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Minireview

MINIREVIEW

Thermoregulation as a disease tolerance defense strategy

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One sentence summary: In this review, the concept of disease tolerance is applied to thermoregulation during infection, inflammation and trauma, and the authors discuss the physiological consequences of thermoregulation during disease including tissue susceptibility to damage, inflammation, behavior and toxin neutralization.

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ABSTRACT

Physiological responses that occur during infection are most often thought of in terms of effectors of microbial destruction through the execution of resistance mechanisms, due to a direct action of the microbe, or are maladaptive consequences of host–pathogen interplay. However, an examination of the cellular and organ-level consequences of one such response, thermoregulation that leads to fever or hypothermia, reveals that these actions cannot be readily explained within the traditional paradigms of microbial killing or maladaptive consequences of host–pathogen interactions. In this review, the concept of disease tolerance is applied to thermoregulation during infection, inflammation and trauma, and we discuss the physiological consequences of thermoregulation during disease including tissue susceptibility to damage, inflammation, behavior and toxin neutralization.

Keywords: tolerance; fever; hypothermia

INTRODUCTION

Perhaps because our view of host defenses is shaped by the importance of anti-microbial strategies, we assume that any physiological change that occurs during an infection does so to support the immunological response is a direct action of microbial products, or is simply a maladaptive consequence of the immune or other host response. Thermoregulation during infection is a prime example of this. Infection elicits changes in the thermoregulatory strategies of a host that occurs through the integration of signals from the immune, metabolic and neural systems to change the thermal regulatory set point and change body temperature (Kluger 1980). In many cases, a higher body temperature is achieved—a response known as fever or hyperthermia. However, with certain infection types, particularly during sepsis or severe systemic inflammation, a lower body temperature is selected for a response known as hypothermia. In certain organisms, such as songbirds, a single infection type can cause circadian-associated patterns of hypothermia and fever (Skold-Chiriac *et al.* 2015). Infection-associated changes in body temperature had long been viewed as an undesirable consequence of host–pathogen interplay. However, because of the widespread occurrence of fever and hypothermia among many different animals in the context of many different types of infectious diseases, and because of the costs to the host associated with thermoregulatory changes, we support the idea that thermoregulation is likely an evolved mechanism that has adaptive value for the host in fighting infections (Kluger 1980, 1986). This initial proposal made by Kluger in the 1970s only considered that the fever response is beneficial for host defenses (Kluger, Ringler and Anver 1975).

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However, we suggest that both fever and hypothermic response are evolved strategies of the host to optimize host defenses during infection and non-infectious diseases in which thermoregulatory strategies of the host are employed. Indeed, there is evidence to suggest that both fever and hypothermic responses are beneficial for host health in the context of an array of diseases. In pioneering studies using the ectotherm *Dipsosaurus dorsalis*, which uses behavioral thermoregulation to generate environmental fevers, Kluger demonstrated that iguanas infected with the pathogen *Aeromonas hydrophila* that were prevented from seeking warmer environments showed significantly increased mortality compared to infected iguanas that were allowed to generate fevers (Kluger, Ringler and Anver 1975; Bernheim and Kluger 1976). The snail, *Lymnaca stagnalis*, exhibited behavioral hypothermia when infected with the trematode species *Diplostomum pseudospathaceum* or *Plagiorchis elegans*. Achieving behavioral hypothermia increased longevity in infected snails compared to those that were maintained at ambient temperatures during infection (Zbikowska 2005).

Similar benefits for infection-associated thermoregulation in endotherms have also been established. Treatment of *Pasteurella multocida*-infected rabbits with an antipyretic was associated with greater mortality than infected rabbits that were allowed to develop fevers (Vaughn, Veale and Cooper 1980). In humans, the therapeutic use of fevers demonstrates the importance of fever in host defenses. *Plasmodium*, the causative agent of malaria, causes cyclical fevers due to the rupture of erythocytic stage schizonts. Wagner-Jauregg discovered that upon infection of neurosyphilus, patients with *Plasmodium* infection developed very high fevers and were cured of the neurosyphilis (Karamanou *et al*. 2013). In the zebra finch, injection with the immune elicitor lipopolysaccharide (LPS) induces a fever response at night but a hypothermic response during the day (Skold-Chiriac *et al*. 2015). These day-to-night differences in body temperature may indicate that there is a tradeoff between the benefit of fever and the possibility of overheating. During the night, when body temperature is lower, the birds are able to achieve a fever to promote host defense but during the day, when body temperatures are normally higher, the birds induce a hypothermic response to offset the costs of fever and prevent overheating. Thus, an additional thermoregulatory strategy may be to use both fever and hypothermic responses in complimentary ways over the course of a single infection to offset the potential maladaptive effects they may have on host health.

Why infection-induced alterations in thermoregulatory strategies are beneficial for the host in combatting infections and the mechanisms by which these protective responses occur remain unknown. The widespread assumption is that thermoregulation protects the host by having a negative impact on pathogen fitness. For example, pathogens may be less able to replicate if the host body temperatures are above or below the optimal temperature for the pathogen. During fever, iron, which is used by many microbes, is sequestered by host tissues (Hacker, Rothenburg and Kluger 1981; Zinchuk and Borisiuk 1997). Thus, changes in body temperature may make the host a less hospitable niche for microbes, yet pathogens vary in their temperature preferences and dependency on nutrient utilization. It has also been proposed that thermoregulation is important for shaping the immune response by optimizing resistance mechanisms and inducing the remobilization of energy stores to fuel microbial killing mechanisms (Hart 1988; Evans, Repasky and Fisher 2015). However, cellular and physiological evidence suggest that temperature-associated defense against infections cannot be readily explained only with the traditional paradigms of microbial killing mechanisms (Table 1).

Infectious diseases cause significant physiological damage to the host. In addition to executing resistance mechanisms to kill microbes, a host must deal with the collateral damage that occurs during host–pathogen interplay in order to survive. Ecologists have long recognized that genetic variation in disease susceptibility exists in plants that can be dissociated from the plants ability to kill a pathogen or pest. This variation is due to a distinct defense strategy encoded by plants, called 'tolerance' that promotes plant fitness in the presence of a given level of pathogen or herbivore. In recent years, the concept of tolerance defenses has been introduced into the field of animal immunology (Raberg, Sim and Read 2007; Ayres and Schneider 2008, 2009, 2012; Ayres, Freitag and Schneider 2008; Ayres, Trinidad and Vance 2012; Medzhitov, Schneider and Soares 2012; Schieber *et al*. 2015). In this context, tolerance is a defense strategy that minimizes the physiological damage that occurs during infection without having a negative impact on microbial fitness. We propose that thermoregulatory mechanisms that both increase and decrease body temperature are adaptive strategies of the host to promote tolerance defenses and survival following infections. There is evidence that fever and heat shock induce cyto- and tissue-protective responses that will work to limit tissue susceptibility to damage.

