An antipyretic role for interleukin-10 in LPS fever in mice

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endogenous antipyretic. We hypothesized that IL-10 knockout mice would develop exacerbated febrile responses to LPS and turpentine compared with their wild-type controls. To induce systemic inflammation, we injected low (50 or 100 µg/kg ip) or high, septiclike (2.5 mg/kg ip) doses of LPS into Swiss Webster (SW) or IL-10 knockout mice. For the induction of local inflammation, mice were injected with a single dose of turpentine (100 µl/mouse sc). We demonstrated that whereas IL-10 functions as an endogenous antipyretic in the regulation of fevers induced by LPS, it may not have a physiological role in the regulation of Tₚ in response to a local inflammation induced by turpentine in mice. Furthermore, inhibition of plasma concentrations of IL-6 in response to a low dose of LPS is a putative mechanism of the antipyretic action of IL-10 in mice.

Because we have shown previously in our laboratory that sepsis induced by the injection of LPS or cecal ligation and puncture (CLP) induces large reductions in body weight and food intake (21, 22), we also examined changes in these variables in the wild-type and IL-10 knockout mice injected with the high dose of LPS. IL-10 knockout mice developed significantly larger reductions in body weight and food intake in response to the high dose of LPS compared with their wild-type counterparts. These differences were paralleled by significant differences in the lethality of sepsis in the wild-type (100% survival) and IL-10 knockout mice (30% survival).

MATERIALS AND METHODS

Experimental animals. For recombinant murine IL-10 (rmuIL-10) injection experiments, specific pathogen-free adult male SW mice weighing 30–35 g were purchased from Taconic Laboratories (Germantown, NY). Additional experiments used adult specific pathogen-free male C57BL/10 wild-type and IL-10 knockout mice weighing ~30–35 g purchased from Jackson Laboratories (Bar Harbor, ME). The original generation of the IL-10 knockout mice and the strategy for inactivation of the IL-10 gene by homologous recombination are described in detail elsewhere (18). Although the development of chronic enterocolitis and other abnormalities have been reported in the IL-10 knockout mice, we did not observe any phenotypic, behavioral, or Tₚ abnormalities in these mice before experimentation.

All mice were housed one per cage in a room maintained at 30°C, with a 12:12-h light-dark cycle (lights on at 0600) and humidity controlled to 30–40%, and provided with ad libitum tap water and laboratory rodent chow (Teklad Rodent Diet W8604). All mice were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care.

Measurement of Tₚ. Core Tₚ (±0.1°C) was monitored using the Datacol 3 telemeterymetry system (Mini-Mitter, Sunriver, OR). Animals were anesthetized with halothane for the intraperitoneal surgical implantation of the Mini-Mitter transmitters (coated with dental wax) and were allowed 7 days to recover from this surgery before the onset of experimentation. The transmitters sent signals whose frequencies vary consistently with Tₚ to receiver boards located under the cages. The receiver boards were connected to an IBM-PC that processed the frequency signals into temperatures using predetermined calibration values. This system allowed the temperature of undisturbed animals to be monitored at 5-min intervals throughout all experiments, beginning at least 24 h before the experimental procedures and continuing until the animals were euthanized. The transmitters were recalibrated after each experiment to ensure that recorded temperatures were accurate. Data from a transmitter that did not recalibrate to within ± 0.1°C of the preimplantation value were excluded from all analysis and presentation.

LPS. Purified lyophylized extract of Escherichia coli endotoxin (0111:B4; Sigma Chemical, St. Louis, MO) was dissolved in 0.9% sodium chloride (saline) to a stock concentration of 2 mg/ml and stored at –20°C. Before injection, the stock solution was warmed to 37°C, diluted in sterile saline to the desired concentration, and intraperitoneally injected at a low dose of 50 µg/kg (C57BL/10 and IL-10 knockout mice) or 100 µg/kg (SW mice) or at a high dose of 2.5 mg/kg (all mice). Control mice were intraperitoneally injected with an equivalent volume of sterile saline. Injection volume did not exceed 0.15 ml/mouse. All injections were performed between 0900 and 0930.

