

Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice

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Leon, Lisa R., Andrew A. White, and Matthew J. Kluger. Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. *Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R269–R277, 1998.*— Interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) have been implicated as key mediators in inflammation, morbidity, and mortality associated with sepsis. We examined the role of IL-6 and TNF- α signaling on hypothermia, fever, cachexia, anorexia, and survival during sepsis induced by cecal ligation and puncture (CLP) in male and female gene knockout mice. Male wild-type mice developed an initial hypothermia and subsequent fever during sepsis. Male IL-6 knockout mice did not develop fever; rather, they maintained a profound hypothermia during sepsis. Male TNF p55/p75 receptor (TNFR) knockout mice had attenuated hypothermia, but developed a virtually identical fever as wild-type mice. Cachexia did not differ between male wild-type and IL-6 or TNFR knockout mice, whereas anorexia was prolonged in IL-6 knockout mice. Due to the rapid lethality of sepsis in female mice, survival was the only variable we were able to statistically compare among female genotypes. Female wild-type mice had significantly decreased survival compared with male wild-type mice. Survival was significantly enhanced in male and female TNFR knockout mice compared with their wild-type controls. Lack of IL-6 did not affect male or female lethality. These data support the hypothesis that IL-6 is a key mediator of fever and food intake, whereas TNF is responsible for the initial hypothermia and lethality of sepsis in both sexes of mice. The enhanced lethality of CLP-treated female mice supports a role for sex steroids during sepsis.

acute phase response; fever; hypothermia; anorexia; cecal ligation and puncture

THE SEPSIS SYNDROME is characterized by such pathophysiological changes as hypotension, hypothermia [i.e., core body temperature (T_b) < 36.5°C] or fever (i.e., core T_b > 38.5°C), metabolic acidosis, pulmonary hemorrhage, and death within several minutes to hours (4). Several lines of evidence support the hypothesis that interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) play a role in the sepsis syndrome. First, administration of IL-6 or TNF- α into several species induces an acute phase response (APR) that consists of sepsislike symptoms (3, 6, 21, 26, 30). Second, high circulating concentrations of IL-6 and TNF- α have been measured during endotoxic shock in several species and are negatively correlated with survival (5, 7, 19, 22). Third, a protective effect of inhibition of IL-6 and TNF- α on sepsis lethality has been demonstrated (15, 28). However, the protective effect of cytokine neutralization is highly influenced by the time course of antibody administration, the species, and the experimental model of sepsis, making the therapeutic efficacy difficult to assess (21, 28).

Fever is a common APR to infection. In response to systemic inflammation induced by the injection of a high, septiclike dose of bacterial lipopolysaccharide (LPS), mice develop an APR that is accompanied by such sickness symptoms as hypothermia, fever, cachexia (i.e., decreased body weight), and anorexia (i.e., decreased food intake) (12, 14). Considerable data support the hypothesis that LPS-induced fever is mediated by IL-6 and TNF- α . IL-6 is thought to act as an endogenous pyrogen and TNF- α as an endogenous antipyretic or cryogen. For example, Chai et al. (6) observed an absence of fever in response to the injection of LPS into mice deficient in IL-6. On the other hand, we have shown that mice deficient in TNF p55 (type I) and p75 (type II) receptors (i.e., the two known signaling receptors for TNF) developed exacerbated fevers to the peripheral injection of a high dose of LPS (14). In addition, Kozak et al. (12) reported attenuated hypothermia in Swiss-Webster mice following coinjection of the soluble TNF receptor or neutralizing antiserum to TNF- α with a high dose of LPS.

Gender differences in the development of fever and the incidence and lethality of sepsis have been reported. Murakami and Ono (20) showed that female rats developed a diminished febrile response to the same dose of LPS injected into male rats. Female rats showed a diminished maximum temperature as well as a different time course to the peak of the fever. Alternatively, Morimoto et al. (17) induced hypothermia in female rats using a dose of intravenously injected LPS that induced fever in male rats. Kozak et al. (13) showed differences in the febrile response of male and female mice to a subcutaneous injection of turpentine. Interestingly, female mice developed larger fevers than males in response to turpentine. These data suggest to us that gender differences in the development of fever are dependent on the inflammatory stimulus. Zellweger et al. (32) showed a higher rate of sepsis survival in female than male mice after cecal ligation and puncture (CLP). A higher occurrence and mortality of male patients with sepsis has also been reported (16). It is unclear whether these studies underestimate the frequency of sepsis in females or whether females are more tolerant to sepsis.

Although injections of LPS or live endotoxin are reasonable models of infection, CLP more closely simulates the clinical situation of a perforated necrotic bowel, inducing several pathophysiological changes observed during a bacterial infection of mixed intestinal flora origin. Many studies have been performed on the role of IL-6 and TNF- α in thermoregulation and lethality, using the peripheral injection of LPS or gram-negative bacteria as a sepsis model. However, these studies used neutralizing cytokine antibodies

whose effects are dependent on diffusion and the time course of injection, thus potentially resulting in limited neutralization of the biological action of IL-6 and TNF- α . Gene knockout mice, on the other hand, provide a unique research tool to investigate the role of cytokines and cytokine receptors in the APR to inflammation due to the elimination of the cytokine's action in all tissues of the body. The present study was designed to examine the thermoregulatory, cachectic, and anorectic responses and lethality to CLP using IL-6 and TNF p55/p75 receptor (TNFR) knockout mice. These mice lack functional genes for IL-6 or TNF p55 and p75 receptors, respectively, in all tissues of the body. Using these mice, we tested the hypothesis that an absence of IL-6 and TNF- α signaling would result in altered sickness symptoms (i.e., hypothermia, fever, cachexia, and anorexia) and survival in gene knockout mice compared with their wild-type controls after CLP. Due to discrepancies in the literature regarding the role of gender in fever and sepsis lethality, we also examined differences in our measured responses between male and female wild-type and gene knockout mice.

