Characterization of the febrile response induced by fibroblast-stimulating lipopeptide-1 in guinea pigs

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Greis A, Murgott J, Rafalzik S, Gerstberger R, Hübschle T, Roth J. Characterization of the febrile response induced by fibroblast-stimulating lipopeptide-1 in guinea pigs. Am J Physiol Regul Integr Comp Physiol 293: R152–R161, 2007. First published May 9, 2007; doi:10.1152/ajpregu.00182.2007.—Recently, it has been shown that the Toll-like receptors-2 and -6 agonist fibroblast-stimulating lipopeptide-1 (FSL-1) have the capacity to induce fever and sickness behavior in rats. Since the mechanisms of the fever-inducing effects of FSL-1 are still unknown, we tested the pyrogenic properties of FSL-1 in guinea pigs and assessed a role for TNF-α and prostaglandins in the manifestation of the febrile response to this substance. Intra-arterial and intraperitoneal injections of FSL-1 caused dose-dependent fevers that coincided with elevated plasma levels of TNF and IL-6, the intraperitoneal route of administration being more effective than the intra-arterial route. Intra-arterial or intraperitoneal injection of a soluble form of the TNF type 1 receptor, referred to as TNF binding protein (TNFβp), together with FSL-1, completely neutralized FSL-1-induced circulating TNF and reduced fever and circulating IL-6. Intra-arterial or intraperitoneal injection of the nonselective cyclooxygenase (COX)-inhibitor diclofenac depressed fever and FSL-1-induced elevations of circulating PGE2. Circulating TNF and IL-6, however, remained unimpaired by treatment with diclofenac. In conclusion, FSL-1-induced fever in guinea pigs depends, in shape and duration, on the route of administration and is, to a high degree, mediated by pyrogenic cytokines and COX products.

Mycoplasma infection; diacylated mycoplasmal lipopeptides; cytokines; prostaglandins

PATHOGEN-ASSOCIATED MOLECULAR patterns (PAMPs) are recognized by the innate immune system via Toll-like receptors (TLRs) that function as pattern-recognition receptors and signal the presence of microbial components to innate immune cells and a variety of other cells (2, 3, 30). For example, LPS from Gram-negative bacteria, the most frequently used agent to induce experimental fever, is recognized by TLR4 (6, 31), and it has recently been shown that all phases of LPS-induced fever depend on TLR4 signaling (43). Meanwhile, several other microbe-associated PAMPs have been tested regarding their capacity to induce fever and other brain-controlled sickness responses. Attempts have been made to elucidate whether there are common or distinct mechanisms for induction of fever and sickness behavior in response to activation of different TLRs by their specific agonists (12, 14, 18, 20, 48, 49). Mycoplasma-specific diacylated lipopeptides, such as fibroblast-stimulating lipopeptide-1 (FSL-1), the NH2-terminal sequence of a lipoprotein from Mycoplasma salivarium, seem to require heterodimerization of TLR2 and TLR6 to stimulate cells implicated in innate immunity (11, 29). We recently reported that systemic treatment of rats with FSL-1 results in a pronounced peripheral formation of the proinflammatory cytokines TNF and IL-6 that was accompanied by fever and sickness behavior (18). In that study, increased levels of circulating cytokines in response to FSL-1 seemed not to be preceded by the onset of fever, as has been reported for bacterial LPS (4, 24). Thus, it might be possible that the cytokines released into the bloodstream after injection of FSL-1 contributed to the observed febrile changes of body temperature (Tb). The guinea pig is frequently used as an animal model in experimental fever research, and even novel concepts on the genesis of endotoxic fever have been introduced that are based on laboratory data obtained in the guinea pig (4). In the first part of the present study, we therefore investigated whether our previous findings in rats (18) are applicable to another species, i.e., the guinea pig. For this purpose, we tested the intra-arterial and intraperitoneal routes of administration and studied the effects of FSL-1 on Tb and on circulating concentrations of TNF, IL-6, and PGE2. We used intra-arterial and intraperitoneal injections since we recently reported that fever in strength and duration strongly depends on the route of administration of a given exogenous pyrogen (49). Additional experiments aimed to get a first mechanistic insight into FSL-1-induced fever. To obtain precise information concerning a putative participation of a given cytokine in the febrile response to FSL-1, substances that are able to neutralize the biological activity of the cytokine are necessary. In this context, the proinflammatory cytokine TNF attracts special interest due to the fact that TNF is regarded as an initial player within the proinflammatory cytokine cascade and that TNF is circulating in increased amounts a short time after a systemic challenge with FSL-1 (18). To obtain insight into the mechanisms responsible for the manifestation of FSL-1-induced fever, we therefore used a dimeric polyethylene glycol-linked form of the 55 kDa TNF type 1 receptor to neutralize endogenously released TNF in response to a fever-inducing dose of FSL-1. We further investigated the putative dependence of FSL-1-induced fever on cyclooxygenase (COX) products by using the nonselective COX inhibitor diclofenac. The results of these experiments should enable us to state whether the mechanisms responsible for the pyrogenic effects of FSL-1 in guinea pigs are similar or different from those that seem to participate at least in the maintenance of LPS-induced fever in this species of experimental animal (34, 35).
MATERIALS AND METHODS

