Putative antihyperpyretic factor induced by LPS in spleen of guinea pigs

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Putative antihyperpyretic factor induced by LPS in spleen of guinea pigs. Am J Physiol Regul Integr Comp Physiol 289: R680–R687, 2005. First published May 26, 2005; doi:10.1152/ajpregu.00022.2005.—We reported previously that the onset of LPS-induced fever, irrespective of its route of administration, is temporally correlated with the appearance of LPS in the liver and that splenectomy significantly increases both the febrile response to LPS and the uptake of LPS by Kupffer cells (KC). To further evaluate the role of the spleen in LPS fever production, we ligated the splenic vein and, 7 and 30 days later, monitored the core temperature changes over 6 h after intraperitoneal (ip) injection of LPS (2 μg/kg). Both the febrile response and the uptake of LPS by KC were significantly augmented. Like splenectomy, splenic vein ligation (SVL) increased the febrile response and LPS uptake by KC until the collateral circulation developed, suggesting that the spleen may normally contribute an inhibitory factor that limits KC uptake of LPS and thus affects the febrile response. Subsequently, to verify the presence of this factor, we prepared splenic extracts from guinea pigs pretreated with LPS (8 μg/kg ip) or pyrogen-free saline, homogenized and ultrafiltered them, and injected them intravenously into splenectomized (Splex) guinea pigs pretreated with LPS (8 μg/kg ip). The results confirmed our presumption that the splenic extract from LPS-treated guinea pigs inhibits the exaggerated febrile response and the LPS uptake by the liver of Splex guinea pigs, indicating the presence of a putative splenic inhibitory factor, confirming the participation of the spleen in LPS-induced fever, and suggesting the existence of a novel antihyperpyretic mechanism. Preliminary data indicate that this factor is a lipid.

Kupffer cells; fluorescein isothiocyanate-labeled lipopolysaccharide; antipyresis; splenic vein ligation

Many studies utilizing different approaches have indicated that the resident macrophages of the liver, the Kupffer cells (KC), are central to the clearance of bacterial endotoxins LPS from the body and for fever production (7, 19, 21). Because pyrogenic cytokines are produced largely by macrophages (6), KC constitute the largest pool of macrophages in the body (2, 4), and the liver is perfused by 25% of the cardiac output (17), the KC are considered to be the principal source of pyrogenic cytokines evoked by circulating LPS. In support, we (15) recently showed that the onset of the febrile response to LPS, irrespective of its intravenous (iv) or intraperitoneal (ip) route of administration, is temporally correlated with the appearance of LPS in the liver and its uptake by KC and that this mutual relationship is abolished by the prior administration of gadolinium chloride, a lanthanide that temporarily inactivates KC.

However, other macrophages in intimate contact with the blood also clear circulating LPS and produce pyrogenic cytokines, e.g., splenic macrophages (SMO), but their relative contribution to fever production and, indeed, whether it is essential, is not entirely clear (11, 27). In a previous study, we (9) found that removal of the spleen of guinea pigs results in significantly enhanced febrile responses to both iv and ip LPS, and that these hyperpyrexias are associated with an increased uptake of LPS by KC. These effects, moreover, are persistent because their robustness is undiminished 30 days after splenectomy. These results agree with clinical experience showing that infected asplenic patients generally exhibit higher fevers than infected eusplenic patients (20). Elevations in postoperative body core temperature (Tc) have also been described in splenectomized patients immediately after surgery (20, 28).

It is possible that this effect simply reflects an increased avidity by the KC for LPS to counterbalance the absence of the SMO. However, were such a compensatory event, the magnitude of the febrile response to LPS should be unaltered rather than augmented, as is actually observed. On the other hand, if the spleen normally exerts an inhibitory influence on the uptake of LPS by the liver, its removal could abrogate this possible action. Indeed, the spleen liberates factors, e.g., PGE2 and transforming growth factor, into the portal circulation that downregulate KC function (4, 18). The removal of such KC-regulatory factor(s) could thus account for the increased febrile response and LPS uptake we observed in splenectomized (Splex) guinea pigs.