MATERIALS AND METHODS

Experimental animals. Adult specific-pathogen-free C57Bl/6 \times 129J F₂ hybrid male and female mice weighing 25–30 g were obtained from Jackson Laboratories (Bar Harbor, ME) to serve as wild-type controls for all experiments. All IL-6 and TNFR knockout mice were age- and sex-matched C57Bl/6 \times 129J random hybrids bred and raised in a facility approved by the American Association for Accreditation of Laboratory Animal Care. IL-6 knockout breeder mice were generously provided by Dr. Valeria Poli (Istituto di Ricerche Molecolare IRBM P. Angeletti, Rome, Italy). These mice do not produce IL-6 in response to a peripheral injection of LPS (24). TNFR breeder mice were kindly provided by Dr. Jacques Peschon (Immunex Research, Seattle, WA). Both lymphoid and myeloid lineage cells from these mice fail to bind TNF- α (23). During all experimentation, mice were individually housed in plastic cages at $30 \pm 1^\circ\text{C}$ ambient temperature and maintained on a 12:12-h light-dark cycle with lights on at 0600. Rodent laboratory chow (Teklad Rodent Diet W8604) and drinking water were provided ad libitum.

Body temperature. Core T_b ($\pm 0.1^\circ\text{C}$) was continuously monitored using the Dataquest III system (Mini-Mitter, Sunriver, OR), as described elsewhere (14). Briefly, each animal was anesthetized with halothane, and a miniature battery-operated, temperature-sensitive transmitter (model VMFH) was implanted intra-abdominally. The frequency of the signal emitted by the transmitter is proportional to the animal's T_b . The signal was received by an antenna underneath each animal's cage and transferred to a peripheral processor connected to an IBM-PC that processed the frequency signals into temperatures using predetermined calibration values. All T_b measurements were collected at 5-min intervals in conscious, unrestrained animals and graphically presented as 1-h averages. All transmitters were calibrated before implantation and at the completion of all experiments to ensure validity of T_b measurements. Data from any transmitter that did not recalibrate to within $\pm 0.1^\circ\text{C}$ of the preimplantation value were excluded from data analysis.

Body weight and food intake measurements. Body weight and food intake were measured on a top-loading balance with accuracy to ± 0.1 g. All measurements were made between

0900 and 1000. Each cage was checked for possible food spillage, and measurements were corrected accordingly. Changes in body weight and food intake were calculated by subtracting the value obtained at each successive 24-h period after CLP or sham surgery from the value obtained immediately before surgery. Therefore, all changes in body weight and food consumption are relative to pre-experimentation values. Any animal observed to be losing weight before the day of CLP or sham surgery was excluded from experimentation.

CLP protocol. CLP was performed ≥ 1 wk after transmitter implantation to ensure recovery of circadian rhythms in T_b , normal body weight, and food consumption before experimentation. Mice were anesthetized with halothane, and their abdomens were shaved and swabbed with Betadine and alcohol. An ~ 1 -cm incision was made through the skin and abdominal muscles lateral to the midline (to avoid the site of the previous incision made during transmitter implantation). The cecum was isolated and ligated with 3–0 silk (Ethicon, Somerville, NJ) below the ileocecal valve to prevent bowel obstruction. The cecum was then punctured once with an 18-gauge needle and gently squeezed to express the cecal contents through the wound. The cecum was replaced into the peritoneal cavity, and the peritoneal wall and skin were closed with interrupted sutures. In sham-operated mice, the cecum was exposed and replaced into the peritoneal cavity without ligation or puncture. No fluid resuscitation or antibiotics were given after CLP or sham surgery. For all mice, the skin incision was dressed with a topical antibiotic (0.2% Nitrofurazone; Durvet, Blue Springs, MO), and the animals were returned to their home cages for recovery with free access to food and water. All CLP and sham surgeries were performed between 0900 and 1030, immediately after measurement of body weight and food intake.

Survival. Each animal was monitored twice daily for survival due to the potentially rapid lethality of the experimental procedure. Any animal that appeared moribund or unresponsive to external stimuli was killed to avoid undue pain and suffering. The survival time of each mouse was recorded every 12 h and analyzed for statistical significance with respect to genotype and experimental manipulation. Thus survival data are presented for each 12-h period following surgery.

Experimental design. For all experiments, we used four groups of mice: wild-type sham, wild-type CLP, knockout sham, and knockout CLP. In some mice, transmitter batteries died, so T_b measurements were not recorded following CLP or sham surgery. However, body weight, food intake, and survival were still recorded in all mice through *night 8* postsurgery. Therefore, differences exist in the sample sizes listed for individual APR measurements in Figs. 1–8.

Statistical analysis. Results are presented as means \pm SE. Survival data are presented as a percentage. To test for statistically significant differences among groups in patterns of T_b and changes in body weight and food consumption, ANOVA with repeated measures followed by post hoc Scheffé's test was performed. Survival curves were analyzed by log-rank analysis using the Savage test and the Wilcoxon test. We used both statistical tests to analyze the survival curves as a whole, because these tests weight the late or early survival times, respectively. A P value of < 0.05 was considered significant.

RESULTS

T_b responses of male wild-type and IL-6 knockout mice after CLP. Before experimentation, wild-type and

IL-6 knockout mice had similar circadian rhythms in T_b , with low daytime and high nighttime values (data not shown).

T_b measurements are shown only through 44-h post-CLP because all mice survived sepsis during this time period. We did not detect any statistically significant correlation between final survival status and 44-h T_b measurements.

