughout the entire observation period. The temperature recordings were sampled during 10 s, whereas the activity recordings were sampled during the entire 2-min period.

Circadian changes in core temperature and motor activity. Core temperature and activity were monitored for two consecutive days. Thereafter, the mice were used for cage-exchange stress experiment and subcutaneous injection of turpentine.

Cage exchange-induced stress response. Cage-exchange stress was evoked by exchanging the home cages of two mice. Control mice were just lifted up and placed back in their home cage.

Subcutaneous injection of turpentine. At least 1 day after the cage-exchange stress experiment, mice were briefly anesthetized with isoflurane (4%) and given a subcutaneous injection of 150 μl of commercially purified turpentine (VWR, Stockholm, Sweden) in the left thigh. Control animals were injected with 150 μl saline.

Intraperitoneal injection of IL-1β. The animals were briefly restrained and injected with 600 ng ip (≈30 μg/kg ip) of recombinant mouse IL-1β expressed in E. coli (R&D Systems; endotoxin content <0.1 ng/1 μg of IL-1β), diluted in 100 μl 0.9% NaCl. Mice in the control groups received an equal volume of saline. None of these mice had been subjected to any previous experiments.

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RESULTS

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**Stress-induced response.** The cage-exchange procedure induced a rapid and pronounced hyperthermia in both wild-type and mPGES-1 knockout mice, with less pronounced responses displayed by the control animals (Fig. 3). Four-way ANOVA of the time period of 2–80 min after cage exchange showed that the temperature increase in cage-exchanged animals was significantly larger than that seen in the control animals ($F_{1,20} = 21.13; P < 0.001$), and single degree of freedom test showed that this difference was significant among the wild-type and knockout animals ($P < 0.01$ and $P < 0.05$, respectively). While there was a tendency that knockout mice displayed somewhat lower temperatures than wild-type mice (Fig. 3), this difference was not statistically significant ($F_{1,20} = 0.30; P = 0.59$).

The temperature responses were associated with increased motor activity, which was much more pronounced in cage-exchanged animals than in control animals (Fig. 4). This difference was statistically significant for cage-exchanged vs. control mice irrespective of genotype ($F_{1,20} = 33.07; P < 0.001$; four-way ANOVA of the time period of 2–80 min after cage exchange), as well as within the wild-type and knockout groups ($P < 0.001$ and $P < 0.05$, respectively; single degree of freedom test). However, the knockout mice displayed significantly less motor activity in response to the treatments than did the wild-type mice (Fig. 4) ($F_{1,20} = 7.91; P < 0.05$), and this difference was statistically significant among cage-exchanged mice ($P < 0.05$; single degree of freedom test) but not among control mice.

**Responses to subcutaneous injection of turpentine.** Subcutaneous injection of turpentine resulted in a strong febrile response in wild-type mice (Fig. 5). The fever period started $\sim 9$ h after injection and persisted throughout the following night-day cycle. While there was no difference in temperature between the turpentine-injected and the saline-injected (control) animals during the second dark period after injection, the turpentine-injected wild-type animals showed an elevated temperature also during the subsequent light period. During the third day after injection, the body temperature curve of the turpentine-injected wild-type animals basically followed that of the control mice.

Four-way ANOVA showed that the observed differences were statistically significant. Thus, during the first two 24-h cycles (starting 7 PM on the day of injection), there were significant temperature differences between the light and dark periods ($F_{1,60} = 119.25; P < 0.001$) and between the first and second cycle ($F_{1,60} = 23.22; P < 0.001$). Further analysis showed that there was a statistically significant difference between treatments (wild-type mice + saline; wild-type mice + turpentine, knockout mice + saline, knockout mice + turpentine) ($F_{3,20} = 28.35; P < 0.001$). Tukey’s pairwise comparisons showed that turpentine-injected wild-type mice differed from all other groups (family error rate 0.05), whereas no significant differences were seen between turpentine-injected knockout mice, saline-injected knockout mice, and saline-injected wild-type mice. The fever response of the turpentine-injected wild-type animals was more pronounced during the first than the second 24-h cycle ($F_{1,15} = 27.72; P < 0.001$).
mice was unrelated to differences in activity, and the turpentine-induced activity depression was independent of mPGES-1.

