Molecular Biology of Thermoregulation
Invited Review: Cytokine regulation of fever: studies using gene knockout mice

LISA R. LEON
Thermal and Mountain Medicine Division, United States Army Research Institute of Environmental Medicine, Natick, Massachusetts 01760-5007

Leon, Lisa R. Invited Review: Cytokine regulation of fever: studies using gene knockout mice. J Appl Physiol 92: 2648–2655, 2002; 10.1152/japplphysiol.01005.2001.—Fever is defined as a regulated rise in body temperature. The regulation of this phenomenon is accomplished by the actions of two types of endogenous cytokines, some functioning as pyrogens and others as antipyretics. Previous data obtained with the use of traditional pharmacological techniques, such as the injection of neutralizing antibodies, implicate interleukin (IL)-1 and IL-6 as endogenous pyrogens or inducers of fever. In almost all instances in which the endogenous actions of IL-1 or IL-6 are antagonized, fevers are attenuated. Other cytokines, such as tumor necrosis factor (TNF) and IL-10, are thought to act as endogenous antipyretics or inhibitors of fever. In several studies, the inhibition of TNF action has enhanced fever. Recently, mice genetically engineered to lack cytokines or their receptors in all tissues of the body have been used to examine the regulation of IL-1, IL-6, TNF, and IL-10 on fever. Data obtained with these mice shed new light on our understanding of cytokine interactions in fever and, in some instances, contradict data obtained with pharmacological methods. This review summarizes the responses of cytokine and cytokine receptor knockout mice to fevers induced by lipopolysaccharide, turpentine, and sepsis.

interleukin-1; interleukin-6; interleukin-10; tumor necrosis factor; thermoregulation

FEVER IS A COMMON RESPONSE to infection, inflammation, and trauma. Clinically, fever is characterized as a rise in body temperature ($T_b$) above the normal range, and it is often met with attempts to eliminate it. We now understand that fever is a complex physiological response that is aimed at facilitating survival of the host. Liebermeister (25) was the first to accurately define fever as a regulated elevation in the thermal set point when he observed a return of febrile individuals to a previous level of $T_b$ after experimental warming or cooling. This distinguishes fever from hyperthermia, which represents an unregulated rise in $T_b$ due to an internal (e.g., exercise) or external (e.g., sauna) heat load that is not triggered by an increased set point.

Fever is the result of communication between the peripheral immune system and the brain. After contact with a pathogen or an inflammatory stimulus, macrophages and other immune cells are activated to release cytokines. Although the pathways used for signaling to the brain are still not clear, cytokines are thought to either cross the blood-brain barrier by saturated transport mechanisms (3) or interact with peripheral neural components of the immune system (e.g., vagus nerve; Ref. 42) to signal the hypothalamus to increase the thermal set point. This subsequently initiates the effector mechanisms that serve to increase $T_b$. Thus, in response to an infectious or inflammatory agent, an organism increases heat production and decreases heat loss in an attempt to actively elevate $T_b$ to match the increased thermal set point. In humans, physiological and behavioral mechanisms used to increase heat production include shivering and the drinking of hot flu-
ids, whereas heat loss is reduced by peripheral vasoconstriction and a reduction of body surface area (e.g., assuming a fetal position).

There are two types of cytokines responsible for the generation of fever. Endogenous pyrogens are cytokines that induce fever and include interleukin (IL)-1, IL-6, IL-8, macrophage-inflammatory protein-1β (MIP-1β), and interferon-γ. The other types of cytokines are endogenous antipyretics, which limit the magnitude and duration of fever and include such substances as IL-10, arginine vasopressin (AVP), α-melanocyte-stimulating hormone (α-MSH), and glucocorticoids. Although AVP, α-MSH, and glucocorticoids are not true cytokines, they still possess endogenous antipyretic properties. Other substances, such as tumor necrosis factor-α (TNF-α), have been shown to have pyrogenic and antipyretic properties, depending on the experimental conditions. Ultimately, it is the sum of the interactions of pyrogenic and antipyretic cytokines that is responsible for the height and duration of a fever response. These cytokine interactions are dependent on a variety of factors, including the species, the model of infection, and the strength of the fever-inducing stimulus. Figure 1 provides a general pathway of fever regulation involving endogenous pyrogens and antipyretics.

