The spleen modulates the febrile response of guinea pigs to LPS

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Feleder, Carlos, Zhonghua Li, Vit Perlik, Allison Evans, and Clark M. Blatteis. The spleen modulates the febrile response of guinea pigs to LPS. Am J Physiol Regul Integr Comp Physiol 284: R1466–R1476, 2003. First published March 6, 2003; 10.1152/ajpregu.00378.2002.—The febrile responses of splenectomized (Splex) or sham-operated (Sham) guinea pigs challenged intravenously or intraperitoneally with lipopolysaccharide (LPS) 7 and 30 days after surgery were evaluated. FITC-LPS uptake by Kupffer cells (KC) was additionally assessed 15, 30, and 60 min after injection. LPS at 0.05 μg/kg iv did not evoke fever in Sham animals but caused a 1.2°C core temperature (Tc) rise in the Splex animals. LPS at 2 μg/kg iv induced a 1.8°C greater Tc rise of the Splex animals than of their controls. LPS at 2 and 8 μg/kg ip 7 days postsurgery induced 1.4 and 1.8°C higher fevers, respectively, in the Splex than Sham animals. LPS at 2 and 8 μg/kg ip 30 days postsurgery also increased the febrile responses of the asplenic animals by 1.6 and 1.8°C, respectively. FITC-LPS at 7 days was detected in the controls within KC 15 min after its administration; the label density was reduced at 30 min and almost 0 at 60 min. In the Splex group, in contrast, the labeling was significantly denser and remained unchanged through all three time points; this effect was still present 30 days after surgery. Similar results were obtained at 60 min after FITC-LPS intraperitoneal injection. Gadolinium chloride pretreatment (~3 days) of the Splex group significantly reduced both their febrile responses to LPS (8 μg/kg ip) and their KC uptake of FITC-LPS 7 days postsurgery. Thus splenectomy increases the magnitude of the febrile response of guinea pigs and the uptake of systemically administered LPS.

FEVER IS A COMMON SIGN of various diseases, and its proper management is still an important issue in modern medicine. Over the past few years, many clinical observations of overwhelming postsplenectomy bacterial infections have been reported (4). Streptococcus pneumoniae is the etiologic agent in ~80% of these cases (9, 10, 27), but gram-negative bacteremia is also implicated in many instances (27). Infected asplenic patients generally exhibit higher fevers than infected eusplenic patients (25). Elevations in postoperative body core temperature (Tc) have also been described after splenectomy (Splex) in patients (25, 41). The basis of these observations has not yet been studied systematically. Indeed, to our best knowledge, the role of the spleen in the febrile response has not yet been investigated, notwithstanding its own fundamental participation and that of fever in the host defenses against infections.

Although pulmonary intravascular macrophages constitute the first filter encountered by intravenously or intraperitoneally injected lipopolysaccharide (LPS), the rate of LPS clearance and detoxification by these cells is slow so that LPS spills over into the general circulation (37). Consequently, neutrophils, monocytes, and other macrophages within the vasculature, including hepatic [Kupffer cells (KC)] and splenic macrophages (SMO), also contribute to the intravascular clearance of LPS (2, 14, 24). Of these, the KC are quantitatively the most important. They constitute 80% of all resident mononuclear phagocytes in the body (24), and the liver is thus considered to be the principal organ responsible for clearing LPS from the blood (24, 27). Hence, the liver is also considered to be the primary source of production of LPS-induced pyrogenic cytokines, the endogenous mediators of fever (11). Conscious guinea pigs challenged with LPS, but pretreated with the KC inhibitor gadolinium chloride, exhibit falls rather than rises in Tc (33), thus supporting the critical intermediary role of KC for LPS fever induction. Hepatic branch vagotomy also inhibits the development of LPS fever (19, 35), further implicating the liver in this response.

