

HYPOTHERMIA IN SYSTEMIC INFLAMMATION: ROLE OF CYTOKINES

Lisa R. Leon

Thermal and Mountain Medicine Division, US Army Research Institute of Environmental Medicine, Natick, Massachusetts

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Experimental Models of Systemic Inflammation
4. Tumor Necrosis Factor
5. Interleukin-10
6. Interleukin-6
7. Interleukin-1beta
8. Interferon-gamma
9. Future Directions
10. Acknowledgements
11. References

1. ABSTRACT

Hypothermia is a thermoregulatory response to systemic inflammation that is often regarded as maladaptive to the host. However, rodents show regulated hypothermia (that is, a selection of cool ambient temperature) during systemic inflammation that correlates with enhanced survival, supporting an adaptive value to this response. The mechanisms regulating hypothermia are not fully understood, but cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukins (ILs) and interferon-gamma have been shown to induce or modulate hypothermia. A review of the literature suggests that TNF-alpha functions as an endogenous cryogen (i.e., induces hypothermia), whereas IL-10 modulates TNF-alpha production and/or release as a mechanism of hypothermia attenuation. IL-1beta and IL-6 are typically regarded as endogenous pyrogens, but may induce hypothermia during viral and bacterial inflammation. A role for endogenous IFN-gamma in hypothermia has not been demonstrated, but injection of this cytokine potentiates hypothermia through augmented production of other cytokines. It is clear that additional research is required in this area. Suggested areas for future research include a determination of the final mediator of hypothermia and its specific anatomical site of action as well as the role of cytokines in the regulation of hypothermia under non-inflammatory conditions.

2. INTRODUCTION

The thermoregulatory response to systemic inflammation is often biphasic in nature, consisting of hypothermia preceding the development of fever. While fever is recognized as an adaptive physiological response aimed at facilitating host survival (1), hypothermia is often regarded as maladaptive, correlating with poor clinical outcome (2, 3). The mortality of hypothermic sepsis patients has been reported as twice that of febrile patients (2, 4), although mechanisms for a detrimental effect of hypothermia have not been identified. Thus, the

adaptive value of this thermoregulatory response during systemic inflammation remains in question.

Regulated hypothermia (also referred to as anapyrexia) represents the condition in which core body temperature (T_c) is lowered in response to a decrease in the thermoregulatory setpoint. A reduction in setpoint evokes a variety of effector mechanisms that promote heat loss, diminish heat production and lower T_c . Several species develop regulated hypothermia in response to food restriction (5), hypoglycemia (6, 7), hypoxia (8, 9), hemorrhage (10), dehydration (11) and infection (12-16). In instances in which regulated hypothermia is prevented, survival is decreased (e.g., 6, 8) supporting a beneficial effect of this response. It is likely that regulated hypothermia functions as a survival mechanism to diminish metabolic demands during conditions of severe energy depletion, tissue injury or infection. The widespread occurrence of regulated hypothermia argues in favor of this hypothesis.

It has been suggested that hypothermia is a nonspecific, secondary response to systemic inflammation that does not represent a regulated phenomenon initiated by a decrease in the thermal setpoint (17). While the former scenario likely exists for extreme cases of circulatory collapse/shock, several studies have shown hypothermia to be a regulated response. It was recognized in bumblebees that infected workers spend significantly more time outside of the nest, experiencing cooler temperatures, than healthy workers (16). When placed into a thermal gradient, which allows the active selection of a range of ambient temperatures (T_a), the parasitized bumblebees displayed cold-seeking behavior that enhanced survival (15, 16). Cold-seeking behavior has also been reported in mammals. Mice inoculated with influenza virus chose cooler ambient temperatures as the infection progressed and the effect on T_c correlated with enhanced survival rates (12). Similar findings have been described for mice and rats injected with

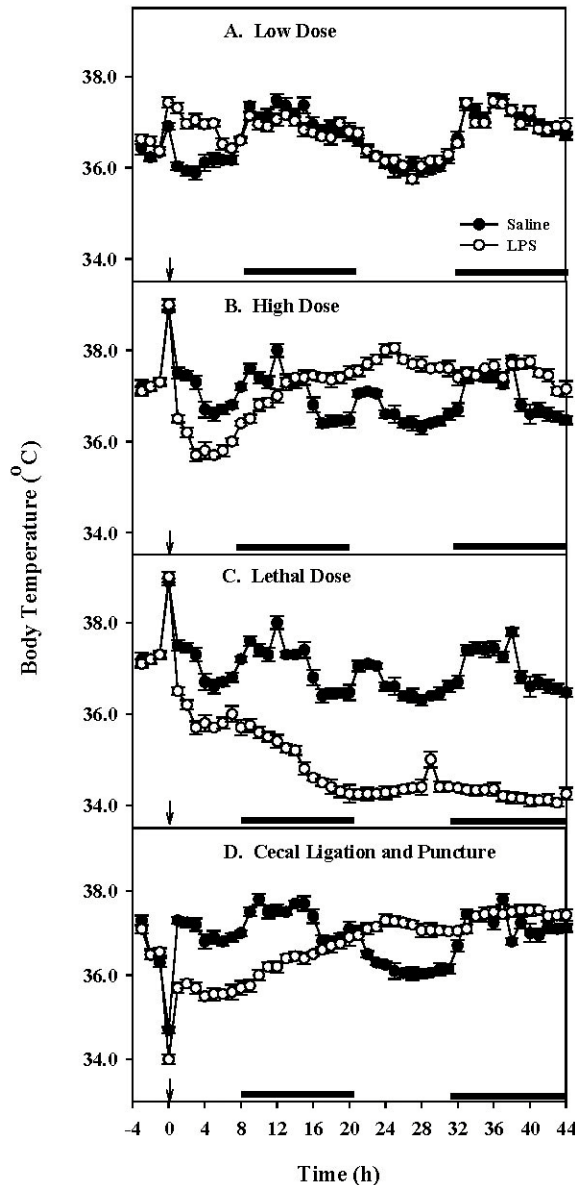


Figure 1. Example of the thermoregulatory profile induced by the IP injection of a (A) low (50 microgram/kg, IP), (B) septic-like (2.5 mg/kg, IP) and (C) lethal dose (10 mg/kg, IP) of lipopolysaccharide (LPS; *E. coli* 0111:B4 serotype) or (D) cecal ligation and puncture (CLP; 18 gauge needle) in mice. Control mice received an equivalent volume of sterile saline or sham surgery. Data are presented as 1h averages. Time 0 (arrow) represents time of injection or CLP. Body temperature was measured by biotelemetry in conscious, unrestrained mice. Black horizontal bars represent lights off period in a 12:12h L:D cycle.

endotoxin (13, 14). On the other hand, the natural development of hypothermia in young mice during septic shock improved survival whereas the development of fever in older individuals was associated with mortality (18). These data support the hypothesis that hypothermia is a regulated response that is evoked during systemic

inflammation to decrease the morbidity and mortality of the infectious state.

The majority of experimental data on hypothermia has been performed in rodents. One facet of rodent research that confounds data interpretation is the profound impact of T_a on thermoregulatory processes. Due to the high surface area to body mass ratio of small rodents which facilitates heat loss, housing at standard laboratory temperatures (typically 20-22°C) may induce hypothermia and mask potential fever responses. For example, pyrogen injection induces hypothermia in rats housed at 24°C (i.e., below their thermoneutral zone; TNZ) and fever at 30°C (within their TNZ; 19). There are several early studies in mammals suggesting that mice and rats, as opposed to larger experimental animals, responded to lipopolysaccharide (LPS; a cell wall component of gram-negative bacteria) with hypothermia rather than fever (20-22). Furthermore, stress-induced elevations in T_c due to restraint may mask fever responses. Thus, to induce fever in rodents, very large pyrogen doses were typically injected. As shown in Figure 1, a large dose of LPS induces hypothermia and, may result in significant mortality following circulatory collapse. With the advent of radiotelemetry to measure T_c in the absence of restraint and the recognition of T_a effects on heat conservation, it is now known that thermoregulatory responses to systemic inflammation are often biphasic in nature, consisting of regulated hypothermia and fever.

