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Kupffer cell-generated PGE\textsubscript{2} triggers the febrile response of guinea pigs to intravenously injected LPS

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Li, Zhonghua, Vit Perlik, Carlos Feleder, Ying Tang, and Clark M. Blatteis. Kupffer cell-generated PGE\textsubscript{2} triggers the febrile response of guinea pigs to intravenously injected LPS. Am J Physiol Regul Integr Comp Physiol 290: R1262–R1270, 2006. First published January 12, 2006; doi:10.1152/ajpregu.00724.2005.—Because the onset of fever induced by intravenously (iv) injected bacterial endotoxic lipopolysaccharides (LPS) precedes the appearance in the bloodstream of pyrogenic cytokines, the presumptive peripheral triggers of the febrile response, we have postulated previously that, in their stead, PGE\textsubscript{2} could be the peripheral fever trigger because it appears in blood coincidentally with the initial body core temperature (T\textsubscript{c}) rise. To test this hypothesis, we injected \textit{Salmonella enteritidis} LPS (2 \textmu g/kg body wt iv) into conscious guinea pigs and measured their plasma levels of LPS, PGE\textsubscript{2}, TNF-\alpha, IL-1\beta, and IL-6 before and 15, 30, 60, 90, and 120 min after LPS administration; T\textsubscript{c} was monitored continuously. The animals were untreated or Kupffer cell (KC) depleted; the essential involvement of KCs in LPS fever was shown previously. LPS very promptly (<10 min) induced a rise of T\textsubscript{c} that was temporally correlated with the elevation of plasma PGE\textsubscript{2}. KC depletion prevented the T\textsubscript{c} and plasma PGE\textsubscript{2} rises and slowed the clearance of LPS from the blood. TNF-\alpha was not detectable in plasma until 30 min and in IL-1\beta and IL-6 until 60 min after LPS injection. KC depletion did not alter the times of appearance or magnitudes of rises of these cytokines, except TNF-\alpha, the maximal level of which was increased approximately twofold in the KC-depleted animals. In a follow-up experiment, PGE\textsubscript{2} antiserum administered iv 10 min before LPS significantly attenuated the febrile response to LPS. Together, these results support the view that, in guinea pigs, PGE\textsubscript{2} rather than pyrogenic cytokines is generated by KCs in immediate response to iv LPS and triggers the febrile response.

IT IS GENERALLY CONSIDERED that the febrile response to systemic infectious pathogens is mediated by the pyrogenic cytokines TNF-\alpha, IL-1\beta, and IL-6, elaborated by mononuclear phagocytes activated by the infectious noxa, and that their messages are transmitted to the fever-controlling center in the ventromedial preoptic-anterior hypothalamic area (POA) either neurally or humorally (for reviews, see Refs. 3, 6, 14, 66, 70, and 90). The neural pathway has been postulated to account for the rapid initiation of fever after, e.g., a bolus, intravenous (iv), low-to-moderate dose injection of bacterial endotoxic LPS; the afferent vagus serves as its link (69, 75, 87). The humoral pathway, on the other hand, depends on the bloodstream for the delivery of these mediators; it is, consequently, slower than the neural pathway (38). However, although cytokines, in particular IL-6, have been demonstrated in plasma correlatively with the onset of fever induced by low-to-moderate doses of LPS administered intraperitoneally, intramuscularly, and subcutaneously (into an air pouch) (~30–60 min), they are not detectable concurrently with the induction of fever provoked by higher doses of LPS or by any dose of LPS injected iv (14). For instance, the body core temperature (T\textsubscript{c}) of conscious guinea pigs rises significantly within 10 min after the injection of 2 \mu g iv of LPS/kg body wt (75, 77), whereas TNF-\alpha, the first of the cytokines to appear in the blood of similarly LPS-challenged guinea pigs, is not detectable until at least 30 min later (42, 59; present study, Fig. 3). This delay, nevertheless, should be anticipated because these cytokines are not expressed constitutively in mononuclear phagocytes, but rather are transcribed, translated, and secreted by these cells in response to the pyrogenic stimulus. Hence, if they are not yet present in the blood, it would seem improbable that circulating cytokines could provide the signals for the very prompt induction of fever after iv LPS or high doses of intraperitoneal LPS.