Turpentine. Commercial-grade steam-distilled turpentine (Sunnyside, Wheeling, IL) was injected subcutaneously into the left hindlimb at a volume of 100 µl/mouse. Control mice received 100 µl of sterile saline subcutaneously into the same injection site. All mice were briefly anesthetized with halothane to relieve the pain of the injection procedure. All injections were performed between 0900 and 0930.

rmuIL-10. rmuIL-10 (E. coli) was a generous gift from Dr. Grace H. W. Wong (Department of Molecular Biology, Genentech, South San Francisco, CA). rmuIL-10 was injected intraperitoneally into SW mice at a concentration of 1.0 µg/mouse (~30 µg/kg). Control mice were intraperitoneally injected with an equivalent volume of sterile saline. Injection volume did not exceed 0.15 ml/mouse. All injections were performed between 0900 and 0930. rmuIL-10 was injected immediately before sterile saline or LPS.

Blood collection for cytokine measurements. Blood for cytokine analyses was collected from anesthetized C57BL/10 and IL-10 knockout mice by cardiac puncture at 1, 4, and 24 h after injection of sterile saline (equivalent volume) or low-dose LPS (50 µg/kg ip). Blood was drawn into heparinized syringes to determine plasma IL-6 and TNFα-like activity. Plasma was separated by centrifugation of the freshly drawn blood and stored at –20°C. Because of a limited supply of rmuIL-10, we were unable to assess plasma cytokine levels in SW mice.

Bioassay for IL-6. The IL-6-dependent mouse B9 hybridoma cell line was used to determine IL-6 activity in plasma. The B9 line was kindly provided by Dr. Lucien Aarden, Amsterdam, The Netherlands (1). Plasma samples to be tested for IL-6 were serially diluted in 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 10 µg/ml streptomycin in Iscove's modified Dulbecco's medium (Sigma), and 100-µl volumes were added in triplicate to 96-well tissue culture plates. Cells were washed and resuspended at 5 × 10⁴ cells/ml in growth medium supplemented with 10% FBS sterile heparin, and 100 µl of cell suspension were added to each well. The plates were incubated for 64–68 h at 37°C, 5% CO₂, and 98% humidity. Then, 0.1 ml of 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/ml, Sigma) were added to each well to determine the amount of proliferation (MTT is a tetrazolium salt that will form dark crystals when combined with metabolizing cells). After being allowed to incubate for an additional 4 h, 150 µl of supernatant were removed from each well, and 100 µl of 10% SDS (Sigma) in 0.01 N HCl were added to dissolve the tetrazolium crystals. The plates were protected from light and left overnight at room temperature before being read at 570 and 630 nm (for
To test for statistical significance, changes in body weight, the normal circadian rhythm in body temperature (Tb), with low daytime and high nighttime values. Mice intraperitoneally injected with recombinant murine interleukin-10 (rmuIL-10) had virtually no effect on the normal circadian rhythm of Tb in Swiss Webster mice (Fig. 2). The transient rises in Tb at 0 and 24 h are stress-induced rises due to injection and weighing procedures, respectively. Injection of the large, septic-like dose of LPS resulted in a biphasic pattern of Tb that differed from the fever induced by 100 µg/kg (compare Figs. 1 and 2). Mice injected with this high dose of LPS had an initial drop of Tb that peaked at ~4 h, lasted throughout the first day of injection, and has been shown previously to be associated with the presence of sepsis-induced fever.

### RESULTS

**Effect of rmuIL-10 on fever induced by a low dose of LPS in SW mice.** Figure 1 depicts 1-h averages of Tb in SW mice injected with rmuIL-10 (1 µg/mouse ip), immediately followed by a low dose of LPS (100 µg/kg ip). Before injection at 0 h, all SW mice had virtually identical Tb. The transient increase in Tb seen in all groups of mice at 0 h represents a stress-induced elevation due to handling during the injection procedure. SW mice injected with rmuIL-10 alone showed the normal circadian rhythm in Tb with low daytime and high nighttime values. Mice intraperitoneally injected with LPS responded with an ~80°C monophasic fever that peaked at ~5 h. Pretreatment of mice with rmuIL-10 resulted in a significant attenuation of LPS-induced fever from 3 to 10 h (P < 0.05). Tb did not differ between groups during the nighttime hours (11–21 h).