The maintenance of normal T_c , particularly under conditions of low T_a , requires significant expenditure of energy. The maintenance of fever during infection is a highly energetic process yet it has been conserved throughout evolution (1), suggesting that the effects of high T_c on immune responses outweigh the high metabolic requirement. Based on the Q_{10} effect (i.e., the factor by which biochemical reaction rate is increased for each 10°C increase in temperature), one can predict that for each 1°C change in T_c , there will be a ~10% change in tissue metabolic requirements (and a subsequent effect on the production of harmful tissue end products). Hypothermia represents the opposite extreme of the thermoregulatory continuum as fever, in that its function is thought to reside in energy conservation. Induced hypothermia has been clinically recognized as a protective treatment strategy under conditions of oxygen scarcity such as cerebral ischemia, cardiopulmonary bypass surgery and stroke (23-25). The beneficial effect of hypothermia under these conditions of oxygen scarcity appears obvious, but does this relationship extend to the inflammatory condition? An energy depletion hypothesis was suggested for the incidence of hypothermia during septic shock (26). This hypothesis states that the adaptive value of hypothermia would be realized under conditions of energy depletion, which prevent the highly energetic cost of fever from being of benefit to the host. In this scenario, hypothermia and fever represent the extremes of a thermoregulatory continuum whose control is dependent on the metabolic capabilities of the host. In accordance with this hypothesis, LPS-induced shock in rats was correlated with the selection of a cool T_a in a thermogradient and a decrease in the T_c

threshold for activation of cold thermogenesis (27). The latter finding is representative of poikilothermic T_c control or a widening of the interthreshold zone for thermal effector activation (i.e., T_c is dependent on T_a) and serves as an effective means of energy conservation.

3. EXPERIMENTAL MODELS OF SYSTEMIC INFLAMMATION

Although several clinical studies have shown an effect of hypothermia on cytokine production and other immune responses (28-30), induced hypothermia was used to lower T_c , making it difficult to extrapolate to the natural occurrence of hypothermia during systemic inflammation. Thus, these studies have provided little insight into the regulatory role of cytokines in the hypothermic response to inflammation.

There are two experimental models of systemic inflammation that have provided the majority of data regarding the role of cytokines in hypothermia. The peripheral injection of LPS is a frequently used model of systemic inflammation. LPS stimulates the release of cytokines from activated macrophages and other cell types and induces a stereotypic T_c response consisting of fever alone (Figure 1A; low dose, 50 microgram/kg, IP), hypothermia preceding fever (Figure 1B; high or septic-like dose, 2.5 mg/kg, IP) or hypothermia that results in mortality, without a subsequent expression of fever (Figure 1C; lethal dose, 10 mg/kg, IP).

Cecal ligation and puncture (CLP) is an experimental model of bacterial infection that simulates the clinical condition of sepsis. The sepsis syndrome is characterized by pathophysiologic symptoms that include hypothermia, fever, hypotension, metabolic acidosis and death (31). These symptoms have been successfully replicated using CLP with a temperature profile similar to that induced by a high (often described as septic-like) dose of LPS (compare Figure 1B and 1D). To induce sepsis, the cecum is isolated, perforated and the contents expressed into the peritoneal cavity where a proliferative infection ensues. Whereas LPS is a non-replicating component of gram-negative bacteria, CLP induces a bacterial infection of mixed intestinal flora that models a perforated necrotic bowel. Interestingly, the cytokines examined using the LPS and CLP models have been shown, by a variety of experimental techniques, to mediate both hypothermia and fever. These data suggest that the classification of a cytokine as an endogenous pyrogen (i.e., fever inducer), antipyretic (i.e., fever reducer) or cryogen (i.e., hypothermia inducer) is not as clear-cut as originally described.

The focus of this review is on cytokines that mediate hypothermia. Most cytokines that have been implicated in the mediation of hypothermia were originally described for their effects on fever. The natural extension of the discovery of a cytokine's ability to reduce T_c during fever (i.e., function as an antipyretic) is to determine its capacity to decrease T_c in the non-febrile organism (i.e., function as an endogenous cryogen). Thus, the description

of each cytokine's role in hypothermia will be prefaced with a brief discussion of its effects on fever. This overview of fever with hypothermia is meant to illustrate that these two processes are often observed as a continuum, rather than as discrete thermoregulatory events and may be mediated by multiple actions of the same cytokine.

4. TUMOR NECROSIS FACTOR

TNF exists in two biologically active forms, termed TNF-alpha (cachectin) and TNF-beta (lymphotoxin). Both forms of the cytokine interact with p55 and p75 receptors to induce physiological effects (32-34). Soluble TNF receptors (sTNFR) circulate in serum and function to neutralize TNF biological activity or inhibit proteolytic degradation (33, 35, 36). TNF-alpha is thought to be the main endogenous form of the cytokine that mediates T_c .

For the past decade or more, controversy has surrounded the role of TNF-alpha in the mediation of T_c during systemic inflammation. One of the principle questions driving the debate is whether endogenous TNF-alpha has pyrogenic, antipyretic or cryogenic actions. Accumulated evidence suggests that TNF-alpha may induce each of these effects, depending on the inflammatory model and species examined.

TNF-alpha has typically been described as an endogenous pyrogen. The injection of TNF-alpha induces fever in mice (37), rats (38-40), rabbits (41, 42), guinea pigs (43) and humans (44). Clearly, the ability of TNF-alpha to induce fever in several species and by several routes of administration (i.e., peripherally and centrally) argues for a generalized pyrogenic role for this cytokine.

The ability of injected TNF-alpha to induce hypothermia in rats (45) and mice (46, 47) suggests that, at least in some species, this cytokine may have cryogenic actions. Due to the ability of TNF-alpha to induce several of the pathophysiologic symptoms of shock, hypothermia may be a secondary response to multiple organ system failure induced by its injection. In rats, hypothermia to central (IIIrd ventricle) TNF-alpha injection was associated with a decrease in sympathetic outflow to brown adipose tissue (BAT). These data suggest a specific effect of TNF-alpha on metabolic thermogenesis rather than a passive heat loss effect from changes in vascular resistance induced by hypotension (45). However, the site(s) of TNF-alpha regulation of BAT activity remain unidentified. That is, the effect of peripheral injection of TNF-alpha on BAT and T_c was not tested. There is also the caveat that the characterization of a cytokine in control of T_c cannot be solely determined from injection studies since these represent pharmacological rather than physiological effects.

The release of endogenous TNF-alpha should precede the development of hypothermia with a time course that suggests a role in the mediation of this phenomenon. In mice and rats, a high or septic-like dose of LPS induces an initial hypothermia within ~1-2h post-injection (see Figure 1B and 1C). While the time course of hypothermia

correlates with enhanced plasma TNF- α levels (60-120 min following LPS injection) in several studies (48-53), there are also instances in which these events are temporally dissociated from one another or hypothermia is observed in the absence of TNF- α release (52, 54). Limited sampling sites and times are likely responsible for some of these negative findings (52, 54).

TNF- α neutralization has been shown to attenuate (55, 56), exacerbate (57, 58) or have no effect (59-61) on fever. The reasons for these divergent results are not well defined, but may be related to the injected dose of LPS or ineffective neutralization of the cytokine using injected antagonists. To test the hypothesis that endogenous TNF- α functions as an antipyretic, we examined fever responses to a low and high dose of LPS in TNF p55/p75 receptor (TNFR) knockout mice (i.e., mice that can produce TNF- α in response to LPS, but do not respond physiologically to the cytokine; 62, 63). Using this animal model, we observed similar fever responses in KO and wild-type (WT) mice to a low dose of LPS, but more rapid initiation of fever in KO mice exposed to a high LPS dose (64). The former result is not particularly surprising since a cytokine's antipyretic effects are expected under conditions in which fever approaches harmful levels and it is likely that a low LPS dose was insufficient to induce that effect. This is particularly relevant to mice, which require much larger LPS doses than rats to develop fever. On the other hand, the more rapid initiation of fever in KO mice in response to a high dose of LPS correlated with reduced plasma IL-10 levels (62, 64) indicating a TNF/IL-10 regulatory loop for antipyresis. While these data do not resolve the issue of endogenous TNF function during fever, they suggest that multiple actions of this cytokine may be elicited depending on the severity of the inflammatory insult and the experimental technique used to neutralize endogenous cytokine activity.