Figure 1 depicts 1-h averages of T_b for male wild-type and IL-6 knockout mice. Immediately after surgery, all groups showed a virtually identical hypothermic effect of surgical anesthesia (0–1 h). By 2 h, sham-operated wild-type and IL-6 knockout mice had recovered their circadian rhythm in T_b , with virtually no difference between groups. CLP-treated mice were hypothermic compared with their sham-treated controls from 2 to 15 h post-CLP ($P < 0.05$), with virtually no differences between wild-type and IL-6 knockout mice.

Starting at 15 h after CLP, T_b in wild-type mice began to increase; the next day they developed fever that was significantly elevated above T_b of their sham-operated controls by 26 h ($P < 0.05$). This febrile response of the wild-type mice was maintained throughout the daytime hours (26–32 h, $P < 0.05$). Due to the nighttime circadian rise in T_b of the sham-operated mice, the fever in response to CLP did not reach statistical significance from 33 to 44 h. IL-6 knockout mice did not develop a fever in response to CLP. Rather, IL-6 knockout mice subjected to CLP were significantly hypothermic compared with their sham-operated controls at several time points (2–21 h and 33–44 h, $P < 0.05$). This hypothermic effect of CLP treatment in the IL-6 knockout mice did not reach statistical significance from 22 to 32 h the day after surgery due to the low daytime circadian T_b of the sham-operated mice. The profound differences in the T_b responses of the CLP-treated wild-type and IL-6 knockout mice (i.e., fever vs. hypothermia, respectively) resulted in significant differ-

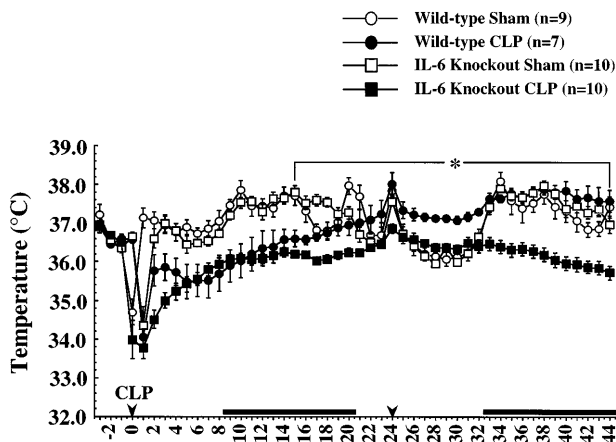


Fig. 1. One-hour averages of body temperature (T_b) responses of male wild-type and interleukin (IL)-6 knockout mice after cecal ligation and puncture (CLP) or sham surgery. Arrowheads indicate time of CLP or sham surgery (0 h) or weighing procedures (0 and 24 h), corresponding to 0900. Sample sizes are indicated in parentheses. Black horizontal bars indicate dark period in 12:12-h light-dark cycle. *Significant difference between CLP-treated wild-type and IL-6 knockout mice at $P < 0.05$.

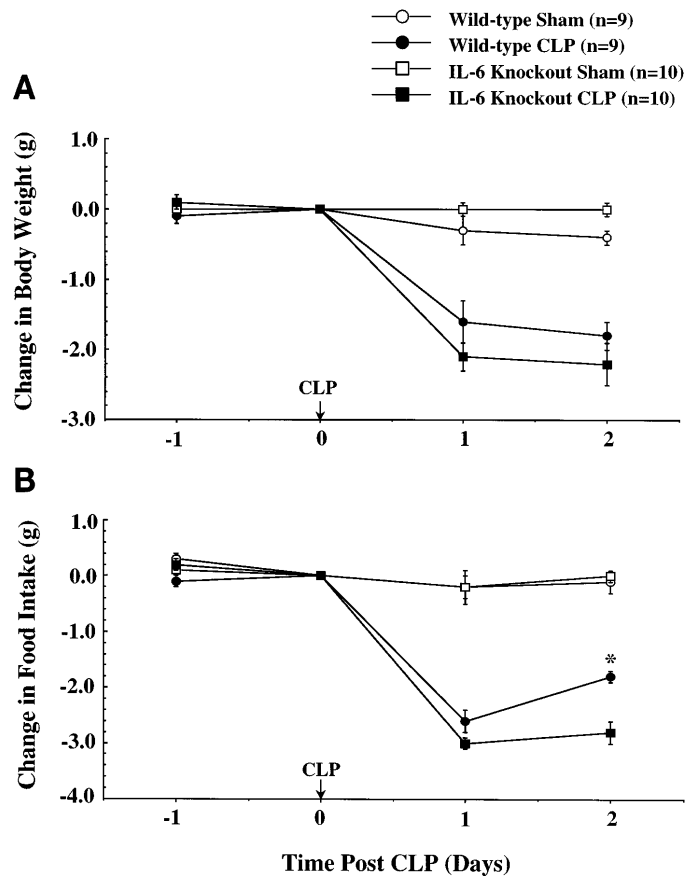


Fig. 2. Change in body weight (A) and food intake (B) of male wild-type and IL-6 knockout mice after CLP or sham surgery. Sample sizes are indicated in parentheses. *Significant difference between CLP-treated wild-type and IL-6 knockout mice at $P < 0.05$.

ences between these groups from 15 to 44 h ($P < 0.05$). The transient increase in T_b seen in all groups of mice at 24 h represents a stress-induced elevation due to weighing procedures.