Traditionally, pharmacological techniques have been used to characterize the role of cytokines in the regulation of fever. For example, cytokines have been peripherally or centrally injected into several experimental animals and shown to induce fever. However, this effect does not warrant the conclusion that the substance functions endogenously as a pyrogen, since many substances may induce fever when injected (e.g., turpentine). Therefore, the injection of neutralizing agents has been used to examine the effect of a cytokine’s antagonism on a fever response induced by a pathogen, such as lipopolysaccharide (LPS; a cell wall component of gram-negative bacteria). The advantage of this technique is that specific anatomic regions implicated in fever regulation can be targeted with the microinjection of a cytokine antagonist. The disadvantage to this method is the uncertainty as to whether the cytokine’s action has been neutralized in all areas of the body that may be important for the regulation of the response. The diffusion properties and the half-life of most injected substances are not measured, and this also often makes interpretation difficult.

The recent development of gene knockout technology provides a valuable tool to extend our investigations into the role of cytokines in fever. These mice lack functional genes for cytokines or cytokine receptors in all tissues of the body. Thus one can study the effect of the absence of a cytokine’s action on the fever response and compare the results with those obtained with the more traditional pharmacological approaches. In addition, one can examine the effect of the absence of one cytokine’s action on the production and regulation of other cytokines involved in the fever pathway. Of course, there are limitations to the gene knockout approach as well. One must assume that the ability of a particular gene knockout mouse to survive to adulthood is an indication of other cytokines possibly compensating for the loss of the deleted cytokine’s action. Although on the one hand this makes the interpretation of negative results from knockout mice problematic, one may also consider this a window of opportunity to further understand the flexibility that is inherent in the physiological networks that regulate fever (and other physiological processes).

The use of knockout mice to study fever is widely used today and has provided important insight into the complex network of cytokine interactions that comprise fever pathways. With this in mind, I will review the current data on cytokines and fever that have been generated with the use of the gene knockout model and, where appropriate, compare and contrast those findings with the results obtained using cytokines and their antagonists. The focus of this review will be mainly on fevers induced by LPS, turpentine, and sepsis.
INTERLEUKIN-1

The IL-1 family of ligands consists of IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1ra). IL-1β is thought to be the primary secreted form of IL-1, whereas IL-1α is thought to remain mainly cell associated (2). The IL-1ra is an inhibitory protein that binds to IL-1 receptors without inducing an intracellular signal, thus acting as a true antagonist of IL-1-inducible effects (6). The two receptors for IL-1 have been cloned and are classified as the type I and type II receptors (38). The type I receptor is thought to be the only signaling receptor for IL-1, whereas the type II receptor is thought to serve as a negative regulator of IL-1 functions (37).

In response to a low dose of LPS, rats and mice develop an ~1°C fever that lasts 4–8 h after injection. Peripheral injection of neutralizing antiserum to IL-1β or central microinjection of neutralizing antibodies to IL-1β or IL-1ra attenuates, but does not eliminate, fevers to a low dose of LPS in rats (14, 26). There are no studies that use IL-1 neutralizing agents that have demonstrated complete inhibition of fever induced by LPS. Is this a consequence of incomplete inhibition of IL-1 actions with the neutralizing agents or does it merely indicate that IL-1 is only one mediator in a complex network of regulation? The latter interpretation appears to be the correct one.

Two studies have examined the fever response of IL-1β knockout mice to the peripheral injection of LPS. In response to a low dose of LPS (100 μg/kg ip), Kozak et al. (17) found virtually identical fevers in IL-1β knockout and wild-type mice. On the other hand, Altheim et al. (1) reported enhanced fevers in IL-1β knockout mice injected with the same dose of LPS. Although these data are contradictory, they support the hypothesis that IL-1β is not essential for the production of fever to a mild dose of LPS. On the other hand, in response to a high, septiclike dose of LPS, fevers were either slightly attenuated (Ref. 17; 2.5 mg/kg ip) or enhanced (Ref. 1; 5 mg/kg ip) in the IL-1β knockout mice. Reasons for discrepancies in the fever responses of these two studies are not apparent but may reflect differences in LPS serotype, LPS dose (see high doses), or the use of different generations and genetic backgrounds of the knockout mice. Although IL-1β is thought to induce fever through the production of IL-6, neither knockout study reported an alteration in LPS-induced plasma levels of IL-6 in the IL-1β knockout mice.

The caveat to using IL-1β knockout mice to study the fever response is that IL-1α is still present in these animals and may be able to bind to the IL-1 type I receptor (IL-1r1) to induce fever, thus compensating for the lack of IL-1β. To address this issue, we used IL-1r1 knockout mice to examine the fever response to LPS. Because the IL-1r1 is the only known signaling receptor for IL-1, neither IL-1α nor IL-1β is able to induce a biological signal in the IL-1r1 knockout mice. We observed virtually identical fevers in the IL-1r1 knockout and wild-type mice in response to both a low (50 μg/kg ip) and high (2.5 mg/kg ip) dose of LPS (21). Others reported a slightly attenuated fever response to a low dose (50 μg/kg ip) of LPS in IL-1r1 knockout mice (19). Despite the discrepancies between the studies, the data from IL-1β and IL-1r1 knockout mice support the hypothesis that IL-1 is not an essential mediator of fever and that cytokine redundancy may be responsible for the lack of effect in some studies.