The spleen also has a role in bacterial clearance. Thus intravenously injected immunoreactive LPS (2.5 μg/g body wt) was detected as intact or fragmented LPS in the SMO of rats; staining gradually decreased from 24 h to 1 wk (13). LPS was almost exclusively localized in the red pulp of the spleen, especially in the marginal zone, where this macrophage population is predominant (13, 14). Significant amounts of LPS were also found bound to splenic tissue (100 ng LPS/g tissue) after intravenous LPS (13 μg/kg) administration to rabbits (32, 40). It has been suggested that the spleen may thus also be a critical clearance organ of injected LPS (13, 40). Indeed, in Splex LPS-treated (1 μg/kg) dogs, plasma endotoxin concentration is higher than that in control dogs, further suggesting that the spleen...
may normally participate in its clearance (26). In support, in the presence of impaired liver function, endotoxin clearance by SMO is increased (16). There is also evidence of a specific 80-kDa LPS-binding protein on murine splenocytes (20).

SMO also affect the ability of macrophages elsewhere to handle pathogens. In particular, it has been shown that Splex alters KC function (1, 36), increasing both their number (7) and affinity (8) for LPS. Splex in mice impairs alveolar macrophage function (18), indicating that the spleen also modulates the activity of these macrophages (17). Moreover, the activities of interleukin (IL)-1β and granulocyte colony-stimulating factor are different depending on whether the mice are splenectomized or eusplenic (4, 15).

It is increasingly recognized that the spleen liberates factors into the portal circulation that modulate KC function (8, 23). For example, SMO produce abundant IL-1β (13). Considerable evidence indicates that the action of IL-1β, the production of which is induced by LPS, is a key event in the generation of fever (11). It has been speculated that it would be advantageous to the host to minimize the production of IL-1β by KC in response to LPS to moderate the host response to inflammation (13). Hence, it would appear that KC and SMO may interact to modulate LPS uptake, and this communication may be mediated by cytokines. Because both KC and SMO produce IL-1β and other cytokines involved in the febrile response, the present study was undertaken to evaluate the possible contribution of the spleen to the febrile response to LPS and its interaction with liver macrophages as regards, particularly, LPS uptake by these cells.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs (300–350 g; 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 in the animal room was 23 ± 1°C; lightness and darkness were alternated, with light on from 0600 to 1800. After quarantine, to moderate the psychological stress and other cytokines involved in the febrile response, the present study was undertaken to evaluate the possible contribution of the spleen to the febrile response to LPS and its interaction with liver macrophages as regards, particularly, LPS uptake by these cells.

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Drugs

FITC-LPS was from Escherichia coli, serotype O111:B4 (lot no. 75H4036; 4.4 μg FITC/mg LPS; Sigma-Aldrich, St. Louis, MO); fluorescein sodium salt (lot no. 26H3407; Sigma-Aldrich) was the control label. LPS was Salmonella enteriditis (batch no. 651628; Difco Laboratories, Detroit, MI). GdCl₃ hexahydrate (lot no. 121K3656) was purchased from Sigma-Aldrich. The vehicle for all the solutions was pyrogen-free saline (PFS, 0.9% NaCl, USP; Abbott Laboratories, Chicago, IL). Heparin was purchased from Elkins-Sinn (Cherry Hill, NJ).

Surgical Procedures

All animals received the antibiotic gentamicin sulfate (6 mg/kg im) prophylactically before any surgical procedure. Sterile techniques were used. For intravenous injections, the animals were prepared by inserting under ketamine-xylazine (35–5 mg/kg im) anesthesia, a siliconized catheter (ID 0.020 in., OD 0.037 in.; Baxter Healthcare, McGraw Park, IL), prefilled with heparinized (10 IU/ml) PFS; through the left jugular vein into the superior vena cava of each guinea pig. The distal end of the catheter was passed subcutaneously and exteriorized on the top of the head, knotted, rolled into a coil, and placed inside a protective polypropylene shield that was fixed to the skull with dental acrylic cement and four self-tapping, miniature stainless steel screws. Gentamicin sulfate was administered during the following 2 days. The catheters were flushed with heparinized (3 IU/ml) PFS daily; 48 h before an experiment, heparinized PFS was replaced with PFS only (38).