The liver is the body’s principal filter of circulating LPS (29, 43, 51, 72); it contains ~80%-90% of all macrophages in the body. Consequently, hepatic macrophages [Kupffer cells (KCs)] are considered to be the principal cell source of pyrogenic cytokines. Indeed, the involvement of KCs in the pathogenesis of fever is supported by a variety of findings (22, 84). In further support, we recently showed that the onset of fever in guinea pigs is linked to the first appearance of LPS in the liver (48) and that splenectomy and vinblastine-induced neutropenia both significantly increase the simultaneous febrile response to LPS and the uptake of LPS by KCs (27, 49). However, because the LPS-induced production of cytokines by KCs, like that of other macrophages, is delayed in relation to fever onset (18, 35, 42, 54), it seems unlikely that KC-generated cytokines could provide the signals that rapidly initiate iv LPS-induced fever. A factor elaborated by KCs earlier than cytokines, i.e., an almost immediate reaction to the presence of LPS, should, therefore, mediate this response.

Although its cell source and the nature of the triggering mechanism that releases it are still in dispute, PGE\textsubscript{2} is generally believed to be the final central mediator of the febrile response (for reviews, see Refs. 3, 10, 41, and 64). It acts on thermoregulatory neurons in the POA, and its levels increase.

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and decrease in this brain region in conjunction with the febrile course. PGE$_2$ levels also increase in the peripheral circulation after the entry of microorganisms or the systemic administration of exogenous and endogenous pyrogens (21, 71, 79). Using an in vivo model for the selective hepatic portal vein infusion of anesthetized guinea pigs, we have recently shown that PGE$_2$ is indeed very quickly generated by KC in response to the intraportal vein injection of LPS (59). Because the peripheral injection of exogenous PGE$_2$ reportedly causes T$_c$ rises in some animals (57, 62, 68), it has been suggested that it could play its mediatory role peripherally rather than centrally (23, 55, 67).

This study was designed to verify this hypothesis in conscious, iv LPS-treated animals. Because the PGE$_2$ generated by KCs under these conditions spills into the inferior vena cava and, thence, into the general circulation (59), and since, moreover, systemically administered LPS invariably appears in the liver (48), we have elected in the present study to inject LPS systemically (into the superior vena cava) rather than directly into the portal vein; this approach, moreover, obviates the trauma of major abdominal surgery and its possible, attendant, inflammatory consequences. Thus we selectively depleted guinea pigs of KCs by pretreatment with gadolinium chloride (GdCl$_3$) and compared the time courses of their febrile and associated PGE$_2$ and pyrogenic cytokine responses to iv LPS with those of untreated controls. To our best knowledge, this is the first report of the simultaneous and coordinated changes in T$_c$ and endogenously produced plasma PGE$_2$ and pyrogenic cytokine levels at close intervals over the first 2 h following iv LPS administration to conscious animals. In a follow-up experiment, we further tested the validity of our hypothesis by pretreating conscious guinea pigs with PGE$_2$ antiserum and evaluating its effect on the animals’ T$_c$ responses to iv LPS.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs (300–350 g body wt on arrival; Charles River Laboratories, Wilmington, MA) were used in these experiments. The animals were quarantined for 1 wk, three to a cage, before any experimental use. Tap water and food (Agway Prolab guinea pig diet) were available ad libitum. The ambient temperature (T$_a$) in the animal room was 23 ± 1°C, the housing T$_a$ recommended by the Institute of Laboratory Animal Resources Commission on Life Sciences (ILAR) (32); light and darkness were alternated, with lights on from 0600 to 1800. After quarantine, to moderate the psychological stress associated with the experiments, the animals were trained to the experimental procedures for 1 wk (daily for 4 h) by handling and placement in individual, locally fabricated, semicircular, wire-mesh confiners designed to prevent their turning around and to minimize their forward and backward movements but without causing excessive restraint stress. All animal protocols were approved by the University of Tennessee Health Science Center Animal Care and Use Committee and fully conformed to the standards established by the U.S. Animal Welfare Act and by the documents entitled “Guiding Principles for Research Involving Animals and Human Beings” (83).

General

All glassware, plasticware, instruments, and cannulas used in these studies were sterilized by autoclaving. Electrochemical grade, high-purity water (Baxter Healthcare, Muskegan, MI) was used exclusively in the preparation of all solutions. Before use, the stock solutions were passed through a sterile 0.22-µm Miller-GS filter unit (Millipore, Bedford, MA), as an added precaution against bacterial contamination. Absence of endotoxic contamination in all fluids not containing LPS by design were verified by the Limulus amebocyte lysate test (Pyrochrome; Associates of Cape Cod, Falmouth, MA).