Turpentine is a model of local inflammation that induces a robust acute phase response (APR) consisting of fever, anorexia, cachexia, and acute phase protein production. Pretreatment of mice with a monoclonal antibody to the IL-1ra attenuates several of the APR responses to turpentine (10, 31). Fever was not measured in those studies but has been examined with the use of both IL-1β and IL-1r1 knockout mice. Subcutaneous injection of turpentine into mice induces a ~2–3°C fever that lasts ~40 h. Fever was shown to be completely abolished in IL-1β (12, 43) and IL-1r1 (21) knockout mice after injection of turpentine. This is the first reported elimination of fever after antagonism of endogenous IL-1 action. Wild-type mice injected with turpentine showed a significant increase in plasma IL-6 levels that were virtually absent in the IL-1β knockout mice (43). In fact, all sickness behaviors associated with the local inflammatory response were abolished in the IL-1β and IL-1r1 knockout mice, including the profound anorexia and weight loss. These data support the hypothesis that the fever and sickness behaviors induced by turpentine are mediated by IL-1β and its type I receptor.

Overall, data obtained with the IL-1β and IL-1r1 knockout mice are consistent with the hypothesis that IL-1 plays only a minor role in fever induced by LPS but is an essential mediator of fever induced by turpentine. Furthermore, IL-6 production in response to turpentine appears to be dependent on the presence of IL-1β, whereas LPS is able to induce IL-6 independently of IL-1β.

INTERLEUKIN-6

Several lines of evidence support the hypothesis that IL-6 is an important mediator of fever. First, plasma and brain concentrations of IL-6 have been shown to rise in response to peripheral injections of LPS and turpentine and in patients with sepsis (10, 14, 32). Second, administration of IL-6 into several species induces fever, although the physiological relevance of the injected doses is uncertain (20, 34). Third, fever in response to the peripheral injection of LPS is attenuated after the injection of an IL-6 antibody into the cerebral ventricle of rats (33). However, experiments that used blocking antibodies to IL-6 have been difficult to interpret due to the reported increase in IL-6 half-life and circulating concentration when the antibody complexes with the cytokine (10, 40).

IL-6 knockout mice have been used to study the role of this cytokine in the fever response to several types of stimuli. IL-6 knockout mice injected with a low dose (50 μg/kg ip) of LPS were resistant to fever (4, 17),
whereas they developed fevers similar to wild-type mice after a high dose (2.5 mg/kg ip) of LPS (17). These data support the hypothesis that the role of IL-6 in the regulation of LPS fever depends on the dose. A high, septiclike dose of LPS may induce the production of additional endogenous pyrogens, which can compensate for a lack of IL-6 in the knockout mice. IL-6 is thought to act centrally to induce fever, and Chai et al. (4) reported the ability of centrally injected IL-6 to induce fever in IL-6 knockout mice, suggesting normal receptor functioning in those mice. These results suggest that other cytokines, including IL-11 and leukemia inhibitory factor, that are known to signal through the IL-6 gp130 receptor may be responsible for fever generation in the IL-6 knockout mice. The alternative to this hypothesis is that other cytokines that do not use the IL-6 gp130 receptor, such as IL-8 or MIP-1β, may produce fever in the IL-6 knockout mice as a compensatory response.

Although the injection of LPS is a reasonable model of bacterial infection, the induction of sepsis by the surgical procedure of cecal ligation and puncture (CLP) more closely simulates a clinically relevant bacterial infection. To induce experimental sepsis with CLP, the cecum is exposed and perforated and the intestinal contents are expressed into the peritoneal cavity. CLP induces a reproducible thermoregulatory response that is characterized by an initial hypothermia and a prolonged fever during the 36 h after surgery. Mice deficient in IL-6 developed the initial hypothermia but did not develop fever during sepsis induced by CLP (24). Surprisingly, mortality was significantly enhanced in IL-6 knockout mice despite reports in the literature of high plasma levels of IL-6 negatively correlating with survival. This suggests a permissive action of low levels of IL-6 for survival from sepsis. The difference in the response of IL-6 knockout mice to the high dose of injected LPS and the CLP model may reflect the fact that LPS is a nonproliferating component of gram-negative bacteria, whereas sepsis is the response to proliferating gram-negative and gram-positive bacteria originating in the gut.