Four-way ANOVA showed that there were significant activity differences between the light and dark periods ($F_{1,60} = 120.39$; $P < 0.001$) during the first two 24-h cycles (starting 7 PM on the day of injection). Further analysis showed that there was a significant difference between treatments ($F_{3,20} = 3.78$; $P = 0.027$), and single degree of freedom test confirmed that there was a difference between turpentine and saline-injected mice ($F_{1,20} = 11.19$; $P = 0.003$), whereas no difference was seen between knockout and wild-type mice ($F_{1,20} = 0.09$; $P = 0.770$). These differences were more pronounced during the first than during the second dark period (two-way ANOVA: $F_{1,20} = 7.17$; $P = 0.014$).

Responses to intraperitoneal injection of IL-1β. All animals, irrespective of genotype or type of injection (IL-1β or saline), displayed an initial stress-induced hyperthermia elicited by the injection procedure (Fig. 7). In IL-1β-injected mice (wild type as well as mutant) this initial hyperthermia was immediately followed by a hypothermic response, which was not seen in saline-injected mice. About 60–90 min after injection, at which time point the animals had returned to a preinjection body temperature, IL-1β-injected mice started to display a febrile response. This peaked at ~4 h after injection and started to disappear after 6–7 h. In contrast, IL-1β-injected mPGES-1 knockout mice did not show any febrile response during the first 4–5 h but displayed a temperature curve that was similar to that displayed by saline-injected wild-type and mutant mice.
PGE2-synthesizing enzyme mPGES-1 is critical for the development of fever in models using peripherally administered or endogenously released cytokines, such as intraperitoneal injection of IL-1β or subcutaneous injection of turpentine. The fever elicited by the aseptic inflammation induced by turpentine is known to be cytokine dependent. Mice deficient in IL-1β or in IL-1 type 1 receptor do not develop fever following turpentine injection, but also IL-6 is necessary for the turpentine-induced fever (18, 27, 30, 35, 58), thus implying that the mPGES-1-mediated febrile response to turpentine is cytokine driven. Taken together with our previous demonstration that mPGES-1 is necessary for endotoxin-induced fever (14), the present findings strongly suggest that mPGES-1-induced PGE2 synthesis is a general and obligatory mechanism for the febrile response to infectious and inflammatory processes.

Only recently, the origin of the inflammatory-induced PGE2 synthesis in the brain has started to become elucidated. Studies using in situ hybridization histochemistry have shown that mPGES-1 is induced in endothelial cells in the venules of the brain vasculature in response to inflammatory stimuli, and the same cells also show an induced COX-2 expression and bear IL-1 type 1 receptors (8, 12, 26, 56). Following immune stimuli, the concentration of PGE2 increases in the brain of wild-type animals but not in mPGES-1 knockout, and this increase is associated with an induced PGE2-synthesizing capacity in the brain and with the capacity to develop immune-induced fever (14). The induced PGE2-synthesizing capacity

However, starting at ~4–5 h after injection, the IL-1β-injected knockout mice showed an increasing body temperature, whereas no significant change was seen in the saline-injected controls before the last hours of the light period when these mice also displayed a temperature increase. While the latter is consistent with the circadian-dependent temperature regulation (cf. Fig. 1), the rise of the body temperature in the IL-1β-injected knockout mice occurred earlier.

The activity recordings (Fig. 8) showed low activity during most of the observation period for all groups (being consistent with the fact that this coincided with the light period). However, as expected, increased activity was seen in association with the injection procedure, as well as at the end of the observation period (corresponding to the end of the light period). Noteworthy, the rapid hypothermia that was displayed by IL-1β-injected mice immediately after the stress-induced hyperthermic response was associated with an equally rapid activity depression. In contrast, the saline-injected mice showed a longer lasting stress-induced activity increase, being consistent with the slower temperature fall seen in these animals after the stress-induced hyperthermia (cf. Figs. 7 and 8).

**DISCUSSION**

The present study demonstrates that the inducible terminal PGE2-synthesizing enzyme mPGES-1 is critical for the development of fever in models using peripherally administered or endogenously released cytokines, such as intraperitoneal injection of IL-1β or subcutaneous injection of turpentine. The fever elicited by the aseptic inflammation induced by turpentine is known to be cytokine dependent. Mice deficient in IL-1β or in IL-1 type 1 receptor do not develop fever following turpentine injection, but also IL-6 is necessary for the turpentine-induced fever (18, 27, 30, 35, 58), thus implying that the mPGES-1-mediated febrile response to turpentine is cytokine driven. Taken together with our previous demonstration that mPGES-1 is necessary for endotoxin-induced fever (14), the present findings strongly suggest that mPGES-1-induced PGE2 synthesis is a general and obligatory mechanism for the febrile response to infectious and inflammatory processes.