The above data showing the effect of systemic inflammation induced by LPS and CLP on T_c indicate that cytokines often function as endogenous pyrogens, antipyretics or cryogens. It has been hypothesized that a reduction in arterial O_2 saturation and its subsequent inhibition of ATP production is sensed by CNS neurons and results in the actions of a cytokine being switched from a pyretic to an antipyretic or cryogenic mode, in an effort to conserve energy (65). This PO_2 hypothesis is particularly attractive given the ability of several of the systemic inflammatory mediators discussed in this review to induce hypoxia, including LPS, TNF- α and influenza virus (65).

Studies of TNF- α neutralization and hypothermia have provided fairly consistent results in favor of cryogenic actions of this cytokine. In rats, LPS-induced hypothermia was attenuated by ~44% using TNF antiserum (66). Given the relatively large LPS dose (500 microgram/kg, iv) used in this study, partial attenuation may be due to the release of other endogenous cryogens not identified, incomplete inhibition of TNF- α by the antiserum, or circulatory collapse. The injection of a neutralizing TNF binding protein did not affect the depth of

LPS-induced hypothermia in another study in rats, but the duration of hypothermia was attenuated such that fever was initiated more rapidly (53). Interestingly, fever peak was enhanced as well, supporting initial cryogenic and subsequent antipyretic effects of TNF- α . In mice, the co-injection of TNF- α (at a dose that had no effect on normal T_c) with LPS exacerbated hypothermia and attenuated fever (46). However, when endogenous TNF was neutralized by the injection of the sTNFR or TNF- α antiserum, hypothermia was blocked, but fever was unaffected (46). These data support the hypothesis that *endogenous* TNF functions as a cryogen and does not have pyrogenic or antipyretic actions in mice. Perhaps more importantly, these results show that the effects of an injected cytokine do not always correlate with those obtained following neutralization of the endogenous cytokine.

There are instances in which TNF- α neutralization has shown no effect on hypothermia and these results may be related to the severity of the inflammatory insult. The injection of an anti-TNF antiserum into mice attenuated LPS-induced mortality, but had no effect on the mortality and hypothermia induced by CLP (67). This study used a very high dose of LPS (200-600 microgram/mouse) such that the thermoregulatory profile is likely representative of septic shock and circulatory collapse. Unfortunately, T_a was not indicated so it is unclear if hypothermia was facilitated by this factor. In this study, T_c dropped to ~22°C within 6h following LPS or CLP and fever was not a component of the T_c profile (67). It is unclear if the antiserum would have been effective at a lower dose of LPS that induced a less pronounced hypothermia. Furthermore, one might predict that the severity of the insult, as indicated by the profound hypothermia, induced several cytokines and/or other factors that were responsible for the deep hypothermia such that TNF- α antagonism alone was insufficient to inhibit the response. However, combined therapy with the sTNFR and IL-1 receptor antagonist was also without effect on CLP-induced hypothermia in mice, despite the alteration of other cytokines, such as IL-6, that have been implicated in the morbidity of sepsis (68). Using the same CLP technique with mice housed at thermoneutrality (T_a =30°C), we examined the effect of TNF neutralization on hypothermia and fever using TNFR knockout mice. Figure 1D provides an example of the temperature profile elicited by CLP in C57BL (WT) mice (one of the background strains used to generate TNFR KO mice). These mice developed ~1.5-2°C hypothermia during the first day following CLP that culminated in ~1°C fever the following day. When we tested the thermoregulatory response to CLP in TNFR KO mice, hypothermia was prevented, fever developed more rapidly than in WT controls and survival was enhanced (69). These data suggest that hypothermia is a detrimental response during sepsis induced by CLP, which contrasts with studies showing beneficial effects of regulated hypothermia (i.e., cold-seeking behavior) following endotoxin injection (13, 14). The reasons for these differences are unknown, but may be related to the severity of the inflammatory insult and/or the complete absence of TNF signaling in these mice. Does CLP induce a regulated

hypothermia in rodents? This question remains unanswered.

Based on the reviewed studies, the actions of endogenous TNF-alpha may be characterized as pyrogenic, antipyretic or cryogenic depending on the species and the type and severity of the inflammatory stimulus. Under conditions in which mild hypothermia is induced, TNF-alpha appears to be at least partially responsible for the decreased T_c . On the other hand, when a rapid, profound hypothermia is the immediate response to inflammation, it is likely that several mediators are involved in the response such that neutralization of any one cytokine is without significant effect. Furthermore, the depth of hypothermia may be a contributing factor to mortality during systemic inflammation, although this hypothesis has not been directly tested. It is clear that the controversy regarding the role of TNF-alpha in fever and hypothermia has not been resolved.

5. INTERLEUKIN-10

IL-10 is a Th2 cytokine originally described as a cytokine synthesis inhibitory factor. IL-10 inhibits the LPS-induced synthesis of a number of cytokines implicated in the regulation of T_c , including IL-1gamma, IL-6, IL-8 and TNF-alpha (70, 71). Elevated plasma IL-10 levels are detected in septicemia (72) and the protective effects of IL-10 against sepsis morbidity and mortality are well recognized, correlating in many cases with the inhibition of TNF-alpha production (72, 73-78).

Based on the ability of IL-10 to inhibit the production of other cytokines implicated in T_c control, a role for this cytokine in fever has been hypothesized. Pharmacologically, IL-10 functions as an antipyretic in rats (79, 80), mice (77) and humans (81), an effect that correlates with reduced production of IL-1beta (79), IL-6 (79, 81, 82) and TNF-alpha (79, 81). Neutralization of IL-10 exacerbated fever to the subcutaneous air pouch injection of endotoxin in rats with augmented production of IL-1beta, IL-6 and TNF-alpha (82). Similarly, IL-10 KO mice showed enhanced fever responses to LPS that correlated with augmented production of IL-6 (77). These studies indicate that the inhibitory effects of IL-10 on cytokine production extend to thermoregulatory processes with IL-10 implicated as an endogenous antipyretic. There are no reports of IL-10 inducing fever or hypothermia.

There are several studies indicating a functional relationship between IL-10 and TNF-alpha in the mediation of hypothermia. IL-10 injection prevented LPS-induced hypothermia through the reduced production of TNF-alpha (73). On the other hand, IL-10 KO mice developed a prolonged decrease in T_c and succumbed to LPS, suggesting a protective effect of IL-10 and detrimental effect of hypothermia (77). It is hypothesized that enhanced TNF-alpha levels (and presumably other cytokines) in IL-10 KO mice are responsible for these effects, although plasma cytokine levels were not determined in this study (77). Others have shown that IL-10 KO mice show

profound hypothermia ($T_c \sim 25^\circ\text{C}$) and decreased survival correlating with enhanced plasma TNF-alpha levels during *Trypanosoma cruzi* infection (74). The specificity of these effects to TNF-alpha was demonstrated by a reversal of hypothermia and prolonged survival following TNF-alpha antibody treatment (74). Based on these results, endogenous IL-10 attenuates hypothermia through the inhibition of endogenous TNF-alpha production.

While studies providing evidence for a role of IL-10 in T_c responses to infection and inflammation are not extensive, the data suggest antipyretic and cryogenic actions of this cytokine. A relationship between IL-10 and TNF-alpha has been demonstrated in hypothermia, but IL-10-induced antipyresis likely results from the inhibited production of several cytokines, including IL-1beta, IL-6 and TNF-alpha.