As was found for IL-1β and IL-1r1 knockout mice, the injection of turpentine into IL-6 knockout mice did not induce fever, anorexia, or body weight loss (17). Thus IL-6 is essential for the production of fever in response to turpentine. It is unknown whether IL-1β was produced in response to turpentine in the IL-6 knockout mice because it was not measured. Fattori et al. (8) showed an increase in IL-1α levels in IL-6 knockout mice injected with turpentine, but others have shown IL-1α knockout mice to develop virtually identical fevers as wild-type mice to the injection of turpentine (12). Thus the role of IL-1α in fever to turpentine is still unresolved.

The data obtained with IL-6 knockout mice support the hypothesis that IL-6 is not essential for all fever responses to bacterial models of infection but is critical for the development of fever, and other APRs, to turpentine.

TUMOR NECROSIS FACTOR

TNF is a proinflammatory cytokine that exists in two forms, TNF-α and TNF-β. Both forms induce a number of similar biological effects, but TNF-α is thought to be the main regulator of fever. TNF interacts with two distinct transmembrane signaling receptors, termed the p55 (type I) and p75 (type II) receptors, to induce its biological effects (35, 39). Soluble TNF receptors (sTNFR) also exist endogenously and act to neutralize the biological activity of TNF (7).

TNF has been implicated as an endogenous pyrogen due to its ability to induce fever after injection into several species (13, 29). This effect of TNF likely represents a pharmacological, rather than physiological, effect of the cytokine. The use of neutralizing agents to TNF has demonstrated both pyrogenic and antipyretic activity of this cytokine. In favor of an antipyretic action of TNF-α, Klir et al. (15) reported attenuated fevers to LPS when rats were treated with a nonpyrogenic dose of TNF-α (a dose that had no effect on Tb alone) and an exacerbated fever when the rats were treated with the soluble receptor, sTNFR. In mice, the early hypothermic phase of fever to a high dose of LPS was exacerbated by TNF-α treatment, whereas the sTNFR attenuated hypothermia (16). Both of these results are consistent with TNF action involving a lowering of Tb. Studies supporting a pyrogenic role for TNF in fever include attenuated fever to turpentine in rats pretreated with a TNF neutralizing antiserum and attenuated LPS fever in rabbits treated with a TNF antibody (5, 28).

Data obtained with knockout mice lacking both the TNF p55 and p75 receptors (TNFR knockout mice) support the hypothesis that endogenous TNF either has little role in fever or functions as an endogenous antipyretic, depending on the experimental conditions. Injection of a low dose (50 μg/kg ip) of LPS resulted in no difference in the fever response between TNFR knockout mice and their wild-type controls (22). This finding is not particularly surprising in that lower doses of LPS are not predicted to elicit as profound, and possibly detrimental, rises in Tb that require antagonism by a putative endogenous antipyretic, such as TNF. This is especially relevant in light of the increased resistance of mice to LPS toxicity (i.e., mice require much higher doses of LPS to elicit a profound fever compared with rats). Injection of a high dose (2.5 mg/kg ip) of LPS led to larger fevers in TNFR knockout mice after a high dose (2.5 mg/kg ip) of LPS (17). These data obtained with knockout mice support the hypothesis that the role of IL-6 in the regulation of LPS fever depends on the dose. A high, septiclike dose of LPS may induce the production of additional endogenous pyrogens, which can compensate for a lack of IL-6 in the knockout mice. IL-6 is thought to act centrally to induce fever, and Chai et al. (4) reported the ability of centrally injected IL-6 to induce fever in IL-6 knockout mice, suggesting normal receptor functioning in those mice. These results suggest that other cytokines, including IL-11 and leukemia inhibitory factor, that are known to signal through the IL-6 gp130 receptor may be responsible for fever generation in the IL-6 knockout mice. The alternative to this hypothesis is that other cytokines that do not use the IL-6 gp130 receptor, such as IL-8 or MIP-1β, may produce fever in the IL-6 knockout mice as a compensatory response.