In addition to fever, the beneficial effects of hypothermia in defense against tissue damage have long been recognized. For example, the ancient Greeks used hypothermia for treating various conditions including hemorrhaging (Diller and Zhu 2009). Hibernating animals are less susceptible to damage induced by ischemia due to their low body temperatures causing reduced oxygen consumption, cardiac function and metabolism rendering tissues less susceptible to damage (Dave *et al*. 2012). In current medical practices, hypothermia is used for its cyto- and tissue-protective effects in the context of trauma in which the metabolic and inflammatory states of the body and microenvironments change frequently making tissues and cells vulnerable to damage, compromising the normal function of organs (Andresen *et al*. 2015; Saigal *et al*. 2015; Usach, Sakopoulos and Razavi 2015; Alkabie and Boileau 2016). Behavioral experiments demonstrated that animals that chose to move to colder environments under conditions in which tissue homeostasis is compromised are healthier than those that do not develop behavioral hypothermia (Romanovsky *et al*. 2005; Almeida *et al*. 2006b). In infections, hypothermia is most often associated with advanced stage septic patients and those with severe systemic inflammation, whom are vulnerable to similar forms of cellular and physiological damage as trauma patients (Perman *et al*. 2014; Chisholm *et al*. 2016; Kohlhauer *et al*. 2015). Hypothermia may be viewed as a host's final effort to limit physiological damage during these disease states but over longer periods of time become maladaptive to the host as is the case with prolonged hyperthermic responses.

The function of thermoregulation during infections is almost inevitably defined in terms of microbial killing mechanisms. Evidence shows, however, that several effects of temperature cannot be readily explained within the paradigm of resistance defenses. In this review, we apply the conceptual framework of tolerance to thermoregulation in animals to illustrate how the physiological responses that occur during hyperthermia and hypothermia can promote health and survival during infectious diseases in animals (Fig. 2).

Table 1. Contribution of thermoregulation to resistance and tolerance defenses. This table summarizes in vitro and in vivo observations of how temperature influences host defense. This table also summarizes data describing how temperature sensitive factors influence various processes and the observed or predicted effects this would have on resistance and tolerance.

Observation	Thermal sensitivity	Apparent effects on host defense strategies/pathogen fitness	Reference
Aeromonas hydrophila-infected iguanas that seek warmer environments were better able to survive infection	Heat sensitive	Undetermined	Kluger, Ringler and Anver (1975); Bernheim and Kluger (1976)
Trematode-infected snails that seek colder environments were better able to survive infection	Cold sensitive	Tolerance	Zbikowska (2005)
Blocking fever in *Pasteurella multocida* infected rabbits increased mortality	Heat sensitive	Undetermined	Vaughn, Veale and Cooper (1980)
LPS challenged zebra finches develop cyclical hypothermia and fever responses	Heat and cold sensitive	Tolerance	Karamanou *et al.* (2013)
Elevated or reduced temperatures impair pathogen growth	Heat and cold sensitive	Impair pathogen fitness	Carmichael, Barnes and Percy (1969); MacKowiak *et al.* (1981)
Sequestration of iron by host	Heat sensitive	Impair pathogen fitness	Hacker, Rothenburg and Kluger (1981); Zinchuk and Borisiuk (1997)
Ancient Greeks used hypothermia to treat hemorrhage	Cold sensitive	Tolerance	Diller and Zhu (2009)
Hibernating animals are less susceptible to ischemic tissue damage	Cold sensitive	Tolerance	Dave *et al.* (2012)
Honeybees develop a brood-comb fever by exerting movement in response to a colonial infection with the fungus *Ascosphaera apis*	Heat sensitive	Impair pathogen fitness	Starks, Blackie and Seeley (2000)
In LPS-treated human monocytes, HSF-1 inhibits transcription of genes encoding proinflammatory cytokines	Heat sensitive	Tolerance	Cahill *et al.* (1996)
Listeria monocytogenes infected *hsf*-1-/- mice exhibited significantly higher levels of serum TNF-α and IFN-γ and rapidly succumbed to infection without a significant difference in liver and spleen *Listeria* burdens	Heat sensitive	Tolerance	Murapa *et al.* (2011)
Heat exposure prevented transcriptional upregulation of proinflammatory cytokines by preventing the release of the damage associated molecular pattern, HMGB1	Heat sensitive	Tolerance	Fiuza *et al.* (2003); Lee and Repasky (2012)
In a mouse model of arthritis, mice exposed to higher temperatures had reduced joint damage	Heat sensitive	Tolerance	Lee *et al.* (2015)
IL-1b causes insulin resistance and the resulting impairment in glucose uptake can limit further IL-1b transcription and ROS production.	Heat sensitive	Tolerance	Jager *et al.* (2007); Wen *et al.* (2011); Benetti *et al.* (2013)
HSP32 detoxifies free heme during malaria and sepsis	Heat sensitive	Tolerance	Larsen *et al.* (2010); Ferreira *et al.* (2011)
Alkaline phosphatase detoxifies LPS	Heat sensitive	Tolerance	Koyama *et al.* (2002); Beumer *et al.* (2003); Tuin *et al.* (2006)
HSP72 inhibits proteotoxicity	Heat sensitive	Tolerance	Mayer and Bukau (2005)
Hypothermia induces expression of cold shock proteins that activate the UPR	Cold sensitive	Tolerance	Rzechorzek *et al.* (2015)
HSP72 maintains cardiac function during sepsis	Heat sensitive	Tolerance	Robert *et al.* (2014)

Table 1. (*Continued*).

Observation	Thermal sensitivity	Apparent effects on host defense strategies/pathogen fitness	Reference
Shift from carbohydrate to lipid metabolism during hibernation and hypothermia limiting ischemic injury	Cold sensitive	Tolerance	Wong (1983); Drew *et al.* (2007); Suozzi, Malatesta and Zancanaro (2009); Hindle *et al.* (2011); Darwazeh and Yan (2013); Xu *et al.* (2013); Quinones *et al.* (2014)
Hypothermia improved coagulopathy in septic patients	Cold sensitive	Tolerance	Johansen *et al.* (2015)
Cold temperatures delay initiation of thrombus formation and speed of clot formation	Cold sensitive	Tolerance	Valeri *et al.* (1987); Patt, McCroskey and Moore (1988); Michelson *et al.* (1994); Ruzicka *et al.* (2012)
Endotherms and ectotherms lower body temperatures under hypoxic conditions	Cold sensitive	Tolerance	Hicks and Wood (1985); Dupre and Owen (1992)
Survival of hypoxic animals increased when put at lower temperatures	Cold sensitive	Tolerance	Gollan and Aono (1973); Hicks and Wood (1985); Gordon (2001)
Hypothermia protects from reperfusion tissue injury	Cold sensitive	Tolerance	Ning *et al.* (2007); Lampe and Becker (2011)
Reduced sensitivity of fibrosarcoma cells to TNFα-mediated cell lysis	Heat sensitive	Tolerance	Gromkowski, Yagi and Janeway (1989)
Incubation of HeLa cells at mild hyperthermic temperatures rendered them resistant to apoptosis when incubated at lethal hyperthermic temperatures	Heat sensitive	Tolerance	Bettaieb and Averill-Bates (2008)
HSP32-deficient mice infected with *Plasmodium* had increased liver damage caused by increased sensitivity of hepatocytes to TNF-mediated apoptosis	Heat sensitive	Tolerance	Seixas *et al.* (2009)
HSP70 and HSP90 inhibit apoptosome formation	Heat sensitive	Tolerance	Beere *et al.* (2000); Pandey *et al.* (2000); Saleh *et al.* (2000)
HSP60 affects caspase-3 activation to inhibit apoptosis	Heat sensitive	Tolerance	Xanthoudakis *et al.* (1999)
Hypothermia has been shown to alleviate pump dysregulation and help maintain intracellular Ca^{2+} homeostasis and may prevent cell death	Cold sensitive	Tolerance	Siesjo *et al.* (1989); Hall (1997)
Cold temperatures delay apoptosis in response to stress by delaying cytochrome c release	Cold sensitive	Tolerance	Goldstein *et al.* (2000)
Heat can directly activate Bax and Bak to induce cytochrome c release	Heat sensitive	Tolerance	Pagliari *et al.* (2005)
HSP90 is required for the induction of necroptosis	Heat sensitive	Tolerance	Jacobsen *et al.* (2016); Zhao *et al.* (2016)
Increased phagocytosis by macrophages	Heat sensitive	Resistance	Evans, Repasky and Fisher (2015)
Increased expression and release of cytokines	Heat sensitive	Resistance or tolerance	Evans, Repasky and Fisher (2015)
Hypothermia protected rats from endotoxemia	Cold sensitive	Trade-offs in resistance and tolerance	Liu *et al.* (2012)
Increased neutrophil release, infiltration and microbial killing	Heat sensitive	Resistance	Evans, Repasky and Fisher (2015)
Enhanced lymphocyte trafficking and proliferation in response to antigens	Heat sensitive	Resistance	Hart (1988)
Increased antibody synthesis	Heat sensitive	Resistance	Hart (1988)