6. INTERLEUKIN-6

IL-6 has been strongly implicated as an endogenous pyrogen based on production of fever following its injection, increased circulating levels of the cytokine during fever and the absence of fever under conditions in which IL-6 has been neutralized (1, 83, 84). There has been little consideration of IL-6 as an endogenous cryogen, although some studies have demonstrated an effect of IL-6 neutralization on hypothermic responses. In IL-6 KO mice, hypothermia to LPS was enhanced in comparison to WT controls and this effect correlated with augmented plasma concentrations of TNF-alpha during the time course of hypothermia (84). These data suggest that endogenous IL-6 functions in a negative feedback loop on TNF-alpha production during infection, much in the same manner as described for IL-10. When IL-6 KO mice were tested during CLP-induced sepsis, hypothermia did not differ from that observed in WT mice through 14h post-surgery (see WT T_c profile of Figure 1D; 69). These data appear to contradict the findings described above in which hypothermia to LPS was enhanced in KO mice. However, as sepsis ensued from 15-48h, the hypothermic response to CLP was enhanced in IL-6 KO mice such that fever never developed and the mice eventually succumbed to the insult (69). These data suggest that fever has a protective role in the sepsis syndrome in mice and that prolonged hypothermia (i.e., beyond the first 14h) could serve as a barometer of morbidity in this model. Although plasma TNF-alpha levels were not measured, it is hypothesized that exacerbated production of TNF-alpha (and perhaps other cytokines) occurred in the absence of IL-6 negative feedback, such that lethality was enhanced (69).

The intranasal inoculation of mice with influenza virus at a thermoneutral T_a results in the development of a $\sim 1-2^\circ\text{C}$ hypothermia, without subsequent development of fever (12, 85, 86). Influenza virus induces hypoxia (87) and an increase in IL-6 levels in lung lavage fluid (85). The hypothermic response to influenza infection is regulated, in that inoculated mice choose a cool T_a in a thermal gradient (12). To test the hypothesis that cytokines are mediating hypothermia during influenza, IL-6 KO mice were

intranasally inoculated with the virus and their T_c profiles compared to that of WT controls. IL-6 KO mice developed *attenuated* decreases in T_c following virus inoculation (86). These results are in direct contrast to the effects obtained in IL-6 KO mice injected with LPS or made septic by CLP in which hypothermia was exacerbated (see above). It appears that endogenous IL-6 modulates hypothermia in several ways, presumably depending on the physiological milieu induced by the inflammatory stimulus. As suggested by the PO_2 hypothesis from Kozak (65), the divergent actions of IL-6 may be directly related to the occurrence of hypoxia during influenza infection.

7. INTERLEUKIN-1beta

The IL-1 family of ligands is comprised of IL-1alpha, IL-1beta and the IL-1 receptor antagonist (IL-1ra). IL-1beta is thought to be the primary secreted form of IL-1 and the main regulator of T_c during inflammation. The IL-1ra is a natural inhibitor of IL-1 actions that binds to IL-1 receptors without inducing an intracellular signal (88). The two receptors for IL-1 have been cloned and classified as the type I and type II receptors (89). The type I receptor (IL-1r1) is thought to be the only signaling receptor for IL-1; the type II receptor is thought to serve as a negative regulator of IL-1 functions (89).

IL-1beta is considered a key mediator of fever. IL-1beta induces fever when injected peripherally or centrally into several species, an effect mediated through the endogenous production of IL-6 (90, 91). Peripheral and central injection of IL-1beta neutralizing antiserum/antibodies or the IL-1ra attenuated, but did not prevent fever to LPS (90, 91) and CLP (92). In studies using IL-1beta or IL-1r1 KO mice, LPS-induced fever was enhanced (93), attenuated (84) or similar (94) to that observed in WT mice. There are currently no studies using the injection of IL-1 neutralizing agents that have demonstrated complete inhibition of fever induced by LPS. These findings indicate that IL-1beta is one of several endogenous pyrogens involved in fever generation.

There are limited data supporting a role for endogenous IL-1 in hypothermia. CLP and LPS-induced hypothermia do not appear to be mediated by endogenous IL-1beta. The injection of a septic-like dose of LPS into IL-1beta KO mice produced hypothermia that was virtually identical to that observed in WT controls (84). Plasma IL-6 levels did not differ between groups at 2h post-injection, which corresponded to the peak of hypothermia (84). These data suggest that (1) IL-1beta does not mediate hypothermia or (2) other factors compensated for IL-1beta deficiency in the KO mice.

More severe inflammatory stimuli such as CLP and influenza virus have elucidated a role for IL-1 in hypothermia. In rats, iv injection of the IL-1ra attenuated hypothermia and hypotension induced by CLP and improved survival (95). Unfortunately, the effects of the IL-1ra alone were not reported. It is not clear if the effects

of IL-1ra on hypothermia were directly related to hemodynamic changes or were specific to the thermoregulatory actions of the cytokine since the profile of changes in blood pressure and T_c were similar following treatment. Intra-hypothalamic injection of a neutralizing IL-1beta antibody had no effect on hypothermia induced by CLP in rats (92), despite proven efficacy of the antibody. In response to intranasal inoculation with influenza virus, hypothermia was attenuated in IL-1beta KO mice compared to their WT counterparts (96). These findings are similar to those reported in IL-6 KO mice, as previously discussed (see Interleukin-6 section). Interestingly, the attenuation of hypothermia on day 5 post-inoculation correlated with the time point at which survival was significantly decreased in the KO mice (96). These data support previous studies showing a protective effect of hypothermia on survival from viral infection in this species (12).

8. INTERFERON-gamma

Interferons are antiviral proteins that have shown pyrogenic effects in humans and experimental animals. That is, the injection of IFNalpha and IFNgamma induced fever when injected peripherally or centrally into a variety of species (reviewed in 1).

IFNgamma is thought to be a key mediator of sepsis lethality due to its potentiation of cytokine production (97). When the thermoregulatory actions of this cytokine were determined in mice, the injection of IFNgamma alone induced $\sim 1.2^\circ\text{C}$ hypothermia of $\sim 6\text{h}$ duration (98). IL-1beta, IL-6 and TNF-alpha did not appear to mediate this effect (98). IFNgamma injection immediately prior to LPS (0h pretreatment) potentiated hypothermia and 8h pretreatment was maximally effective, producing $\sim 7.0^\circ\text{C}$ decrease in T_c . The latter effect was related to the augmented production of IL-1beta and TNF-alpha (98). Although these data provide evidence for an effect of exogenous IFNgamma on T_c , further studies are required to identify endogenous effects of this cytokine on hypothermia.

9. FUTURE DIRECTIONS

It is difficult to assess the impact of hypothermia on infection outcome and the role of cytokines in the regulation of this response, based on current experimental data. There is considerable controversy regarding TNF-alpha in the regulation of hypothermia, although this cytokine appears to function as an endogenous cryogen in most instances. Unfortunately, few data are available on the effects of other cytokines on hypothermia and more research is required in this area. Most importantly, research beyond the effects of exogenous cytokines on T_c is required to delineate an endogenous role of any cytokine in thermoregulation. Figure 2 provides a hypothetical model of cytokine interactions that mediate hypothermia in response to an inflammatory stimulus. This model is based on our current understanding of the mediation of hypothermia by the cytokines discussed in this review.