Although the injection of LPS is a reasonable model of bacterial infection, the induction of sepsis by the surgical procedure of cecal ligation and puncture (CLP) more closely simulates a clinically relevant bacterial infection. To induce experimental sepsis with CLP, the cecum is exposed and perforated and the intestinal contents are expressed into the peritoneal cavity. CLP induces a reproducible thermoregulatory response that is characterized by an initial hypothermia and a prolonged fever during the 36 h after surgery. Mice deficient in IL-6 developed the initial hypothermia but did not develop fever during sepsis induced by CLP (24). Surprisingly, mortality was significantly enhanced in IL-6 knockout mice despite reports in the literature of high plasma levels of IL-6 negatively correlating with survival. This suggests a permissive action of low levels of IL-6 for survival from sepsis. The difference in the response of IL-6 knockout mice to the high dose of injected LPS and the CLP model may reflect the fact that LPS is a nonproliferating component of gram-negative bacteria, whereas sepsis is the response to proliferating gram-negative and gram-positive bacteria originating in the gut.

As was found for IL-1β and IL-1r1 knockout mice, the injection of turpentine into IL-6 knockout mice did not induce fever, anorexia, or body weight loss (17). Thus IL-6 is essential for the production of fever in response to turpentine. It is unknown whether IL-1β was produced in response to turpentine in the IL-6 knockout mice because it was not measured. Fattori et al. (8) showed an increase in IL-1α levels in IL-6 knockout mice injected with turpentine, but others have shown IL-1α knockout mice to develop virtually identical fevers as wild-type mice to the injection of turpentine (12). Thus the role of IL-1α in fever to turpentine is still unresolved.

The data obtained with IL-6 knockout mice support the hypothesis that IL-6 is not essential for all fever responses to bacterial models of infection but is critical for the development of fever, and other APRs, to turpentine.

TUMOR NECROSIS FACTOR

TNF is a proinflammatory cytokine that exists in two forms, TNF-α and TNF-β. Both forms induce a number of similar biological effects, but TNF-α is thought to be the main regulator of fever. TNF interacts with two distinct transmembrane signaling receptors, termed the p55 (type I) and p75 (type II) receptors, to induce its biological effects (35, 39). Soluble TNF receptors (sTNFR) also exist endogenously and act to neutralize the biological activity of TNF (7).

TNF has been implicated as an endogenous pyrogen due to its ability to induce fever after injection into several species (13, 29). This effect of TNF likely represents a pharmacological, rather than physiological, effect of the cytokine. The use of neutralizing agents to TNF has demonstrated both pyrogenic and antipyretic activity of this cytokine. In favor of an antipyretic action of TNF-α, Klir et al. (15) reported attenuated fevers to LPS when rats were treated with a nonpyrogenic dose of TNF-α (a dose that had no effect on Tb alone) and an exacerbated fever when the rats were treated with the soluble receptor, sTNFR. In mice, the early hypothermic phase of fever to a high dose of LPS was exacerbated by TNF-α treatment, whereas the sTNFR attenuated hypothermia (16). Both of these results are consistent with TNF action involving a lowering of Tb. Studies supporting a pyrogenic role for TNF in fever include attenuated fever to turpentine in rats pretreated with a TNF neutralizing antiserum and attenuated LPS fever in rabbits treated with a TNF antibody (5, 28).

Data obtained with knockout mice lacking both the TNF p55 and p75 receptors (TNFR knockout mice) support the hypothesis that endogenous TNF either has little role in fever or functions as an endogenous antipyretic, depending on the experimental conditions. Injection of a low dose (50 μg/kg ip) of LPS resulted in no difference in the fever response between TNFR knockout mice and their wild-type controls (22). This finding is not particularly surprising in that lower doses of LPS are not predicted to elicit as profound, and possibly detrimental, rises in Tb that require antagonism by a putative endogenous antipyretic, such as TNF. This is especially relevant in light of the increased resistance of mice to LPS toxicity (i.e., mice require much higher doses of LPS to elicit a profound fever compared with rats). Injection of a high dose (2.5 mg/kg ip) of LPS led to larger fevers in TNFR knockout mice during the early phase (3–15 h) of the febrile response. The enhanced fever in the TNFR knockout mice correlated with decreased plasma levels of endogenous IL-10 at 8 h after LPS injection (11), corresponding to the time of the maximal difference in fever between the TNFR knockout and wild-type mice. These data support the hypothesis that the antipyretic action of TNF during LPS fever is mediated by endogenous IL-10 (see interleukin-10, below).

The biphasic Tb response to CLP was differentially altered in the TNFR knockout mice. TNFR knockout mice showed attenuated hypothermia but developed a
virtually identical fever as that shown in wild-type mice after CLP (24). These data extend the findings by Kozak et al. (16) to implicate further endogenous TNF in the natural lowering of $T_b$ during the early phase of the fever response. Survival was significantly enhanced in TNFR knockout mice, which corresponds to the reported protective effect of TNF inhibition on the lethality of sepsis in several species (27, 36). These data support the hypothesis that endogenous TNF is responsible for the initial hypothermia and lethality to a bacterial infection in mice. An unaffected fever response in the TNFR knockout mice treated with CLP most likely reflects the late time course for fever development in response to this type of infection. TNF is usually detectable at early time points ($\sim 1-2$ h), but fever did not develop until $\sim 26$ h after CLP. It is likely that the endogenous effects of TNF had dissipated by this time point such that the absence of TNF action in the knockout mice was without effect.