Induction of cachexia and anorexia in male wild-type and IL-6 knockout mice by CLP. Figure 2A depicts the changes in body weight (i.e., cachexia) of sham and CLP-treated wild-type and IL-6 knockout mice. Sham surgery did not affect changes in body weight of wild-type and IL-6 knockout mice on *day 1*, whereas on *day 2* there was a small but significant difference between groups (Fig. 2A; $P < 0.05$). Wild-type and IL-6 knockout mice showed a significant cachectic response to CLP on *days 1* and *2*, compared with their sham-operated controls (Fig. 2A; $P < 0.05$). Wild-type and IL-6 knockout mice did not differ on any day with respect to their cachectic response to CLP.

Changes in food intake (i.e., anorexia) of sham and CLP-operated wild-type and IL-6 knockout mice are depicted in Fig. 2B. Sham surgery did not significantly affect the daily change in food intake of wild-type or IL-6 knockout mice on any day. Therefore, the decrease in body weight seen in the sham-operated wild-type mice on *day 2* was not due to a reduction in food intake (compare open circles in Fig. 2, A and B). In response to CLP, wild-type and IL-6 knockout mice displayed an-

orexia compared with their sham controls on *days 1* and *2* ($P < 0.05$). A direct comparison of CLP-operated wild-type and IL-6 knockout mice revealed a significant difference between groups on *day 2* only ($P < 0.05$); that is, CLP-operated wild-type mice began to increase their food intake on this day.

Survival status of male wild-type and IL-6 knockout mice after CLP. Figure 3 shows the percent survival of male wild-type and IL-6 knockout mice for each 12-h period after CLP. All sham-operated mice survived the entire time course of the experiment (data not shown). Using log-rank analysis, we did not detect a significant difference in survival between male wild-type mice and IL-6 knockout mice during sepsis (Savage test $P = 0.18$; Wilcoxon test $P = 0.44$; Fig. 3).

APR of female wild-type and IL-6 knockout mice after CLP. Due to the short-term survival of female mice in response to CLP (most mice only survived 12 h; see Fig. 4), we could not statistically compare daily changes in T_b or cachectic or anorectic responses of female mice. However, we did assess 12-h changes in survival (see below).

Survival status of female wild-type and IL-6 knockout mice after CLP. Figure 4 depicts the 12-h changes in survival of female wild-type and IL-6 knockout mice after CLP. All sham-operated mice survived the entire time course of the experiment (data not shown). A direct comparison using log-rank analysis of the survival times of female wild-type and knockout mice revealed no significant differences between groups during any 12-h time period (Savage test $P = 0.71$; Wilcoxon test $P = 0.70$; Fig. 4).

T_b of male wild-type and TNFR knockout mice after CLP. Male wild-type and TNFR knockout mice did not differ with respect to their circadian rhythms in T_b before experimentation. All mice demonstrated normal low daytime and high nighttime T_b values (data not shown).

T_b measurements are shown only through 44-h post-CLP because all mice survived sepsis during this time period. We did not detect any statistically significant

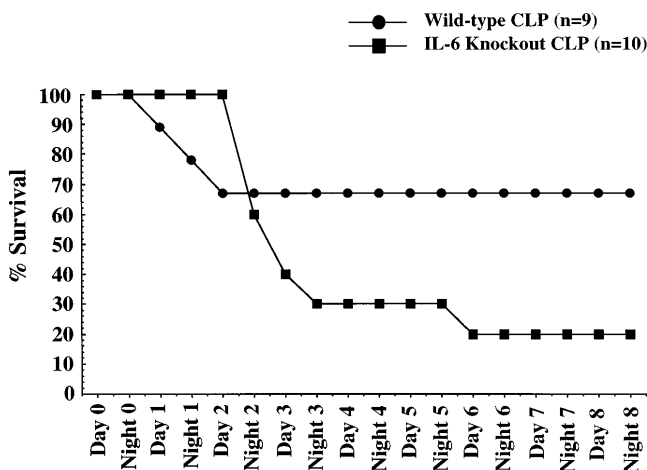


Fig. 3. Twelve-hour changes in survival of male wild-type and IL-6 knockout mice after CLP or sham surgery. All surgeries were performed on *day 0*. Sample sizes are indicated in parentheses.

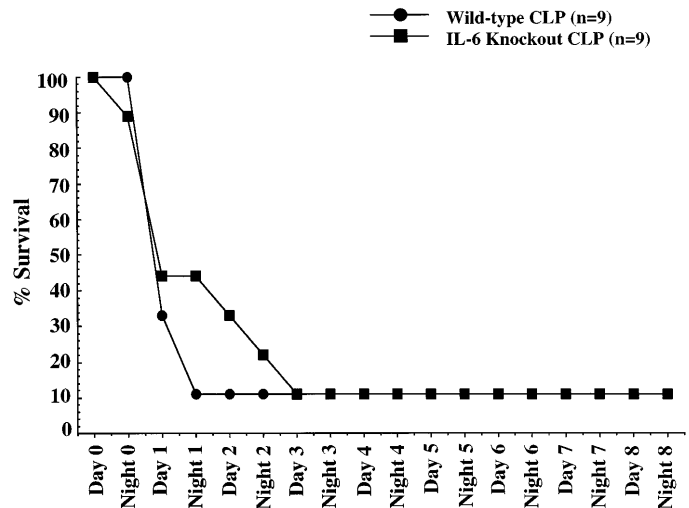


Fig. 4. Twelve-hour changes in survival of female wild-type and IL-6 knockout mice after CLP or sham surgery. All surgeries were performed on *day 0*. Sample sizes are indicated in parentheses.

correlation between final survival status and 44-h T_b measurements.