THERMOREGULATORY MECHANISMS IN ANIMALS

Initiation of fever in endotherms occurs upon central and peripheral recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors on innate immune cells including macrophages and dendritic cells (Jounai *et al.* 2012). In addition, multiple cell types in the brain including endothelial cells, microglial cells and neurons can respond to PAMPs (Rivest 2009; Hernangomez *et al.* 2014). The fever response has been best characterized in the context of LPS challenge and we will focus our discussion around these studies. Activation of Toll-like receptor 4 (TLR4) by LPS centrally and peripherally induces the production of host-derived pyrogens including IL-1β, TNF-α and IL-6. These cytokines circulate through the lymphatic system and signal to the brain (Kluger *et al.* 1995; Romanovsky, Steiner and Matsumura 2006; Zhang *et al.* 2008; Yamawaki *et al.* 2010; Nakano *et al.* 2015; Poon *et al.* 2015). Il-1β has been long thought to be the primary pyrogen responsible for fever induction; however, accumulating evidence using genetic knockout and neutralization studies as well as clinical data now suggests that IL-6 is the essential mediator for the febrile response (Hart 1988; Evans, Repasky and Fisher 2015). The febrile response is due to these systemic and locally produced cytokines acting on the hypothalamus in the brain to change the thermoregulatory set point. This set point is raised so that what was once a thermal neutral temperature is now subjectively cold to the host. The host reaches a new thermal equilibrium by a number of mechanisms. Among these are brown adipose tissue thermogenesis driven by noradrenaline release and increasing metabolism to induce shivering to produce heat. Vasoconstriction is also induced to reduce blood flow to peripheral fevers preventing heat loss (Almeida *et al.* 2006a; Nakamura and Morrison 2011). Endotherms will also exhibit behavioral changes including seeking warmer environments and curling up to reduce the amount of body surface exposed to the environment to minimize heat loss. The mechanism by which endogenous pyrogenic cytokines induce the hypothalamus to raise the thermogenic set point involves their induction of cyclooxygenase 2 (COX2) to induce the conversion of arachidonic acid into prostaglandin E2 (PGE2). Peripheral synthesis of COX2 and PGE2 is important for initiation of fever. In a LPS fever model, recognition of LPS by TLR4 on lung and hepaptic hematopoietic cells induces transcriptional upregulation of COX2 leading to production of PGE2 to signal to the brain (Steiner *et al.* 2006). Injection of a cyclooxygenase inhibitor in the preoptic area (POA) has shown inconsistent results, but seems to mainly attenuate activation of median preoptic and arcuate hypothalamus suggesting that there may also be a central mechanism that regulates COX2 activity in fever induction (Nadjar *et al.* 2010). PGE2 binds to the PGE2 receptors (EP), on thermoregulatory neurons in the hypothalamus (Oka *et al.* 2000; Nakamura *et al.* 2002; Oka 2004; Lazarus *et al.* 2007). Signals are then sent through neurons to the dorsomedial hypothalamic nucleus to elicit sympathetic thermogenesis in peripheral effector organs (Nakamura *et al.* 2005). The cytokine receptor activator of NF-kB, TNFSF11, found on astrocytes in the hypothalamus, also activates COX2 and PGE2 production to activate EP3 (Hanada *et al.* 2009). Other EP receptors including EP1 and EP4 may contribute to different phases of fever suggesting functional redundancy of EP receptors in fever induction (Oka *et al.* 2000). Drugs that suppress fever including salicylic acid do so through the inhibition of prostaglandin synthesis (Vane and Botting 2003). Antipyretics are non-specific and can also influence resistance mechanisms. This must be considered when examining how such drugs promote host defenses during infection.

Recent evidence suggests that the microbiota, the trillions of beneficial microbes that colonize the body surfaces exposed to the environment, can also regulate hyperthermic responses. Cold temperatures can induce ecological perturbations in the intestinal microbiota composition that are apparently adaptive for the host. These ecological changes are associated with intestinal tissue remodeling and changes in adipose tissue physiology that cause white adipose tissue to become beige, which has thermogenic properties similar to brown adipose tissue, and cause a rise in body temperature (Chevalier *et al.* 2015). This is a complex relationship in that environmental conditions cause a host response that perturbs the microbiota, which in turn influences host physiology to return to homeostasis.

While ectotherms such as amphibians, reptiles and insects do not generate endogenous pyrogenic chemical responses, they can generate fevers through behavioral regulation of body temperature during infections. For example, ectotherms will move to warmer environments in order to exogenously induce fever in response to an LPS injection and this movement is required to increase survival during challenge (Vaughn, Bernheim and Kluger 1974). Honeybees develop a brood-comb fever by exerting movement in response to a colonial infection with the fungus *Ascosphaera apis*. This pathogen is heat sensitive and in this case it seems that this social fever response promotes host defense by creating a less hospitable niche for the pathogen (Starks, Blackie and Seeley 2000). Thus, the recognition of infections and microbial-derived products in ectotherms appears to induce behavioral changes that result in thermoregulatory responses to promote host survival by promoting mechanisms that can have either a negative or neutral/positive impact on pathogen fitness.