Turpentine induced virtually identical fevers in TNFR knockout and wild-type mice (22). In fact, none of the APRs, including anorexia or cachexia, was altered in TNFR knockout mice. Data obtained with TNFR knockout mice do not support the hypothesis that TNF regulates the APR to turpentine, but cytokine redundancy may have developed in these mice.

In summary, studies that used TNFR knockout mice support an endogenous antipyretic role for TNF in fever. Given a strong systemic stimulus, such as a high dose of LPS or sepsis, TNF functions to lower $T_b$, thus attenuating fever or producing hypothermia, respectively. TNF does not appear to function in the regulation of a less robust fever, such as that induced by a low dose of LPS. On the other hand, there is little evidence to suggest that endogenous TNF mediates fever to turpentine in mice.

**INTERLEUKIN-10**

IL-10 is a protein product of T helper 2 subset cells that was originally described as a “cytokine synthesis inhibitory factor.” IL-10 inhibits the LPS-induced production of many cytokines implicated in fever, including IL-1$\beta$, IL-6, and TNF-\(\alpha\) (9).

On the basis of the ability of IL-10 to inhibit the production of pyrogenic and antipyretic cytokines, many studies have been designed to examine the role of IL-10 as a regulator of fever. Pharmacologically, IL-10 functions as an antipyretic. In rats, the central injection of IL-10 inhibits fever to a peripheral injection of LPS (30). Similarly, pretreatment of mice with an intraperitoneal injection of IL-10 significantly attenuates the fever response to a low (100 \(\mu g/kg\) ip) and high (2.5 mg/kg ip) dose of LPS (23). To examine the endogenous role of IL-10 in fever, we injected IL-10 knockout mice with LPS and turpentine. IL-10 knockout mice injected with a low dose of LPS (50 \(\mu g/kg\) ip) developed an exacerbated and prolonged fever response (23). Wild-type mice developed the typical $\sim 4-8$ h fever in response to this low dose of LPS, whereas the IL-10 knockout mice showed a fever response both the first and second day after injection. To examine the mechanism(s) responsible for the exacerbated fevers in the knockout mice, we measured plasma levels of IL-6 and TNF-\(\alpha\) at 4 and 24 h postinjection. These time points corresponded to the time of maximal fevers in the IL-10 knockout mice. Enhanced plasma levels of IL-6 in the knockout mice at 4 h postinjection correlated with the exacerbated fever at this time point, whereas the second-day fever did not correlate with changes in plasma levels of IL-6 or TNF-\(\alpha\). These data support the hypothesis that IL-10 has endogenous antipyretic action during LPS-induced fever due to its ability to inhibit the production of endogenous IL-6. Interestingly, these data correspond to the lack of a fever response to a low dose of LPS in IL-6 knockout mice, again implicating IL-6 as a mediator of fever in this experimental model (see Ref. 17 and INTERLEUKIN-6, above). On the other hand, the lack of an alteration of plasma TNF-\(\alpha\) levels in response to the low dose of LPS correlates with the inability to detect differences in fever to this same dose of LPS in TNFR knockout mice (see TUMOR NECROSIS FACTOR, above). The mechanism for the prolonged, second-day fever response in the IL-10 knockout mice is currently unresolved.

Fever in response to a high dose (2.5 mg/kg ip) of LPS resulted in a profound hypothermia in the IL-10 knockout mice that lasted $\sim 41$ h after injection (23). Mortality was significantly enhanced in the knockout mice such that only three animals survived the injection. These data confirm earlier findings of a protective role of IL-10 in septic shock (41).

Data obtained in IL-10 knockout mice support the hypothesis that IL-10 functions as an endogenous antipyretic during LPS fever due to its inhibitory actions on the production of IL-6. IL-10 does not appear to play a role in the regulation of fever to a local inflammation induced by turpentine because IL-10 knockout mice and wild-type mice developed virtually identical fevers (23).

**CONCLUSIONS**

Fever is a complex physiological process. The generation of fever is accomplished by the interaction of multiple endogenous mediators, some acting as pyrogens and others as antipyretics. With continuing research on the role of numerous cytokines in fever, our understanding of the regulation of this phenomenon is constantly evolving.