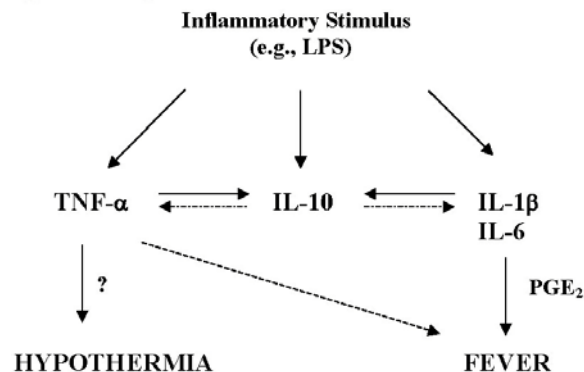


Figure 2. Hypothetical model of cytokine interactions that regulate hypothermia during systemic inflammation. Exposure to an inflammatory stimulus, such as lipopolysaccharide (LPS), induces the production of IL-1 β , IL-6, TNF- α and IL-10 from macrophages and other cell types. IL-1 β and IL-6 stimulate hypothalamic production of prostaglandin E₂ (PGE₂) through a variety of proposed signaling pathways. PGE₂ increases the thermal setpoint, resulting in the production of fever. TNF- α stimulates a decrease in T_c that is thought to be a regulated response, although the final mediator of this response remains unidentified. TNF- α also functions as an endogenous antipyretic in several species, thus limiting the ultimate magnitude of fever. IL-10 is produced in response to LPS and other cytokines and serves as a negative regulator of cytokine production, thus limiting the ultimate magnitude of fever and hypothermic responses. This model ignores the contribution of IFN γ to T_c responses since a role for this endogenous cytokine has not been identified in hypothermia. This model does not identify other endogenous substances that have effects on T_c, such as nitric oxide or arginine vasopressin. Solid line denotes stimulatory pathways; Dashed line denotes inhibitory pathways.

Hypothermia is clinically described as a detrimental symptom of sepsis, whereas several animal studies have shown a protective effect of a regulated decrease in T_c (i.e., cold-seeking behavior in a thermal gradient). In gene knockout mice, attenuated hypothermia was associated with enhanced (TNFR KO mice; 69) or decreased (IL-1 β and IL-6 KO mice; 86, 96) survival during bacterial and viral infection, respectively. Whether these differences in study outcome are a direct consequence of a decrease in T_c or other cytokine actions is unknown, but it suggests that the type of inflammatory insult is important. Although experimental data indicate that the depth of hypothermia may have the most profound impact on infection outcome, additional studies are required to directly test this hypothesis.

Many of the studies presented in this review were conducted using gene knockout mice. It is important to recognize that while gene knockout technology provides a unique approach to thermoregulation studies, it also can complicate data interpretation. Due to the absence of a gene product throughout development, several unrecognized redundancies have developed that likely impact the thermoregulatory processes being examined. In general

terms, gene knockout studies are performed on animals that were capable of surviving in a gene's absence. Unfortunately, in most cases the resultant changes in an animal's basic thermoregulatory process due to a gene's absence during development have not been assessed. It is anticipated that the use of time-dependent and tissue-specific gene knockout strategies, as well as the recently developed gene knockout rat (99), will permit an examination of cytokine actions in the regulation of hypothermia in a more natural animal model and in species other than the mouse.

There are several clinical conditions that could directly benefit from a better understanding of the mechanisms regulating hypothermia. Forced hypothermia, in which T_c is physically decreased using cooling blankets or other methods, is used clinically during cardiopulmonary bypass surgery and as treatment for brain ischemia and stroke to reduce metabolic demands of injured tissue (23-25, 100, 101). The benefit of hypothermia treatment for these conditions is thought to reside in the inhibitory effect of reduced T_c on tissue metabolism and cytokine production. However, it is likely that the efficacy of this treatment is not fully realized when T_c is being physically reduced in the absence of a change in the thermoregulatory setpoint. Under these conditions, a variety of physiologic effector mechanisms are evoked to resist body heat loss and the resultant increase in metabolism is expected to counter the desired benefit of the treatment. On the other hand, the manifestation of poikilothermia as an adaptive thermoregulatory strategy suggests that a breakdown in T_c control precision may be of benefit to the host under severe pathological conditions such as septic shock (27). It is hoped that the identification of additional inflammatory conditions that evoke this response and an understanding of the mechanisms of cytokine regulation of hypothermia will provide treatment strategies that can be used to maximize patient benefits during several injurious and inflammatory states.

While data are accumulating on the role of cytokines in hypothermia, significant gaps still exist in our current knowledge. A few proposed areas for future research include:

1. Determination of the final mediator of hypothermia. Several studies have examined prostaglandins (PG) as the final mediator of hypothermia, but the results have been contradictory. While hypothalamic PGD₂ levels were reported to increase during LPS-induced hypothermia in rats (102), prostaglandin synthesis inhibitors, such as indomethacin attenuate (103), exacerbate (50) or have no effect (17) on hypothermia. More studies are required in this area to determine the final mediator as well as the specific anatomical location for regulation of this response.
2. Examination of cytokine antagonism on thermoregulatory setpoint changes. An alteration of hypothermia using cytokines/antagonists is assumed to represent a direct effect on the thermal setpoint, but this has not been verified. Are cytokine effects on hypothermia associated with the appropriate thermoeffector mechanisms that are indicative of a change in the thermoregulatory

setpoint? Do cytokine antagonists alter cold-seeking behavior during infection-induced hypothermia? Do gene knockout mice behave differently in a thermal gradient during infection than their wild-type counterparts?

3. A study of cytokine regulation of hypothermia during non-infectious states. Do the effects of cytokines on T_c extend to non-inflammatory states? Clearly, a more global understanding of cytokine thermoregulatory effects is needed.

Finally, it is important to note that the majority of studies on cytokines and hypothermia have been performed on rodents. There remains the question of the applicability of cytokine effects to the human condition since regulated hypothermia is poorly understood in this species. Given the large difference in surface area to body mass ratio between rodents and humans and the reduced sensitivity of mice and rats to bacterial products such as LPS (i.e., much larger doses are required to elicit fever in rodents, even within the TNZ), extrapolation between species is difficult. Furthermore, there is the concern that the thermoregulatory actions of a cytokine may be beneficial, whereas other physiological effects may be harmful, thus complicating our assessment of potential beneficial treatment effects. Only through careful analysis of a cytokine's actions in several models of systemic inflammation, with attention to hypothermia as well as fever, can these obstacles be identified and overcome.

10. ACKNOWLEDGEMENTS

I am grateful to David A. DuBose and Michael N. Sawka for their critical review of the manuscript.

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of Army position, policy, or decision unless so designated by official documentation.

11. REFERENCES

- Kluger, M. J: Fever: role of pyrogens and cryogens. *Physiol Rev* 71, 93-127 (1991)
- Clemmer, T. P, C. J. Fisher Jr., R.C. Bone, G. J. Slotman, C. A. Metz and F. O. Thomas: Hypothermia in the sepsis syndrome and clinical outcome. *Crit Care Med* 20, 1395-1401 (1992)
- Marik, P. E, G. P. Zaloga and the Norasept II study investigators: Hypothermia and cytokines in septic shock. *Intensive Care Med* 26, 716-721 (2000)
- Sprung, C. L, P. N. Peduzzi, C. H. Shatney, R. M. Schein, M. F. Wilson, J. N. Sheagren and L. B. Hinshaw: Impact of encephalopathy on mortality in the sepsis syndrome. The Veterans Administration Systemic Sepsis Cooperative Study Group. *Crit Care Med* 18, 801-806 (1990)
- Gavrilova, O, L. R. Leon, B. Marcus-Samuels, M. M. Mason, S. Refetoff, C. Vinson and M. L. Reitman: Torpor in mice is induced by both leptin-dependent and – independent mechanisms. *Proc Natl Acad Sci* 96, 14623-14628 (1999)
- Buchanan, T. A, P. Cane, C. C. Eng, G. F. Sipos and C. Lee: Hypothermia is critical for survival during prolonged insulin-induced hypoglycemia in rats. *Metabolism* 40, 330-334 (1991)
- Graf, R, S. Krishna and H. C. Heller: Regulated nocturnal hypothermia induced in pigeons by food deprivation. *Am J Physiol* 256, R733-R738 (1989)
- Malvin, G. M and S. C. Wood: Behavioral hypothermia and survival of hypoxic protozoans *Paramecium caudatum*. *Science* 255, 1423-1425 (1992)
- Rausch, R. N, L. I. Crawshaw and H. L. Wallace: Effects of hypoxia, anoxia and endogenous ethanol on thermoregulation in goldfish, *Carassius auratus*. *Am J Physiol* 278, R545-R555 (2000)
- Henderson, R. A, M. E. Whitehurst, K. R. Morgan and R. G. Carroll: Reduced metabolic rate accompanies the hemorrhage-induced hypothermia in conscious rats. *Resuscitation* 44, 129-138 (2000)
- Ibuka, N. and K. Fukumura: Unpredictable deprivation of water increases the probability of torpor in the Syrian hamster. *Physiol Behav* 62, 551-556 (1997)
- Klein, M. S, C. A. Conn and M. J. Kluger: Behavioral thermoregulation in mice inoculated with influenza virus. *Physiol Behav* 52, 1133-1139 (1992)
- Romanovsky, A. A, O. Shido, S. Sakurada, N. Sugimoto and T. Nagasaka: Endotoxin shock-associated hypothermia: how and why does it occur? *Ann NY Acad Sci* 813, 733-737 (1997)
- Lagerspetz, K. Y. H and T. Vaatainen: Bacterial endotoxin and infection cause behavioral hypothermia in infant mice. *Comp Biochem Physiol* 88A, 519-521 (1987)
- Heinrich, B and M. J. E. Heinrich: Heterothermia in foraging workers and drones of the bumblebee *Bombus terrestris*. *Physiol Zool* 56, 563-567 (1983)
- Muller, C. B and P. Schmid-Hempel: Exploitation of cold temperature as defence against parasitoids in bumblebees. *Nature* 363, 65-67 (1993)
- Feldberg, W and P. N. Saxena: Prostaglandins, endotoxin and lipid A on body temperature in rats. *J Physiol* 249, 601-615 (1975)
- Habicht G. S: Body temperature in normal and endotoxin-treated mice of different ages. *Mechanisms Aging Devel* 16, 97-104 (1981)
- Derijk, R. H., M. van Kampen, N. van Rooijen and F. Berkenbosch: Hypothermia to endotoxin involves reduced