To remove the spleen, a lateral, 3-cm subcostal incision was made on the left side after the jugular cannulation. This approach facilitated the removal of the greater omentum from the left upper quadrant and allowed displacing the stomach away from the spleen. The spleen was lifted gently by placing its body over a blunt grasper across its inferior pole. The gastrospenic ligament was depressed, the splenocolic and phrenocolic ligaments were dissected, and all local vessels were ligated using 4–0 silk suture. As the inferior pole was freed, the propping grasper was moved gradually more anteriorly, thus allowing the spleen to be dissected in segments, and elevated, starting at the lower pole and working toward the hilar vessels. The splenic hilar vessels and short gastric vessels were isolated, divided, and ligated. The proximal splenic artery was also ligated. The spleen was freed completely and removed from the abdominal cavity. The abdomen was then reexplored, with attention to the pedicle vessels, short gastrics, and diaphragmatic bed. The operative field was cleaned, and all port sites were closed.

The sham operation was performed similarly except that, after the displacement of the stomach and spleen, these organs were returned to the abdominal cavity, and the wound was closed. The animals were allowed to recover for 48 h. They were then trained to the experimental procedure described above until the experiments were performed.

Temperature Recording

Seven and 30 days after this surgery, the guinea pigs, fully conscious in the individual confiners to which they had been trained, were placed under a plastic hood (free air circulation through open ports) to prevent undue disturbances from noise and fluctuations in ambient temperature (23 ± 1°C). Tc of the guinea pigs were monitored constantly and recorded at 2-min intervals for the duration of the experiments (6 h) on a Macintosh Plus 1Mb microcomputer through an analog-to-digital converter, using precalibrated copper-constantan thermocouples inserted 5 cm in 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 the measurements. To obviate possible effects of circadian variations,
all the experiments were begun at the same time of day (0830).

**Fluorescent Microscopy**

PFS (0.3 ml), fluorescein sodium salt (0.0007 μg/μl PFS); this dose is equivalent to the amount of fluorescein in the FITC-LPS conjugate), or 75 μg FITC-LPS/kg was injected intravenously or intraperitoneally. At 15, 30, and 60 min after administration, the animals, under ketamine-xylazine anesthesia, were attached to a perfusion tray, the jugular veins were exposed, and, using a 28-g ½ needle and a tuberculin syringe, 0.1 ml heparin (10 IU/ml) and 1 ml of 1% sodium nitrite were injected. Venous blood (5 ml) was collected; leukocytes-rich plasma was prepared by centrifuging (1,500 rpm, 15 min) the whole blood. The abdomen was incised (1 cm) on the midline, and 10 ml Hanks’ balanced salt solution was pipetted into the abdominal cavity, swirled and mixed, and then collected. This peritoneal cavity lavage solution and the leukocyte-rich plasma were dropped to slides, incubated at 37°C for 40 min, and then fixed with 2% paraformaldehyde at -40°C for 15 min. The slides were mounted with anti-fade mounting medium (Vectorshield; Vector Laboratories, Burlingame, CA) and covered with coverslips for later analysis. For the collection of tissue 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. Paraformaldehyde (250 ml of 4%) was then infused for 20 min, after which the guinea pigs were laparotomized and a 0.5 × 0.5 × 0.5 cm cube was excised from the liver (middle left lobe) of each animal. Tissue samples were similarly collected from the brain (hypothalamic region), lungs (middle left lobe), left kidney (upper one-third), and mesenteric lymph nodes. All the tissues were stored in 20% sucrose-4% paraformaldehyde solution for later cryostat sectioning. Slices (10 μm thick) were cut and mounted on glass slides, using Vectashield anti-fade mounting medium, and covered with coverslips. The slides were viewed, and images were collected using a fluorescent microscopy system consisting of a Nikon Diaphot microscope with a fluorescein filter (488 λ) coupled to a MacQuadra 950 computer system with a Power Mac processor 601, Vaytek software for deconvolution, and IP Lab Spectrum software for image collection in conjunction with a cooled charge-coupled device camera (Photometric model 250 CH). Digital processing of the images was done using Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA). For quantitative analysis, five areas of 25 × 25 mm² each were selected randomly, and the fluorescent patches in these areas were counted.