The results reported here are consonant with such a possibility. They indicate that the enhanced febrile response to LPS and its associated increased uptake of LPS by KC exhibited by Splex guinea pigs are reproduced by splenic vein ligation (SVL). However, these effects persist only until collateral vessels develop. An extract prepared from the spleens of LPS-treated guinea pigs attenuates both the exaggerated fever and the KC LPS uptake of Splex LPS-treated recipients. Preliminary, crude characterization of this putative splenic inhibitory factor suggests that it is a lipid.

Materials and Methods

Animals

Male Hartley guinea pigs (300–350 g 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 (Ta) in the animal room was 23 ± 1°C, the housing Ta recommended by the Institute for...
Laboratory Animal Research, Commission on Life Sciences, National Research Council (12); 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 movement, without causing restraint stress. All the 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” (1).

**General**

All glassware, plasticware, instruments, and cannulas used in these studies were sterilized by autoclaving. Electrochemical grade, high-purity water (Baxter Healthcare, Muskegon, 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. The absence of endotoxin contamination in all fluids not containing LPS by design was verified by the Limulus amebocyte lysate test (Pyrochrome; Associates of Cape Cod, Falmouth, MA).

**Drugs**

FITC-labeled LPS was purchased from Sigma-Aldrich (cat. no. F-3665, lot no. 75H4036; St. Louis, MO). It was from *Escherichia coli* 0111:B4, the only kind currently available commercially and also the same as that used in our previous studies (9); fluorescein sodium salt (FSS; cat. no. F-6377, lot no. 26H3407; Sigma-Aldrich) was the control label. Unlabeled LPS was *Salmonella enteritidis* LPS B (batch no. 651628; Difco Laboratories, Detroit, MI), the same LPS batch we used in our previous studies (9, 15). The vehicle for all solutions was pyrogen-free saline (PFS, 0.9% NaCl; Abbott Laboratories, Chicago, IL). Heparin was purchased from Elkins-Sinn (Cherry Hill, NJ).

**Injection Procedures**

For iv injections, the guinea pigs received a prophylactic subcutaneous (sc) injection of the antibiotic chloramphenicol (20 mg/kg) before the surgery and, 1 h later, were anesthetized with ketamine-xylazine (35 and 5 mg/kg, respectively) intramuscularly (im). Under aseptic conditions, usually the right jugular vein was exposed and freed from adhering tissue. A sterile siliconized catheter (inner diameter 0.020 in., outer diameter 0.037 in.; Baxter Healthcare, Deerfield, IL) containing heparinized (10 IU/ml) PFS was inserted and gently freed from adhering tissue. A sterile siliconized catheter (inner diameter 0.020 in., outer diameter 0.037 in.; Baxter Healthcare, Deerfield, IL) containing heparinized (10 IU/ml) PFS was inserted and gently guided into the inferior vena cava. The free end of the catheter was pulled under the skin to the head, exteriorized onto the top of the head, knotted, and rolled into a coil. This coil was placed inside a 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 covered with its screwed-on cap. The neck wound was sutured and cleansed with 10% povidone-iodine solution and treated with nitrofurazone powder. After this surgery, the animals received a bolus 10-ml PFS sc injection and pain medication (butorphanol, 0.05 mg/kg sc), and, for two more days, sc chloramphenicol. The catheters were flushed with heparinized (3 IU/ml) PFS daily, except on the last day before an experiment, when PFS alone was used (26). During flushing, the performance of the catheters was verified by 1) unimpeded withdrawal of blood into the catheter, 2) low resistance to injection, and 3) absence of locomotor and vocal responses from the animals to the injection. One week was allowed for recovery from this surgery before beginning an experiment.

For ip injections, the drugs were injected directly with a sterile 23-gauge 5/8 needle and tuberculin syringe into the peritoneum of the conscious animals.

