Fluid extravasation from spleen reduces blood volume in endotoxemia

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Andrew, Peter, Yiming Deng, and Susan Kaufman. Fluid extravasation from spleen reduces blood volume in endotoxemia. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R60–R65, 2000.—We recently demonstrated that fluid is filtered out of the splenic circulation and into the lymphatic system. The current experiments were designed to investigate the importance of this route of fluid extravasation in endotoxemia. Lipopolysaccharide (LPS) was infused into conscious intact and splenectomized rats (150 µg·kg⁻¹·h⁻¹·iv for 18 h). In the intact rats, mean arterial pressure (MAP) fell from 101 ± 2.4 to 88 ± 3.9 mmHg (n = 7) and then stabilized at about 90 mmHg. Hematocrit rose from 41 ± 0.9 to 45 ± 0.4% at 40 min, at which time plasma volume had fallen from 4.7 ± 0.12 to 4.0 ± 0.05 ml/100 g body wt. In the splenectomized rats MAP did not fall and hematocrit did not rise. There also was no change in plasma volume, i.e., splenectomy prevented the hypotension and hemoconcentration customarily induced by LPS. In a second series of experiments, splenic arterial and venous blood flows were simultaneously measured in anesthetized rats infused with LPS (150 µg·kg⁻¹·h⁻¹). LPS increased splenic fluid efflux. We conclude that during endotoxemia the initial fall in circulating blood volume may be attributed to fluid extravasation from the splenic vasculature.

lipopolysaccharide; blood pressure; plasma volume

SEPTIC SHOCK, THE LEADING cause of death in intensive care units throughout North America (31), is most commonly a consequence of gram-negative bacteremia. It results from complex interactions with endogenous chemical mediators, such as interleukin-1 and tumor necrosis factor (9, 18, 31), released by the host when challenged with lipopolysaccharide (LPS), a component of the bacterial cell wall. These endogenous chemical mediators exert their effects at the microcirculatory level to bring about changes in nutritional blood flow, microvascular permeability, and cell metabolism (20). These changes result in impaired tissue perfusion and organ failure (26). A prominent feature of septic shock is the severe and intractable hypotension coupled with a primary reduction in circulating blood volume, which leads ultimately to circulatory collapse.

We have previously reported that the spleen may influence blood volume by controlling the efflux of protein-rich fluid from the intravascular compartment into the lymphatic system (6, 23, 24). Although decreased plasma volume has been demonstrated with induction of septic shock, the role that the spleen might play has been ignored. We determined therefore to investigate the effect of splenectomy on the changes in plasma volume and mean arterial pressure (MAP) induced by infusion of LPS (8, 10). We reasoned that if fluid extravasation from the splenic circulation does indeed contribute to the loss of fluid from the blood, splenectomy should reduce or prevent the hemoconcentration customarily observed after administration of LPS (7, 34). In Study A, conscious splenectomized and intact rats were given a continuous nonlethal infusion of LPS for 18 h, using a protocol based on the model developed by Gardiner et al. (16); changes in MAP, hematocrit, and plasma volume were monitored. In a second series of experiments (Study B), the effect of LPS on fluid extravasation was investigated by simultaneously measuring splenic arterial and venous blood flows in anesthetized rats infused with the same low dose of LPS.

MATERIAL AND METHODS

The experiments described herein were examined by the local Animal Welfare Committee and were found to be in compliance with the guidelines issued by the Canada Council on Animal Care. Unless otherwise stated, all animals were killed with an anesthetic overdose at the completion of the studies.

Animals and housing. Male Long Evans rats (450–600 g) were obtained from Charles River (St. Foy, PQ). They were held in the University Animal Facility for at least 1 wk before surgical or experimental procedures, exposed to 12:12-h light-dark cycle, in a humidity- and temperature-controlled environment, and maintained on a 0.3% sodium diet and water ad libitum.