Animals

This study was performed in male guinea pigs (Cavia aperea porcellus) with a body weight of 380–415 g at the day of surgery. After surgery (see Surgery) animals were housed individually in a temperature- and humidity-controlled room (22 ± 1°C and 50% humidity). The animals had access to food and water ad libitum. Twice a week the reservoirs were filled with fresh pellet food and water and also at the same time the cages were changed. About 10 days after surgery the animals were used for the respective experiment. Within the last 3 days before the experiment, the animals were habituated at least twice to the experimental handling procedures. Single animals that did not develop a normal body weight gain within the days before the scheduled experiment were not used. The national guidelines for experiments with vertebrate animals were followed, and local ethics committee approval was obtained from the regional government for the experimental protocols (ethics approval no. GI 18/2 - Nr. 59/2003).

Substances

The diacylated lipopeptide named FSL-1 represents the NH₂-terminal sequence of the 44-kDa lipoprotein of Mycoplasma salivarium (28, 29, 41). Synthetic FSL-1 was purchased as a mixture of the RR and RS stereoisomers (prod. no. L7000; EMC Microcollections, Tübingen, Germany). According to EMC Microcollections, test samples of this synthetic product that were tested with a sensitive limulus amebocyte lysate assay have never shown contamination with endotoxins. The lyophilized powder was diluted in sterile PBS at concentrations of 100 or 1,000 µg/ml. Doses of 100 or 1,000 µg/kg FSL-1 were used for systemic injections on the basis of experience from a previous study in rats (18).

TNF binding protein (TNFbp), a synthetic dimeric polyethylene-glycol-linked form of the type 1 soluble receptor of TNF [PEG-rsTNF-R1]₂ was kindly provided by Dr. Dave Martin (AMGEN, Boulder CO). This substance effectively neutralizes bioactive TNF (33, 35, 42, 47). A stock solution was diluted with 0.9% sterile saline to a final concentration of 5 mg/ml. From this stock solution, aliquots of 100 µg TNFbp were prepared for intra-arterial or intraperitoneal injections. An amount of 100 µg of TNFbp in a volume of 200 µl sterile saline (or an equivalent volume of sterile saline alone) was used for each injection along with FSL-1 or its solvent (PBS) in accordance with the neutralizing capacity of this compound shown in own previous studies (33, 35).

Diclofenac sodium (Calbiochem, LaJolla, CA) was dissolved at a concentration of 500 µg/ml in a vehicle that consisted of 95% sterile saline and 5% ethanol in accordance with a previous study (39). Diclofenac was injected at a dose of 500 µg/kg. Control animals were injected with an equivalent volume of vehicle (1 ml vehicle/kg) together with FSL-1 or PBS.