Figure 5 depicts 1-h averages of T_b for male wild-type and TNFR knockout mice. All groups showed a virtually identical hypothermic effect of anesthesia from 0 to 1 h. By 2 h, sham-treated mice resumed their circadian rhythm in T_b , with virtually no differences between groups (Fig. 5). In response to CLP, wild-type mice developed a profound hypothermia compared with their sham-treated controls from 2 to 21 h ($P < 0.05$), followed by a fever from 26 to 31 h ($P < 0.05$). In contrast, CLP-operated TNFR knockout mice were significantly hypothermic compared with their sham-operated controls from 3 to 9 h only ($P < 0.05$). From 18 to 31 h and 39 to 44 h, TNFR knockout mice developed

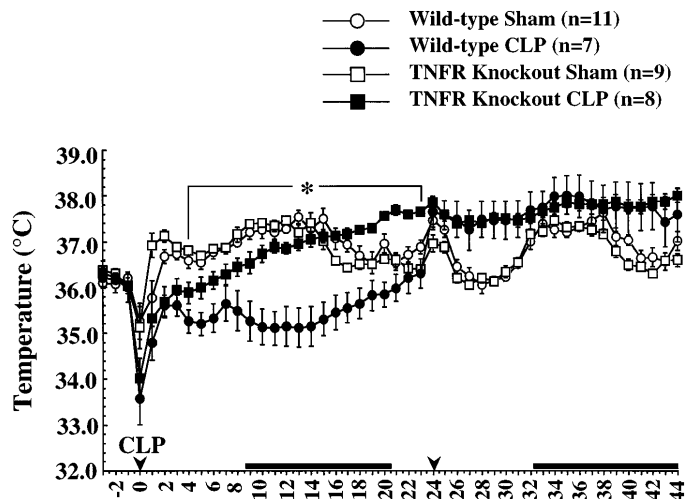


Fig. 5. One-hour averages of T_b responses of male wild-type and tumor necrosis factor p55/p75 receptor (TNFR) knockout mice after CLP or sham surgery. Arrowheads indicate time of CLP or sham surgery (0 h) or weighing procedures (0 and 24 h), corresponding to 0900. Sample sizes are indicated in parentheses. Black horizontal bars indicate dark period in 12:12-h light-dark cycle. *Significant difference between CLP-treated wild-type and TNFR knockout mice at $P < 0.05$.

fever following CLP that was significantly elevated above the T_b of their sham-operated knockout controls ($P < 0.05$). A direct comparison of these T_b changes in the wild-type and knockout mice revealed a significant difference from 4 to 23 h ($P < 0.05$). That is, a lack of TNF signaling resulted in a significantly attenuated hypothermia and earlier febrile response to CLP compared with wild-type mice. However, the febrile response of the CLP-treated wild-type and knockout mice was virtually identical from 26 to 31 h and 39 to 44 h ($P > 0.05$). The transient increase in T_b seen in all groups of mice at 24 h postsurgery represents a stress-induced elevation due to weighing procedures.

Induction of cachexia and anorexia in male wild-type and TNFR knockout mice by CLP. Changes in body weight of male wild-type and TNFR knockout mice after CLP or sham treatment are shown in Fig. 6A. Sham surgery did not affect the 24-h changes in body weight of male wild-type and knockout mice. CLP induced a significant cachectic response in wild-type and TNFR knockout mice on *days 1* and *2*, with virtually no difference between groups on either day.

Figure 6B shows the changes in food intake of male wild-type and TNFR knockout mice. Sham surgery did

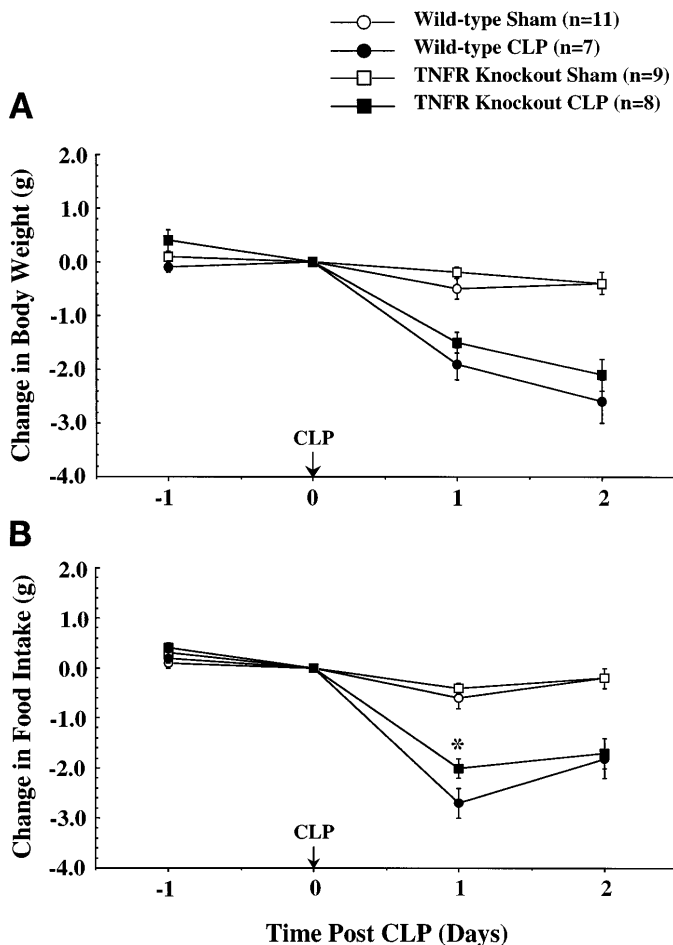


Fig. 6. Change in body weight (A) and food intake (B) of male wild-type and TNFR knockout mice after CLP or sham surgery. Sample sizes are indicated in parentheses. *Significant difference between CLP-treated wild-type and TNFR knockout mice at $P < 0.05$.