Study A

Surgery. Pentobarbital sodium (62 mg/kg body wt it) was administered, followed by penicillin (0.1 ml im, Ethacillin Rogas/STB, London, ON) and atropine (0.1 ml, 0.4 mg/ml). Buprenorphine (0.01 mg/kg) was given after the completion of surgery. Throughout the surgical procedures, the rats were maintained on a Deltaphase isothermic heating pad (Braintree Scientific), which maintained body temperature at ~37°C. Isotonic saline (4 ml/h iv) was infused into each animal throughout the surgery.

The jugular vein was cannulated using Silastic tubing (0.51 mm ID × 0.94 mm OD). A midline laparotomy was then performed. Two canulas were placed nonocclusively into the

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injected into the jugular vein. Blood pressure was recorded continuously on line throughout the experiment and later analyzed (Windaq, DATAQ Instruments, Akron, OH). MAP was derived from data collected the day before the experiment (basal), for 10 min immediately before the LPS infusion (time 0), and for the 10-min period before each time hematocrit sample. Serial measurements of hematocrit were made before (the previous day), and at 0 min, 20 min, 40 min, 60 min, 90 min, 180 min, 8 h, and 18 h post-LPS infusion into the jugular vein. There were four investigative groups: 1) nonsplenectomized rats given LPS (n = 9), 2) nonsplenectomized rats given isotonic saline (n = 3), 3) splenectomized rats given LPS (n = 7), and 4) splenectomized rats given isotonic saline (n = 3).

Study B

Surgery. Under pentobarbital sodium anesthesia (62 mg/kg body wt) plus atropine (0.1 ml, 0.4 mg/ml), the rats were implanted unilaterally with femoral vein (Silastic, 0.51 mm ID, 0.94 mm OD) and artery (PE-50, 0.58 mm ID, 0.97 mm OD) cannulas. The duodenal artery was ligated, and a Silastic cannula (0.31 mm ID, 0.64 mm OD) was placed in the gastric artery so that its tip lay just above the junction with the splenic artery. The vascular arc serving the spleen was isolated by ligating all vessels leading to or from other vascular beds. Flow probes (size 1 RB, Transonic Systems, Ithaca, NY) were placed around the splenic artery and vein. At the end of each experiment, dye was infused into the gastric artery to confirm that it perfused only the spleen and that the blood from the spleen drained exclusively into the splenic vein, i.e., that the probes did accurately measure all blood supplying and leaving the splenic circulation.

Experimental protocol. Saline was infused through the femoral vein cannula at a rate of 3 ml/h. A supplementary dose of Inactin [ethyl-(1-methylpropyl)-malonyl-thio-urea; 60 mg sc] was administered to maintain a level plane of anesthesia. Blood pressure was recorded on line (Windaq, DATAQ Instruments) through the femoral arterial cannula. After a 45-min stabilization period, basal blood pressure and blood flows were recorded for a further 30 min. The last 5 min of this period was accepted as the basal values for the experiment. The LPS infusion (150 μg·kg⁻¹·h⁻¹) was then started via the femoral venous cannula. Arterial blood pressure and splenic and arterial blood flows were monitored for the next 2 h.

Statistical analysis. The significance of changes across time were analyzed by repeated measures ANOVA, followed by the Tukey test to identify the individual points of significance. If the data were not normally distributed, a repeated measures ANOVA on ranks was used. The significance of differences between the intact and splenectomized animals was analyzed by Student’s t-test (parametric) or the rank sum test (nonparametric). Significance was accepted at P < 0.05.

RESULTS

Study A

There were no significant differences between the basal measurements of MAP in any of the four groups of animals, nor were there any changes in MAP in either the intact or splenectomized, saline-infused groups (Fig. 1B). However, LPS infusion caused a significant decrease in MAP throughout the duration of the experimental period in the intact animals, relative both to the basal values and compared with the saline-infused intact group (Fig. 1A). Splenectomy abolished this response, i.e., there was no significant change in MAP in the splenectomized rats infused with LPS compared with the saline-infused splenectomized group. Blood pressure was significantly lower in the intact animals, relative both to the basal values and compared with the saline-infused intact group (Fig. 1A). Splenectomy abolished this response, i.e., there was no significant change in MAP in the splenectomized rats infused with LPS compared with the saline-infused splenectomized group. Blood pressure was significantly lower in the intact rats than in the splenectomized rats at all points following initiation of the LPS infusion.