Studies that used traditional pharmacological techniques have supported the hypothesis that IL-1$\beta$ and IL-6 are important mediators of fever to LPS. In almost all instances when IL-1$\beta$ is pharmacologically antagonized (e.g., injection of an antibody), fever in response to LPS is attenuated. Furthermore, this attenuation is commonly correlated with a reduction in IL-6 levels, suggesting that IL-1 induces fever through the production of IL-6. One criticism of the use of antagonists is that it is never clear whether the injected substance neutralizes the action of the cytokine in all areas of the body important for the regulation of the physiological...
response, in this case fever. Furthermore, in many instances, high-quality antagonists for a cytokine are not commercially available. The use of gene knockout mice to study fever circumvents these technological limitations and allows the effect of the elimination of a cytokine’s action on fever to be examined.

Data obtained on fever from gene knockout mice have revealed a redundancy in cytokine action that was not previously recognized. The pathways depicted in Fig. 2 summarize the data obtained with gene knockout mice to study fever in response to LPS and turpentine. Of note, the oversimplification of the fever pathway to these models of inflammation is only meant to represent the essential components as identified in gene knockout mice. Undoubtedly, other mediators are involved in fever regulation. To develop fever to a low dose of LPS, IL-6 and IL-10 are required. That is, IL-6 knockout mice are resistant to fever (17), whereas IL-10 knockout mice develop exacerbated fevers that correlate with enhanced plasma levels of IL-6 (23). Thus, endogenous IL-6 functions as a pyrogen, whereas IL-10 inhibits the production of IL-6 to act as an endogenous antipyretic. TNF and IL-1 are not essential mediators of fever to this dose of LPS, because TNFR, IL-1β, and IL-1r1 knockout mice developed virtually identical fevers as their wild-type controls (17, 21, 22). Figure 2B shows that a high dose of LPS or sepsis induces a different pattern of cytokine regulation. In this model of fever, IL-1 interacts with the IL-1r1 to produce IL-6 and fever. The inclusion of IL-1 in this model is based on the attenuated fever response in IL-1β knockout mice (17). However, there are instances in which IL-1 action is absent yet fevers are normal, such as in the IL-1r1 knockout mice (21). Under these conditions, fevers are likely due to cytokine redundancy possibly via the direct production of IL-6 by LPS or the interaction of other cytokines with the IL-6/gp130 receptor complex (not shown). This latter explanation is likely responsible for the normal fevers to a high dose of LPS in IL-6 knockout mice as well, since they still maintain functional gp130 receptor complexes. The inclusion of IL-6 in this model of fever reflects the contribution of this cytokine to fever development during sepsis induced by CLP. IL-6 knockout mice did not develop fever in response to sepsis (24). The difference between fever in response to a high dose of LPS and sepsis likely reflects the presence of proliferating bacteria in the sepsis model. To inhibit fevers that occur in response to a high dose of LPS or sepsis, TNF interacts with its p55/p75 receptors. TNFR knockout mice develop exacerbated fevers in response to a high dose of LPS (22). This antipyretic action of TNF may be due to the inhibition of IL-6 production by IL-10, although this hypothesis is difficult to test because of the enhanced sensitivity of IL-10 knockout mice to sepsis mortality (23). TNFR knockout mice were also resistant to sepsis-induced hypothermia, further implicating endogenous TNF in the natural lowering of T_{b}.

The predominant feature of the LPS fever model(s) is the presence of multiple pathways of cytokine production. The presence of parallel pathways of cytokine release makes the regulation of fever to LPS conducive to redundancy. This is in direct contrast to the type of pathway responsible for fever in response to turpentine. As shown in Fig. 2B, data obtained with gene knockout mice support a serial pathway of cytokine production in the regulation of turpentine fever such that the elimination of any component in the pathway eliminates the response. Turpentine induces a serial pathway of fever regulation. IL-1 interacts with the IL-1r1 to induce IL-6 and produce fever and function as an endogenous pyrogen in this model of fever. In some instances, LPS can produce fever independently of IL-1 by directly stimulating the production of IL-6. TNF interacts with its p55/p75 receptors to inhibit fever and act as an endogenous antipyretic. Turpentine induces a serial pathway of fever regulation. IL-1 interacts with the IL-1r1 to induce IL-6 and produce fever. IL-1 and IL-6 are essential in this fever model. IL-1β knockout mice do not develop fever or a rise in plasma IL-6 to turpentine. IL-1r1 and IL-6 knockout mice also do not develop fever in response to turpentine. TNF and IL-10 are not involved in turpentine fever.