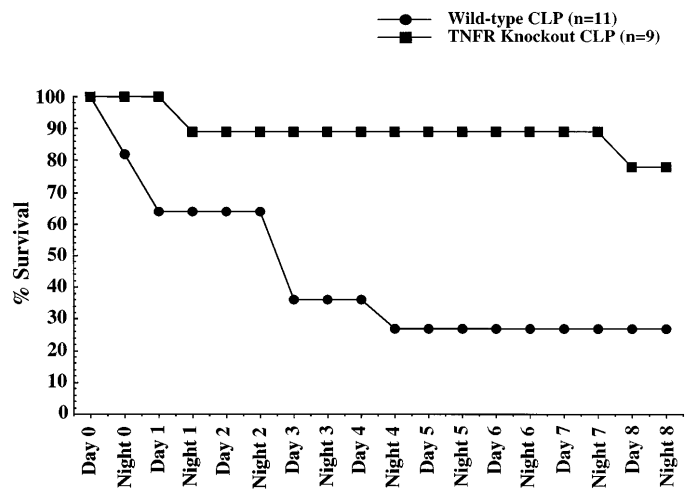


Fig. 7. Twelve-hour changes in survival of male wild-type and TNFR knockout mice after CLP or sham surgery. All surgeries were performed on *day 0*. Sample sizes are indicated in parentheses.

not affect the 24-h changes in food intake. CLP-treatment resulted in virtually identical anorectic responses in wild-type and TNFR knockout mice on *days 1* and *2* ($P < 0.05$). On *day 1*, TNFR knockout mice showed a tendency toward a blunted anorectic response compared with their CLP-operated wild-type controls (Fisher, $P < 0.05$).

Survival status of male wild-type and TNFR knockout mice after CLP. Figure 7 shows 12-h changes in survival of male wild-type and TNFR knockout mice after CLP. All sham-operated mice survived the entire time course of the experiment (data not shown). TNFR knockout mice had significantly enhanced survival in response to CLP compared with their wild-type counterparts (Savage test $P = 0.02$; Wilcoxon test $P = 0.02$; Fig. 7). A direct comparison between the CLP-treated male wild-type mice used as controls for the IL-6 knockout mice (Fig. 3) and those used with the TNFR knockout mice (Fig. 7) shows 30 vs. 70% final survival. Using log-rank analysis to compare the curves as a whole, we did not detect any statistically significant difference between the two male wild-type groups (Savage $P = 0.13$; Wilcoxon $P = 0.14$).

APR of female wild-type and TNFR knockout mice after CLP. Due to the short-term survival of female wild-type mice in response to CLP (most mice only survived 12 h; see Fig. 8), we could not statistically compare daily changes in T_b or cachectic or anorectic responses of female wild-type and TNFR knockout mice. However, we did assess 12-h changes in survival (see below)

Survival status of female wild-type and TNFR knockout mice after CLP. Figure 8 depicts the 12-h changes in survival of female wild-type and TNFR knockout mice after CLP. All sham-operated mice survived the entire time course of the experiment (data not shown). As was seen with the male TNFR knockout mice, the absence of TNF signaling resulted in a significantly enhanced survival of the female knockout mice compared with their wild-type counterparts (Savage test $P = 0.001$; Wilcoxon test $P = 0.0013$; Fig. 8).

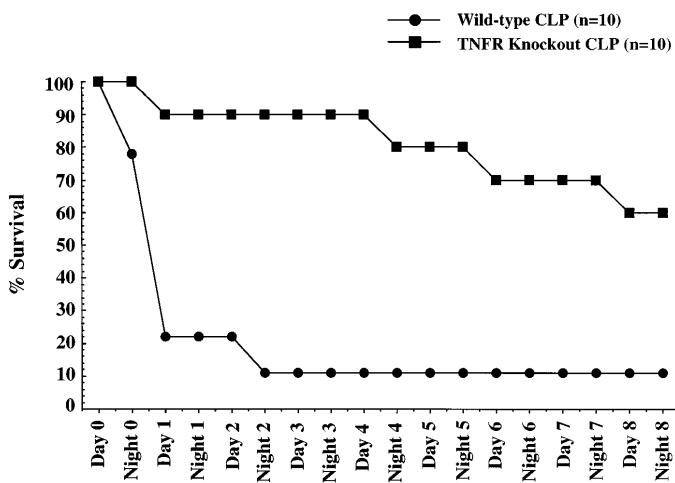


Fig. 8. Twelve-hour changes in survival of female wild-type and TNFR knockout mice after CLP or sham surgery. All surgeries were performed on *day 0*. Sample sizes are indicated in parentheses.

DISCUSSION

The results of the present study demonstrate that sepsis induces a complex APR in mice, consisting of hypothermia, fever, cachexia, anorexia, and lethality. We used male and female gene knockout mice to examine the role of endogenous IL-6 and TNF- α signaling on these responses. IL-6 knockout mice developed hypothermia, but not fever, during sepsis, whereas TNFR knockout mice developed fever, but minimal hypothermia. These data indicate that during sepsis in mice IL-6 functions as an endogenous pyrogen, whereas TNF- α functions as an endogenous antipyretic. On the other hand, cachexia was unaffected and anorexia only transiently affected by a lack of IL-6 or TNF- α signaling, supporting a role for other, perhaps redundant, cytokine mechanisms in the control of these responses. Interestingly, female wild-type and knockout mice succumbed to the lethality of sepsis sooner than males, such that 24-h changes in T_b , cachexia, and anorexia could not be assessed. The reason for these gender differences is unclear but supports a role for sex steroids in the ability of the host to recover from a septic insult. Interestingly, these data contradict earlier reports of female mice in proestrus showing more tolerance to sepsis (CLP) than their male counterparts (32). In the present study, lethality during sepsis was also significantly decreased due to a lack of TNF signaling, but not IL-6, in female and male mice. These data are in contrast to previous reports of both TNF and IL-6 antagonism having a protective effect on sepsis lethality (15, 28). This disparity may result from the different methodologies used (i.e., pharmacological antagonism vs. gene knockout studies).