**Splenic Vein Ligation**

All the animals received the antibiotic chloramphenicol (20 mg/kg sc) prophylactically before a surgical procedure. To ligate the splenic vein, a lateral 3-cm incision was made on the left subcostal side under ketamine-xylazine (35 and 5 mg/kg im, respectively) anesthesia and aseptic conditions. This approach facilitated the removal of the greater omentum from the left upper quadrant and allowed displacement of the stomach away from the spleen. The spleen was lifted gently by placing its body over a blunt grasper across its inferior pole. The splenic vein was ligated with 4-0 silk suture. After ligation, the stomach and spleen were returned to the abdominal cavity. The wound was closed in two layers, with a running 4-0 silk suture. Sham operations were performed in the same manner without ligating the blood vessel. The postoperative measures were the same as described for the iv injections above.

**Splenic and Muscle Extract Preparation**

To verify the presence of a splenic inhibitory factor, we prepared under aseptic conditions splenic extracts from guinea pigs. Using a method previously demonstrated to successfully yield a functional, hypotensive splenic extract (14), we removed the spleen and a psosas muscle (the control tissue) 5, 15, and 30 min after the administration of LPS or PFS from deeply anesthetized (ketamine-xylazine) guinea pigs, cleared the tissues of blood by perfusing with PFS, and rinsed them in ice-cold PBS; the same weight of tissue was used for each extraction. The tissues were then homogenized and centrifuged at 4,500 g, 4°C for 20 min. The supernatant was centrifuged at 14,000 g, 4°C for a further 10 min. Because our initial working hypothesis was that the splenic factor could be either a small peptide or a lipid, the supernatant was ultrafiltered with a Centriprep-10 centrifugal concentrator (mol mass cutoff 10 KDa; Amicon, Beverly, MA), and the rest was discarded; 1.5 ml of spleen extract was normally obtained per donor animal.

To determine the chemical identity of the splenic factor, i.e., whether it is a peptide or a lipid, 15-min splenic extracts were prepared as above and incubated at 90°C for 80 min to denature peptides or passed through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) to remove nonpolar compounds, mainly lipids. The collections were stored at −70°C.

**Temperature Recording**

A 90-min stabilization period to achieve thermal equilibrium preceded all the measurements. The Tc of the conscious guinea pigs, loosely restrained in the individual confiners to which they had been trained, were monitored continuously from 90 min before to 300–360 min after an injection. The data were recorded at 2-min intervals for the duration of the experiments on a Macintosh Plus 1 Mb microcomputer through an analog-to-digital converter, using precalibrated copper-constantan thermocouples inserted 5 cm into the colon. They were displayed digitally on a monitor, printed on an ImageWriter printer, and stored on a diskette. Fever indices (F) were derived from the area under the 360-min fever curve by imaging with a cooled charge-coupled device (CCD) camera (Photometric model 250 CH) and then scanning the images with NIH Image version 1.61. These values were converted to degrees Celsius by interpolating from a calibrated standard area that was 1°C high and 1-h long.

**Fluorescent Microscopy**

As described previously (15), an animal, under deep ketamine-xylazine anesthesia, was attached to a perfusion tray 60 min after FITC-LPS administration. The left jugular vein was exposed in the neck, and 0.1 ml of heparinized PFS and 1 ml of 1% sodium nitrite were injected with a 25-gauge 5/8 needle and a tuberculin syringe. Subsequently, for collection of liver samples, the animal’s thorax was opened and normal saline was perfused through the left ventricle until the fluid exiting the right atrium was clear of blood; 250 ml of 4% paraformaldehyde was then infused for 20 min. Next, the guinea pig was...
laparotomized and a 0.5-cm × 0.5-cm × 0.5-cm cube was excised from the middle part of the left liver lobe of each animal. The tissue was immediately stored in 20% sucrose-4% paraformaldehyde solution, for later cryostat sectioning. Ten-micrometer-thick slices were cut, mounted on glass slides with antifade mounting medium (Vectashield; Vector Laboratories, Burlingame, CA), and covered with coverslips.