**Effect of rmuIL-10 on fever induced by a high dose of LPS in SW mice.** Figure 2 depicts the 1-h averages of Tb in SW mice injected with rmuIL-10 (1.0 µg/mouse ip) or sterile saline (equivalent volume) immediately before injection of a high dose of lipopolysaccharide (LPS; 100 µg/kg ip). Sample sizes are indicated in parentheses. Black horizontal bars represent lights-off period in a 12:12-h light-dark cycle. Arrowhead indicates time of injection. *Significant difference between LPS-injected mice pretreated with saline or rmuIL-10 (P < 0.05).
high circulating levels of biologically active TNF-\(\alpha\) (4, 15). \(T_b\) increased during the first night following injection and resulted in an \(\sim 0.90^\circ\)C fever by 24 h postinjection compared with preinjection \(T_b\) (\(P < 0.05\)). Due to the stress-induced rise in \(T_b\) as a consequence of weighing at 24 h, this peak in fever represented only an \(\sim 0.5^\circ\)C rise above the \(T_b\) of the animals injected with rmuIL-10 alone. \(T_b\) of the group injected with LPS alone was afibrate during the second night after injection. The pretreatment of SW mice with rmuIL-10 significantly attenuated both the hyperthermic (2–9 h; \(P < 0.05\)) and febrile phase (16–33 h; \(P < 0.05\)) of the \(T_b\) response to this high dose of LPS. Fever in response to a low dose of LPS in IL-10 knockout mice. To examine the effect of the elimination of endogenous IL-10 signaling on the febrile response elicited to a low dose of LPS, we used IL-10 knockout mice of C57BL genetic background (18) and their sex- and age-matched wild-type counterparts. Before injection, all groups of mice had virtually identical \(T_b\) (Fig. 3). Wild-type and IL-10 knockout mice injected with sterile saline showed similar biphasic circadian \(T_b\), with low daytime and high nighttime values, indicating that endogenous IL-10 is not a critical regulator of the normal circadian rhythm of \(T_b\). Wild-type mice injected intraperitoneally at 0 h with LPS at a dose of 50 \(\mu\)g/kg developed an \(\sim 1.6^\circ\)C fever that was significantly elevated above their saline-injected controls from 1 to 5 h during the first day after injection (\(P < 0.05\)). Starting at 11 h and lasting until the end of the experimental observation period, wild-type mice injected with LPS had virtually identical \(T_b\) responses as their saline-injected controls. Thus, this low dose of LPS elicited a relatively transient (\(-5\) h) fever in the wild-type mice. IL-10 knockout mice injected with the same dose of LPS showed an exacerbated and prolonged febrile response. During the first day following injection, LPS-injected knockout mice developed a fever that was significantly elevated above their LPS-injected wild-type controls from 4 to 11 h (\(P < 0.05\)). IL-10 knockout mice manifested an additional febrile response the second day after injection from 20 to 28 h that was absent in the LPS-injected wild-type mice (\(P < 0.05\)). Subsequent to this latter time point, wild-type and IL-10 knockout mice injected with LPS maintained similar \(T_b\) profiles. Plasma IL-6 and TNF-\(\alpha\) levels in IL-10 knockout mice injected with a low dose of LPS. Plasma IL-6- and TNF-\(\alpha\)-like activity was measured in wild-type and IL-10 knockout mice at 1, 4, and 24 h after injection of a low dose of LPS (50 \(\mu\)g/kg ip) or sterile saline (equivalent volume). At all time points tested after injection of sterile saline, wild-type and IL-10 knockout mice showed low plasma IL-6-like activity that did not differ between groups (Fig. 4A). Wild-type mice injected with the low dose of LPS showed a peak elevation in plasma IL-6-like activity at 1 h postinjection compared with their saline-injected controls (Fig. 4A; \(P < 0.05\)). Although the plasma IL-6-like activity of the LPS-injected wild-type mice began to decrease by 4 h, it was still significantly elevated above the level of their saline-injected controls (\(P < 0.05\)). By 24 h, the plasma IL-6 levels of the LPS-injected wild-type mice did not differ from their saline-injected controls. IL-10 knockout mice injected with LPS showed a virtually identical rise of plasma IL-6-like activity as the wild-type mice at 1 h. However, at 4 h the LPS-injected knockout mice maintained a high level of plasma IL-6-like activity that was significantly elevated above that of their LPS-injected wild-type counterparts (ANOVA, Fisher’s \(P < 0.05\)). That is, the lack of IL-10 in the knockout mice resulted in a sustained elevation of plasma IL-6-like activity at 4 h. By 24 h, LPS-injected wild-type and knockout mice had virtually identical plasma IL-6-like activity. Figure 4B shows the plasma TNF-\(\alpha\)-like activity in response to the low dose of LPS injected into wild-type and IL-10 knockout mice. Injection of sterile saline did not induce a significant elevation in plasma TNF-\(\alpha\)-like activity in either wild-type or IL-10 knockout mice at any time point following injection. In both wild-type and IL-10 knockout mice, injection of a low dose of LPS led to a significant rise in plasma TNF-\(\alpha\)-like activity at 1 h postinjection compared with saline-injected controls (\(P < 0.05\)). However, the increase in circulating TNF-\(\alpha\) did not differ between the LPS-injected wild-type and IL-10 knockout mice at 1 h. At 4 and 24 h, plasma TNF-\(\alpha\)-like activity was no longer detectable in the LPS-injected wild-type and IL-10 knockout mice, and there were no statistically significant differences between groups. Fever in response to a high dose of LPS in IL-10 knockout mice. Figure 5 shows the \(T_b\) response of wild-type and IL-10 knockout mice intraperitoneally injected with the same high, septiclike dose of LPS (2.5 mg/kg) that was injected into SW mice shown in Fig. 2. Before injection at 0 h, all groups had virtually identical \(T_b\). The stress-induced rise in \(T_b\) at 0 and 24 h was in response to weighing and injection procedures. Due to
the increased sensitivity of the IL-10 knockout mice to this dose of LPS, several of the knockout mice did not survive through the first day following injection. Therefore, only the Tb of the survivors of the 48-h observation period are presented (wild type = 100%, IL-10 knockout = 30%; data not shown). Wild-type and IL-10 knockout mice injected with sterile saline showed the normal circadian rhythm in Tb with low daytime and high nighttime values, with virtually no difference between groups. Wild-type mice intraperitoneally injected with the high dose of LPS developed an ~0.6°C fever that was significantly elevated above their saline-injected controls from 17 to 31 h (P < 0.05). Interestingly, wild-type mice developed a different Tb profile of fever to this high dose of LPS compared with the SW mice shown in Fig. 2. IL-10 knockout mice intraperitoneally injected with the high dose of LPS did not develop a fever at any time point. Starting at 10 h, knockout mice developed a profound hypothermia compared with their LPS-injected wild-type controls that was maintained through 41 h (P < 0.05).