Surgery

Guinea pigs were chronically implanted with intra-arterial catheters for blood sampling and injections, as well as intra-abdominal radio transmitters for the measurement of body core temperatures. Briefly, the guinea pigs were anesthetized with 0.25 mg/kg medetomidine hydrochloride (Pfätzer, Karlsruhe, Germany) injected intramuscularly, followed by intraperitoneal injection of 100 mg/kg ketamine hydrochloride (Pharmacia Upjohn, Erlangen, Germany). A polyethylene catheter (0.4 mm ID, 0.8 mm OD; Portex, Kent, UK) was inserted through the left carotid artery until it reached the aortic arch. Slow aspiration of blood with a syringe indicated the correct position of the catheters. The catheter was then fixed with two sutures. The muscle layer and the skin were closed separately with sutures. The catheter was flushed with sterile heparinized saline and sealed by heating.

Finally, a midline laparotomy was performed in each animal for implantation of a free-floating temperature transmitter into the peritoneal cavity. Muscle layer and skin were sutured separately to close the abdominal incision.

Sampling of Blood Plasma

During the acute experiment, single blood samples with a volume of 0.5 ml were slowly (within 1 min) drawn via the implanted catheter into heparinized sterile syringes (heparin sodium 25000; Merckel, Blaubeuren, Germany) by aspiration, put into chilled polypropylene tubes, and immediately centrifuged. The catheter was flushed with saline and closed by heating. The blood plasma was stored at −70°C for later determination of cytokines or PGE2. Within the week before the experiment, the animals were accustomed to the blood sampling procedure once or twice, which then did not cause excitement during experiments.

Measurement of Tb

Abdominal temperature was measured by using the intra-abdominally implanted biotelemetry transmitters (model PDT-4000 E-Mitter; Mini-Mitter, Sunriver, OR). Output (frequency in Hz) was monitored by an antenna placed under each cage (model ER-4000 radio receivers; Mini-Mitter). A data acquisition system (Vital View; Mini Mitter) was used for automatic control of data collection and analysis. Tb was monitored and recorded at 5-min intervals. For the analysis and graphical documentation, temperature data at time intervals of 15 min were used.

Cytokine Bioassays

Cytokine analysis was performed with plasma samples from guinea pigs stimulated either with FSL-1 or the respective control vehicle (PBS). Determination of TNF was achieved by a bioassay based on the cyttotoxic effect of TNF on the mouse fibrosarcoma cell line WEHI 164 subclone 13 (13). The assay was performed in sterile, 96-well microtiter plates. Serial dilutions of biological samples or different concentrations of a murine TNF standard (code 88/532; National Institute for Biological Standards and Control, South Mimms, UK) were incubated for 24 h in wells that had been seeded with 50,000 actinomycin D-treated WEHI cells. The number of surviving cells after 24 h was measured by using the dimethylthiazol-diphenyl tetrazolium bromide (MTT) colorimetric assay (17). Plasma samples were prediluted so that serial dilution of samples and standard dilution curves were parallel. The detection limit of the assay, after considering the dilution of samples into the assays, was 6 pg/ml TNF.

Determination of IL-6 was achieved by a bioassay based on the dose-dependent growth stimulation of IL-6 on the B9 hybridoma cell line (1). The assay was performed in sterile, 96-well microtiter plates. In each well, 5,000 B9 cells were incubated for 72 h with serial dilutions of biological samples or with different concentrations of a human IL-6 standard (code 89/548; National Institute for Biological Standards and Control, South Mimms, UK). Plasma samples were prediluted so that serial dilution of samples and standard dilution curves were parallel. The number of cells in each well was measured by using the MTT assay (see above). The detection limit of the assay, after considering the dilution of samples into the assays, was 3 IU of IL-6/ml.

PGE2 Assay

PGE2 was determined by using a commercially available enzyme immunoassay (Assay Designs, Ann Arbor, MI). The assay is based on the competitive binding technique in which PGE2 in a sample competes with a fixed amount of alkaline phosphatase-labeled PGE2 for
sites on a mouse monoclonal antibody. During incubation, the mouse monoclonal antibody was bound to a goat anti-mouse antibody that coated the wells of a microplate. After the sample was washed once to remove excess conjugate and unbound sample, a substrate solution was added to the wells for determination of the bound enzyme activity. Immediately after color development, the absorbance was read at 405 nm on a microplate reader (Digiscan; Asys Hitech, Eugendorf, Austria). The color intensity inversely correlated with the PGE2 concentration in the sample. According to the manufacturer’s product information, the sensitivity of the PGE2 assay was 8.3 pg/ml.