Slides were viewed and images were collected with a fluorescent microscopy system consisting of a Nikon Diaphot microscope with a fluorescein filter (488 Å) coupled to a MacQuadra 950 computer system with a Power Mac processor 601, Vaytek software for deconvolution, and IPLab Spectrum software for image collection in conjunction with a cooled CCD camera. Digital processing of the images was done with Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA). The fluorescent patches were counted over five randomly selected 0.25 mm × 0.25 mm areas of tissue; no adjustment was made for the size or intensity of the individual fluorescent patches.

**Experimental Protocols**

**Experiment 1: effect of SVL on Tc.** Seven and thirty days after surgery, sham-operated and SVL guinea pigs were challenged ip with LPS at a dose of 2 μg/kg or with its vehicle (PFS, 0.5 ml/animal). This dose and route of LPS administration were used to replicate the conditions under which the augmenting febrigenic and KC LPS uptake effects of splenectomy were the most manifest in our previous study (9). Tc was monitored continuously for 360 min.

**Experiment 2: effect of SVL on FITC-LPS uptake by KC.** To visualize the expected, differential distribution of LPS in sham-operated and SVL guinea pigs, the uptake of its fluorescent analog, FITC-LPS (75 μg/kg ip), by the liver was evaluated in separate experiments 7 and 30 days after surgery, 60 min after its ip injection. This protocol was chosen because the density of fluorescent labeling normally present in KC 60 min after iv or ip FITC-LPS injection at this dose is minimal but is significantly increased after splenectomy (9, 15); 75 μg of FITC-LPS/kg ip is approximately pyrogenetically equivalent to 2 μg of native LPS/kg ip (15). FSS was the control label. After each experiment, the guinea pigs’ spleen and surrounding tissues were examined to verify that the splenic vein had successfully been completely ligated and to determine whether new collateral veins had meanwhile developed around the ligated splenic vein.

**Experiment 3: effects of splenic and muscle extracts on Tc, and FITC-LPS uptake by KC.** To verify the presence of a splenic inhibitory factor, splenic and psoas muscle extracts prepared as described above were injected iv (one donor extract per recipient) into sham-operated and Splex guinea pigs pretreated ip with 8 μg of LPS/kg. This dose is our standard ip dose for intact guinea pigs; it induces a fever not different from that caused in Splex animals by 2 μg of LPS/kg ip (9). Tc was monitored continuously from 90 min before to 360 min after drug treatment. FITC-LPS (75 μg/kg ip) uptake by the liver was evaluated in separate experiments 60 min after its ip injection 7 days after surgery.

**Experiment 4: effects of protein- and lipid-free extracts on Tc.** The extracts obtained after the injection of LPS as described above were injected into recipient guinea pigs treated with PFS or LPS, as before. Again, Tc was monitored continuously from 90 min before to 300 min after LPS treatment.

**Statistical Analysis**

Results are reported as means ± SE. The values of Tc are changes from basal values [Tc (initial Tc); 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 data were evaluated by a repeated-measures analysis of variance model, where factor 1 was the between-group factor (the experimental treatment) and factor 2 the within-subject factor (the different sampling periods). The analyses were performed with Instat 3 (GraphPad software; Instant Biostatistics, San Diego, CA); each variable was considered to be independent. Latencies of fever onset were defined as the intervals (in min) between the time of LPS injection (0 min) and that of the first Tc rise >0.2°C (i.e., the SD of the Tc of PFS-treated sham-operated guinea pigs) that continued uninterrupted beyond 0.5°C. The unpaired Student’s t-test was used to evaluate the differences in fluorescent density of livers of the various groups. The 5% level of probability was accepted as statistically significant in all experiments.

**RESULTS**

**Experiment 1: Effect of SVL on Tc**

Seven days after surgery, LPS at 2 μg/kg ip induced low-magnitude ΔTc rises in the sham-operated guinea pigs, as pre-
viously described (9). These fevers had onset latencies of ~80 min and peak magnitudes of ~0.5°C occurring at ~160 min (Fig. 1B). In contrast, the same dose of LPS caused significant and long-lasting Tc increases in the SVL guinea pigs (P < 0.05). Tc began to rise at 30 min, reached a ~1.2°C peak at ~190 min, and then gradually returned to basal Tc; recovery was essentially complete by the end of the experiment. Thirty days after surgery, the fevers of the LPS-treated sham-operated and SVL guinea pigs were not significantly different from each other over the 6-h duration of these experiments and were similar to those of the sham-operated animals 7 days after surgery (Fig. 1C). PFS did not affect the Tc of either the sham-operated or SVL animals 7 days after surgery (Fig. 1A); this test was not repeated 30 days after surgery.