**Erythrocyte Sedimentation Rate**

Erythrocyte sedimentation rate (ESR) was measured in all the animals using the Westergren method, a rapid, accepted, and simple indicator of latent infection (6a). It was assessed 60 min before an experiment.

**Experimental Design**

**Experiment 1: effects of intravenous LPS on Tc.** Sham and Splex guinea pigs were challenged with a low (0.05 μg/kg) or a high (2 μg/kg) dose of LPS in 0.9 ml PFS or PFS (0.9 ml/kg) administered intravenously 7 days after the surgery. Tc was monitored continuously from 90 min before to 360 min after drug treatment.

**Experiment 2: effects of intraperitoneal LPS on Tc.** A low (2 μg/kg) or a high (8 μg/kg in 0.9 ml PFS) dose of LPS or PFS (0.9 ml/kg) was administered intraperitoneally 7 and 30 days after the surgery to other Sham and Splex guinea pigs. The latter treatment day was chosen postfacto to determine whether the responses observed at 7 days were transitory or permanent. Tc was measured continuously as in experiment 1.

**Experiment 3: effects of Splex on FITC-LPS uptake.** To visualize the presumptive differential distribution of LPS in Sham and Splex guinea pigs, the uptake of its fluorescent analog, FITC-LPS (75 μg/kg iv or ip), by the liver, lungs, brain, kidneys, peritoneal macrophages, lymph nodes, and leukocytes of Sham and Splex guinea pigs was evaluated 7 and 30 days after the surgery at 15, 30, and 60 min after its intravenous or intraperitoneal injection. This dose was selected on the basis of its intravenous and intraperitoneal pyrogenic potencies, determined in preliminary studies to suitably approximate those of 2 μg unlabeled LPS/kg delivered intravenously and intraperitoneally (our standard pyrogenic iv dose is 2 μg/kg; see, e.g., Ref. 21) in intact, conscious animals (Fig. 1, A and B). Although 37.5 μg FITC-LPS, in fact, approximated more closely the febrile course induced by 2 μg of unlabeled LPS when both were delivered intravenously (Fig. 1A), it was not pyrogenic, and its fluorescence was not detectable in tissues after its intraperitoneal injection (22); consequently, this dose was not used for this experiment.

**Experiment 4: effects of GdCl3 on FITC-LPS uptake and Tc.** To verify a posteriori whether KC in particular were the cell types that bound FITC-LPS and accounted for the associated

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Fig. 1. Effects of unlabeled lipopolysaccharide (LPS; 2 μg/kg) and FITC-LPS (37.5 and 75 μg/kg) injected iv (A) or ip (B) on the core temperatures (Tc) of conscious, unoperated guinea pigs. Tc values are expressed as differences (∆Tc) relative to their initial level (Ti); average of the Ti over the last 10 min before the injection of pyrogen-free saline (PFS) or LPS. Values are means ± SE; n = no. of animals.
Tc changes observed in this study, Sham and Splex guinea pigs were injected 7 days postoperatively with 75 μg/kg FITC-LPS/kg iv, 3 days after the intravenous injection (in the penile vein, under light metofane anesthesia) of 7.5 mg GdCl3/kg (21). The animals were killed 60 min after the FITC-LPS injection and perfused transcardially, their livers were excised and processed, and the fluorescent patches were counted as before. The intraperitoneal route and 60-min end point were chosen because the enhancement of the febrile response of the Splex animals to unlabeled LPS at 8 μg/kg ip was more manifest than that to LPS at 2 μg/kg iv (Figs. 2C and 3B) and because the density of FITC-LPS staining in these animals remained unchanged at 60 min after its intraperitoneal injection, whereas it was abating at this time after its intravenous injection (Fig. 4A). Other, similarly GdCl3-pretreated Sham and Splex guinea pigs received 8 μg/kg unlabeled LPS intraperitoneally; their Tc were measured for 6 h, as before.