There were no significant differences between the basal values of hematocrit in any of the four groups of animals, nor were there any significant changes in hematocrit in either the intact or splenectomized, saline-infused groups (Fig. 2B). In the intact animals, LPS infusion resulted in a significant increase in hematocrit
at 20, 40, 60, and 90 min post-LPS infusion relative both to the basal-0 values and compared with the saline-infused, intact control group (Fig. 2A). In contrast, LPS infusion failed to induce any change in hematocrit in the splenectomized group. Hematocrit was significantly higher in the intact rats than in the splenectomized rats at 20, 40, 60, and 90 min after initiation of the LPS infusion. After 40 min LPS infusion, plasma volume was significantly reduced in the intact animals, but remained unchanged in the splenectomized group (Fig. 3). During the period of determination of plasma volume (40–90 min post-LPS) there was no significant change in hematocrit (repeated measures ANOVA). It may thus be assumed that plasma volume was in a steady state at this time.

**DISCUSSION**

These results demonstrate that splenectomy is able to blunt the decline in MAP, the increase in hematocrit, and the reduction in plasma volume that is observed in intact animals infused with a low dose of LPS. It is important to emphasize that the increase in hematocrit observed in the intact animals results from the reduction in plasma volume (Fig. 3) not from splenic contrac-
tion and discharge of high hematocrit blood into the circulation. The rat spleen neither is contractile nor does it have storage capacity (32). Moreover, we have previously demonstrated that within the intrasplenic circulation there appears to be a mechanism enabling the efflux of iso-oncotic fluid from the blood into the lymphatic system (24). We propose therefore that during endotoxemia this fluid efflux is exaggerated, resulting in hemoconcentration and hypotension. The second experiment (Study B) supports this suggestion. Despite the initial fall in blood pressure caused by LPS, splenic arterial flow was maintained due to an increase in splenic vascular conductance. However, venous effluent fell so that the A-V flow differential increased, denoting an increase in fluid extravasation from the splenic circulation.

We are not denying that ultimately LPS causes a generalized increase in capillary permeability (11). However, we have specifically examined the mechanisms underlying the early changes in blood pressure and volume before there is endothelial damage and endothelin release (17). It should be pointed out that, in contrast to our studies and those of Gardiner and co-workers (15, 38), many researchers in this field have used acute (bolus) administration of very much higher doses of endotoxin (3, 27, 29) or have not recorded data during the early stages of endotoxemia (14). The model we used, namely low-dose LPS infusion in conscious animals, was chosen because we believe it to closely replicate the initial phase of clinical sepsis, where there is an insidious and progressive onset of cardiovascular anomalies (28, 37). Cecal ligation and perforation (CLP) is arguably the model of sepsis that most closely resembles the clinical situation of bowel perforation. However, there are major differences between CLP and infusion of endotoxin with respect to the roles of cytokines such as tumor necrosis factor-α (12), a factor known to be a key mediator of endotoxic shock (31).

This is probably due to a different site of infection (peritoneal vs. intravenous) (4) and to the fact that the pathogenic agent is bacterial rather than endotoxic (5). One study, which has compared these two models, concluded that the plasma levels of LPS achieved 1 h after an intraperitoneal bolus injection of endotoxin were 40 times higher than those found 2 h after CLP (35). However, it must be pointed out that the dose of LPS used in that study was 50 mg/kg, 200 times higher than the total LPS administered to our animals after 1 h of infusion. Moreover, the local (intraperitoneal) titers of endotoxin achieved by intraperitoneal injection...
of E. coli are very much higher than the plasma levels (25). Although we may conclude that the systemic load of LPS achieved in our study was probably no greater than those found in animals subjected to CLP, the differences in the distribution of that load and of the mechanisms underlying the development of cardiovascular derangement and circulatory shock make it difficult to compare the changes in blood volume obtained in our study with those obtained using CLP (36), where blood volume was reported to be reduced only after 20 h.