Experiment 2: Effect of SVL on FITC-LPS Uptake by KC

Only normal autofluorescence was observed 7 days after surgery in the livers of the sham-operated and SVL guinea pigs after ip injection of PFS (not illustrated; see Ref. 9) or FSS, the latter at a dose equivalent to its amount in the FITC-LPS conjugate (15). On the other hand, abundant labeling was observed as granular fluorescent patches within presumptive KC in the hepatic sinusoids and hepatocytes of SVL guinea pigs 60 min after FITC-LPS injection. However, in contrast, the fluorescent labeling was virtually absent 30 days after surgery (Fig. 2A). Figure 2B quantifies this difference (P < 0.05).

Complete ligation of the splenic vein was verified post-mortem on days 7 and 30 after surgery. No vessels were visible 7 days after surgery around the ligated portal-splenic vein, but new collateral veins were evident 30 days after surgery (not illustrated). The gross morphology of the SVL spleen appeared normal on both test days. There was also no evidence of splenomegaly or hematemesis.

Experiment 3: Effects of Splenic and Muscle Extracts on Tc and FITC-LPS Uptake by KC

As shown in Fig. 3A, LPS at 8 μg/kg ip 7 days after surgery induced Tc rises of ~0.9°C in the sham-operated guinea pigs, as previously described (9); their onset latencies were ~45 min. Tc reached its single maximum at ~170 min and, thereafter, gradually decreased throughout the remainder of the experimental period, returning to nearly its basal levels by ~5 h. In contrast, LPS at the same dose caused significantly greater Tc increases in the Splex guinea pigs (~1.6°C, P < 0.05; Fig. 3B), which, however, peaked at the same time as those in the sham-operated controls. Thereafter, Tc gradually returned toward basal levels, but recovery was not complete by the end of the experiment.

Pssoas muscle extracts from intact guinea pigs treated with LPS injected iv into Splex guinea pigs similarly treated with LPS 7 days after their surgery did not modify the exaggerated febrile response of the recipient animals (Fig. 3C). The iv
injection of 5-min splenic extracts from the donor guinea pigs also did not affect the febrile response to LPS of the recipient Splex animals, but the 15- and 30-min splenic extracts significantly reduced the magnitudes of the exaggerated fevers of these Splex guinea pigs (Fig. 3D), returning them to the levels of the sham-operated animals. Tc in the latter did not begin to rise until ~60 min after the injection, reached a ~1.0°C peak at ~160 min, stabilized for ~1.5 h, and then gradually returned to basal Tc; recovery was not fully complete by the end of the experiment. These febrile curves, however, were not significantly different from those observed in the sham-operated guinea pigs treated with ip LPS (Fig. 3, A and D). These results are summarized as FI in Fig. 4.

Seven days after surgery, FITC-LPS fluorescent patches were abundant within the sinusoids and hepatocytes of the recipient Splex guinea pigs 60 min after iv injection of the 15-min muscle extracts [Fig. 5A, left; the same results were obtained 7 days after splenectomy + FITC-LPS alone (9)], but this fluorescent labeling was significantly reduced in the livers of the Splex guinea pigs treated with the 15-min splenic extracts (Fig. 5A, right).

Experiment 4: Effects of Protein- and Lipid-Free Extracts on Tc

The iv coadministration of PFS and both the lipid- and protein-free splenic extracts had no effect on the animals’ Tc (Fig. 6, A and B). In contrast, the coadministration of LPS and both extracts caused an initial, transient ~0.5°C fall in the Tc of the Splex recipients (Fig. 6, C and D); its nadir was

Fig. 3. Effects of LPS (8 µg/kg ip) on the Tc of conscious Sham (A) and Splex (B) guinea pigs and Splex guinea pigs that additionally received 1.5 ml iv of the 15-min psoas muscle (C) and splenic (D) extracts 7 days after surgery. Abbreviations and conventions as in Fig. 1. *P < 0.05, relative to Sham.