**Fig. 2.** Effects of PFS (A; 0.9 ml/kg) and LPS [0.05 (B) and 2 (C) μg/kg] injected iv on Tc of conscious Sham and splenectomized (Splex) guinea pigs 7 days after surgery. Conventions as in Fig. 1. *P < 0.05 relative to Sham.

**Fig. 3.** Effects of PFS (0.9 ml/kg; A) and LPS [2 (B) and 8 (C) μg/kg] injected ip on the Tc of conscious Sham and Splex guinea pigs 7 days after the surgery. Conventions as in Fig. 1. *P < 0.05.
Statistical Analyses

The results are reported as means ± SE. The values of $T_c$ are changes from basal values ($T_c(\text{initial})$, the $T_c$ at 2-min intervals averaged over the last 10 min of the preceding 90-min stabilization period) plotted at 6-min intervals. Latencies of fever onset were defined as the intervals (in min) between the time of LPS injection (0 min) and that of the first $T_c$ rise greater than 0.2°C (i.e., the SD of the $T_c$ of PFS-treated guinea pigs) that continued uninterruptedly beyond 0.5°C. A two-way ANOVA followed by a Tukey-Kramer multiple comparisons test were used to compare the thermal courses between groups. The 5% level of probability was accepted as statistically significant.

RESULTS

ESR

No abnormality, i.e., no evidence of infection, was found in the Sham and the Splex groups (0.7 ± 0.3 and 0.8 ± 0.4 mm/h, respectively). No guinea pig died during the experiments.

Experiment 1: Effects of Intravenous LPS on $T_c$

The $T_c$ values of both the Sham and Splex guinea pigs given PFS did not vary significantly over the 6-h duration of these experiments (Fig. 2A).

LPS at 0.05 μg/kg did not induce a statistically significant $T_c$ rise in the Sham animals, but it did cause a significant biphasic $T_c$ increase in the Splex guinea pigs (Fig. 2B). The first febrile peak was higher than the second, reaching ~1.2°C ~80 min after the LPS injection; the second peak occurred ~150 min after the injection. The return to basal $T_c$ was gradual but essentially completed by ~270 min after LPS administration.

LPS at 2 μg/kg induced fever in the Sham guinea pigs with an onset latency of 10–12 min (Fig. 2C). The first febrile peak of ~1.3°C occurred at ~40 min after LPS administration and the second, also of ~1.3°C, at ~60 min after the first. The return to $T_c(\text{initial})$ was gradual but essentially completed by ~250 min after LPS injection. The magnitude of the fever produced by this...
dose of LPS was significantly augmented in the Splex animals. Thus the first febrile rise was higher, to \(\sim 1.8^\circ C\), and the postfebrile recovery stabilized at a higher level than that of the Sham group (Fig. 2C). However, the onset latencies and the febrile courses were not significantly different in the Sham and Splex groups.

**Experiment 2: Effects of Intraperitoneal LPS on \(T_c\)**

PFS (7 and 30 days postsurgery) did not affect the \(T_c\) values of either the Sham or Splex animals (Figs. 3A and 5A).

As shown in Fig. 3B, LPS at 2 \(\mu g/\text{kg}\) 7 days postsurgery induced a significant \(T_c\) rise of \(\sim 1.4^\circ C\) in the Splex animals. The rising phase began at \(\sim 30\) min after LPS injection and culminated at 200 min. Thereafter, the return to \(T_c\) was gradual and nearly complete by the end of the experimental period, 6 h later. In contrast, this dose of LPS caused only a small \(\sim 0.5^\circ C\) increase in the \(T_c\) of the Sham animals; its onset latency was \(\sim 60\) min. \(T_c\) reached its single maximum at \(\sim 150\) min and gradually decreased throughout the remainder of the experimental period.