Changes in body weight and food intake in response to a high dose of LPS in IL-10 knockout mice. Figure 6 depicts changes in body weight and food intake of

Fig. 4. Effect of injection of a low dose of LPS (50 µg/kg ip) or sterile saline (equivalent volume) on 1-, 4-, and 24-h plasma levels of IL-6 (A) or tumor necrosis factor (TNF)α-like (B) activity in wild-type (C57BL/10) and IL-10 knockout mice. Sample sizes are indicated in parentheses. *Significant difference between LPS-injected wild-type and IL-10 knockout mice (P < 0.05).

Fig. 5. Body temperature response (1-h averages) of wild-type (C57BL/10) and IL-10 knockout mice injected with a high, septic-like dose of LPS (2.5 mg/kg ip) or sterile saline (equivalent volume). Sample sizes are indicated in parentheses. Black horizontal bars represent lights-off periods in a 12:12-h light-dark cycle. Arrowheads indicate time of injection (0 h) and weighing procedures (0 and 24 h). *Significant difference between wild-type and IL-10 knockout mice injected with LPS (P < 0.05).
wild-type and IL-10 knockout mice injected with a high dose of LPS (2.5 mg/kg ip) or sterile saline (equivalent volume). Only those IL-10 knockout mice that survived the entire 4-day weighing period are shown. Wild-type and IL-10 knockout mice responded with virtually no change in body weight (A) and food intake (B) in wild-type (C57BL/10) and IL-10 knockout mice. Sample sizes are indicated in parentheses. Arrowhead indicates time of injection. d, Days. *Significant difference between LPS-injected wild-type and IL-10 knockout mice (P < 0.05).