Drugs

LPS was Salmonella enteritidis LPS B (batch no. 651628; Difco Laboratories, Detroit, MI), the same LPS batch we have used in all of our previous studies. GdCl$_3$ hexahydrate was purchased from Sigma-Aldrich (cat. no. G-7532, lot no. 121K3655; St. Louis, MO). Heparin was purchased from Elkins-Sinn (Cherry Hill, NJ). The vehicle for these solutions was pyrogen-free saline (PFS; 0.9% NaCl, USP; Abbott Laboratories, Chicago, IL). Rabbit monoclonal PGE$_2$ antisera and its vehicle, phosphate buffer containing 0.1% sodium azide (Na$_3$N) and bovine serum gamma globulin (BSG), were procured from Assay Designs (cat. no. 905–025, lot no. 01E073A; Ann Arbor, MI) and Sigma-Aldrich (cat. no. S2002), respectively.

Jugular Vein Cannulation

In preparation for iv injections and blood collections, all animals received the antibiotic chloramphenicol (20 mg/kg body wt sc) prophylactically 1 h before the surgical procedure. Under ketamine-xylazine (35/5 mg/kg body wt im) anesthesia and aseptic conditions, a siliconized cannula (0.020 in. ID, 0.037 in. OD; Baxter Healthcare, McGraw Park, IL), prefilled with heparinized (10 IU/ml) PFS, was inserted into the left jugular vein and gently guided into the superior vena cava of each guinea pig. The free end of the cannula was passed subcutaneously toward the head, exteriorized on the top of the head, knotted, and rolled into a coil. This coil was then placed inside a protective polypropylene shield (a centrifuge microtube with a screw cap, with its cone cut off) that was fixed to the skull with dental acrylic cement and four self-tapping, miniature stainless steel screws. The neck wound was sutured and cleansed with 10% povidone-iodine solution and treated with nitrofurazone powder. Immediately after surgery, the animals received a bolus (10 ml PFS sc) injection and pain medication (0.05 mg/kg body wt butorphanol) and, for two more days, chloramphenicol subcutaneously. To maintain the patency of the inserted cannulas, the cannulas were flushed with 0.5 ml of heparinized (3 IU/ml) PFS every day after surgery until 3 days before an experiment, when PFS alone was used because of the confounding effect of heparin on complement activation and cytokine production (88). Experiments were performed 7 days after this surgical procedure, when the animals had recovered. Retraining was performed during the latter 4 days of this recovery period.

Temperature Recording

Beginning at 0800 of the experimental day, the T$_c$s of the conscious guinea pigs, loosely restrained in the individual confiners to which they had been trained, were monitored constantly and recorded at 2-min intervals for 2 h on a Macintosh Plus 1 Mb microcomputer through an analog-to-digital converter, using precalibrated copper-constantan thermocouples inserted 5 cm into the colon. The data were displayed digitally on a monitor, printed on an ImageWriter printer, and stored on a diskette. A 90-min stabilization period to achieve thermal equilibrium preceded all measurements.

Blood Collections

Blood (0.4 ml) was collected at predetermined intervals from the preinserted cannulas before and after PFS or LPS injection. PFS (~0.5 ml) was used to flush blood from the cannulas and replace plasma volume following its withdrawal. Heparin (0.05 ml, 10%) was added to all samples; they were then swirled and centrifuged (3,000 g, 10 min, at 4°C). Aliquots of the plasma were stored at −70°C for assay later.
Assays

LPS. LPS concentrations in the plasma of guinea pigs were measured using a chromogenic Limulus amebocyte lysate assay (Pyrochrome; Associates of Cape Cod), according to the supplier’s instructions. The First International Standard for Endotoxin (code 84/650: World Health Organization) was used as a reference; endotoxin concentrations were expressed as endotoxin units per milliliter (EU/ml). The detection limit of this assay was 0.005 EU/ml.

PGE2. The PGE2 levels in the plasma of guinea pigs were analyzed using a commercial enzyme immunoassay kit (High Sensitivity PGE2 ELA Kit model 931–001; Assay Designs), according to the manufacturer’s instructions. The PG synthetase inhibitor indomethacin (10 µg/ml) was added to all blood samples immediately after collection. All of the samples were diluted before analysis in the assay buffer system provided by the manufacturer, according to the manufacturer’s instructions. All of the samples were analyzed in duplicate. The detection limit of this assay was 8.26 pg/ml.