- thermogenesis, macrophage-dependent mechanisms and prostaglandins. *Am J Physiol* 266, R1-R8 (1994)
20. Connor, D. G. and E. H. Kass: Effect of artificial fever in increasing susceptibility to bacterial endotoxin. *Nature Lond* 190, 453-454 (1961)
21. Feldberg, W. and P. N. Saxena: Prostaglandins, endotoxins and lipid A on body temperature in rats. *J Physiol Lond* 249, 601-615 (1975)
22. Miert, Van, A. S. J. P.A. M. and J. Frens: The reaction of different animal species to bacterial pyrogens. *Zentralbl Veterinarmed* 15, 532-543 (1968)
23. Dietrich, W. D and J. W. Kuluz: New research in the field of stroke: therapeutic hypothermia after cardiac arrest. *Stroke* 34, 1051-1053 (2003)
24. Feigin, V. L, C. S. Anderson, A. Rodgers, N. E. Anderson and A. J. Gunn: The emerging role of induced hypothermia in the management of acute stroke. *J Clin Neurosci* 9, 502-507 (2002)
25. Marion, D. W, L. E. Penrod, S. F. Kelsey, W. D. Obrist, P. M. Kochanek, A. M. Palmer, S. R. Wisniewski and S. T. DeKosky: Treatment of traumatic brain injury with moderate hypothermia. *New Engl J Med* 336, 540-546 (1997)
26. Romanovsky A. A. and M. Szekely: Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Med Hypotheses* 50, 219-226 (1998)
27. Romanovsky, A. A., O. Shido, S. Sakurada, N. Sugimoto and T. Nagasaka: Endotoxin shock: thermoregulatory mechanisms. *Am J Physiol* 270, R693-R703 (1996)
28. Marion, D. W: Therapeutic moderate hypothermia and fever. *Curr Pharm Des* 7, 1533-1536 (2001)
29. Nandate, K., A. Vuylsteke, A. E. Crosbie, S. Messahel, A. Oduro-Dominah and D. K. Menon: Cerebrovascular cytokine responses during coronary artery bypass surgery: specific production of interleukin-8 and its attenuation by hypothermic cardiopulmonary bypass. *Anesth Analg* 89, 823-828 (1999)
30. Tassani, P., A. Barankay, F. Haas, S. U. Paek, M. Heilmair, J. Hess, R. Lange and J. A. Richter: Cardiac surgery with deep hypothermic circulatory arrest produces less systemic inflammatory response than low-flow cardiopulmonary bypass in newborns. *J Thor Cardiovasc Surg* 123, 648-654 (2002)
31. Bone, R. C, C. J. Fisher, T. D. Clemmer, G. J. Slotman, C. A. Metz and R. A. Balk: Sepsis syndrome: a valid clinical entity. *Crit Care Med* 17, 389-393 (1989)
32. Loetscher, H, Y. Pan, H. Lahm, R. Gentz, M. Brockhaus, H. Tabuchi and W. Lesslauer: Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. *Cell* 61, 351-359 (1990)
33. Schall, T. J, M. Lewis, K. J. Koller, A. Lee, G. C. Rice, G. H. Wong, T. Gatanaga, G. A. Granger, R. Lentz and H. Raab: Molecular cloning and expression of a receptor for human tumor necrosis factor. *Cell* 61, 361-370 (1990)
34. Smith, C. A, T. Davis, D. Anderson, L. Solam, M. P. Beckmann, R. Jerzy, S. K. Dower, D. Cosman and R. G. Goodwin: A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. *Science* 248, 1019-1023 (1990)
35. Engelmann, H, D. Akerka, M. Rubinstein, D. Rotman and D. Wallach.: A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. *J Biol Chem* 264, 11974-11980 (1989)
36. Kern, W. V, A. Engel and P. Kern: Soluble tumor necrosis factor receptors in febrile neutropenic cancer patients. *Infection* 23, 64-65 (1995)
37. Sundgren-Andersson, A. K, P. Ostlund and T. Bartfai: IL-6 is essential in TNF-alpha-induced fever. *Am J Physiol* 275, R2028-R2034 (1998)
38. Kettelhut, I. C. and A. L. Goldberg: Tumor necrosis factor can induce fever in rats without activating protein breakdown in muscle or lipolysis in adipose tissue. *J Clin Invest* 81, 1384-1389 (1988)
39. Rothwell, N. J: Central effects of TNFalpha on thermogenesis and fever in the rat. *Biosci Rep* 8, 345-352 (1988)
40. Stefferl, A, S. J. Hopkins, N. J. Rothwell and G. N. Luheshi: The role of TNF-alpha in fever: opposing actions of human and murine TNF-alpha and interactions with IL-1beta in the rat. *Br J Pharmacol* 118, 1919-1924 (1996)
41. Hashimoto, M, Y. Sakakibara, M. Iriki, F. Goto and Y. Shimada: Does interleukin-1 mediate tumour necrosis factor alpha-induced fever in rabbits? *Pflugers Arch* 427, 365-372 (1994)
42. Nakamura, H, Y. Seto, S. Motoyoshi, T. Kadokawa and N. Sunahara: Recombinant human tumor necrosis factor causes long-lasting and prostaglandin-mediated fever, with little tolerance, in rabbits. *J Pharmacol Exp Ther* 245, 336-341 (1988)
43. Goldblach, J.-M, J. Roth, B. Storr and E. Zeisberger: Influence of pentoxifylline on fevers induced by bacterial lipopolysaccharide and tumor necrosis factor- α in guinea pigs. *Eur J Pharmacol* 319, 273-278 (1997)
44. Michie, H. R, D. R. Spriggs, K. R. Manogue, M. L. Sherman, A. Revhaug, S. O'Dwyer, K. Arthur, C. A. Dinarello, A. Cerami, S. M. Wolff, D. W. Kufe and D. W. Wilmore: Tumor necrosis factor and endotoxin induce

similar metabolic responses in human beings. *Surgery St Louis* 104, 280-286 (1988)

45. Holt, S. J, R. F. Grimble and D. A. York: Tumour necrosis factor-alpha and lymphotoxin have opposite effects on sympathetic efferent nerves to brown adipose tissue by direct action in the central nervous system. *Brain Res* 497, 183-186 (1989)