Experimental Protocols

Experiment 1. This experiment was performed to test the capacity of FSL-1 to induce fever and circulating TNF and IL-6 when administered intra-arterially or intraperitoneally. FSL-1 was dissolved at concentrations of 100 or 1,000 μg/ml and injected at doses of 100 or 1,000 μg/kg. For intraperitoneal injections, the injection volume was adjusted to 1 ml by adding PBS to achieve a better distribution of the drug within the abdominal cavity. Control groups were injected with equivalent volumes of PBS (1 ml/kg i.a. or 1 ml/animal i.p.). From all animal groups, blood samples were collected at selected intervals from the time of injection and assayed for bioactive TNF and IL-6. In guinea pigs, injected intra-arterial blood was collected 60 min before, as well as 60 and 180 min after treatment with FSL-1 or PBS. In animals injected intraperitoneally, blood samples were taken 60 min before, as well as 60 or 120 min after the respective injection. This slight modification was chosen because we considered the possibility of a delay of the initial formation of TNF (18) in intraperitoneal-treated compared with intra-arterial-injected animals. To ensure we obtained information about the FSL-1-induced formation of TNF, we collected blood after 2 h instead of 3 h after the respective intra-arterial injection. Due to the observation that intraperitoneal injections of the high dose of FSL-1 caused greatly extended fever duration, we performed an additional pilot experiment to test whether circulating cytokines were still elevated during this late phase. In this pilot experiment, blood was collected 8, 10, and 12 h after intraperitoneal injection of 1,000 μg/kg FSL-1. All injections were performed between 10:45 and 11:15 AM.

Experiment 2. To determine the effects of TNFbp on FSL-1-induced fever and circulating TNF and IL-6, 100 μg/kg TNFbp dissolved in 0.2 ml sterile saline or 0.2 ml solvent alone were injected together with 100 μg/kg FSL-1 or an equivalent volume of PBS (solvent for FSL-1) intra-arterially or intraperitoneally. For intraperitoneal injections, the injection volume was adjusted to 1 ml by adding PBS to achieve a better distribution of the drugs within the abdominal cavity. Blood samples were collected according to the schedules introduced in the protocol for experiment 1.

Evaluation and Statistics

In graphs of the thermal responses to injections of FSL-1 or sterile saline, the mean abdominal temperatures were plotted against time and expressed as means ± SE at each time point. Abdominal temperatures of different groups of guinea pigs were compared by two-way repeated-measures ANOVA followed by Scheffé’s post hoc test. Circulating levels of TNF-α, IL-6, or PGE2 were compared by one-way ANOVA and the Scheffé test. Because the values for cytokine concentrations are not normally distributed, a log transformation of the cytokine values was performed prior to the statistical calculation. All calculations were carried out on an Apple Macintosh computer by using the software package StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Experiment 1: FSL-1-Induced Fever and Cytokines in Guinea Pigs Depends on Dose and on the Route of Administration

Intra-arterial injections of 100 or 1,000 μg/kg FSL-1 induced distinct thermal and cytokine responses in guinea pigs as shown in Fig. 1. Within a short time after intra-arterial injection of 100 μg/kg FSL-1, guinea pigs developed a moderate fever which rose by 1.2°C to a peak at 60–75 min after injection. Thereafter, Tb declined again but stayed elevated for another 2 h compared with the preinjection values. In response to the higher dose of FSL-1 (1,000 μg/kg), Tb was rising with a slower slope to a higher peak (1.5°C) at 165 min after injection. Thereafter, Tb declined slowly so that the baseline temperature was not yet reached at the end of the lights-on period. The overall strength of fever was more pronounced in response to the higher dose of FSL-1.