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can solely be ascribed to induced mPGES-1 expression, because the expression of the other PGE₂-synthesizing enzymes that so far have been identified, cytosolic PGE synthase and mPGES-2, are either unaffected or downregulated in the brain after inflammatory stimuli (14, 17). These data, taken together with the observation that mPGES-1, in contrast to COX-2, has little constitutive expression in the brain and, with the possible exception of cells in the paraventricular hypothalamic nucleus (12), is induced only in the endothelial cells, strongly suggest that these cells are the likely source of the inflammation-induced central PGE₂-synthesis.

In addition to the central production of PGE₂, peripheral PGE₂ synthesis may also play a role for the febrile response, especially during its early phases (21). Thus studies in rats have shown that intravenous injection of a pyrogenic dose of LPS induces an initial expression upregulation of mPGES-1 in peripheral organs such as liver and lungs that precedes the upregulation of this enzyme in the brain (20). The PGE₂ produced in peripheral organs through the enzymatic action of mPGES-1 may influence the brain both via a neural route (42)—possibly by the activation of the vagus nerve (9)—or via a blood-borne carrier-mediated transport mechanism (22, 43).

The effects of the PGE₂ that is synthesized in response to a peripheral inflammatory stimulus are exerted via its specific receptors. The EP₃ receptor seems to be critical for the febrile response, since mice with a deletion of the EP₃ receptor gene do not develop fever in response to subcutaneous turpentine or to systemic or intraperitoneal injection of lipopolysaccharide or IL-1β (39, 53), and they are also unresponsive to intracerebrally delivered PGE₂ (14, 53). Experiments with restricted injection of PGE₂ into the brain have shown that the fever-producing region is localized along the ventromedial aspect of the preoptic area of the hypothalamus, and local injection of a cyclooxygenase inhibitor into the same sites can alleviate the fever produced by peripherally administered endotoxin (48). The preoptic region is richly vascularized, shows a dense IL-1 type 1 receptor expression, and displays a strong NFKB and COX-2 response after immune stimulation, indicating a high rate of prostaglandin synthesis (26). The preoptic region is also rich in EP₂ receptors (7, 40), many of which are expressed on inhibitory GABAergic neurons that project to the brain stem raphe pallidus nucleus, where they are supposed to exert a tonic inhibitory effect (38). Because EP₃ receptor activation results in lowered levels of cAMP, the GABAergic inhibition is thought to be released on PGE₂ binding, resulting in fever development via activation of sympathetic effectors by the raphe neurons (38, 57).

Subcutaneous injection of turpentine in wild-type mice resulted in a biphasic febrile response that lasted ~48 h. Thus a high core temperature was seen during the first dark/light cycle following injection and then during the subsequent light period, whereas it did not differ from that recorded in control animals during the intervening second dark period. Some previous studies using turpentine have shown less pronounced suppression of the normal circadian variation in core temperature than of an actual febrile response (39). These differences may be due to differences in the ambient temperature at which the experiments were performed (44). The present study was carried out at an ambient temperature of ~27°C, which is close to the thermoneutral zone of mice (29).

In contrast to the wild-type mice, mPGES-1 mutant mice injected with turpentine displayed a temperature curve that was similar to that displayed by saline-injected wild-type and mutant mice. However, whereas in the control animals the core temperature decreased rapidly at the end of the dark period, this circadian related fall in body temperature was less pronounced, especially following the first, but to some extent also following the second dark period after injection. The significance of this difference is unclear. It may indicate the presence of some additional temperature-regulating mechanism that is mPGES-1 independent (6, 13), but it could also be secondary to, e.g., changes in the circadian activity patterns of these animals. Thus both the wild-type and mutant mice that were injected with turpentine showed very little activity during the first dark period after injection, and attenuated activity during the subsequent dark periods, indicating that the activity depression was mPGES-1 independent. A similar dissociation between fever and activity has been found for IL-6 knockout mice. While IL-6 mutants did not develop fever after turpentine administration, the IL-6 gene deletion did not prevent the lethargy associated with the turpentine abscess (28). It is possible that the activity depression is mediated by a different prostaglandin, such as PGD₂ (50, 51).