Cytokines. Cytokine levels in guinea pig plasma were assayed by established bioassay techniques. All samples were analyzed in triplicate. TNF-α was evaluated based on the cytotoxic effect of TNF-α on the mouse fibrosarcoma cell line WEHI 164 subclone 13 (26). The assay was conducted using sterile, 96-well microtiter plates. Serial dilutions of biological samples or different concentrations of TNF-α standard [code 88/532; National Institute for Biological Standards and Control (NIBSC), South Mimms, UK] were incubated for 24 h in wells that had been seeded with 50,000 actinomycin-D-treated WEHI 164 cells. The number of surviving cells after 24 h was measured by use of the MTT colorimetric assay.

The determinations of IL-1β and IL-6 were performed by bioassays based on the dose-dependent growth stimulation of the D10 and B9 hyridroma cell lines, respectively (36, 37). These assays were conducted using sterile, 96-well microtiter plates. In each well, 5,000 D10 or B9 cells were incubated for 72 h with serial dilutions of biological samples or with different concentrations of IL-1β or IL-6 standards (codes 86/680 and 89/548, respectively; NIBSC). The number of cells in each well was measured by use of the MTT assay.

Experimental Design

Experiment 1. Effect of LPS on the Tc, and plasma LPS, PGE2, and cytokine levels of untreated and GdCl3-pretreated guinea pigs. Seven days after surgery, the guinea pigs were randomly divided into four treatment groups: 1) PFS (0.9 ml/kg; n = 4), 2) LPS (2 µg/kg; n = 5), 3) GdCl3 (7.5 mg/kg) + PFS (n = 4), and 4) GdCl3 + LPS (n = 5). GdCl3 was injected into the animals’ cannulated jugular veins 3 days before PFS or LPS. GdCl3 is a lanthanide rare earth metal that, when injected at 7.5 mg/kg body wt iv (12, 33, 48, 76), inactivates the macrophages within the vasculature, i.e., hepatic, splenic, and pulmonary intravascular phagocytes. Repopulation of splenic and pulmonary macrophages starts at day 1 and is complete in 2–3 days; repopulation of KC begins 4 days after GdCl3 injection (33, 48). Hence, the present experiments were performed on day 3 post-GdCl3, when the KC, the principal clearinghouse of LPS and source of pyrogenic mediators (see Introduction), were still nonfunctional, i.e., when their affinity for LPS was significantly reduced (48). GdCl3 at this dose has no demonstrable effect on hepatic stellate and sinusoidal endothelial cells, which also release PGE2, but in smaller quantities (73).

On the experimental day, the conscious animals were placed in their confiners and connected to the Tc recording system. After the 90-min stabilization period, 2 µg of LPS/kg body wt in 0.9 ml of PFS/kg body wt or the same volume of PFS was injected via the implanted jugular vein cannulas. Tc was monitored continuously for the following 2 h. Just before time 0 and 15, 30, 60, 90, and 120 min after PFS or LPS administration, 0.4 ml of blood was collected, prepared, and stored as described above, for later analysis.

Experiment 2. Effect of LPS on the Tc of untreated and PGE2 antiserum-pretreated guinea pigs. To substantiate that PGE2, rapidly released by KC in response to LPS (59), is indeed the trigger that initiates the febrile response to LPS, conscious guinea pigs, in their confiners and after their 90-min stabilization period, received via their implanted jugular vein cannulas PGE2 antiserum (1 ml/kg) or its vehicle, 0.1% NaNO3 (1 ml/kg body wt in PBS containing BSG), 10 min before PFS (0.9 ml/kg; n = 6 and 5, respectively) or LPS (2 µg/kg body wt in 0.9 ml of PFS/kg; n = 6 and 6, respectively) by the same route. The dose of the antiserum was based on previous data in the literature regarding its PGE2 neutralizing activity in vivo and in vitro (31, 44, 60, 61); its solvent, NaNO3, an inhibitor of oxidative phosphorylation, is a neurotoxicant that transiently reduces Tc (15, 30). The antiserum was raised in rabbits; its cross-reactivity was 50, 1.6, and <0.1% against PGE1, PGF2α, and PGD2, respectively, other PGs with thermoregulatory activities. Tc was monitored continuously for the following 5 h.