Plasma levels of TNF and IL-6 were measured 60 min before injection and 60 and 180 min after injection of FSL-1 or solvent (Fig. 1, C and D). TNF was not detected prior to injection of FSL-1 or PBS and remained undetectable 1 and 3 h after administration of PBS. However, intra-arterial injection of both doses of FSL-1 caused the appearance of pronounced amounts of TNF in plasma within 60 min, the higher dose of 1,000 μg/kg being more effective than the lower dose (F = 12.65, P = 0.0074). Bioactive IL-6 was detected in plasma even under basal conditions. Prior to injection of FSL-1 or PBS and 1 and 3 h after administration of PBS, mean circulating IL-6 levels in the range of 30–80 IU/ml were measured. Intra-arterial injections of 100 or 1,000 μg/kg FSL-1 caused significant elevations of IL-6 in plasma compared with controls. At both investigated intervals from the time of injection, the higher dose of FSL-1 had a stronger effect on the endogenous release of IL-6 (60 min: F = 34.7, P = 0.0004; 180 min: F = 58.5, P < 0.0001).

Intraperitoneal injections of FSL-1 caused fevers of longer duration compared with the respective responses to intra-arterial administrations of this pyrogen (Fig. 2). The febrile response to the lower dose of FSL-1 started with a delay of 60 min and rose to a first peak between 105 and 135 min from the time of injection. Thereafter, we observed a further increase to a second peak at 210 min, followed by a continuous decline of Tb within the following hours. In response to intraperitoneal injections, the higher dose of FSL-1 induced a biphasic elevation of Tb, the first phase lasting about 4 h, while the second phase ended not earlier than 23 h after the time of injection. The cytokine responses within the first hours after intraperitoneal administration of FSL-1 were similar as seen after the respective intra-arterial injections (Fig. 2, C and D), the higher dose of FSL-1 being more effective in inducing elevations of circulating TNF and IL-6. Due to the greatly extended fever duration in response to intraperitoneal injections of the higher dose of FSL-1, we performed a pilot experiment in another three guinea pigs to test whether there might be elevated cytokine levels throughout this phase. In these animals injected
intraperitoneally with 1,000 μg/kg FSL-1, blood samples were collected at 8, 10, and 12 h after the injection. Bioactive TNF was not detected at any of these intervals from the time of injection. IL-6 was still elevated. In two animals, 1,100–3,700 IU/ml of IL-6 were measured, while just 200–450 IU/ml of IL-6 were detected in the third guinea pig. These values were considerably lower than those detected during the early phase of fever in response to intraperitoneal injections of the high dose of FSL-1 (Fig. 2D). All further experiments were performed with the lower dose of 100 μg/kg FSL-1.

Experiment 2: Neutralization of FSL-1-Induced Bioactive TNF Dampens Fever and Circulating IL-6

Fever induced by intra-arterial injection of 100 μg/kg FSL-1 was strongly impaired by coadministration of 100 μg TNFbp (Fig. 3A). Namely, the early strong rise of Tb from 30–120 min after the time of injection was significantly depressed by coinjection of TNFbp 60 min after injection (Fig. 3B). All further experiments were performed with PBS together with TNFbp or its solvent, bioactive IL-6 in plasma remained at its baseline level at the corresponding intervals from the time of injection (not shown).

We repeated this experiment, using the intraperitoneal route of administration, and obtained similar results (Fig. 4). FSL-1-induced fever was significantly depressed by coinjection of TNFbp from 135–300 min after the injection (F = 12.1, P = 0.0061; Fig. 4A). FSL-1-induced bioactive TNF was completely neutralized by treatment with TNFbp (Fig. 4C), and this treatment caused a significant attenuation of FSL-1-induced circulating IL-6 at both investigated intervals from the time of injection (Fig. 4D).