There is also evidence to suggest that in addition to endotherms, ectotherms use PGE2 in order to induce behavioral fevers. Treatment of the toad, *Bufo paracnemis,* with the COX inhibitor indomethacin rendered the toads unable to produce fevers in response to an LPS challenge (Bicego *et al.* 2002). Histamine, hemoglobin, corticosterine and temperature-sensitive transient receptor potential channels have also presented as possible factors in mediating ectothermal fever (Hutchison and Spriestersbach 1986; Wiggins and Frappell 2000; Preest and Cree 2008; Saper, Romanovsky and Scammell 2012). Thermoregulatory behavior requires a complex neuronal circuitry of initiating, organizing, performing and controlling motor actions. Our current understanding of the neural pathways regulating behavioral thermoregulation is limited, and neural pathways controlling thermoregulatory behaviors differ from those controlling autonomic thermoeffectors, making work in these areas more complex (Nagashima *et al.* 2000; Flouris 2011). Recent studies involving neural thermal stimulation have provided evidence as to which central thermosensors are involved with behavioral thermoregulation; these include the medulla oblongata, pons, midbrain, and amygdala, and the orbitofrontal, insular and somatosensory cortex. Further studies in the toad *B. paracnemis* have demonstrated that lesions in the POA of the hypothalamus prevented toads from developing a febrile response triggered by LPS challenge, although regular thermoregulatory abilities were not affected. These data suggest that the POA may be important in fever generation in ectotherms (Bicego and Branco 2002).

Hypothermia or anapyrexia has been most studied as a clinical therapeutic and less focus has been placed on mechanistically understanding the induction of the cryogenic response triggered by infection and immune stimulation. Studies of hypoxia-induced anapyrexia, which may serve as a strategy

to avoid severe hypoxia by reducing metabolic demands, have unraveled some mechanisms that may apply to other forms of hypothermia. Centrally, the glutamatergic action of the lateral POA of the hypothalamus has been implicated in the control of hypoxia-induced hypothermia in endotherms. This appears to be mediated by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and ionotropic GABA receptors in the rostral parapyramidal region of the medulla oblongata, as neurons directly project between these parts of the brain (Yoshida *et al.* 2009; Osaka 2014). Connecting the brainstem to the rest of the brain, the medulla oblongata contains respiratory, cardiac and vasomotor centers, positioning itself as a reasonable culprit of thermoregulatory action. While endogenous regulation of the hypothermic response involves the POA, the behavioral responses that mediate hypothermia appear to involve the neuronal bodies located in the dorsomedial nucleus and neural fibers passing through the paraventricular nucleus of the hypothalamus (Almeida *et al.* 2006a).

In certain contexts, proinflammatory cytokines that typically act as pyrogens can also act as cryogens. For example, the proinflammatory cytokines IL-1β, IL-6 and TNF-α have been shown to act as cryogens in animal models of infection and systemic inflammation. In a mouse model of antibiotic-induced dysbiosis and intestinal injury, administration of broad spectrum antibiotics induced the expansion of a multi-antibiotic-resistant *Escherichia coli* strain. Upon disruption of the intestinal barrier with the sulfated polysaccharide dextran sulfate sodium (DSS), this *E. coli* translocated to extraintestinal tissues causing a sepsis-like disease characterized by multi-organ dysfunction and hypothermia. The authors found that this hypothermic response was dependent on activation of a component of the innate immune system called the NLRC4 inflammasome by this *E. coli* strain, which resulted in an overly exuberant response mediated by IL-1β (Ayres, Trinidad and Vance 2012). The hypothermic response seen in this model and many other mouse infection models is likely due to the animals being thermally stressed. While pyrogenic infections cause fever at thermoneutrality and above, subneutral temperatures elicit a hypothermic response in response to similar agents in animals (Ivanov *et al.* 2003). Most animal vivariums that conduct research house their mice at subthermal temperatures (~19°C–26°C; Speakman and Keijer 2012), despite knowledge that the rodent's thermal neutral zone is ~30°C, causing thermal stress (Watkinson and Gordon 1993; Swoap *et al.* 2008). Due to this, biological studies of infectious diseases and in general have been in the context of hypothermia. This hypothermic rather than hyperthermic response may result from various inflammatory stimuli including proinflammatory cytokines, differences in pathogen load, route/site of administration, ambient temperatures, circadian timing and other factors that influence host temperature differently when animals are under thermal neutral and thermal stressed conditions (Nomoto 1996; Szelenyi *et al.* 2004). With this in mind, it is important to recognize what we can learn from these 'mistakes' and use them as observations for relevant studies.

Other cryogens potentially exist and take part in LPS-induced hypothermia, including the cytokines IL-10 and IFNγ (Leon 2004). 3-Iodothyronamine (T_1AM), an endogenous derivative of thyroid hormone, induces robust hypothermia in mice and rhesus monkeys, possibly related to hypothermic neuroprotective effects during stroke (Scanlan *et al.* 2004; Panas *et al.* 2010). Hypothalamic cysteinyl-LT (CysLT), an arachidonate metabolism product, 5-lipoxygenase and epoxygenase-derived eicosanoids have also been indicated as promoters of anapyrexia. This may suggest that LPS-induced hypothermia may be mediated by leukotrienes (Paul, Fraifeld and Kaplanski 1999; Kozak and Fraifeld 2004). A few cryogens have been identified in the context of hypoxic-induced hypothermia. Nitric oxide in the POA is a potential promoter of hypothermia induced by hypoxia (Steiner and Branco 2003). Simultaneous increases in the levels of cyclic adenosine monophosphate (cAMP) and cGMP in the POA may play a role in hypoxia-induced hypothermia due to increases in production and/or release of serotonin and nitric oxide (Steiner and Branco 2003). An intracellular cascade of adenylate cyclase, protein kinase A and cAMP caused by high levels of hydrogen sulfide in the POA may play an essential role in the occurrence of the hypothermia in response to hypoxia (Kwiatkoski *et al.* 2012). It appears that carbon monoxide may downregulate hypoxia-induced hypothermia, as studies using heme-oxygenase (CO-synthesizing enzyme) inhibition showed that hypothermia was attenuated (Paro *et al.* 2002). There have been indications of human cryogens, but the specific identity of these molecules remains to be determined (Shido *et al.* 2004; Shido and Sugimoto 2011).

Methods by which tolerance can be measured have been described in several recent reviews (Raberg, Sim and Read 2007; Ayres and Schneider 2008, 2011). The relative contributions of resistance and tolerance to host defense against infection can be distinguished in any host microbe system by examining the relationship between host health and pathogen levels. Using these parameters, and assuming health of the host when uninfected is equivalent between different host populations (vigor), a dose response curve can be generated to determine how host health changes as microbial levels change. Changes in health as microbe levels change would shift the host along the diagonal and indicate differences in resistance (Fig. 1). Changes in health without a change in microbe levels would shift the curve along the y-axis and would indicate differences in tolerance (Fig. 1). The more tolerant a host, the shallower the slope of the dose response curve would be. This method can be used to determine how different factors including environmental and genetic factors can influence host defenses. For example, mice infected with pathogen A that differ in the ability to thermally regulate their bodies may differ in how the shift within this health-by-microbe space with respect to each other, revealing how temperature regulates resistance and tolerance in the context of pathogen A infection. Ayres and Schneider (2008, 2012) reported the point tolerance method, which uses microbial levels at a single defined time point to reveal how a single mutation in a component of the fly immune response can impact resistance and tolerance in response to different infection challenges (Fig. 1). Using a mouse malaria model, Raberg, Sim and Read (2007) reported that range tolerance, which measures host health at various pathogen doses, can reveal variations in resistance and tolerance (Fig. 1). Both models are based on the assumption that these relationships are linear and have been useful at identifying environmental, microbial and genetic factors that influence tolerance defenses. These relationships however are likely more complex and further experimentation and data points, for example, measuring pathogen burden over the course of the infection will reveal how differences in host populations influence resistance and tolerance at different stages of the infection.

