Attenuated febrile response to lipopolysaccharide in rats with biliary obstruction

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McCullough, L. K., Y. Takahashi, T. Le, Q. J. Pittman, and M. G. Swain. Attenuated febrile response to lipopolysaccharide in rats with biliary obstruction. Am J Physiol Gastrointest Liver Physiol 279: G172–G177, 2000.—Patients with biliary tract obstruction have unexplained, inordinately high rates of perioperative morbidity and mortality, whereas cholestatic animals display abnormal hypothalamic responses to pyrogenic stimuli. We asked if obstructive cholestasis was associated with abnormal fever generation. Male Sprague-Dawley rats (250 g) underwent laparotomy for implantation of thermistors and either bile duct resection (BDR) or sham operation. After recovery, temperatures were recorded by telemetry and conscious, unrestrained rats in each group were injected intraperitoneally with either interleukin-1β (IL-1β; 1 μg/kg) or Escherichia coli lipopolysaccharide (LPS; 50 μg/kg). Baseline temperatures in both groups were similar. Febrile responses after IL-1β injection in BDR and sham groups were not significantly different. However, in response to LPS injection, BDR rats showed an initial hypothermia with a subsequently attenuated febrile response. Administration of anti-tumor necrosis factor-α (TNF-α) antibody 2 h before LPS injection blocked the LPS-induced hypothermia seen in BDR animals. However, serum levels of TNF-α were not significantly different between sham and BDR animals after LPS injection at any time point measured (0, 1.5, and 3 h).

FEVER MAY BE DEFINED AS AN elevation in body temperature in response to infection, injury, or inflammation. It is one of the most clinically important indicators of disease and is a critical part of the acute phase response, which is composed of a large spectrum of physiological alterations (3). Hypothermia, or the inability to generate fever in response to infection or inflammation, is a poor prognostic factor in certain disease states (11, 15, 33). Ectothermic animals that are infected with Gram-negative bacteria and are prevented from developing fever have markedly increased mortality compared with animals that generate a febrile response (21), and fever is now thought to be an important component of the acute phase response. It has been shown that various components of the immune response such as T cell activation, T cell helper function, and generation of cytotoxic T cells are augmented at higher temperatures (17).

Cholestatic patients have high rates of perioperative morbidity and mortality, but the pathophysiology behind these increases are unknown. Surgical mortality rates are much higher than expected, and this increase is seen independent of whether the biliary obstruction is secondary to benign or malignant causes (1, 31). A variety of physiological abnormalities are seen in obstructive cholestasis, including delayed wound healing with increased rates of wound infection, increased propensity to renal failure, increased plasma endotoxin levels, and biliary bacteremia (4, 9, 10, 14, 19, 20, 29). Many studies (8, 30, 31) have attempted to correlate preoperative factors to operative outcome; however, the underlying pathophysiology remains unclear.

Cytokine-mediated fever induction is in general felt to be prostaglandin dependent (27). Obstructive cholestasis in the rat is associated with decreased hypothalamic production of PGE_2 in response to the cytokine interleukin-1β, (IL-1β) (35). Furthermore, inflammatory mediator-induced central activation of the hypothalamic-pituitary-adrenal axis is defective in rats with obstructive cholestasis (35, 36).

The role of tumor necrosis factor-α (TNF-α) in both cholestasis and thermoregulation is controversial. Some studies (6, 22, 28, 34) report increased levels of serum TNF-α in cholestasis, whereas others (5, 23, 31) report no difference. Whether TNF-α acts as a cyrogen or pyrogen is also controversial (13, 23), but the majority of studies (25) point to enhanced fever generation when the actions of TNF-α are blocked.

The possible presence of abnormalities in thermoregulatory mechanisms that may occur in obstructive cholestasis has not been studied. Given that it is already known that cholestatic animals have an attenuated activation of the hypothalamic-pituitary-adrenal axis in response to endogenous pyrogens (37), we hypothesized that cholestatic rats may have an abnormal thermoregulatory response to pyrogenic stimuli. The fol-
following experiments were undertaken to determine if indeed fever generation is altered in rats with early obstructive cholestasis.