Statistical Analysis

The results are reported here as means ± SE. The values of Tc are changes (ΔTc) from basal values [Tci (initial), the Tc at 2-min intervals averaged over the last 10 min of the preceding 90-min stabilization period, plotted at 6-min intervals]. The PGE2 data are expressed as changes relative to their values before a treatment (P0). The data were evaluated by a repeated-measures ANOVA (Instat 3, GraphPad Software; Instant Biostatistics, San Diego, CA), where factor 1 was the between-group factor (the experimental treatment) and factor 2 the within-subject factor (the different sampling periods). Each variable was considered to be independent. Latencies of fever onset were defined as the intervals (in minutes) between the time of LPS injection (0 min) and that of the first Tc rise greater than 0.2°C (the SD of the mean Tci) that continued uninterruptedly beyond 0.5°C. The 5% level of probability was accepted as statistically significant in all experiments.

RESULTS

Experiment 1

The responses of Tc, plasma LPS, PGE2, and cytokine levels of untreated and GdCl3-pretreated guinea pigs to the iv injection of LPS are illustrated in Figs. 1–3. Figs. 1A and 2A illustrate the courses of the plasma LPS levels of all animals following LPS challenge. In all cases, the plasma LPS concentration reached its maximum 15 min after LPS administration, declined rapidly to about half its maximum by 30 min, and then slowly decreased toward its original level by 120 min (P < 0.01 relative to their corresponding PFS controls). GdCl3 pretreatment did not affect the rise and fall of plasma LPS except during the last 30 min, when its rate of fall slowed significantly (P < 0.05 relative to LPS, Fig. 1A vs. Fig. 2A).

The courses of the plasma PGE2 levels of the untreated and GdCl3-pretreated guinea pigs in response to PFS or LPS are shown in Figs. 1B and 2B. In the untreated guinea pigs, the plasma PGE2 level rose to its first maximum within 15 min after LPS administration, declined to near control at about 60 min, then rose again and reached a second maximum at 90 min (P < 0.01 relative to PFS, Fig. 1B). The LPS-induced plasma PGE2 rise was essentially eliminated in the GdCl3-pretreated guinea pigs compared with their untreated LPS controls (P < 0.05 relative to LPS, Fig. 1B vs. Fig. 2B). PFS injection did not significantly affect the PGE2 levels of the untreated and GdCl3-pretreated guinea pigs, although they tended to decrease a little over time.
The Tc of the untreated guinea pigs began to rise within 6 min after the injection of LPS, reaching its first maximum (\(1.0 \pm 0.1^\circ C\)) at about 48 min. It then decreased slightly over the following 10 min, then rose again toward a second peak at 2 h post-LPS (\(P < 0.01\) relative to PFS, Fig. 1A). GdCl3 pretreatment prevented the LPS-induced Tc rise and even caused an \(-0.4 \pm 0.1^\circ C\) fall at 60 min after the LPS injection (\(P < 0.01\) relative to LPS, Fig. 1A vs. Fig. 2A). PFS administration had no significant effect on the Tcs of the untreated and GdCl3-pretreated guinea pigs.

The plasma cytokine responses to PFS and LPS of the untreated and GdCl3-pretreated guinea pigs are shown in Fig. 3. After LPS administration, TNF-\(\alpha\) first appeared in plasma at 30 min, reached its maximum at 60 min, and then returned to its baseline at 120 min (\(P < 0.01\) relative to PFS; Fig. 3A). KC depletion did not change the levels of plasma TNF-\(\alpha\) during the first 30 min and 120 min after LPS administration, but it significantly increased them at 60 and 90 min (\(P < 0.01\) relative to LPS, Fig. 3A). The plasma IL-1\(\beta\) (Fig. 3B) and IL-6 (Fig. 3C) were undetectable until 60 min after LPS administration when their levels began and then continued to rise during the rest of the experimental period (\(P < 0.01\) relative to their corresponding PFS controls). GdCl3 pretreatment did not alter the responses of these two cytokines to LPS. No pyrogenic cytokine was detectable in the plasma of the PFS-treated animals.

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characteristically biphasic (Fig. 4A). The initial hypothermic effect of NaN₃ was similarly present in the animals treated with PGE₂ antiserum before LPS. However, in this group, the febrile response to LPS was abrogated (Fig. 4D).