Fever in response to turpentine in IL-10 knockout mice. To induce a local inflammation, wild-type and IL-10 knockout mice were subcutaneously injected with turpentine (100 µl/mouse) into the left hindlimb. Control mice received a subcutaneous injection of sterile saline (equivalent volume) that did not alter the normal circadian rhythm of Tb (Fig. 7). Before injection, all groups of mice had virtually identical Tb. The peak in Tb immediately following injection at 0 h is a stress-induced rise due to the pain of the injection procedure. Wild-type and IL-10 knockout mice injected with turpentine responded with an ~1.5°C fever that did not differ between groups at any time point. This fever represented an ~1.0°C difference in Tb compared with their saline-injected controls. This fever peaked at ~16 h, at which time Tb began to defervesce. Wild-type and IL-10 knockout mice injected with turpentine resumed their normal circadian rhythm in Tb the second night after injection, at which time all four groups of mice had virtually identical Tb.

DISCUSSION

We used two different murine models to examine the role of IL-10 in the Tb response to peripherally injected LPS and turpentine. Pretreatment of SW mice with rmuIL-10 significantly attenuated the febrile response to the intraperitoneal injection of a low and a high, septiclike dose of LPS. Furthermore, the hypothermia induced by a high dose of LPS was attenuated by IL-10 pretreatment. These results support the hypothesis that, pharmacologically, IL-10 can antagonize LPS-induced hypothermia and fever in mice. To test the endogenous actions of this cytokine, we examined the Tb response of IL-10 knockout mice to the peripheral injection of a low and a high, septiclike dose of LPS. These mice lack a functional gene for IL-10 in all tissues of the body and have never been exposed to the LPS-injected wild-type controls from days 2–4 and days 2–3, respectively (P < 0.05).
endogenous actions of this cytokine during development. IL-10 knockout mice injected with a low dose of LPS developed an exacerbated and prolonged febrile response compared with their LPS-injected C57BL/10 (wild-type) counterparts. These results suggest that endogenous IL-10 functions as an antipyretic during LPS-induced fevers in mice. On the other hand, IL-10 knockout mice showed an enhanced sensitivity to the injection of a high, septiclike dose of LPS in terms of their T<sub>b</sub>, body weight, and food intake responses. Whereas wild-type mice developed a fever 24 h after a high dose of LPS, IL-10 knockout mice either succumbed to the infection (30% survival in IL-10 knockout mice vs. 100% survival in wild-type mice) or developed a prolonged hypothermia. In addition, in response to the high dose of LPS, IL-10 knockout mice showed sustained reductions in body weight and food intake compared with their wild-type counterparts. These results confirm earlier findings of a protective role of IL-10 in septic shock (8, 10, 36).

Release of IL-10 in response to LPS has been reported. For example, sepsis induced by CLP or LPS injection induced elevated plasma levels of IL-10 in mice (36). Other studies have demonstrated IL-10 production from human hypothalamus and pituitary, supporting a role in bidirectional communication between the neuroendocrine and immune systems (31). Recently, two studies showed a role for IL-10 in the febrile response to endotoxin injection. Rats treated intracerebroventricularly with IL-10 developed attenuated fevers to the peripheral injection of LPS (27). These data support a role for central IL-10 in the regulation of fever during systemic inflammation induced by a bacterial stimulus. Opp et al. (29) similarly showed central inhibitory effects of rmuIL-10 on spontaneous sleep in rats. Many of the cytokines implicated in the regulation of sleep have also been shown to regulate fever (29). An antipyretic action of IL-10 on LPS fever in human volunteers has also been reported (30).

IL-10 pretreatment, but not posttreatment, has been shown to attenuate fever and cytokine release in response to endotoxin injection in humans (30). Howard et al. (10) reported protection of mice from the lethality of endotoxemia only when mice were treated no later than 30 min after the LPS injection. In the present study, we chose to inject IL-10 into SW mice immediately before the injection of LPS. We observed an immediate (~1–3 h) inhibition of hypothermia and fever in response to the different doses of LPS. Although we did not measure plasma cytokine levels in those mice, it is presumed that the antagonism of LPS effects by IL-10 was due to reduction in the plasma concentration of IL-6 or TNF-α. Several studies have reported similar time courses of effects of IL-10 on cytokine release in vivo (30, 35).