Experiment 3: Treatment With Diclofenac Depresses Fever and Circulating PGE2 but Has no Influence on Circulating Cytokines

Figure 5 shows the febrile and cytokine responses of guinea pigs to intra-arterial injection of 100 μg/kg FSL-1 with solvent or 500 μg/kg diclofenac. Injection of solvent or diclofenac
together with PBS had no effect on Tb (Fig. 5B). Injection of FSL-1 with solvent caused a febrile response that is characteristic for this species of experimental animals (see Figs. 1 and 3). Administration of 500 μg/kg diclofenac with FSL-1 abolished the FSL-1-induced rise of Tb (60–240 min: F = 31.1, P = 0.0002), compared with guinea pigs injected with FSL-1 and solvent (Fig. 5A).

Figure 5 also summarizes the circulating levels of TNF (Fig. 5C) and IL-6 (Fig. 5D) in response to FSL-1 injected with diclofenac or solvent. FSL-1-induced TNF and IL-6 in plasma were not affected by the treatment with diclofenac. In the control groups injected with PBS together with diclofenac or solvent, TNF was not detected, and IL-6 remained at its baseline level (not shown). We further measured PGE2 in plasma in this experiment to test the capacity of FSL-1 to cause an elevation of PGE2 in plasma and to study the effect of the administered diclofenac on circulating PGE2 (Fig. 6).

At 60 and 180 min after intra-arterial injection of FSL-1 together with solvent, we observed elevated concentrations of ~600 pg/ml PGE2 in plasma compared with the baseline value of ~100 pg/ml PGE2. The FSL-1-induced increase of PGE2 in plasma was completely blocked by the treatment with diclofenac at both time intervals after the respective injection. We repeated this experiment using the intraperitoneal route of administration and obtained similar results. The biphasic fever evoked by intraperitoneal injections of 100 μg/kg FSL-1 was completely abrogated by coadministration of diclofenac (Fig. 7A). Again, the depression of fever by treatment with diclofenac was not related to any significant alteration in endogenous release of TNF and IL-6 into the systemic circulation (Fig. 7, C and D).

Intraperitoneal injections of FSL-1 with solvent resulted in a moderate increase of PGE2 in plasma to mean values that were about twice as high as we observed in the respective control group treated with PBS and solvent. This rather moderate, but still significant, elevation of circulating PGE2 was completely blocked in guinea pigs injected with FSL-1 and diclofenac. In those animals, just the basal values of ~100 pg/ml PGE2 were determined (Fig. 8).

In summary, the treatment with the nonselective COX-inhibitor diclofenac abolished FSL-1-induced fever and the FSL-1-induced increase of PGE2 in plasma, indicating that COX products are essential for the manifestation of a febrile response to mycoplasma-specific diacylated lipopeptides.

**DISCUSSION**

*Mycoplasmas* form a large group of prokaryotic microorganisms that can be distinguished from ordinary bacteria by their small size and their total lack of a cell wall (37). Diacylated lipopeptides from these *Mycoplasmas* act as PAMPs and stimulate the innate immune system by TLR2- and -6-mediated activation of intracellular signal transduction pathways, which finally leads to the formation of the proinflammatory cytokines TNF and IL-6 in vitro (27, 45, 46) and in vivo (11, 15, 18). We recently reported that systemic treatments of rats with FSL-1 causes fever and sickness behavior in a similar fashion as it has been reported for other
PAMPs, such as the TLR4 agonist LPS (for a review, see Refs. 4, 25, and 36), the TLR9 agonist bacterial DNA (20), or double-stranded RNA, which acts as a TLR3 agonist (14, 48, 49). In this study, we confirmed in another species of experimental animals that FSL-1 has dose-dependent pyrogenic properties. In guinea pigs, fever is stronger in its height and duration when FSL-1 is injected at a dose of 100 μg/kg ip compared with the intra-arterial route of administration. With.