CATABOLISM AND ENERGETIC COSTS OF THERMOREGULATION

Fever responses caused by many forms of insult including sepsis induce a hypermetabolic state in the host (Frankenfield *et al.* 1997). Induction of a hypermetabolic state increases heat production to raise and maintain body temperature at the new

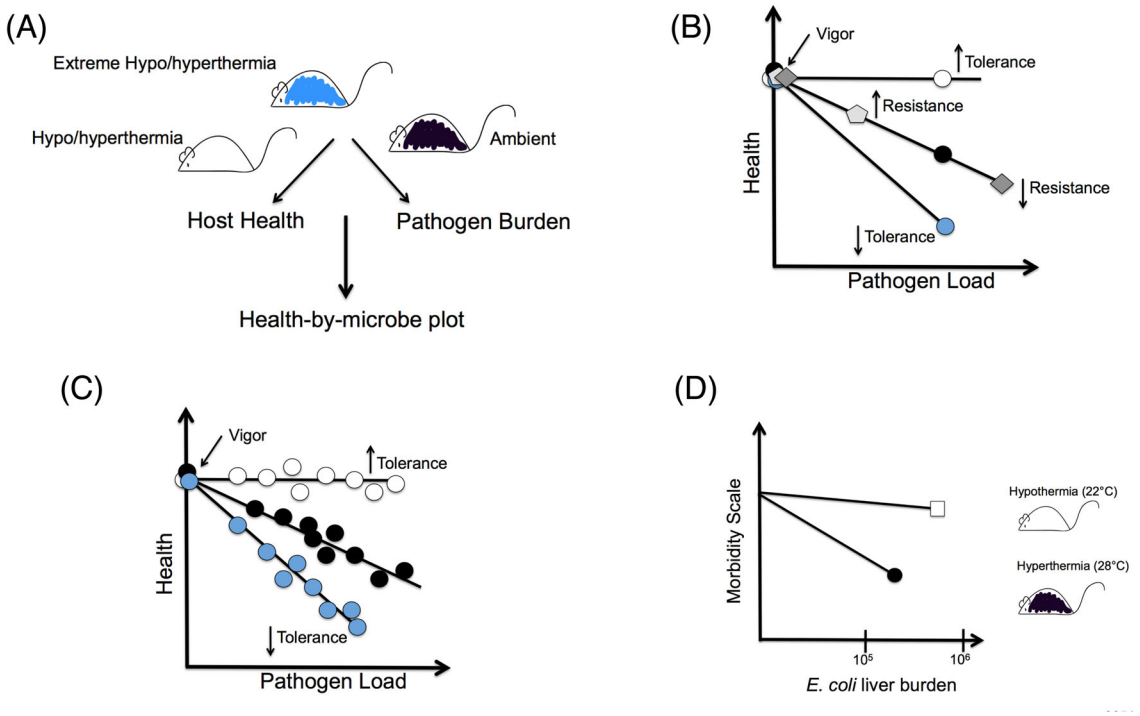

Figure 1. Measuring the effects of thermoregulation on resistance and tolerance. (A) Host health and pathogen burdens of infected host populations that differ in their thermoregulatory responses are measured. Using these values, a health-by-pathogen plot is generated in (B) and (C) to determine how these host populations move in space with respect to each other. (B) Point tolerance. The relationship between host health and pathogen levels at a defined time point is examined. Shifts along the y-axis indicate health is changing as microbe levels are changing and indicate changes in resistance. Shifts along the y-axis indicate changes in tolerance. The steeper the slope the more tolerant the host. Adapted from Ayres and Schneider, (2008, 2012). (C) Range tolerance. The relationship between host health and different levels of pathogen are examined. Adapted from Raberg, Sim and Read (2007). (D) Wisteria rats were infected intraperitoneally with a clinical *E. coli* isolate and placed in either hyperthermic or hypothermic conditions. Morbidity and *E. coli* burdens in livers at 5 h post infection were measured. Shown are the data represented as point tolerance. Animals at 28°C have an increase in resistance. On the other hand, animals at ambient temperature that were allowed to develop hypothermia had lessened morbidity as characterized by decreased lung inflammation and neutrophil infiltration. Decreased tolerance is represented by a less steep slope. Data from Liu *et al.* (2012).

elevated thermoregulatory set point. Studies in humans have suggested that the induction of fever causes a 30%–50% increase in metabolism and the average percent increase in metabolism for every 1°C of fever is ~13% (Mackowiak and Plaisance 1998; Kluger *et al.* 1998; Kluger 2002). Consequences of the hypermetabolic response during fever are muscle proteolysis and a negative nitrogen balance in the host that results clinically in the wasting of energetic body tissues. While anabolic responses occur during infection, the magnitude of catabolic responses tends to be greater and results in the depletion of body tissues. The function of muscle wasting is not understood but possibly its function may serve to generate a source of amino acids that are mobilized to other parts of the body that are used for the anabolic requirements of the host response. Catabolism of body tissues does not become evident until the fever has been fully reached. Consistent with the notion that active thermoregulation during infection is beneficial for host defenses, muscle catabolism should also be beneficial for the host. Given that there are costs to mounting resistance responses (Iseri and Klasing 2014), the current hypothesis is that this muscle wasting occurs to fuel the resistance response. However, we propose that muscle catabolism is also necessary to fuel tolerance defense mechanisms that are induced by fever (Fig. 2). For example, defense responses that negatively regulate inflammatory responses and the induction of cytoprotective and tissue repair pathways are also energetically costly (Ayres and Schneider 2011).

The pathophysiology of muscle wasting is complex and incompletely understood. Factors including severity of the primary disease, proinflammatory cytokines such as TNFα, IL-1β and IL-6, severity of the anorexic response, hormones, metabolism and pathogen factors are all believed to be the main drivers of skeletal muscle catabolism that can lead to the induction of atrophy-dependent programs in muscle (Beutler *et al.* 1985; Goodman 1991, 1994; Zamir *et al.* 1992; Costelli *et al.* 1993). The intestinal microbiota is also a critical regulator of skeletal muscle wasting in response to infectious and inflammatory diseases (Schieber *et al.* 2015). Colony born C57Bl/6 mice were protected from muscle wasting caused by DSS-induced intestinal injury compared to mice from Jackson Labs. Colony born mice harbored an *Escherichia coli* O21:H+ strain in the intestine that was absent in the mice from Jackson Labs. According to Koch's postulates, administration of this commensal to Jackson mice resulted in protection from wasting induced by intestinal injury. This protection could be extended to oral infection with the pathogen *Salmonella* Typhimurium and lung infection caused by *Burkholderia thailandensis*. *Escherichia coli* O21:H+ mediates its protective effect by preventing infection/inflammation-induced systemic drop in circulating levels of IGF-1 in an NLC4 inflammasome-dependent manner without impacting pathogen infection levels indicating that this *E. coli* promotes tolerance. This protection was associated with *E. coli* O21:H+ occupying a new niche—white adipose tissue—during challenged states. In this case, muscle wasting