The responses to intraperitoneal injection of IL-1β were similar to those which we previously demonstrated in the same strains of mice after intraperitoneal lipopolysaccharide injection (14). After the initial restraint-induced hyperthermia, IL-1β-injected animals showed a rapid hypothermic response that was not present in saline-injected controls. This hypothermia is probably elicited by an IL-1β-induced release of TNF-α and consequent peripheral vasodilatation (34). The wild-type mice injected with IL-1β showed a subsequent monophasic fever, whereas the mutant IL-1β-injected mice displayed a temperature curve that with the exception of the later time points was similar to that seen in saline-injected animals. Since the end of the observation period coincides with the end of the light period, the elevation of the body temperature seen at the late time points in the saline-injected controls is consistent with a circadian-dependent temperature increase (cf. Fig. 1). However, because the late temperature increase seen in the IL-1-1β-injected knockout mice began earlier than that seen in the controls, it seems to be an unrelated phenomenon. While it thus may represent some additional IL-1β-elicited temperature-regulating mechanism that is mPGES-1 independent, similar to that suggested by the turpentine experiment, it should be recalled that bolus injection of IL-1β represents an artificial situation that results in cytokine concentrations and profiles that may not occur during naturally appearing infectious and inflammatory conditions (33).

In contrast to the dependence of mPGES-1 for the inflammatory-induced febrile response that is demonstrated in the present study, mPGES-1 does not seem to be critical for the normal body temperature or for maintaining normal circadian temperature variations. Thus wild-type and mutant mice showed similar baseline temperature curves and activity patterns (Figs. 1 and 2). A normal circadian temperature rhythm was seen also in EP₁ and EP₃ receptor mutant mice (39), and in EP₂ receptor mutant mice (L. Engström and A. Blomqvist, unpublished observations). Unless the EP4 receptor will be shown to be involved, these data seem to indicate that the normal temperature regulation is PGE₂ independent.
Similar to the hyperthermia elicited by the restraint during the IL-1β injection, a pronounced temperature response was elicited by the cage-exchange experiment in both wild-type and knockout mice (Fig. 3), showing that mPGES-1-produced PGE2 is not critical for the psychogenic stress-induced temperature response. Because also this response is independent of the EP1 and EP3 receptors (39), as well as of the EP2 receptor (L. Engström and A. Blomqvist, unpublished observations), these data, taken together, indicate that the mechanism of psychological stress-induced hyperthermia is distinct from that of immune challenge-induced fever. This is consistent with observations that lesions of the anteroventral third ventricle region, including the organum vasculosum of the lamina terminals, attenuated pyrogen- but not stress-induced fever (4, 19). However, the temperature peak elicited by the cage exchange was somewhat lower in the knockout mice than in the wild-type mice, and a similar difference between the two genotypes was also seen in the control group (Fig. 3). These observations lend support to previous observations, showing that the COX-inhibitor indomethacin attenuated the increase in body temperature induced by cage-exchange stress (25, 36, 46, 49). However, in contrast to previous work that did not show any effect of indomethacin on the cage exchange-induced activity increase (25, 36, 46, 49), in the present study cage-exchanged knockout mice displayed lower activity than cage-exchanged wild-type mice (Fig. 4), indicating that PGE2 synthesis by mPGES-1 contributes to this response. However, because of its very rapid character, being elicited within minutes after the stimulus, it is unlikely that it involved de novo synthesis of PGE2 and rather indicates that constitutive PGE2 plays a facilitating role in the involved neuronal structures.

In summary, we have shown that mPGES-1 is critical for the development of fever during endotoxin challenge (14), turpentine-induced abscess, and intraperitoneal injection of IL-1β (present study). Taken together with previous observations showing induction of mPGES-1 during adjuvant-induced arthritis (12) and reduced sensitivity of the mPGES-1 knockout mice to collagen-induced arthritis (52), the present findings demonstrate that mPGES-1 plays a major role in both central and peripheral inflammatory responses. Thus they provide support for mPGES-1 as an important novel drug target for treatment of fever and other inflammation-induced disease symptoms.

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