Fig. 3. Fever and IL-6 induced by IA injection of FSL-1 are attenuated by treatment with TNF binding protein (TNFbp), while bioactive TNF is neutralized. Guinea pigs were injected IA with 100 μg/kg FSL-1 (A) or vehicle (PBS; B) together with TNFbp or vehicle (saline) at time 0. Tb in response to the respective injections is shown as means ± SE. Time intervals are labeled with significance bars at which the treatment with TNFbp caused a significant attenuation of fever (P < 0.05). Plasma TNF (C) and IL-6 (D) concentrations in response to 100 μg/kg FSL-1 injected IA together with TNFbp or solvent are shown. Plasma samples were collected and analyzed 60 min before and 60 min and 180 min after the respective injections. All columns are means ± SE. *Significant increases compared with the preinjection values (measured at t = −60 min; P < 0.05). At a given time from injection, any columns with the same letter are not significantly different from each other, while any columns with different letters are significantly different (P < 0.05; differences between both groups). Bioactive TNF was not detectable in all samples collected after injection of FSL-1 together with TNFbp.

Fig. 4. Fever and IL-6 induced by IP injection of FSL-1 are attenuated by treatment with TNFbp, whereas bioactive TNF is neutralized. Guinea pigs were injected IP with 100 μg/kg FSL-1 (A) or vehicle (PBS; B) together with TNFbp or vehicle (saline) at time 0. Tb in response to the respective injections is shown as means ± SE. Time intervals are labeled with significance bars, at which the treatment with TNFbp caused a significant attenuation of fever (P < 0.05). Plasma TNF (C) and IL-6 (D) concentrations in response to 100 μg/kg FSL-1 injected IP together with TNFbp or solvent are shown. Plasma samples were collected and analyzed 60 min before and 60 min and 120 min after the respective injections. All columns are means ± SE. *Significant increases compared with the preinjection values (measured at t = −60 min; P < 0.05). At a given time from injection, any columns with the same letter are not significantly different from each other, while any columns with different letters are significantly different (P < 0.05; differences between both groups). Bioactive TNF was not detectable in all samples collected after injection of FSL-1 together with TNFbp.
regard to the larger dose of FSL-1 used in this study, the initial fever peak (i.e., within the first 4 h) is larger after the intra-arterial injection. However, the duration of a second, later peak of fever, after intraperitoneal stimulation with the higher dose of FSL-1 and, thereby, the overall strength of the febrile response, is greatly increased and extended. This observation was surprising in so far as guinea pigs respond to intra-arterial injections of LPS in a more sensitive fashion compared with intraperitoneal injections of this pyrogen. A possible reason for this discrepancy may be a more effective access of FSL-1 to cells bearing the TLR-2 and -6 receptors within the abdominal cavity compared with the arterial circulation. Consequently, there might have been a subsequent localized intraperitoneal formation of inflammatory mediators, which entered, in part, the systemic circulation and may have evoked additional local effects, which possibly contributed to the manifestation of fever. Indeed, pilot measurements indicated that at least IL-6 was still elevated up to 12 h after intraperitoneal injections of 1,000 μg/kg FSL-1. In this context, it seems remarkable that guinea pigs did not develop a phase of hypothermia, prior to fever in response to the treatment with the high dose of FSL-1. The same dose of FSL-1 (1,000 μg/kg) readily induced hypothermia when administered to rats (18). The reason for this species-specific discrepancy may be a higher sensitivity of rats to this pyrogen. On the other hand, distinct zones of thermoneutrality in rats and guinea pigs may have been responsible for the hypothermic response of rats (18) and its lack in guinea pigs (Figs. 1 and 2). Studies in rats and mice clearly showed that the dose of a given pyrogen and the ambient temperature (thermoregulation vs. subneutral ambient temperature) are both critical determinants for the developing thermoregulatory response (22, 32, 38). Our previous study in rats (18) was performed at an ambient temperature of 23°C, which can be classified as “subthermoneutral” for this species (32). With regard to the thermoneutral zone of guinea pigs, a few informative studies were performed more than 20 years ago. Laughter and Blatteis (21), as well as Gordon (16), determined the preferred ambient temperature of guinea pigs in a thermal gradient and observed that this preferred air temperature was in the range of 28.5–30°C. Interestingly, this preferred air temperature corresponds to the upper limit of thermoneutrality in guinea pigs (16). In this study, the upper and lower ends of the thermoneutral zone were determined in the guinea pig, which starts to increase evaporative water loss at an ambient temperature of 28°C and metabolic heat production at an ambient temperature lower than 20°C (16). These data indicate that our experiments were performed at an ambient temperature within the thermoneutral range and may, at
least in part, explain why we did not observe a phase of hypothermia in response to the high dose of FSL-1.