LPS at 8 \(\mu g/\text{kg}\) 7 days after the surgery induced a significant \(T_c\) rise of \(\sim 1.8^\circ C\) in the Splex animals (Fig. 3C). The latency of onset was \(\sim 45\) min, and the \(T_c\) peak was reached at \(\sim 170\) min. \(T_c\) then decreased gradually to \(\sim 110\) min and gradually decreased throughout the remainder of the experimental period.

LPS at 2 \(\mu g/\text{kg}\) 30 days postsurgery induced a significant \(T_c\) rise of \(\sim 1.6^\circ C\) in the Splex animals (Fig. 5B); the latency of onset was \(\sim 45\) min, and the \(T_c\) peak was reached at \(\sim 210\) min. \(T_c\) decreased toward its basal level over the next 150 min. By contrast, the Sham group exhibited a maximum \(T_c\) rise of only \(\sim 0.7^\circ C\). The onset latency was \(\sim 45\) min, and the \(T_c\) peak was reached at \(\sim 170\) min. \(T_c\) then decreased slowly until the end of the experimental period.

LPS injected at 8 \(\mu g/\text{kg}\) 30 days postsurgery induced in the Splex animals a significant \(T_c\) rise of \(\sim 1.8^\circ C\), beginning promptly after the injection (Fig. 5C). The time to peak was \(\sim 170\) min. The \(T_c\) then gradually fell until the end of the experiment. Again, the Sham group exhibited a lower \(T_c\) increase (\(\sim 0.9^\circ C\)); the onset latency was \(\sim 35\) min, and the \(T_c\) peak was reached at \(\sim 170\) min. \(T_c\) then declined gradually until the end of the experimental period.

**Experiment 3: Effects of Splex on FITC-LPS Uptake**

Only normal autofluorescence was observed after the intravenous and intraperitoneal injections of fluorescein sodium salt at a dose equivalent to its amount in the FITC-LPS conjugate (data not shown). The distribution and density of FITC-LPS fluorescence in the lungs, brain, kidneys, leukocytes (delivered intravenously or intraperitoneally), and peritoneal macrophages and mesenteric lymph nodes (intraperitoneally only) of Sham and Splex guinea pigs 7 and 30 days postsurgery were not different in the two groups (data not shown). On the other hand, differences were apparent between the livers of the two groups. Thus intravenous FITC-LPS appeared in the livers of Sham and Splex guinea pigs 7 days postoperatively at 15, 30, and 60 min as patches of granular fluorescence within,
presumptively, KC in the liver sinusoids (Fig. 6). At 15 min, there was no demonstrable difference in the density of fluorescence between the two groups. In the Sham animals, the density of fluorescence decreased at 30 min and was virtually zero at 60 min, whereas in the Splex animals the labeling remained unchanged over the first 30 min and then decreased by ~40% at 60 min. These differences are expressed quantitatively in Fig. 4A.

FITC-LPS administered intraperitoneally 7 days postsurgery similarly appeared in the livers of the Splex guinea pigs 15, 30, and 60 min later as patches of granular fluorescence in the sinusoids but persisted at the same density throughout all three time points (Figs. 4A and 7). This enhanced uptake of FITC-LPS was associated with rising $T_c$ values (Fig. 4B). In contrast, no such $T_c$ elevations occurred in the Sham animals, although a little, but statistically insignificant, fluorescent labeling was evident at 60 min. The livers of the Splex guinea pigs still contained dense labeling 30 days postoperatively 60 min after intraperitoneal FITC-LPS; the density of these fluorescent patches was not significantly different from that 7 days postoperatively (Fig. 8). These results are illustrated quantitatively in Fig. 4A. The livers of Sham and Splex animals 7 days postoperatively, 15, 30, and 60 min after intravenous or intraperitoneal PFS showed only small amounts of diffuse autofluorescence; examples are shown in Figs. 8 and 9.