46. Kozak, W, C. A. Conn, J. J. Klir, G. H. W. Wong and M. J. Kluger: TNF soluble receptor and antiserum against TNF enhance lipopolysaccharide fever in mice. *Am J Physiol* 269, R23-R29 (1995)

47. Waage, A and T. Espevik: Interleukin 1 potentiates the lethal effect of tumor necrosis factor alpha/cachectin in mice. *J Exp Med* 167, 1987-1992 (1988)

48. Blaque, R, C. Meakin, S. Millet and C. R. Gardner: Hypothermia as an indicator of the acute effects of lipopolysaccharides: comparison with serum levels of IL1beta, IL6 and TNFalpha. *Gen Pharmacol* 27, 973-977 (1996)

49. Dogan M. D, H. Ataoglu and E. S. Akarsu: Characterization of the hypothermic component of LPS-induced dual thermoregulatory response in rats. *Pharmacol Biochem Behav* 72, 143-150 (2002)

50. Kozak, W, D. Soszynski, K. Rudolph, L. R. Leon, C. A. Conn and M. J. Kluger: Soluble tumor necrosis factor alpha receptor prevents decrease of body temperature in mice treated with indomethacin and lipopolysaccharide. *Ann NY Acad Sci* 813, 264-271 (1997)

51. Paul, L, V. Fraielfeld and J. Kaplanski: Evidence supporting involvement of leukotrienes in LPS-induced hypothermia in mice. *Am J Physiol* 276, R52-R58 (1999)

52. Schotanus, K, G. M. Holtkamp, N. van Rooijen, F. J. H. Tilders and F. Berkenbosch: Circulating tumor necrosis factor-alpha does not mediate endotoxin-induced hypothermia in rats. *Am J Physiol* 268, R989-R996 (1995)

53. Tollner, B, J. Roth, B. Storr, D. Martin, K. Voigt and E. Zeisberger: The role of tumor necrosis factor (TNF) in the febrile and metabolic responses of rats to intraperitoneal injection of a high dose of lipopolysaccharide. *Pflugers Arch* 440, 925-932 (2000)

54. Alexander, H, R. B. C. Sheppard, J. C. Jensen, H. N. Langstein, C. M. Buresh, D. Venzon, E. C. Walker, D. L. Fraker, M. D. Stovroff and J. A. Norton: Treatment with recombinant human tumor necrosis factor-alpha protects rats against the lethality, hypotension and hypothermia of gram-negative sepsis. *J Clin Invest* 88, 34-9 (1991)

55. Kwiatkowski, D, M. E. Molyneux, S. Stephens, N. Curtis, N. Klein, P. Pointaire, M. Smith, R. Allan, D. R. Brewster, G. E. Grau and B. M. Greenwood: Anti-TNF

therapy inhibits fever in cerebral malaria. *Quart J Med* 86, 91-98 (1993)

56. Nagai, M, T. Saigusa, Y. Shimada, H. Inagawa, H. Oshima and M. Iriki: Antibody to tumor necrosis factor (TNF) reduced endotoxin fever. *Experientia* 44, 606-607 (1988)

57. Klir, J. J, J. L. McClellan, W. Kozak, Z. Szelenyi, G. H. W. Wong and M. J. Kluger: Systemic, but not central, administration of tumor necrosis factor alpha attenuates LPS-induced fever in rats. *Am J Physiol* 268, R480-R486 (1995)

58. Long, N. C, S. L. Kunkel, A. J. Vander and M. J. Kluger: Antiserum against tumor necrosis factor enhances lipopolysaccharide fever in rats. *Am J Physiol* 258, R332-R337 (1990)

59. LeMay, L. G, A. J. Vander and M. J. Kluger: The effects of pentoxifylline on lipopolysaccharide (LPS) fever and plasma interleukin-6 (IL-6) and tumor necrosis factor (TNF) in the rat. *Cytokine* 2, 300-306 (1990)

60. Roth, J, B. Storr, D. Martin, K. Voigt and E. Zeisberger: The role of local induction of tumor necrosis factor by LPS with a subcutaneous air pouch in the development of a febrile response in guinea pigs. *Neuroimmunomodulation* 7, 169-176 (2000)

61. Zabel, P, F.U. Schade and M. Schlaak: Inhibition of endogenous TNF formation by pentoxifylline. *Immunobiol* 187, 447-463 (1993)

62. Gourine, A. V., L. R. Leon, K. Rudolph, J. Tesfaigzi and M. J. Kluger: Cytokine cascade induced by endotoxin in TNF double receptor knockout mice: evidence supporting a role for IL-10 in mediating antipyretic action of TNF. *J Therm Biol* 25, 21-27 (2000)

63. Peschon, J. J, D. S. Torrance, K. L. Stocking, M. B. Glaccum, C. Otten, C. R. Willis, K. Charrier, P. J. Morrissey, C. B. Ware and K. M. Mohler: TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. *J Immunol* 160, 943-952 (1998)

64. Leon, L. R, W. Kozak, J. Peschon and M. J. Kluger: Exacerbated febrile responses to LPS, but not turpentine, in TNF double receptor-knockout mice. *Am J Physiol* 272, R563-R569 (1997)

65. Kozak, W: Regulated decreases in body temperature. In: Fever: Basic Mechanisms and Management. Ed: Mackowiak PA, Lippincott-Raven, Philadelphia 467-478 (1997)

66. Derijk, R.H. and F. Berkenbosch: Hypothermia to endotoxin involves the cytokine tumor necrosis factor and the neuropeptide vasopressin in rats. *Am J Physiol* 266, R9-R14 (1994)

67. Remick, D., P. Manohar, G. Bolgos, J. Rodriguez, L. Moldawer, G. Wollenberg: Blockade of tumor necrosis

factor reduces lipopolysaccharide lethality, but not the lethality of cecal ligation and puncture. *Shock* 4, 89-95 (1995)

68. Remick, D. G., D. R. Call, S. J. Ebong, D. E. Newcomb, P. Nybom, J. A. Nemzek and G. E. Bolgos: Combination immunotherapy with soluble tumor necrosis factor receptors plus interleukin 1 receptor antagonist decreases sepsis mortality. *Crit Care Med* 29, 473-481 (2001)

69. Leon, L. R., A. A. White and M. J. Kluger: Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. *Am J Physiol* 275, R269-R277 (1998)

70. De Waal Malefyt, R., J. Abrams, B. Bennett, C. G. Figdor and J. E. de Vries: Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174, 1209-1220 (1991)

71. Fiorentino, D. F., A. Zlotnik, T.R. Mosmann, M. Howard and A. O'Garra: IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 147, 3815-3822 (1991)

72. Marchant, A., J. Deviere, B. Byl, D. de Groote, J. L. Vincent and M. Goldman: Interleukin-10 production during septicemia. *Lancet* 343, 707-708 (1994)

73. Gerard, C., C. Bruyns, A. Marchant, D. Abramowicz, P. Vandenabeele, A. Delvaux, W. Fiers, M. Goldman and T. Velu: Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J Exp Med* 177, 547-550 (1993)

74. Holscher, C., M. Mohrs, W.J. Dai, G. Kohler, B. Ryffel, G.A. Schaub, H. Mossman and F. Brombacher: Tumor necrosis factor alpha-mediated toxic shock in *Trypanosoma cruzi*-infected interleukin 10-deficient mice. *Infect Immun* 68, 4075-4083 (2000)

75. Howard, M., T. Muchamuel, S. Andrade and S. Menon: Interleukin 10 protects mice from lethal endotoxemia. *J Exp Med* 177, 1205-1208 (1993)

76. Jansen, M. J. J. M., T. Hendriks, B.M. de Man, J. W. M. van der Meer and J. A. Goris: Interleukin 10 mitigates the development of the zymosan-induced multiple organ dysfunction syndrome in mice. *Cytokine* 11, 713-721 (1999)