METHODS

Male Sprague-Dawley rats (250 g; Charles River, Pointe Claire, QC, Canada) were housed in a light-controlled room with a 12:12-h light-dark cycle and given free access to food and water. All experiments were performed according to the University of Calgary Animal Care Committee guidelines. The animals were anesthetized with inhalational halothane, and a laparotomy was performed. In bile duct-resected (BDR) animals, the bile duct was identified, isolated, doubly ligated with silk suture, and then resected between the ligatures. Sham-resected animals had their bile ducts identified and dissected free, but not divided. A precalibrated temperature telemetry device (model VMHF, Mini-mitter, Sunriver, OR) was then implanted in the peritoneal cavity. The rats were handled, and given sham injections of PBS (200 μl), or lipopolysaccharide (LPS; endotoxin) (Escherichia coli serotype 026:B6; 50 μg/kg; Sigma Chemical, St. Louis, MO; dose found in preliminary experiments to be consistently pyrogenic in control rats). As a control, to estimate the magnitude of hyperthermia from the stress response to injection, sham injections of PBS were done 2 days later only in the group of rats that received LPS injection, on postoperative day 7 (i.e., these animals had received injection of LPS 2 days earlier). All injections were done between 9 and 11 AM to avoid effects due to circadian rhythm in temperature regulation.

A second set of experiments were carried out to measure serum TNF-α levels after LPS injection. A separate cohort of 36 animals underwent bile duct ligation and 36 underwent sham operation, as described above. On day 5 postsurgery, LPS was injected at 50 μg/kg ip, and the animals were then killed and serum was drawn by cardiac puncture. Serum TNF-α levels were measured at 0-, 1.5-, and 3-h time points, (n = 6 BDR and sham animals per group). A TNF-α ELISA kit (Biosource, Montreal, QC, Canada) was used to measure serum levels of TNF-α at each time point according to the manufacturer’s instructions (assay lower limit of detection 4 pg/ml).

A third cohort of animals (n = 8 BDR; n = 6 sham) were injected with rabbit anti-TNF-α antibody (a kind gift from Dr. S. Kunkel) (0.5 ml/kg ip) 2 h before injection of LPS on day 5 postlaparotomy. This antibody has been well characterized, and this dose of antibody effectively neutralizes endogenous TNF-α production. (24, 34)

For all groups, baseline temperatures were calculated for each individual animal as the mean of the body temperature values taken during the 1-h interval before injection. The results were expressed as the change from this average above or below pretreatment values. Grouped scores were expressed as means ± SE. For statistical analysis, overall significance between groups was assessed by two-way ANOVA (time and treatment as variables), and differences were identified using a Student-Newman-Kuels post hoc test. P < 0.05 was used for all comparisons, at which level the null hypothesis was rejected.

RESULTS

Baseline temperatures were similar in all groups (P > 0.05). BDR animals had significantly less weight gain than sham animals and, on average, lost weight compared with sham animals (P < 0.001; data not shown). BDR rats showed similar fever generation in response to IL-1β injection (1 μg/kg ip) compared with sham-operated animals (peak change in baseline temperature 0.7 ± 0.2°C for sham vs. 0.9 ± 0.2°C for BDR; Fig. 1). BDR rats (n = 8) generated a fever response profile almost identical to that of sham animals (n = 9, P > 0.05) (Fig. 1).

However, in response to LPS injection, BDR animals (n = 8) showed a significant postinjection hypothermic response initially and subsequently failed to generate as great a magnitude of a febrile response over an 8-h

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Table 1. Serum biochemistries in BDR and sham-resected animals 5 days after surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>BDR</th>
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<tr>
<td>Bilirubin, mg/dl</td>
<td>0.22 ± 0.04</td>
<td>8.23 ± 0.43*</td>
</tr>
<tr>
<td>Aspartate aminotransferase, IU/l</td>
<td>74.45 ± 11.59</td>
<td>134.83 ± 9.15†</td>
</tr>
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Values are means ± SE for n = 4 animals/group. BDR, bile duct resected. *P ≤ 0.02, †P ≤ 0.05.
time course compared with sham-operated animals ($n = 9$, $P \leq 0.05$) (Fig. 2). At 1.5 h postinjection, BDR rat temperatures had reached their lowest point, dropping an average of 0.9°C, and the animals remained hypothermic until the 2-h time point, at which time their temperatures began to recover. The BDR rats subsequently generated a small fever. In contrast, sham animals showed a typical fever response to LPS. At 1.5 h after injection, mean temperature in sham animals was 0.5°C above baseline, reaching a maximum febrile response 2.25 h after injection (1.3°C above baseline). Temperatures in BDR rats were significantly different from those in sham animals at all time points between 1.5 and 2.5 h after injection ($P < 0.05$). There was clearly a lesser magnitude of febrile response in the BDR animals throughout all subsequent time points, which intermittently attained significance (Fig. 2). There was no mortality in any group of animals treated with PBS, IL-1β, LPS, or anti-TNF-α antibody.