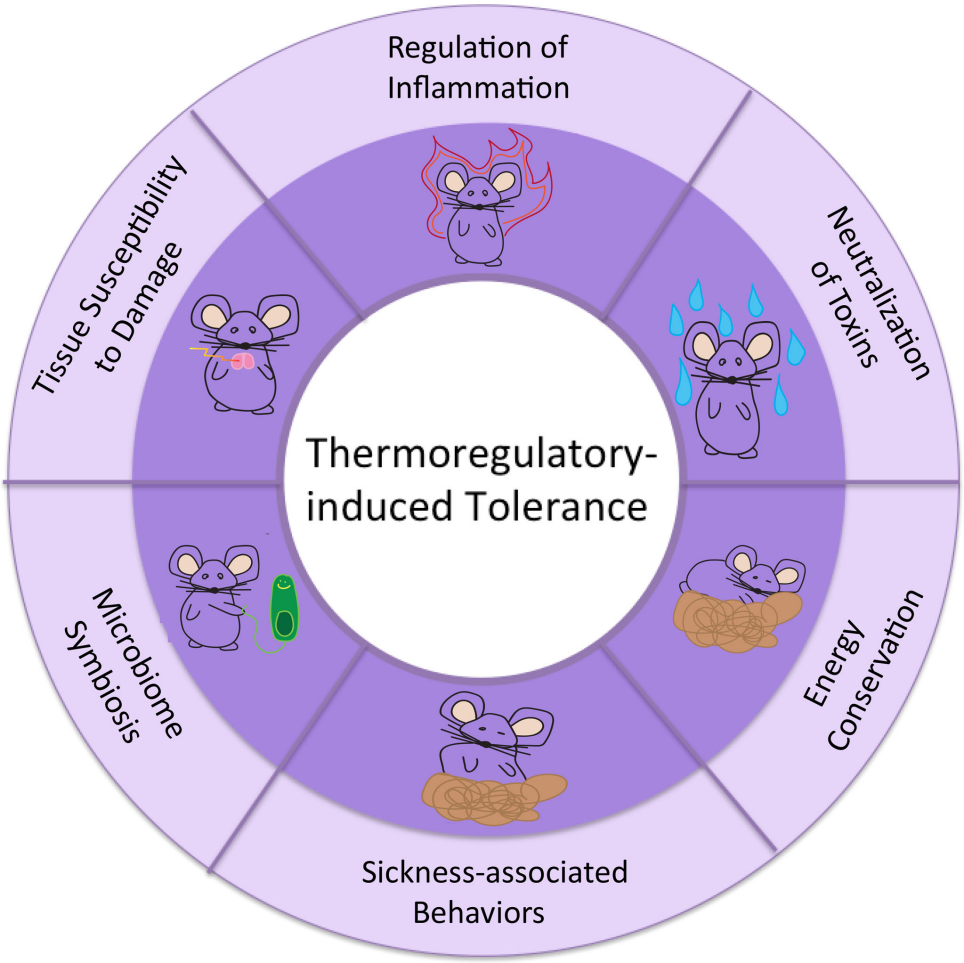

Figure 2. Thermoregulatory induced tolerance mechanisms.

appears to be maladaptive to the host by reducing host tolerance (Schieber *et al.* 2015). Clearly, this is inconsistent with the idea that wasting provides resources that enhance both resistance and tolerance mechanisms, as mentioned above. One possible explanation is that these studies were done in animals housed under thermally stressed conditions and that wasting in the absence of a febrile response is maladaptive. The effects of muscle wasting on tolerance defenses are likely to be complex and context dependent and will require further investigation.

REGULATION OF INFLAMMATION

The most obvious way to promote tolerance would be to regulate the degree and duration of an inflammatory response during an infection. In doing so, the energetic costs associated with mounting an immune response would be minimized to avoid excessive and potentially pathogenic skeletal muscle catabolism. Furthermore, the potential tissue damage caused by the inflammatory response would be reduced. Elevated body temperatures induce the expression of heat shock factor 1 (HSF-1), which is involved in the acute heat response in mammals to activate expression of cytoprotective genes (Hasday and Singh 2000). HSF-1 has been demonstrated to act as a negative transcriptional regulator of inflammatory responses. In LPS-treated human monocytes, HSF-1 inhibits transcription of genes encoding proinflammatory cytokines (Cahill *et al.* 1996). Consistent with this, when *hsf-1*$^{-/-}$ mice were infected with *Listeria monocytogenes*, they exhibited significantly higher levels of serum TNFα and IFNγ and rapidly succumbed to infection without a significant difference in liver and spleen *Listeria* burdens (Murapa *et al.* 2011). Neutralization of TNFα promoted survival of infected animals deficient for HSF-1 demonstrating that inhibition of the inflammatory response by HSF-1 promotes tolerance (Murapa *et al.* 2011). Heat exposure appears to act as a negative regulator of inflammation in activated macrophages and this is dependent on HSF-1 transcriptional repressive behavior. Heat exposure also prevents transcriptional upregulation of proinflammatory cytokines by preventing the release of the damage-associated molecular pattern, HMGB1, reducing transcript stability and the recruitment of NF-kB to promoter regions of cytokine genes (Fiuza *et al.* 2003; Lee and Repasky 2012). In a mouse model of arthritis, mice exposed to higher temperatures had reduced joint damage associated with lower levels of circulating levels of TNFα and increased levels of the anti-inflammatory cytokine IL-10 in the joints (Lee *et al.* 2015). Downregulation of inflammation may also occur through the metabolic effects caused by the fever response. For example, IL-1β causes insulin resistance, and the resulting impairment in glucose uptake can limit further IL-1β transcription and reactive oxygen species (ROS) production (Jager *et al.* 2007; Wen *et al.* 2011; Benetti *et al.* 2013).

Hypothermia can also reduce inflammation to promote tolerance. For example, Wistar rats infected with a clinical *Escherichia coli* isolate that were maintained under hypothermic conditions displayed less endotoxemia, organ dysfunction and lung neutrophil recruitment despite higher liver bacterial burden (Fig. 1) (Liu *et al.* 2012). Thus, shifts in the thermostatic set point of a host appear to provide a negative feedback mechanism that downregulates proinflammatory cytokine production to prevent excessive tissue damage.