77. Leon, L. R., W. Kozak, K. Rudolph and M. J. Kluger: An antipyretic role for IL-10 in LPS fever in mice. *Am J Physiol* 276, R81-R89 (1999)

78. Van der Poll, T., A. Marchant, W. A. Buurman, L. Berman, C. V. Keogh, D. D. Lazarus, L. Nguyen, M. Goldman, L. L. Moldawer and S. F. Lowry: Endogenous IL-10 protects mice from death during septic peritonitis. *J Immunol* 155, 5397-5401 (1995)

79. Ledebor, A., R. Binnekade, J. J. P. Breve, J. G. J. M. Bol, F. J. H. Tilders and A.-M. Van Dam: Site-specific

modulation of LPS-induced fever and interleukin-1beta expression in rats by interleukin-10. *Am J Physiol* 282, R1762-R1772 (2002)

80. Nava, F., G. Calapai, G. Facciola, S. Cuzzocrea, M. C. Marciano, A. De Sarro and A. P. Caputi: Effects of interleukin-10 on water intake, locomotor activity and rectal temperature in rat treated with endotoxin. *Int J Immunopharmac* 19, 31-38 (1997)

81. Pajkrt, D., L. Camoglio, M. C. M. Tiel-van Buul, K. de Bruin, D. L. Cutler, M. B. Affrime, G. Rikken, T. van der Poll, J. Wouter ten Cate and S. J. H. van Deventer: Attenuation of the proinflammatory response by recombinant human IL-10 in human endotoxemia. *J Immunol* 158, 3971-3977 (1997)

82. Cartmell, T., C. Ball, A. F. Bristow, D. Mitchell and S. Poole: Endogenous interleukin-10 is required for the defervescence of fever evoked by local lipopolysaccharide-induced and *Staphylococcus aureus*-induced inflammation in rats. *J Physiol* 549, 653-664 (2003)

83. Chai, Z., S. Gatti, C. Toniatti, V. Poli and T. Bartfai: Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever responses to lipopolysaccharide or IL-1beta: a study on IL-6-deficient mice. *J Exp Med* 183, 311-316 (1996)

84. Kozak, W., M. J. Kluger, D. Soszynski, C. A. Conn, K. Rudolph, L. R. Leon and H. Zheng: IL-6 and IL-1beta in fever. Studies using cytokine-deficient (knockout) mice. *Ann NY Acad Sci* 856, 33-47 (1998)

85. Conn, C.A., J. L. McClellan, H. F. Maassab, C. W. Smitka, J. A. Madje and M. J. Kluger: Cytokines and the acute phase response to influenza virus in mice. *Am J Physiol* 268, R78-R84 (1995)

86. Kozak, W., V. Poli, D. Soszynski, C. A. Conn, L. R. Leon and M. J. Kluger: Sickness behavior in mice deficient in interleukin-6 during turpentine abscess and influenza pneumonitis. *Am J Physiol* 272, R621-R630 (1997)

87. Sidwell, R. W., J. H. Huffman, J. Gilbert, B. Moscon, G. Pedersen, R. Burger and R. P. Warren: Utilization of pulse oximetry for the study of the inhibitory effects of antiviral agents on influenza virus in mice. *Antimicrob Agents Chemother* 36, 473-476 (1992)

88. Eisenberg, S. P., M. T. Brewer, E. Verderber, P. Heimdal, B.J. Brandhuber and R. C. Thompson: Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: evolution of a cytokine control mechanism. *Proc Natl Acad Sci USA* 88, 5232-5236 (1991)

89. Sims, J. E., J. G. Giri and S. K. Dower: The two interleukin-1 receptors play different roles in IL-1 actions. *Clin Immunol Immunopathol* 72, 9-14 (1993)

90. Klir, J. J., J. L. McClellan and M. J. Kluger: Interleukin-1beta causes the increase in anterior hypothalamic

interleukin-6 during LPS-induced fever in rats. *Am J Physiol* 266, R1845-R1848 (1994)

91. Luheshi, G., A. J. Miller, S. Brouwer, M. J. Dascombe, N. J. Rothwell and S. J. Hopkins: Interleukin-1 receptor antagonist inhibits endotoxin fever and systemic interleukin-6 induction in the rat. *Am J Physiol* 270, E91-5 (1996)

92. Gourine, A. V., K. Rudolph, J. Tesfaigzi and M. J. Kluger: Role of hypothalamic interleukin-1beta in fever induced by cecal ligation and puncture in rats. *Am J Physiol* 275, R754-R761 (1998)

93. Alheim, K., Z. Chai, G. Fantuzzi, H. Hasanvan, D. Malinowsky, E. Di Santo, P. Ghezzi, C. A. Dinarello and T. Bartfai: Hyperresponsive febrile reactions to interleukin (IL) 1alpha and IL-1beta and altered brain cytokine mRNA and serum cytokine levels, in IL-1beta-deficient mice. *Proc Natl Acad Sci USA* 94, 2681-2686 (1997)

94. Leon, L. R., C. A. Conn, M. Glaccum and M. J. Kluger: IL-1 type I receptor mediates acute phase response to turpentine, but not lipopolysaccharide, in mice. *Am J Physiol* 271, R1668-R1675 (1996)

95. Alexander, H. R., G. M. Doherty, D. J. Venzon, M. J. Merino, D. L. Fraker and J. A. Norton: Recombinant interleukin-1 receptor antagonist (IL-1ra): effective therapy against gram-negative sepsis in rats. *Surgery* 112, 188-194 (1992)

96. Kozak, W., H. Zheng, C. A. Conn, D. Soszynski, L. H. van der Ploeg and M. J. Kluger: Thermal and behavioral effects of lipopolysaccharide and influenza in interleukin-1 beta-deficient mice. *Am J Physiol* 269, R969-77 (1995)

97. Heinzel, F. P: The role of IFN-gamma in the pathology of experimental endotoxemia. *J Immunol* 125, 2920-2924 (1990)

98. Blaque, R., C. Meakin, S. Millet and C. R. Gardner: Dual mechanisms of action of interferon-gamma in potentiating responses to LPS in mice: IL1, TNFalpha and IL6 production in serum and hypothermia. *Gen Pharmacol* 32, 453-61 (1999)

99. Smits, B. M. G., J. Mudde, R. H. A. Plasterk and E. Cuppen: Target-selected mutagenesis of the rat. *Genomics* 83, 332-334 (2004)

100. Qing, M., J. F. Vazquez-Jimenez, B. Klosterhalfen, M. Sigler, K. Schumacher, J. Duchateau, B. J. Messmer, G. von Bernuth and M.-C. Seghaye: Influence of temperature during cardiopulmonary bypass on leukocyte activation, cytokine balance and post-operative organ damage. *Shock* 15, 372-377 (2001)

101. Zhi, D., S. Zhang and X. Lin: Study on therapeutic mechanism and clinical effect of mild hypothermia in patients with severe head injury. *Surg Neurol* 59, 381-385 (2003)

102. Ueno, R., S. Narumiya, T. Ogorochi, T. Nakayama, Y. Ishikawa and O. Hayaishi: Role of prostaglandin D₂ in the

hypothermia of rats caused by bacterial lipopolysaccharide. *Proc Natl Acad Sci* 79, 6093-6097 (1982)

103. Dogan, M. D., H. Ataoglu and E. S. Akarsu: Effects of selective cyclooxygenase enzyme inhibitors on lipopolysaccharide-induced dual thermoregulatory changes in rats. *Brain Res Bull* 57, 179-185 (2002)

Key Words: Tumor necrosis factor, Interleukin-10, Interleukin-6, Interleukin-1, Interferon, Body temperature, Anaprexia, Fever, Review

Send correspondence to: Lisa R. Leon, Ph.D., US Army Research Institute of Environmental Medicine, Thermal and Mountain Medicine Division, Kansas Street, Building 42, Natick, Massachusetts 01760, Tel: 508-233-4862, Fax: 508-233-5298, E-mail: lisa.leon@na.amedd.army.mil