Wild-type mice developed an initial hypothermia and a subsequent fever during the 44-h time period after CLP (Figs. 1 and 5). Mice deficient in IL-6 developed the initial hypothermia but did not develop fever. These data support the hypothesis that IL-6 mediates fever, but not hypothermia, during sepsis in mice. CLP induces an intra-abdominal infection of gram-negative

and gram-positive bacterial origin, and a pyrogenic role for IL-6 has been implicated using other bacterial models of fever. For example, it has been shown in mice that neutralization of IL-6 activity abrogates fever in response to the peripheral injection of LPS (6). However, in the present study IL-6 knockout mice did not simply resume their circadian variation in T_b as a consequence of the abrogated fever. Instead, these mice developed a pronounced hypothermia that persisted throughout the 44-h observation period after CLP. These data suggest to us that a lack of IL-6 signaling results in the release of an endogenous cryogen in these mice that attenuates the normal circadian rise in T_b .

Considerable data indicate that a number of endogenously produced factors have antipyretic properties *in vivo*. These include such factors as α -melanocyte-stimulating hormone (27), glucocorticoids (18), and TNF- α (12, 14). Although TNF- α is often considered an endogenous pyrogen, data in the literature support the interpretation that under certain circumstances TNF- α acts as an endogenous antipyretic or cryogen. For example, Alexander et al. (2) reported attenuated hypothermia in rats treated with recombinant TNF- α (and thus made tolerant to the cytokine's actions) before CLP. Similarly, Kozak et al. (12) reported attenuated hypothermia in mice treated with the soluble TNF receptor or neutralizing TNF- α antiserum before the injection of a high, septiclike dose of LPS. Furthermore, we recently reported exacerbation of the early phase of the febrile response to LPS injected peripherally in TNFR knockout mice (14). All of these studies suggest that the endogenous action of TNF- α and its p55 and p75 receptors is cryogenic.

In the present study, we extended our earlier observations of a cryogenic action of endogenous TNF- α signaling using CLP as a model of sepsis. We report here that the initial hypothermia, but not the subsequent fever, in response to CLP was attenuated in TNFR knockout mice. These data are in contrast to the report by Remick et al. (25) indicating that neutralizing TNF- α antiserum did not affect hypothermia induced by LPS or CLP in CD-1 mice. Perhaps these discrepancies represent the effect of partial neutralization of TNF- α using antiserum (25) versus total neutralization of TNF- α action in the receptor knockout mice (present study). Alternately, a strain difference may explain these different results.

IL-6 inhibits endotoxin-induced production of TNF- α . Aderka et al. (1) demonstrated an inhibition of LPS-induced TNF- α release by IL-6 both *in vitro* and *in vivo*. In a study by Fattori et al. (9), serum TNF- α induction by LPS was found to be approximately threefold higher in IL-6 knockout mice compared with their wild-type controls. Similarly, van der Poll et al. (31) measured enhanced plasma and organ (i.e., liver) levels of TNF- α in C57Bl/6 mice treated with an anti-IL-6 monoclonal antibody (MAb) before CLP. TNF- α levels were similar in control and MAb-treated mice at 2, 6, and 12 h post-CLP. These time points correspond to the virtually identical hypothermic responses seen in the wild-type and IL-6 knockout mice used in the present study.

However, in the study by van der Poll et al. (31), plasma TNF- α levels were exacerbated at 24 h after anti-IL-6 MAb treatment, a time point that corresponds to observed differences in the thermoregulatory responses of CLP-treated wild-type and IL-6 knockout mice used in the present study (i.e., when IL-6 knockout mice were hypothermic and wild-type mice were febrile; see Fig. 1). Unfortunately, plasma and peritoneal levels of TNF- α in septic wild-type and anti-IL-6 MAb-treated mice were not reported between 12 and 24 h or beyond 24 h in the study by van der Poll et al. (31). In any case, on the basis of these data, we speculate that the lack of IL-6 in the knockout mice resulted in the exacerbated release of TNF- α and its cryogenic activity such that T_b was significantly lower in these mice during sepsis. Studies are currently ongoing to assess plasma levels of TNF- α at several time points after CLP in the IL-6 knockout mice.

In addition to changes in T_b , common phenomena associated with bacterial infection include cachexia and anorexia or hypophagia. Wild-type mice did not differ from IL-6 or TNFR knockout mice with respect to their cachectic responses. Similar results were reported by Fattori et al. (9), in which LPS injection into IL-6 knockout mice produced cachexia and anorexia similar to that observed in wild-type mice. These data suggest that IL-6 and TNF- α signaling are not required for these host defense responses to CLP and may indicate the development of cytokine redundancy *in vivo*. Cytokine interactions *in vivo* may play an important role in infection-induced APR such that the absence of one cytokine throughout development (i.e., as in the knockout mice) may be compensated for by another. Other investigators have reported the ability of antibodies against IL-6 to partially block LPS-induced weight loss (29).