SICKNESS-INDUCED BEHAVIORS

The fever response in animals and humans is accompanied by stereotypical behavioral modifications including anorexia, sleep disturbances, social withdrawal and grooming disturbances that are known as sickness-induced behaviors (Hart 1988) (Fig. 2). These behaviors appear to be highly conserved as they are found in both invertebrates and vertebrates. Similar to fever, the general assumption regarding these sickness-associated behaviors is that they are maladaptive consequences of the host response to the infection. However, because these behaviors are ubiquitous among animal species during infection it is likely that these are evolved behavioral adaptations to increase the chance of survival during infection (Hart 1988; Ayres and Schneider 2009, 2012; Ayres 2013). Hart (1988) proposed that the main purpose of these behaviors is to facilitate the febrile response to better promote resistance defenses. While there is evidence to suggest that resistance defenses are influenced by sickness-induced behaviors (Hart 1988; Morag *et al.* 1998; Ayres and Schneider 2009), we propose that fever induces tolerance defenses in part by promoting these behaviors. Given the energetic costs of fever, the induction of anorexia and the reduced consumption of food during infection seem counterintuitive. Foraging and capture of food is energetically demanding and a reduced motivation to eat may promote tolerance by conserving energy stores for host defense and minimize wasting pathology. Calorically restricted states induce stress responses in animals that may reduce the susceptibility of tissues to damage during infection (Koella and Sorense 2002; Partridge, Piper and Mair 2005; Burger *et al.* 2007; Kristan 2007; Libert *et al.* 2008; Mair and Dillin 2008; Ayres and Schneider 2009). Consistent with this, in *Drosophila*, mutations in the gustatory receptor Gr28b render flies constitutively anorexic. When infected with *Salmonella* Typhimurium, these flies have enhanced tolerance. Similarly, when flies are calorically restricted they have increased tolerance to infection compared to flies fed a standard diet (Ayres and Schneider 2009). These same parameters, however, rendered flies more susceptible to infection with *Listeria monocytogenes* due to resistance defects (Ayres, Freitag and Schneider 2008). Thus, the effects of sickness-induced anorexia on resistance and tolerance defenses will be context dependent.

Infected animals typically exhibit sleepiness or fatigue and have a tendency to sleep during periods that they would otherwise be awake. Increasing sleep may be an additional means to conserve energy for the host defense response. Disruptions in sleep during infection lead to mortality in animal studies, for example, sleep deprived mice were more susceptible to infection with *Plasmodium* (Lungato *et al.* 2015). In humans, workers are more susceptible to infection (Pietroiusti *et al.* 2006). The circadian timing system in animals regulates the wake-sleep cycle and synchronizes biological processes and is important for host defense against infections (Shirasu-Hiza *et al.* 2007; Stone *et al.* 2012; Allen *et al.* 2016). In ectotherms, both light and external temperatures regulate the circadian clocks (Garrity *et al.* 2010; Romeijn *et al.* 2012; Villamizar *et al.* 2012; Fan, Stuart-Fox and Cadena 2014). In endotherms, peripheral tissues have independent clocks that are regulated by the master clock in the brain called the suprachiasmatic nucleus (SCN) in the hypothalamus. The master clock is regulated by light-dark cycles and is resistant to temperature changes. The peripheral clocks respond to temperature changes (Reppert and Weaver 2002; Buhr, Yoo and Takahashi 2010). It appears that the SCN drives core body temperature rhythms that are sensed by the peripheral clocks. This suggests that fever and hypothermic responses may induce clock-dependent responses in peripheral tissues that influence host defenses.

Reduced grooming and social withdrawal are additional sickness-associated behaviors in animals. Similar to sleep and changes in feeding, reduced grooming may serve as an energy conservation mechanism. Social withdrawal likely serves a beneficial function for a group rather than an individual. Workers of the ant Temnothorax unifasciatus infected with the fungal pathogen *Metarhizium anisopliae* leave their social network prior to death (Heinze and Walter 2010). In this context, social withdrawal would protect the group due to avoidance defenses. The exact function of social withdrawal and the extent to which it and other sickness-induced behaviors influences tolerance defenses and how this relates to temperature requires further exploration.

NEUTRALIZATION OF TOXINS

Infection-induced tissue damage can release host-derived toxic compounds that must be dealt with to minimize further tissue damage. Mechanisms that do so would operate as tolerance mechanisms because they target the toxin rather than the pathogen (Fig. 2). For example, infection with *Plasmodium*, the causative agent of malaria, causes hemolysis freeing host-derived hemoglobin into circulation. Under inflammatory conditions, free hemoglobin is oxidized resulting in the release of its prosthetic heme groups into circulation, which is toxic to the host. Heat shock protein 32 (HSP-32, also known as heme oxygenase-1 HO-1) is induced during *Plasmodium* infection and in response to heat shock and catalyzes the degradation of free heme resulting in carbon monoxide, labile iron and biliverden (Seixas *et al.* 2009). Individuals hemizygous for the sickle cell mutation contain a point mutation in the beta chain of hemoglobin and have elevated basal levels of free heme and HSP32 expression and are less susceptible to *Plasmodium* infection (Ferreira *et al.* 2011). Using a transgenic mouse model hemizygous for the human sickle cell trait, HbS, investigators found that animals had increased circulating levels of free heme, which activated HSP32 expression via the Nrf2 transcription factor. Evidence suggests that the CO produced during the heme detoxification reaction by HSP32 prevented further release of cell-free hemoglobin into circulation during infection and the pathogenic effects of CD8+ T cells in cerebral malaria (Ferreira *et al.* 2011). This protection conferred by the HbS trait did so without influencing *Plasmodium* levels; thus, the detoxification mechanism of HSP32 promotes tolerance of malaria infection. In a mouse cecal ligation and puncture sepsis model, HSP32 was required for survival without an apparent difference in systemic bacterial levels (Larsen *et al.* 2010). A caveat of this model however is that the authors only measured culturable levels of bacteria which only represents a small fraction of the members of the intestinal microbiota.

In addition to host-derived toxic compounds, mechanisms that neutralize microbial-derived toxins will promote tolerance of the host. Although we have evolved mechanisms to use LPS as an elicitor of tissue protective responses, systemic recognition of LPS by TLR4 leads to severe systemic inflammation. Alkaline phosphatases have been shown to modify LPS by dephosphorylating the lipid A moiety, which confers LPS toxicity (Koyama *et al.* 2002; Beumer *et al.* 2003; Tuin *et al.* 2006). Mild heat shock (39°C–41°C) has been shown to induce expression of alkaline phosphatase (Shui and Scutt 2001), as well as enhance enzymatic activity (Trieb, Blahovec and Kubista 2007). In a zebrafish model, intestinal alkaline phosphatase was required to prevent intestinal inflammation and pathology caused by the microbiota (Bates *et al.* 2007). Therefore, temperature regulation of alkaline phosphatase may represent a host-encoded strategy to promote tolerance by detoxification of microbial products.