Sham injections of PBS (200 μl), done 48 h post-LPS injection (day 7 postlaparotomy), were not associated with a change in temperature in either BDR or sham-operated animals at any time point (Fig. 3).

Injection of anti-TNF-α antibody 2 h before injection of LPS in BDR rats resulted in fevers that were significantly different from those of BDR rats not treated with the antibody ($P < 0.05$, Fig. 4). When responses were compared in anti-TNF-α antibody-treated sham and BDR rats, the fevers observed were identical. There were no significant differences in fever generation between the two groups given the anti-TNF-α antibody at all time points ($P > 0.05$; Fig. 5).

Serum levels of TNF-α did not differ significantly between BDR and sham animals at any of the three time points (0, 1.5, and 3 h) post-LPS injection (Table 2).

**DISCUSSION**

The development of a febrile response has been shown to be a tightly regulated physiological phenomenon (21) that is highly conserved across a diverse range of mammals and is felt to be of critical importance for survival. A wide variety of derangements in homoeostatic physiology are associated with the development of obstructive cholestasis in both animal models and humans. Patients with obstructive cholestasis have an unexplained high rate of surgical mortality and morbidity; however, defects in thermoregulation and fever have not been assessed in this group of patients, although prior studies (34) have found abnormalities in the cytokines thought to be important for thermoregulation. In the current study, we have identified an altered febrile response to LPS in rats with...
obstructive cholestasis and, in addition, have found that this can be reversed by prior treatment with anti-TNF-\( \alpha \) antibody. We suggest that this defect in fever generation in cholestasis may contribute, at least in part, to the increased surgical mortality identified in cholestatic patients.

In contrast to the reduced febrile response to LPS, the febrile response after administration of IL-1\( \beta \) was identical in both BDR and sham animals. To understand why responses to these two pyrogens are different, it is instructive to examine differences in the mechanisms of action of LPS and IL-1\( \beta \). In animals and patients developing fever, it is thought that exogenous pyrogens such as LPS, derived from the cell coat of Gram-negative bacteria, induce the production of pyrogenic cytokines from macrophages and other cells of the immune system. Cytokines are immunoregulatory peptides that have a variety of functions in mediating the febrile and acute phase response. In the brain, prostaglandin production is induced by cytokines, and prostaglandins then mediate the fever response via their actions on hypothalamic neurons (21). From the above schema, it would seem that the febrile response to IL-1\( \beta \) and LPS would be identical, but in fact the literature suggests that some of the mechanisms of fever induction may be different. For example, Bishai and Coccaeni (7) found increased PGE\( _2 \) synthesis in isolated brain tissue in response to peripheral endotoxin injection, but not in response to IL-1\( \beta \). There may also be differences in the peripheral responses to IL-1\( \beta \) and LPS; LPS causes the elaboration and secretion of many other substances in addition to IL-1\( \beta \). Significant among these is the production of the cytokine TNF-\( \alpha \) (13).

The role of TNF-\( \alpha \) in fever has been intensively investigated. Although under some conditions it may potentiate the effects of IL-1\( \beta \), there is also good evidence that it is cryogenic (12, 24). In keeping with this, animals becoming hypothermic during sepsis have elevated TNF-\( \alpha \) levels, and interference with the actions of TNF-\( \alpha \) mitigates the hypothermic response (12, 23, 24) and also attenuates the rise in plasma corticosterone in other models of inflammation (34). Indeed, there is considerable evidence that obstructive cholestasis is associated with augmented TNF-\( \alpha \) release in response to LPS in cholestasis (6, 16, 22, 28), although other studies (5) contradict these findings.