After intravenous (60 min) but not after intraperitoneal FITC-LPS, fluorescent patches also appeared in the hepatocytes of both the Sham and Splex animals (Figs. 6–8).

Experiment 4: Effects of GdCl$_3$ on FITC-LPS Uptake and $T_c$

GdCl$_3$ pretreatment 7 days postsurgery reduced the febrile responses of both the Sham and Splex guinea pigs to 8 $\mu$g LPS/kg, injected intraperitoneally 3 days after the GdCl$_3$, to the same levels (Fig. 10), but those of the Splex animals were depressed quantitatively more than their counterparts not pretreated with GdCl$_3$ (Fig. 3C). Thus LPS-induced rises of the $T_c$ values of both groups of animals were delayed significantly in onset (~60 vs. ~45 min) and virtually abolished (~0.4 vs. ~1.5°C), although they peaked at the same time (~170 min). The reductions of these responses were associated with parallel, significant decreases in the densities of FITC-LPS labeling in the livers of these GdCl$_3$-pretreated guinea pigs (Figs. 4A and 9) compared with their untreated Splex counterparts (Fig. 7).

DISCUSSION

The present results show that significantly higher fevers occurred in Splex guinea pigs than in their Sham controls 7 and 30 days after their surgery in
Fig. 7. Autofluorescence of hepatocytes and FITC-LPS labeling of KC in livers of Sham and Splex guinea pigs 7 days postsurgery, 15, 30, and 60 min after FITC-LPS (75 μg/kg ip). Magnification: ×200. Conventions as in Fig. 6.

Fig. 8. Autofluorescence of hepatocytes and FITC-LPS labeling of KC in livers of Sham and Splex guinea pigs 7 and 30 days postsurgery, 60 min after FITC-LPS (75 μg/kg ip). Magnification: ×100. Conventions as in Fig. 6.
response to both intravenous and intraperitoneal LPS. Indeed, doses minimally pyrogenic in Sham-operated animals caused greatly enhanced fevers in Splex guinea pigs. Furthermore, this effect of Splex was still robustly present 30 days postsurgery, indicating that it was not a transitory consequence of the procedure itself. These results further show that the observed, intensified fevers were associated with a significantly larger uptake of LPS by KC. Thus FITC-LPS injected intravenously labeled more KC and remained in these cells for a longer time in Splex than in Sham guinea pigs. Similarly, FITC-LPS injected intraperitoneally was taken up by these phagocytes more rapidly in Splex than in Sham guinea pigs. Again, this effect was still present 30 days post-Splex. In contrast, the uptake of LPS by other mononuclear phagocytes was not differentially affected by Splex. Elimination of KC by pretreatment with GdCl3 attenuated both the febrile responses and the intensity of the labeling in the livers of both Sham and Splex animals, further implying a close link between LPS-induced fever and the liver.