We have shown previously in our laboratory that the hypothermic effect of LPS in mice is regulated by endogenous TNF-α. SW mice pretreated with the soluble TNF receptor or TNF antiserum developed an attenuated hypothermia in response to the same high, septiclike dose of LPS used in the present study (15). Similarly, in response to sepsis induced by CLP, TNF p55/p75 receptor knockout mice developed an attenuated hypothermia compared with their wild-type controls, whereas fever was not affected (22). Although injection of pharmacological doses of TNF-α results in fever in several species, there is little evidence that TNF-α mediates fever in mice. On the other hand, IL-6 is thought to be a key mediator of LPS fevers. Choi et al. (3) found that IL-6-deficient mice failed to develop fever to the intraperitoneal injection of a low dose of LPS (50 µg/kg ip). In our laboratory, IL-6 knockout mice injected with the same high, septiclike dose of LPS used in the present study developed normal fevers (16). Together, these data indicate that the role of these cytokines in the febrile response may differ depending on the injected dose of LPS. In the present study, we were unable to assess plasma levels of IL-6 and TNF-α in the SW mice due to a lack of availability of rmuIL-10.

In response to a low dose of LPS, IL-10 knockout mice developed an exacerbated and prolonged fever compared with their wild-type controls. To examine the mechanism(s) responsible for the exacerbated fevers in the knockout mice, we measured plasma levels of IL-6 and TNF-α-like activity at several time points following injection. Our results show a correlation between a sustained elevation in plasma IL-6 levels at 4 h postinjection in the IL-10 knockout mice and their early (i.e., 4–11 h) exacerbated febrile response. On the other hand, the late (i.e., 20–28 h) febrile response in the knockout mice did not correlate with an alteration in the plasma level of IL-6 or TNF-α. Interestingly, plasma TNF-α-like activity did not differ in the wild-type and knockout mice at any time point following LPS. These results correlate with our earlier findings of an inability to detect differences in the T<sub>b</sub> responses of wild-type and TNF p55/p75 receptor knockout mice to a low dose of LPS (21). Thus TNF-α does not appear to mediate fever to a low dose of LPS in mice, and other putative mediators of fever may be responsible for the altered febrile response of the knockout mice in the present study. The mechanism responsible for the late fever in the IL-10 knockout mice was not elucidated in this study. We have injected this same low dose of LPS (50 µg/kg ip) in several types of wild-type and knockout mice in our laboratory and never detected a fever 24 h after injection, indicating an enhanced sensitivity of the IL-10 knockout mice to this dose. Similar data were reported in a study by Berg et al. (2) in which lethality was induced at a 40-fold lower dose of LPS in IL-10 knockout compared with wild-type mice. Enhanced plasma levels of TNF-α, IL-12, IL-1α, and IFN-γ were detected in the IL-10 knockout mice by Berg et al. (2). The production of IL-12 during septic shock appears to be partially responsible for tissue injury and death (37).

In light of these reports, we must consider the possibility that alterations in the plasma levels of one or more of these cytokines, or others perhaps not yet identified, may be responsible for the altered fevers and other acute phase responses in the IL-10 knockout mice (or in the SW mice in response to IL-10 pretreatment) in response to LPS.
An additional putative mechanism of IL-10 antipyretic action could be through the release of endogenous glucocorticoids, or other putative endogenous antipyretics. IL-10 has been shown to stimulate secretion of glucocorticoids in humans (31). Glucocorticoids have been shown to inhibit the in vitro and in vivo production of IL-6 (26, 34). In addition, several studies have demonstrated exacerbated fevers in rats exposed to LPS or psychological stress following treatment with the glucocorticoid II receptor antagonist RU-38486 (25, 26). The exacerbated fevers to LPS correlated with increased plasma concentration of IL-6 (26). These effects of glucocorticoids on LPS-induced fevers have also been shown to be centrally mediated in the rat (25). Because we did not measure the level of glucocorticoids in our murine model, we are unable to assess the contribution of this steroid on the observed changes in the febrile response to LPS in our knockout mice.