Interestingly, despite the lack of a difference in the cachectic responses of male wild-type and IL-6 knockout mice treated with CLP, we did detect a significant difference in their anorectic responses on *day 2* (Fig. 2B). That is, male wild-type mice had a significant attenuation of anorexia on *day 2*, whereas IL-6 knockout mice showed a similar degree of anorexia as seen on *day 1*. Although these data suggest that IL-6 may be involved in the recovery of food intake after CLP, we are cautious in our interpretation of these data because we did not monitor changes in this variable beyond the 48 h after surgery. Thus the long-term effects of an absence of IL-6 or TNF- α signaling on the cachectic and anorectic responses cannot be elucidated in the present study and require further characterization. Although TNFR knockout mice showed a tendency toward a more pronounced anorectic response to CLP than wild-type mice, this difference only reached statistical significance using the less conservative post hoc Fisher's test. We have monitored changes in body weight and food intake in wild-type and TNFR knockout mice for up to 6 days after the injection of a high dose of LPS and found no difference between groups, again indicating the possibility of cytokine redundancies in these mice (14).

Survival data in the current study support a role for TNF- α and IL-6 interactions in the lethality of sepsis. TNF inhibitors have been shown to protect against sepsis lethality (15). Male and female TNFR knockout mice showed prolonged survival in response to CLP compared with their wild-type controls. On the basis of these data, we conclude that TNF- α contributes significantly to lethality during sepsis. A direct effect of high plasma TNF- α levels on the lethality of sepsis has been similarly reported in studies that demonstrate a protective effect of IL-10 treatment. IL-10 is a pleiotropic cytokine that blocks the *in vitro* production of TNF- α , as well as other cytokines, by LPS-activated monocytes/macrophages (8). Howard et al. (11) showed a protective effect of IL-10 treatment in endotoxemic mice that correlated with a reduction in serum TNF- α levels. Interestingly, mice injected with IL-10 before a high dose of LPS showed attenuated hypothermia and lethality, responses that corresponded with reduced plasma TNF- α levels (10). These data further support a role of cytokine interactions *in vivo* and the importance of modulation of TNF- α levels for the control of hypothermia and lethality of sepsis in mice. Similarly, high serum levels of IL-6 have been correlated with the severity and poor outcome of sepsis (5, 7, 22). The systemic administration of IL-6 inhibitors has been demonstrated to protect mice and rats against septic shock induced by the injection of gram-negative bacteria (15). However, in the present study, survival of the male and female IL-6 knockout mice was similar, rather than enhanced, compared with their wild-type controls. Similarly, van der Poll et al. (31) found no effect of anti-IL-6 MAb treatment on survival of mice, despite enhanced TNF- α levels in those mice at the 24-h time point after CLP. The reason for discrepancies between the IL-6 knockout survival data and previously published observations using IL-6 inhibitors is unclear.

Gender differences with respect to the lethality of CLP were also evident in the present study. One of the main findings of this study was that female mice were more susceptible to the lethality of CLP compared with their male counterparts. In all of our experiments, we found that female mice began to succumb to the lethality of CLP within 12 h, whereas male mice survived through 44 h. These data suggest that sex steroids may play a pivotal role in susceptibility of the host to sepsis, although an examination of the specific hormones responsible for the gender differences in survival was beyond the scope of our objectives. Interestingly, our data are in contrast to previous reports in the literature of higher rates of sepsis mortality among males than females. For example, Zellweger et al. (32) examined female mice in the proestrus cycle and reported enhanced survival and reduced immunosuppression compared with male mice after CLP. However, the use of 12-h-fasted mice that were saline-resuscitated after CLP and the different strain of mice used may account for the differences between the survival data reported by Zellweger et al. (32) and our studies. It is intriguing to note, however, that despite the rapid lethality rates

of the female mice used in the present study, the absence of TNF signaling still proved beneficial to survival (Fig. 8), thus implicating TNF- α as a critical cytokine in the lethality of sepsis, regardless of gender. Clearly, additional studies are required to determine the mechanisms responsible for the observed gender differences in survival rates during sepsis.

In the past several years our laboratory has been actively involved in experimentation with several different types of knockout mice for the study of thermoregulatory processes. The use of genetically engineered mice in physiological studies of inflammatory responses circumvents the problems inherent with the injection of pharmacological agents. Certainly, the most significant contribution of this model to the study of fever (and related sickness behaviors) is the ability to study the effect of the elimination of a cytokine's action from all tissues of the body. However, there is a caveat to the knockout approach in that the generation of negative results, or the inability to confirm the results using a more traditional approach (i.e., antibody administration), suggests that redundancies may have developed that can mask the true function of the cytokine in vivo. Results obtained using knockout mice that contradict data obtained via "traditional" pharmacological means may be difficult to interpret because knockout mice have never been exposed to the cytokine's actions. Gene knockout mice may use compensatory mechanisms to elicit physiological and behavioral responses to an inflammatory insult that are indistinguishable from those seen in wild-type mice. Thus experiments using pharmacological and genetic approaches are both important in developing a greater understanding of the complex relationship between cytokines and the physiological responses they control.

Perspectives

Sepsis induces a wide variety of pathophysiological changes thought to be detrimental to survival of the host. IL-6 and TNF- α have been implicated as key mediators of the sepsis syndrome. However, there has been difficulty establishing a specific role for these cytokines in the regulation of sickness behaviors during infection due to the complex interaction of cytokines with overlapping biological activities. Although use of the mouse model in the study of the APR during the sepsis syndrome does not allow us to make a direct comparison to the clinical condition of sepsis, we believe that the present study further elucidates the role of IL-6 and TNF- α in several of the APR and the lethality of this bacterial condition. Whereas our data provide additional support for a pyrogenic role for IL-6 and an antipyretic role for TNF- α in the febrile response to sepsis, our survival data suggest more complex regulatory mechanisms are involved in sepsis tolerance. It is clear that gender differences cited in the literature for the control of fever and tolerance to sepsis need to be examined more thoroughly to reconcile the apparent controversies that exist between species and models of clinical drug intervention.

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