Low-dose, long-term infusion of LPS, as used in this study, has been reported elsewhere to cause hemodynamic changes similar to those we observed (15, 38). Specifically, there is an initial reduction in cardiac and stroke index (heart rate increased), a transient fall in MAP, but no change in total peripheral conductance (16). Later, between 4 and 8 h after initiation of LPS treatment, cardiac output recovers and MAP is restored toward normal, despite a gradual increase in total peripheral conductance. Still later, hypotension gradually worsens, despite further increases in cardiac performance (heart rate, stroke index, cardiac index) (16). Our proposal that LPS or its mediators cause an exaggerated efflux of fluid into extrascular spaces from the intrasplenic circulation is not inconsistent with these data.

The effect of LPS on fluid extravasation from the splenic circulation was monitored by measuring the change in the A-V differential of flow. According to the published specifications for the transonic flow probes (1 RB), the smallest detectable difference in flow that can be measured is 0.05 ml/min for a relative accuracy of ±2%. We thus have confidence in our reported values shown in Fig. 6. However, the question will arise as to whether the differences in splenic blood flow that we have reported have physiological significance. At 60-min LPS was associated with an increase in A-V difference of nearly 0.5 ml/min. This means that during the initial 90-min period, LPS would have caused an additional extravasation of about 20 ml of fluid from the blood into the lymphatic system, assuming an average increase in fluid efflux of 0.3 ml/min above basal levels. Most of this fluid would have been returned to the vascular system. However, the capacity of the lymphatic system is increased by such agents as atrial natriuretic factor (30), the circulating levels of which rise in endotoxic shock (1). This would allow for storage of the ~3.5 ml that disappeared from the circulation after infusion of LPS (Fig. 3). Considering that the blood volume of these rats is only about 35 ml (2), it is apparent that splenic fluid extravasation could contribute greatly to the hemoconcentration and hypotension observed after administration of LPS; LPS-induced hypotension has been attributed at least in part to the reduction in blood volume (8, 13).

Perspectives

Two questions arise: 1) what is the mechanism of this fluid extravasation and 2) why should the spleen respond in this way to endotoxemia? The increase in fluid efflux within the splenic circulation does not appear to involve changes in capillary permeability. The splenic vascular system is normally freely permeable to albumin (6). The control must lie instead at precapillary sphincters controlling the route that blood takes once it enters the spleen. Under basal conditions, a large proportion of the splenic blood flow is shunted through A-V anastomoses, the so-called fast route (19). By altering the tone of selective sphincters, blood can be directed through the "slow route." We propose that by altering the pre- and/or postcapillary resistance, changes can be brought about in the capillary filtration pressure within the spleen, in much the same way as afferent and/or efferent arteriolar vasoconstriction or dilatation controls glomerular filtration rate in the kidney. As a consequence of this alteration in splenic capillary filtration pressure, fluid moves from the blood into the extravascular space, i.e., into the splenic lymphatic system.

In septic shock, the reason for this fluid extravasation may lie with the nature of the response to bacteremia, i.e., with the immune assault on the infectious agent. T cells will be activated and released into the circulation. The major site of such activity is the spleen; the activated T cells are disseminated not by release directly into the venous outflow from the spleen but through lymphatic drainage (21, 33). An increase in lymph flow thus facilitates the immune response. Unfortunately, this fluid derives from the plasma. As a result, if the increase in lymph flow is very large there will be a significant reduction in plasma volume. We propose that this balance of fighting the infection and preserving the integrity of the circulation is perturbed in septic shock.

Septic shock remains the condition associated with the largest mortality rate in intensive care units in North America (31), and cardiovascular collapse is the major factor responsible for this statistic. The results of this study indicate that the spleen may be crucially involved in controlling blood volume during the initial stages of endotoxemia (and perhaps beyond). Because of the dual roles of the spleen (immunological and cardiovascular) it would not be desirable to splenectomize patients in septic shock. However, it should be feasible to develop therapeutic agents that would limit fluid extravasation from the spleen, while still conserving its immunological function.

We thank Ryan Sigurdson for the contribution in developing some of the techniques used in this study, and to the Alberta Heritage Foundation for Medical Research for his summer studentship.

This research was supported by the Medical Research Council of Canada.

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Received 19 March 1999; accepted in final form 3 August 1999.

REFERENCES