To our best knowledge, this is the first experimental study demonstrating that Splex augments the febrile response to LPS. These results are consonant with clinical findings in which infected asplenic patients manifest higher fevers than infected eusplenic patients (25). Elevations in postsurgery Tc without any prior apparent infection have also been reported after Splex (25, 40), analogous to the data shown in Figs. 2, 3, and 5, B and C. A similar increase in the hepatic uptake of radiolabeled heat-killed Escherichia coli after their systemic administration was reported in Splex rats (6, 34). Also, in Splex mice with bone marrow irradiation-induced monocytopenia, both the number of KC and their autoradiographic uptake of tritiated thymidine were increased significantly (39). It has also been reported that the loss of splenic phagocytosis after removal of the spleen may be compensated by increased phagocytic activity of KC in rats (1, 9). However, Splex did not cause any change in the number of KC in rats (8), although, in other studies using mice, Splex did increase this number (7, 28). Hence, it is possible that the increased uptake of LPS by KC observed in the present study simply represents an effort by the KC to counterbalance the absence of the SMO. However, were this the case, one might expect that the magnitude of the febrile response would be unaffected or perhaps reduced a little but not enhanced as was observed. These data suggest, therefore, that the spleen may exert a modulating influence, presumably inhibitory, on the uptake of LPS by the liver. The present data alone do not allow determination of whether the increased density of fluorescence indicates an increase in the total number of activated KC, in the number of LPS receptors/KC, or in the functional activity of individual KC. Indeed, despite its high resolution, a difficulty inherent in the present imaging technique is that it causes blurs and halos from fluorescence emitted above or below the focal plane, thereby not allowing discrimination between fluorescence in or on the KC. This difficulty is compounded when the
labeled cells are aggregated, as probably was the case here.

The mechanism by which the spleen may modulate, i.e., inhibit, the uptake of LPS by KC remains speculative. Several alternatives are possible. For example, SMO in the marginal zone of the spleen reportedly secrete quantitatively more IL-1α and IL-1β in response to LPS and to gram-negative bacteria (13). Because the induction of fever is thought to be mediated by the release of IL-1β in response to exogenous pyrogens (11) and if SMO, like KC, were another large source of this IL-1β, it might be advantageous to the host to normally minimize IL-1β production by the liver to limit fever height. Hence, the absence in Splex guinea pigs of an inhibitory influence on KC by IL-1β from the spleen could result in an increased production of hepatic IL-1β, thereby augmenting the febrile response. In this regard, KC are, in fact, directly exposed to splenic products via the portal vein (23). Among the factors that influence hepatic macrophagic activation state are PGE2, transforming growth factor-β, IL-10, IL-6, tumor necrosis factor (TNF)-α, and interferon-γ. These could thus participate in the cross-talk between the spleen and the liver (2, 25). Indeed, it has been shown that in vitro pretreatment of KC with PGE2 significantly decreases subsequent KC responses to LPS (2, 8). Hepatic TNF-α production is increased in Splex dogs compared with in control dogs, inferring an inhibitory influence by the spleen on hepatic TNF-α production (26). Splex reportedly also decreases the LPS-induced plasma IL-6 levels of rats more than can be attributed to the absence of splenic IL-6 production alone, hence suggesting a decrease in total, nonsplenic IL-6 levels (3). Other data have also implicated the spleen as an important mediator in the activation of nonsplenic IL-6 secretion by LPS (26). Because IL-6 is a feedback inhibitor of TNF secretion (11, 12), the lack of splenic IL-6 production (26) may produce more febrile cytokines and/or factors, and/or possibly could express more cells, more LPS receptors/KC, or a greater functional activity than unprimed KC.

In conclusion, the present data indicate that LPS induces higher fevers in Splex than in Sham guinea pigs. This is associated with, and possibly causally related to, an increased LPS uptake by individual KC and/or an increased number of LPS-reactive KC. A factor released by the spleen could modulate KC LPS uptake and, hence, cytokine production. Whether such a factor is secreted by the spleen in the splenic vein and whether it carries signals to the KC remains to be clarified. However, regardless of the particular splenic factor responsible or of its mechanism of action, these data show that Splex is associated with higher fevers after LPS administration, indicating a participation of the spleen in the course of LPS-induced fever. Taken together, these data suggest that the apparently enhanced avidity of KC for bacterial endotoxin could account for the higher fevers of the Splex guinea pigs to a given dose of LPS, whether administered intravenously or intraperitoneally, compared with the febrile responses of Sham guinea pigs. Moreover, the absence of differences in the density of fluorescence in the brain, lungs, kidneys, lymph nodes, peritoneal macrophages, and monocytes between the Splex and Sham animals would further support the pivotal role of the liver in LPS-induced fever production, as suggested by previous observations (22, 31).

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