Plasma TNF-\( \alpha \) levels are also increased in patients with biliary tract obstruction (2, 37, 38), possibly due to elevations in plasma LPS levels (19, 29). In association with these elevations in serum TNF-\( \alpha \) levels, cholestatic patients are predisposed to septic complications, which are often contributing factors to the high mortality seen in these patients. In neonates and the elderly, hypothermia, as opposed to fever, is often seen in response to infectious stimuli. The incidence of hypothermia accompanying sepsis has been reported to vary between 1% and 10% and approximately doubles the rate of mortality (11, 15, 33). Because of this evidence for both a role for TNF-\( \alpha \) as a cryogen and elevated levels of TNF-\( \alpha \) in obstructive cholestasis, we considered the possibility that the hypothermia after LPS in BDR rats could be due to elevated levels of TNF-\( \alpha \). To test this hypothesis, we pretreated BDR animals with an anti-TNF-\( \alpha \) antibody to interfere with the actions of endogenously released TNF-\( \alpha \). In keeping with the possible involvement of TNF-\( \alpha \) in the transient hypothermic response to LPS, rats pretreated with the anti-TNF-\( \alpha \) antibody did not exhibit the atypical hypothermia. Indeed, these anti-TNF-\( \alpha \) antibody-pretreated BDR animals displayed fevers that were identical to sham animals.

As an additional way to investigate a role for TNF-\( \alpha \) in the atypical febrile response in BDR rats in response to LPS, we also measured serum TNF-\( \alpha \) levels after LPS. However, although serum TNF-\( \alpha \) levels were elevated after LPS administration in both groups of rats, there was no difference between levels observed in BDR and sham animals. The finding of no significant differences between BDR and sham animals in the absolute serum values of TNF-\( \alpha \) after LPS administration is not necessarily surprising. Although some prior studies (6, 16, 22, 28) have found elevations in serum TNF-\( \alpha \) levels after LPS in BDR compared with sham rats, often these studies had differences in experimental protocol, such as higher doses of LPS, longer duration of cholestasis, and different techniques of med-

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**Table 2. Serum TNF-\( \alpha \) levels in BDR vs. sham animals at 0, 1.5, and 3 h after LPS injection**

<table>
<thead>
<tr>
<th>Time After LPS Injection</th>
<th>TNF-( \alpha ) Serum Level, pg/ml</th>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
</tr>
<tr>
<td>0 h</td>
<td>4.27 ± 2.75</td>
</tr>
<tr>
<td>1.5 h</td>
<td>1,488.82 ± 462.39</td>
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<tr>
<td>3 h</td>
<td>290.40 ± 111.9</td>
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Values are means ± SE for \( n = 6 \) animals/time point. Lipopolysaccharide (LPS) was injected at 50 \( \mu \)g/kg. TNF-\( \alpha \), tumor necrosis factor-\( \alpha \). \( P > 0.05 \), sham vs. BDR at all time points.
semenure of TNF-α. In addition, TNF-α may act as a cryogen in cholestasis due to an increased central TNF-α sensitivity as opposed to an increase in serum TNF-α levels. It has been suggested that elevations of TNF-α in biliary obstruction are rapidly inactivated by soluble plasma receptors. (6)

Overall, the literature seems to support the presence of enhanced immune function at higher temperatures with a decrease in the body’s ability to resist infectious agents at lower temperatures. Although hypothermia in sepsis may merely be a marker of how ill the patient is, as opposed to an actual contributor to increased mortality, all evidence suggests that the body’s capabilities in fighting infection are compromised in the absence of fever. This study has shown an abnormal thermoregulatory response in cholestatic rats, which is then blocked by administration of an anti-TNF-α antibody. Although it is difficult to ascribe a deleterious effect of the transient hypothermia seen in BDR rats in the present experiments, it should be noted that a single intraperitoneal injection of LPS is a transient stimulus, whereas sepsis in cholestatic patients is an ongoing process. If cholestatic patients were to show a similar abnormal thermoregulatory response to LPS, the reduction in fever and accompanying hypothermia could be one contributor to their increased morbidity and mortality. Further research is needed to further elucidate the roles of TNF-α and other cytokines in fever generation in cholestasis.

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REFERENCES