Fig. 4. Three hundred sixty-minute fever index (FI360) of conscious Sham and Splex guinea pigs that received 1.5 ml iv of splenic extract obtained from donor guinea pigs killed 5, 15, and 30 min after LPS challenge (8 µg/kg ip). Abbreviations and conventions as in Fig. 1. *P < 0.05, relative to Sham.
reached 15 min after injection. This hypothermic effect was presumably due to an interaction of LPS (but not PFS) and these preparations, the nature of which is undetermined at this time. The Tc of both the lipid- and protein-free-treated guinea pigs increased immediately thereafter. The Tc of the lipid-free extract-treated animals reached a peak of \(1.0^{\circ}\text{C}\) at \(210\) min. It then gradually decreased throughout the remainder of the experimental period; recovery, however, was not complete by the end of the experiment (Fig. 6C). In contrast, the Tc rise exhibited by the Splex guinea pigs treated with the protein-free extract reached only \(0.5^{\circ}\text{C}\). This rise was significantly smaller than that caused by the lipid-free extract (\(P < 0.05\); Fig. 6D). Both rises, however, peaked at the same time and subsequently also decreased at the same rate.

**DISCUSSION**

The present results show that significantly higher fevers occurred in response to ip LPS in SVL guinea pigs than in their sham-operated controls 7 days after surgery. However, 30 days after surgery, when new collateral vessels apparently draining from the spleen into the portal vein became evident around the ligated splenic vein, these effects were considerably moderated. These results also show that the observed intensified febrile response 7 days after SVL was associated with a significantly increased uptake of LPS by KC, but that this uptake was again normal 30 days later. These findings suggest, therefore, that a factor released by the spleen and flowing to the liver via the splenic-hepatic portal vein modulates KC uptake of LPS and hence the febrile response. Because the splenic extracts from ip LPS-treated donor guinea pigs injected iv into Splex guinea pigs also challenged with ip LPS significantly attenuated their exaggerated febrile response and their elevated uptake of LPS, both to levels essentially identical to those of sham-operated controls similarly treated, the production of a putative splenic counterregulatory factor may be inferred. Preliminary, crude characterization of the factor indicates that it is a lipid. Thus these results add further support to our earlier suggestion (9) that the spleen may normally exert a limiting effect on the uptake of LPS by KC, so that, in its absence, the magnitude of LPS-induced fever is greatly augmented. The present results also reinforce the notion that KC are central to the febrile response to LPS (15).

In validation of the hypothesis that the spleen may release a factor that limits LPS uptake by KC and the febrile response, extracts prepared from the spleens of intact donor guinea pigs...
15 and 30 min after ip injection of LPS, injected iv into recipient Splex guinea pigs also treated with ip LPS, attenuated the exaggerated febrile response of the latter to LPS, as well as the augmented LPS uptake by KC. On the other hand, the extracts collected from donors 5 min after the injection of LPS did not affect these enhanced responses of the recipient Splex guinea pigs. Because the inhibitory activity of the extracts was not detectable in samples collected earlier than 15 min, it may be conjectured that the effective splenic factor probably does not exist in a preformed state but rather is produced and released after a relatively short delay in response to the presence of LPS in the blood. The shortness of this interval would suggest that it could be a lipid mediator rather than a protein mediator, because the transcription and translation of the latter would be expected to require more time. Indeed, our preliminary and very crude analysis of the chemical nature of this compound suggests that it is a lipid. It is unlikely that this factor is an artifact of the extraction procedure because the 5-min extract was not effective but the subsequent extracts, obtained by the same procedure, were effective.