**DISCUSSION**

To validate our hypothesis, the rise of T_c and plasma PGE₂ levels induced by iv LPS in untreated guinea pigs should occur nearly simultaneously, but not at all in KC-depleted animals; plasma cytokine levels should be irrelevant to the initiation of the febrile response. The hypothesis is well supported by the responses observed in this study. Thus the results show that the onset of fever in response to LPS injected iv into untreated guinea pigs was initiated in close coincidence with the appearance in plasma of significantly increased amounts of PGE₂, both occurring within the first 15 min after LPS administration, whereas TNF-α became detectable between 15 and 30 min and IL-1β and IL-6 after 30 min post-LPS. The iv administration of PGE₂ antiserum (which should not cross the blood-brain barrier [BBB]) before LPS significantly attenuated the subsequent LPS-induced T_c rise. Furthermore, the selective ablation of KCs by pretreatment with GdCl₃ prevented the rises of both T_c and plasma PGE₂, enhanced the production of TNF-α, but did not affect the rises of plasma IL-1β and IL-6; these latter results are in agreement with previous findings (63, 65). The clearance of LPS was, however, somewhat slower in this group compared with that in the untreated group, as would be expected from the inactivation of this central LPS clearinghouse (27, 48, 49). The combined effects of their inactivation on T_c and plasma PGE₂ would thus support the role of KCs as the cellular source of the LPS-induced plasma PGE₂, and the temporal separation of the appearance in plasma of endogenous PGE₂ and pyrogenic cytokines would thus implicate KC-generated PGE₂ and exclude circulating cytokines as the signaling molecules of the febrile response to iv LPS.

The role of PGE₂ as an essential mediator of fever production is well established. Thus it is generally considered that its synthesis is catalyzed by cyclooxygenase (COX)-2 and microsomal PGE₂ synthase (mPGES)-1 selectively upregulated by pyrogenic cytokines released into the circulation by mononuclear phagocytes activated by exogenous pyrogens and that it acts in the POA (for reviews, see Refs. 10 and 41). The involvement of KCs as the principal phagocytic cell source of pyrogenic cytokines is also well recognized (22, 43, 48, 76, 84); the present results again demonstrate the critical importance of these cells in LPS fever production. However, not being constitutively expressed in macrophages, the de novo production of cytokines by KCs in response to LPS involves a delay that is significantly longer than the latency of the onset of iv LPS-induced fever. Hence, as also demonstrated by the present results, KC-generated cytokines cannot account for the rapid development of the febrile response to iv LPS. This would infer, therefore, that its prompt beginning is mediated by a different, KC-derived product very quickly elaborated in response to the presence of LPS. We suggested in 1997–1998 (9, 11–13) that PGE₂ could be this mediator because it is synthesized by KCs in response to LPS (for a review, see Ref.

**Experiment 2**

The effects of PGE₂ antiserum and its vehicle, NaN₃, administered iv 10 min before PFS or LPS by the same route on the thermal responses to these agents are illustrated in Fig. 4. NaN₃ produced in the PFS controls an initial, transient, −0.5°C fall in T_c that reached its nadir in ~15 min, but T_c quickly recovered thereafter, returning to its basal level by 30 min and stabilizing there for the remainder of the experimental period (Fig. 4A). This effect was expected (15, 30). NaN₃ caused a similar initial and transient T_c fall in the animals challenged with LPS 10 min later but did not affect the magnitude and course of the subsequent fever, which were characteristically biphasic (Fig. 4B). The transient hypothermic effect of the vehicle was also evident in the guinea pigs treated with PGE₂ antiserum before PFS, but, in this case, T_c "rebounded" ~0.7°C above basal by ~100 min. It decreased over the next ~100 min, then more slowly, but did not fully return to its initial value by the end of the experiment (Fig. 4C). The initial hypothermic effect of NaN₃ was similarly present in the animals treated with PGE₂ antiserum before LPS. However, in this group, the febrile response to LPS was abrogated (Fig. 4D).