In response to the high, sepsis-like dose of LPS, IL-10 knockout mice showed a virtually identical reduction in body weight and food intake as their wild-type controls on day 1. Whereas LPS-treated wild-type mice began to recover their normal body weight and food intake by day 2, IL-10 knockout mice showed a sustained reduction in body weight and a more gradual recovery of food intake through day 4. Previous studies in our laboratory have been unable to detect dramatic differences in the body weight and food intake responses of wild-type and knockout mice (e.g., IL-1β, IL-6, TNF-α/p55/p75 receptor knockout mice) to the injection of LPS or sepsis induced by CLP (17, 21, 22). Despite evidence in the literature for a role of these cytokines in the regulation of body weight and food intake during infection, our previous data suggested the development of redundancies in the control of these variables in many types of knockout mice. Because we only measured survival through day 5 post-LPS, it is unclear whether the dramatic differences detected were an indication of eventual lethality in these mice or simply a dysregulation of these variables due to the lack of endogenous IL-10 control.

IL-10 knockout mice showed a prolonged hypothermia and enhanced lethality in response to the high dose of LPS. Van der Poll et al. (35, 36) have shown a protective role of IL-10 for mice during sepsis induced by CLP, a finding that correlated with inhibition of plasma concentrations of TNF-α (35, 36). Surprisingly, anti-TNF treatment of the mice did not provide protection from the lethality of CLP, perhaps due to methodological complications related to the timing of anti-TNF treatment. Because we have shown that TNF-p55/p75 receptor knockout mice are protected from the hypothermia and lethality of sepsis induced by CLP (22), it is reasonable to hypothesize that the lethality we observed in the IL-10 knockout mice was due to enhanced plasma levels of TNF-α. This would correlate with the enhanced hypothermia observed in these mice as well (see discussion above). Unfortunately, the rapid lethality of sepsis prevented us from measuring plasma cytokine levels in the IL-10 knockout mice.

In response to the local inflammatory stimulus turpentine, wild-type and IL-10 knockout mice developed virtually identical fevers. These data suggest that either 1) endogenous IL-10 is not involved in the febrile response or 2) redundancies have developed in the regulation of fevers to turpentine in the knockout mice. To the best of our knowledge, the role of endogenous IL-10 in the T<sub>q</sub> response to turpentine in mice has not been investigated. We hypothesized that IL-10 knockout mice would develop an exacerbated febrile response to turpentine on the basis of the reported ability of IL-10 to inhibit the production of several cytokines, including IL-1β and IL-6. Although TNF-α production is also inhibited by IL-10 and has been implicated in fevers to turpentine in rats, there are currently no data to support the hypothesis that TNF-α regulates turpentine fevers in mice. In fact, we have shown virtually identical fevers in wild-type and TNF-p55/p75 receptor knockout mice injected subcutaneously with turpentine (21). However, we cannot eliminate the possibility that TNF-α is involved in turpentine fevers in wild-type mice while absence of its action is effectively compensated for in the TNF-p55/p75 receptor knockout mice. In any case, TNF-α appears to be an important regulator of fevers to a systemic inflammation induced by LPS, but not to a local inflammation in response to turpentine in mice. On the other hand, IL-1β and IL-6 are critical mediators of the febrile response to turpentine. Mice deficient in IL-1β (38), the IL-1 type I receptor (the only known signaling receptor for IL-1; Ref. 20), or IL-6 (16) are resistant to fevers, body weight loss, and anorexia induced by the same dose of turpentine used in the present study. These data are similar to those reported by Oldenburg et al. (28) and Gershewald et al. (9) demonstrating the efficacy of antibody against IL-6 or the IL-1 type I receptor, respectively, in reducing several of the acute phase responses to turpentine in mice. Although we were unable to detect any differences in the febrile response to turpentine in the wild-type and IL-10 knockout mice, this again may simply be a reflection of redundant cytokine actions in vivo in the mediation of this response. Over the past several years our laboratory has been actively involved in experimentation with several types of knockout mice for the study of thermoregulatory mechanisms in response to inflammation. Certainly, the most significant contribution of this model to the study of fever and sickness behaviors is the ability to study the effect of the elimination of a cytokine’s action from all tissues of the body. However, the caveat to this approach is that these mice have never been exposed to the cytokine’s action during their development. Therefore, it is informative to use more traditional pharmacological approaches, such as antibody administration, to confirm negative results generated with the use of gene knockout mice.

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REFERENCES