Although the specific identity of this factor is still uncertain, the fact that the active principle passed through a microporous filter having a nominal molecular mass cutoff of 10 kDa suggests that it is small, perhaps a prostanoid. SMO in the marginal zone of the organ secrete PGE2 in response to LPS and gram-negative bacteria (11), consequently directly exposing KC to splenic PGE2 via the portal vein (18). PGE2 is also released by the spleen into the splenic vein during hemorrhagic and septic shock (22, 24). It has been shown in vitro that PGE2 inhibits LPS-induced TNF-α and nitric oxide formation via prostaglandin receptors EP2 and EP4 on KC in time- and concentration-dependent manners (3, 10). Because fever is thought to involve the release of cytokines by KC in response to exogenous pyrogens (7), the delivery of splenic PGE2 to the liver via the splenic-portal vein could be a mechanism, whereby cytokine production by the liver could be modulated; i.e., it could be a counterregulatory mechanism that limits fever height. The specific effect of PGE2 on KC LPS uptake, however, is unknown.

The modulatory effect of the factor putatively released by the spleen reappeared after collateral vessels connecting the spleen and the liver had developed between 7 and 30 days after SVL. Hence, a direct vascular communication between the spleen and the liver would appear to be necessary for this factor to exert its effect on KC, inferring that the amount released by the spleen under normal conditions could be very small. It has been reported in dogs that, although the volume of the spleen and its venous pressure rise immediately after SVL, the splenic parenchymal pressure gradually returns to control levels over the following hours because of the dilation of short, collateral vessels that drain the venous blood into the left gastric and gastroepiploic veins, preventing hematemesis and splenic enlargement (13); the spleens of the present SVL guinea pigs also appeared normal. Because these vessels, in turn, empty into the splanchnic venous circulation, which then drains into the systemic circulation, its dilution in the general circulation could reduce its efficacy, thus rendering it ineffective under the present experimental conditions. The fact that the bolus iv injection of the splenic extract (presumably representing a large dose of the splenic factor) was effective in inhibiting the LPS-induced hyper-

Fig. 6. Effects of iv injection of 1.5 ml of the splenic lipid-free (A and C) and protein-free (B and D) extracts on the Tc of Splex guinea pigs coinjected with PFS (0.5 ml, A and B) or LPS (8 μg/kg ip, C and D) 7 days after surgery. Abbreviations and conventions as in Fig. 1. *P < 0.05, relative to A.
pyrexia of the present Splex guinea pigs would support this interpretation. However, the restoration of the full blood supply from the spleen to the liver 30 days after SVL still needs to be demonstrated specifically. Indeed, the possibility that no factor was released at 7 days after SVL because of functional impairment of the spleen consequent to the original, acute insult provoked by the SVL cannot yet be ruled out.

It is noteworthy that this putative inhibitory factor did not abrogate the entire fever response to LPS and restore the basal Tc, but rather returned the exaggerated fever to its usual febrile level. This antihyperpyretic action is therefore different from the antipyretic actions of, e.g., arginine vasopressin or glucocorticoids, which act in the periphery by inhibiting the production of pyrogenic cytokines, but different from them by limiting LPS uptake by KC. This antihyperpyretic action is therefore different from the antipyretic actions of, e.g., arginine vasopressin or glucocorticoids, which act in the periphery by inhibiting the production of pyrogenic cytokines, but different from them by limiting LPS uptake by KC.

In conclusion, these data confirm our previous findings in Splex guinea pigs that the spleen may modulate the febrile response to LPS. It remains to be determined which cells release this factor and what activates them. One possibility is that circulating LPS itself could be the trigger that directly stimulates the release of the splenic factor, presumably from SMO. Although LPS at 8 μg/kg is cleared rather slowly from the peritoneum, it nevertheless appears in the blood, its level gradually increasing during the first 60–90 min after ip injection (5, 21). Another possibility is that this factor could be induced by the autonomic nervous system reflexively activated by LPS (25). It is now generally recognized that, in the spleen, this system promotes the release of anti-inflammatory mediators (8, 23). Norepinephrine or ACh released by autonomic afferents to the spleen could therefore potentially stimulate the rapid release of this factor.

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