**Fig. 3.** Corresponding courses of the levels of TNF-α (A), IL-1β (B), and IL-6 (C) in the plasma of the animals depicted in Figs. 1 and 2. Values are means ± SE; number in parentheses is number of animals; *P < 0.05 relative to corresponding PFS ± GdCl₃-pretreated groups.
and it rises in venous and, to a lesser extent, in arterial blood very quickly after the peripheral administration of both exogenous and endogenous pyrogens (21, 59, 71, 79). Moreover, the peripheral injection of exogenous PGE2 has been reported to cause Tc rises (1, 20, 69, 79), prompting the suggestion that the PGE2 that acts in the POA in response to peripheral pyrogenic stimuli could, in fact, be PGE2 that enters it from the blood (20, 23, 55, 67). It is controversial, however, whether PGE2 can actually pass from the blood into the brain and, especially, whether PGE2 entering the brain in this way can raise Tc (57, 78). But be that as it may, the present data are, to our best knowledge, the first to show in conscious animals that endogenous PGE2 specifically released by the liver’s KCs in response to iv LPS is coincident with and critical to the appearance of the febrile response.

To our knowledge, the antipyretic effect of PGE2 antiserum demonstrated in this study is the first report directly implicating circulating PGE2 as a mediator of the febrile response to LPS, although, as already noted, elevations of PGE2 in the blood following the administration of pyrogens have been described previously by various authors. However, in those studies, with few exceptions (57, 78), the pyrogenic action of plasma PGE2 was generally ascribed to its diffusion into and action within the POA (for a review, see Ref. 10). Attenuations of fever similar to the one produced in this study have likewise been observed previously in other studies (for reviews, see Refs. 67, 70, and 90) after the iv administration of nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors, which prevent the synthesis of PGE2. These agents, however, being lipophilic, cross the BBB, therefore leaving unclear whether it is the blockade of the peripheral or the central PGE2 that accounts for their antipyretic action. It is improbable, by contrast, that the present PGE2 antiserum would cross the BBB. Because it was injected directly into the jugular vein, it probably bound the majority of the PGE2 produced by the KCs, hence reducing the circulating level of free PGE2. Indeed, cross-reactivity results reported by the supplier indicate that this antiserum is directed almost exclusively against the PGE2 structure (see MATERIALS AND METHODS and Ref. 16), and previous dose-response studies of a similar antagonist (56) have indicated that it neutralizes the functional activity of PGE2 in a concentration-dependent manner. Although the levels of plasma PGE2 were not assayed in experiment 2, the dose we chose had been demonstrated previously to be antagonistic to PGE2 in vivo (31, 44, 60, 61); it clearly was effective also in this experiment. On this basis, we would postulate that its observed antipyretic effect was due to its specific neutralization of PGE2 synthesized by the liver, and hence, that the observed attenuation of the fever resulted from the blockade of a peripheral rather than a central mode of action of PGE2. Because, according to the neural concept of afferent pyrogenic signaling (3, 6–8), the first site of action of PGE2 would be hepatic vagal sensory terminals, their activation would thus not occur, preventing the manifestation of fever; or alternatively, according to the humoral concept (66–68), antiserum-free PGE2 not being available, its transport to the brain would be precluded to the same effect.

The effect on Tc of blocking the synthesis or activity of PGE2 in the periphery has apparently not been investigated previously, presumably due to the paucity of pharmacological agents that antagonize PGE2 selectively in vivo, but perhaps even more so due to the little incentive to consider a possible role of peripheral PGE2 in temperature regulation. According to the conventional view of its central action, the selective, in vivo blockade of peripheral PGE2 would not seem conceptually warranted. In view of its demonstrated antipyretic effect, the rise of Tc produced by PGE2 antiserum (PGE2Az) in the control animals would seem, a priori, paradoxical. We would submit, however, that it is consistent. Thus it is well established that PGE2 inhibits the release of norepinephrine (NE)
from sympathetic prejunctional nerve endings and, hence, modulates the response of target tissues to this neurotransmitter (24, 34, 50). Conversely, the inhibition of PGE2 synthesis has been shown to augment NE turnover in a number of organs and tissues (50, 82). A major thermoregulatory function controlled by NE signaling in rodents is brown adipose tissue (BAT) thermogenesis, activated through $\beta_3$-adrenoceptors (for a review, see Ref. 17). We suggest that a competitive interaction between noradrenergic stimulation and PGE2 inhibition of BAT function could underlie the observed Tc rise. That is, the elimination of circulating free PGE2 that resulted from its specific neutralization by its antiserum caused the gradual disinhibition of the peripheral sympathetic nervous system in experiment 2, allowing the continual release of NE from its terminals, thus stimulating the metabolism of brown adipocytes and thereby increasing their rate of heat production, hence raising Tc (for reviews, see Refs. 17 and 89); it is pertinent in this regard that the Tc rise in this experiment developed relatively slowly and had not fully returned to control levels by the end of the experiment. One example in support of this proposition comes from earlier studies showing that PGE2 secreted by the placenta into the circulation of cooled fetal lambs inhibits BAT thermogenesis before birth, whereas the disappearance of PGE2 after placental separation at birth allows its initiation (32). Other peripheral thermoeffectors responsive to sympathetic stimulation, e.g., cutaneous vasculature, adrenal medulla, could also have contributed to the observed Tc rise. An interesting implication of these data, parenthetically, is that PGE2 may be important not only for fever production, but also for the maintenance of normal Tc.