In this context, there is experimental evidence that the hypothermia, which may develop during systemic inflammation under specific conditions, is, at least in part, mediated by TNF (19, 22, 26, 47). The role of TNF in the manifestation of fever, on the other hand, seems to be equivocal according to some conflicting data from the literature. A propyretic or an antipyretic role of TNF in fevers induced by a variety of pyrogenic or inflammatory stimuli may depend on the stimulus (7, 19, 23), the dose of the stimulus (23), and the investigated species of the experimental animal (35, 47). In a previous study in guinea pigs, we observed that the second phase of the biphasic febrile responses to LPS or muramyl-dipeptide was attenuated by neutralization of TNF with TNFbp (35). The impact of neutralizing bioactive TNF on FSL-1-induced fever was even stronger in so far as the entire febrile response to this pyrogen was significantly attenuated by treatment with TNFbp (Figs. 3 and 4). This effect was not necessarily caused by the lack of bioactive TNF per se. It should be noted that the removal of TNF from the FSL-1-induced cytokine cascade consequently led to a reduced formation of IL-6, for which a pyrogenic role is well established (5, 40). Other anti-TNF-strategies selectively attenuated the endogenous formation of TNF, whereas the level of IL-6 in plasma was not impaired, exclusively antagonized the hypothermic, but not the febrile component of the thermoregulatory responses to LPS (26).

Regardless of whether the contribution of TNF to FSL-1-induced fever in guinea pigs is of direct or indirect origin, we could clearly demonstrate that the cytokines, which are produced and released after systemic stimulation with FSL-1, significantly contribute to the manifestation of the febrile response to this novel exogenous pyrogen.

Several strong arguments support a role for PGs, particularly PGE2, as key mediators in the manifestation of fever (for a review, see Refs. 4 and 36), although some pyrogens don’t seem to require PGs to elicit a febrile response (8, 50). Several PAMPs have been tested in their capacity to induce increased levels of PGE2 in the blood, such as LPS (9, 20, 24), bacterial DNA (20), or double-stranded RNA (9). Here we report for the first time that intra-arterial and, to a smaller degree, intraperitoneal injections of the TLR-2/6 agonist FSL-1 cause a significant increase of PGE2 in the plasma of guinea pigs (Figs. 6 and 8). The absolute values of circulating PGE2, which we observed under basal (~100 pg/ml) or under stimulated conditions (200–600 pg/ml), are in good agreement with data obtained in the guinea pig (24) and in other species of experimental animals (9, 20). It is a matter of debate whether
peripheral PGE2 is generated via a prior formation of cytokines or by direct effect of a given PAMP on Kupffer cells in the liver (4, 24). However, there is increasing evidence for the view that peripherally generated PGE2 provides an important contribution to the initiation of fever, while PGE2 generated within the brain plays a crucial role in the maintenance of fever (4, 44). Data from this study suggest that PGE2 is critically involved in FSL-1-induced fever, due to the fact that treatment with diclofenac resulted in total abrogation of the febrile response (Figs. 5 and 7) and in a depression of the increased level of PGE2 in the blood (Figs. 6 and 8). We are, however, not able to exclude the possibility that the fever-suppressing effects of diclofenac were mediated by the action of this drug within the brain, since diclofenac reportedly crosses the blood-brain barrier (10) and might thus have readily blocked the central formation of PGE2, in addition to its peripheral effect.

In this study, it was demonstrated that peripheral administration of the TLR-2 and -6 activator FSL-1 results in fever, which resembles that induced by other PAMPs with regard to the peripheral onset of the TLR-2 and -6 activator FSL-1 results in fever, in addition to its peripheral effect. It thus seems that activation of distinct receptors of the innate immune system finally results in the manifestation of rather similar brain-intrinsic mechanisms, which may be responsible for a stereotypic pattern of brain-controlled illness responses including fever.

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GRANTS

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REFERENCES