**Fig. 2.** Summary of fever data obtained with gene knockout mice. Solid arrows represent stimulatory pathways; dashed arrows represent inhibitory pathways. A: IL-6 and IL-10 were shown in gene knockout mice to be essential for fevers to a low dose of lipopolysaccharide (LPS). IL-6 knockout mice do not develop fever in response to a low dose of LPS, thus implicating IL-6 as an essential pyrogen in this fever model. IL-10 knockout mice respond with exacerbated fever that correlates with enhanced plasma IL-6 levels. Thus, IL-10 functions as an endogenous antipyretic in this fever model through the inhibition of IL-6 levels. IL-1β, IL-1 type I receptor (IL-1r1), and TNF p55 and p75 receptor (TNFR) knockout mice developed fever virtually identical to wild-type mice, implicating these cytokines as nonessential mediators of fever to a low dose of LPS. B: A high dose of LPS induces the release of IL-1, IL-6, and TNF independently. IL-1 interacts with IL-1r1 to induce IL-6 to produce fever and function as an endogenous pyrogen in this model of fever. In some instances, LPS can produce fever independently of IL-1 by directly stimulating the production of IL-6. TNF interacts with its p55/p75 receptors to inhibit fever and act as an endogenous antipyretic. Turpentine induces a serial pathway of fever regulation. IL-1 interacts with the IL-1r1 to induce IL-6 and produce fever. IL-1 and IL-6 are essential in this fever model. IL-1β knockout mice do not develop fever or a rise in plasma IL-6 to turpentine. IL-1r1 and IL-6 knockout mice also do not develop fever in response to turpentine. TNF and IL-10 are not involved in turpentine fever.
injection (43). Similarly, IL-1rt1 and IL-6 knockout mice are resistant to turpentine fever (18, 21). Redundancy is probably not a feature of cytokine regulation of turpentine fever. Although it can be argued that the normal turpentine fever in TNFR and IL-10 knockout mice is the result of cytokine redundancy, there are few data in the literature to suggest that TNF or IL-10 regulates turpentine fever in mice.

Gene knockout mice provide a unique animal model to study thermoregulatory processes. Several studies have utilized cytokine knockout mice in the study of fever to different stimuli, including LPS, turpentine, and sepsis. It has always been assumed in these studies that the lack of a fever response is the direct result of the missing cytokine's action in these mice. However, an equally plausible explanation for an absent fever response may be the inability of the knockout mouse to develop a fever because of a direct effect of the genetic manipulation on the thermoregulatory controlling center. A critical question to be addressed in any study that uses these mice is whether they are physiologically able to thermoregulate in response to a stimulus. For example, is the absence of fever the result of a deficiency in the afferent pathway (e.g., cytokine signaling) or the efferent pathway (e.g., shivering, vasocostriction) of thermoregulation? If a particular gene knockout mouse were placed in a thermal gradient to use behavioral thermoregulation to develop a fever, would it show the same fever deficiency to a particular stimulus as it does in the home cage? Although it is assumed that the fever response will be independent of ambient temperature, it is unclear whether the fever responses observed in the gene knockout mice are a direct reflection of alteration in the hypothalamic thermoregulatory set point. However, in most published studies that used cytokine knockout mice, there have been observations of a normal fever response to one stimulus and an altered or absent fever response to another stimulus. These data alone support the hypothesis that the fever-generating mechanisms in these mice are intact and the absence of a response is specific to the missing gene product.

One of the consistent characteristics of gene knockout mice is the absence of gross developmental or phenotypic abnormalities. Circadian rhythms of Tb, reproductive status, and body weight regulation appear to be unaffected in many knockout mice. These observations suggest a redundancy in many of the cytokine actions that have been implicated in developmental processes and normal physiological functioning. Redundancy in cytokine action has been the driving force behind the argument against the use of gene knockout technology to study physiological processes. However, as outlined in this review, there are many circumstances in the same gene knockout mice in which the endogenous actions of a cytokine being studied are compensated in response to one stimulus (e.g., LPS) but not to another (e.g., turpentine). The absence of redundancy under some circumstances may be viewed as an indication of the evolutionary significance of that cytokine's action in the regulation of the response. Our understanding of the complex interaction of cytokines is only enhanced by the use of both pharmacological techniques and gene knockout mice, and these techniques should continue to be used in conjunction to expand our understanding of Tb regulation.

The views, opinions, and/or findings contained in this manuscript are those of the author and should not be construed as an official Department of Army position, policy, or decision.

REFERENCES


15. Klir JJ, McClellan JL, Kozak W, Szelenyi Z, Wong GHW, and Kluger MJ. Systemic, but not central, administration of