It is generally agreed that the production of PGE2 induced by LPS is initiated by the LBP-mediated transfer of LPS to the receptor complex CD14/TLR4/MD2 (2, 19, 53). The LPS-TLR4 complex is also the system that induces the production of pyrogenic cytokines. Although, as already mentioned, it is clear that the increased biosynthesis of PGE2 thus induced is selectively catalyzed by COX-2 and mPGES-1 (58, 80, 86), the transcription and translation of these inducible enzymes also involve a significant delay. In rats, the ex vivo expression of genes encoding COX-2 and mPGES-1 in liver is not strongly enhanced until 0.5 h after iv LPS (40, 41), i.e., after the onset of fever. This implies, therefore, that the prompt elevation of plasma PGE2 levels in response to iv LPS cannot be accounted for by its COX-2/mPGES-1-mediated production in KCs. Therefore, again, another factor, very quickly released in response to LPS, must mediate the rapid appearance of PGE2 into the blood before and independently of the occurrence of these PGE2-synthesizing enzymes in the liver. We have recently demonstrated that this factor is complement (C), in particular its anaphylatoxic component 5a (C5a; for reviews, see Refs. 4, 7, 8). To wit, the C cascade is activated immediately upon contact with LPS (85). KCs express C5a receptors (C5aR1) (73, 74), and the release of PGE2 is stimulated within 0 to 2 min after C5a administration to in situ-perfused rat livers and isolated rat KCs (28, 74). The addition of C alone or in combination with LPS, but not that of LPS alone, to freshly harvested mouse and guinea pig KCs provokes the virtually immediate, near-maximal release of PGE2 by these cells (7, 59). This response is catalyzed nondifferentially by COX-1 and COX-2 (4), which are both constitutive in KCs. Cytokines are released significantly later. C5a induced by LPS, not LPS per se, is the trigger of the early release of PGE2 from the livers of conscious guinea pigs (59). Decomplementation abrogates all of these effects. Finally, the presence of C5a is necessary for the febrile response of guinea pigs and mice to LPS (7, 8, 45–47). An impairment of the uptake of LPS by KCs because of the insufficiency of C in those studies is excluded by subsequent findings that the uptakes of LPS by the livers of C-sufficient and C-insufficient guinea pigs are not different (Li Z and Blatteis CM, unpublished observations), consistent with an earlier report in rhesus monkeys and rabbits (52). The virtually instantaneous release of PGE2 induced by C5a has been accounted for by its binding to C5aR1, a G protein-linked receptor that acts by increasing intracellular inositol-1,4,5-trisphosphate and Ca2+, rapidly activating COX-1-catalyzed PGE2 production (for a review, see Ref. 73). COX-1 is functionally coupled mainly with cPGES and, therefore, prepared to quickly synthesize PGE2 (81).

Taken together, therefore, these findings further substantiate the importance of KCs in initiating the febrile response of guinea pigs to iv LPS and validate the notion that the fever is triggered by peripheral PGE2 rapidly generated by KCs presumptively stimulated by LPS-activated C5a; cytokines are not involved in this initial febrigenic process. It was not addressed in this study how the PGE2 thus released may inform the POA. It could, indeed, be transported to it by the circulation and eventually penetrate it (23, 68). Or, alternatively, it could activate sensory vagal terminals in the liver [PGE2 receptors are widely distributed on sensory neurons, including vagal afferents (25)]; these could transmit its signals without delay to the POA and thus account for the prompt onset of the febrile response to iv LPS (3–6).

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