TISSUE SUSCEPTIBILITY TO DAMAGE

Tissue susceptibility to damage during an infection is largely dependent on a cell's ability to mount an appropriate compensatory stress response that will contribute to tolerance defenses (Fig. 2). In the absence of such responses, cells and tissues become vulnerable to host–pathogen interplay. For example, the host response to an infection places heavy demands on a cell's protein synthesis machinery and can lead to the accumulation of damaged proteins as well as misfolded and unfolded proteins that leads to proteotoxicity. In *Drosophila*, pcmt, which encodes an L-isoaspartyl methyltransferase that is important for the damaged protein repair response, is required for tolerance of infection with the lethal intracellular bacterium *Listeria monocytogenes* (Ayres, Freitag and Schneider 2008). In *Caenorhabditis elegans*, the transcription factor xbp-1 that is a critical component of the unfolded protein response (UPR) promotes tolerance in worms challenged with the pathogen *Pseudomonas*, by limiting the accumulation of unfolded protein species (Richardson, Kooistra and Kim 2010). Changes in body temperature are sensed and trigger activation of stress response transcriptional programs, which include protection from proteotoxicity. In human cortical neurons, hypothermia induces the expression of cold shock proteins and endoplasmic reticulum stress leading to activation of the UPR. This can lead to cross-protection against oxidative stress in neurons (Rzechorzek *et al.* 2015). HSF-1 induces expression of a variety of genes including HSP-72, which inhibits proteotoxicity by facilitating the proper folding of newly translated or misfolded proteins (Mayer and Bukau 2005). In a variety of septic models, mice deficient for HSF-1 presented with impaired cardiac contraction and relaxation that was associated with increased production of immune effectors in cardiomyocytes, suggesting that HSF-1 may promote tolerance by maintaining cardiac function during sepsis (Robert *et al.* 2014). In a forward genetic ENU screen in mice, *Kcnj8*$^{mydy/mydy}$ mice have a null allele for Kir6.1 which encodes an ATP-sensitive potassium channel, and are hypersusceptible to challenges with LPS and MCMV infection without differences in viral titers compared to challenged wild-type mice. During infection, this channel maintains coronary homeostasis and prevents coronary artery vasoconstriction induced by the inflammatory response to maintain cardiac tonicity and prevent death (Croker *et al.* 2007).

HSF-1 likely promotes tolerance via mechanisms independent of the protein stress response and downregulation of inflammation. In another study, T cells isolated from *hsf*-1$^{-/-}$ mice exhibit defective proliferation at febrile temperatures and this was due to an inability of HSF-1-deficient mice to restore ROS levels after heat challenge (Murapa *et al.* 2007). Thus, regulation of cellular stress responses by body temperature may represent a general tolerance mechanism that is protective against an array of infections.

Cellular and tissue health is dependent on proper circulation of the body's blood supply to provide oxygen and nutrients to the tissue. In the clinical setting of sepsis and in severe inflammatory disorders, dysregulated activation of the coagulation cascade and the inhibition of fibrinolysis can lead to disseminated intravascular coagulation that ultimately can cause multi-organ failure due to hypoxia caused by perturbations in the microcirculation (Levi, van der Poll and Schultz 2012). This causes ischemia of the tissue due to the limited amount of oxygen available for cellular aerobic respiration leading to a reduction in ATP and phosphocreatine levels and a switch in intracellular metabolism to anaerobic glycolysis. In anaerobic glycolysis, glucose is transformed into lactate generating 2 ATP molecules per glucose molecule. The resulting accumulation of hydrogen ions and lactate leads to the production of lactic acid and a drop in extracellular and intracellular pH, a condition called lactic acidosis. Patients who develop severe sepsis or septic shock have elevated circulating levels of lactic acid and lactic acidosis, and this is a marker of the severity of the infection. Early lactate normalization within the first six hours of resuscitation is a strong predictor of sepsis survival (Puskarich *et al.* 2013). In hibernating mammals, the internal thermostat is lowered and tissues function at lower temperatures. There is a shift from carbohydrate to lipid metabolism during hibernation limiting anaerobic metabolism and ischemic injury (Suozzi, Malatesta and Zancanaro 2009; Hindle *et al.* 2011; Xu *et al.* 2013; Quinones *et al.* 2014). In the hypothermic brain, metabolism also shifts from glucose to lipid utilization (Wong 1983; Drew *et al.* 2007; Darwazeh and Yan 2013). Thus, changing substrate utilization induced by temperature changes may represent a tolerance mechanism to prevent lactate build-up. An obvious mechanism to prevent tissue hypoxia in a septic patient would be to prevent dysregulation in the coagulation pathway to maintain blood flow to tissues. In a randomized controlled trial, mild hypothermia induction improved coagulopathy in septic patients (Johansen *et al.* 2015). In experimental studies, temperatures below 33°C reduce the synthesis and kinetics of clotting enzymes and plasminogen activator inhibitors as well as delay the initiation of thrombus formation and speed of clot formation (Valeri *et al.* 1987; Patt, McCroskey and Moore 1988; Michelson *et al.* 1994; Ruzicka *et al.* 2012), suggesting that cold temperatures may provide a defense strategy against severe coagulopathy.

Once hypoxia occurs, however, the body must employ mechanisms to combat this stress. Increasing cardiac output and ventilation are possible defense strategies; however, these methods are energetically costly to the critically ill host. An alternative mechanism is to induce hypothermia to reduce the oxygen demand of tissues. Consistent with this, physiological mechanisms that promote hypothermia are induced in mammals during hypoxic conditions. Typically endotherms produce a thermogenic response when ambient temperatures are below thermoneutrality. However, in hypoxic rodents, the thermogenic response to cold temperatures is depressed (Tattersall and Milsom 2003; McAllen 2009). Furthermore, there is decreased heat production in hypoxic animals due to allocation of blood supply away from brown adipose tissue (McAllen 2009). Thus, hypoxic mammals appear to reduce the temperature threshold at which they begin their shivering response to generate heat for thermoneutrality. Both ectotherms and endotherms

including lizards, mice and rats chose to maintain lower body temperatures under hypoxic conditions through behavioral thermoregulation (Hicks and Wood 1985; Dupre and Owen 1992). The discovery that animals exhibit behavioral hypothermia in response to hypoxia provides support that hypothermia likely provides physiological protection under oxygen stressed conditions. Indeed, survival studies demonstrate that hypothermia induces a number of physiological benefits under oxygen limiting conditions. Survival of hypoxic mice was increased when animals were maintained at 35°C and reduced when housed at 40°C (Gordon 2001). Hypoxic lizards that were allowed to seek cold temperatures exhibited 100% survival. By contrast, 100% of hypoxic lizards that were not allowed to seek cold temperatures died (Hicks and Wood 1985). Rabbits that are anemic exhibited increased survival when maintained at hypothermic conditions presumably due to cold temperatures shifting the oxyhemoglobin dissociation curve to the left influencing oxygen accessibility (Gollan and Aono 1973). Thus, the induction of hypothermia in response to hypoxia may promote tolerance by reducing the oxygen demand by tissues, altering the affinity of hemoglobin for oxygen and avoiding the energetic costs that are associated with increasing cardiac output and ventilation.

After ischemic episodes, restoration of circulation to tissues can be detrimental to tissue health and result in reperfusion injury. Cellular metabolites accumulate during ischemia that become oxidized upon the reintroduction of molecular oxygen to the tissues when blood flow is reestablished resulting in the accumulation of ROS. This activates an inflammatory response in the tissue that leads to tissue damage and death. Evidence from therapeutic hypothermia studies suggests that cold body temperatures can prevent reperfusion induced tissue injury. This protection is likely due to the fact that hypothermia reduces the levels of free radicals and stabilizes cell membranes, cellular swelling and edema (Ning *et al.* 2007; Lampe and Becker 2011).

A wide variety of pathogens can cause tissue damage by induction of host cell death either by direct activation of host cell death machinery or indirectly through the host response to the infection. The dysregulated physiological responses that occur in severe systemic inflammatory conditions and sepsis, such as ischemia and reperfusion, cause cell death and lead to multiorgan failure. The cytokine TNFα is a central mediator of inflammation and induction of the febrile response (Jiang *et al.* 1999). TNFα has also cytolytic effects via the extrinsic apoptosis pathway involving activation of caspase-8 downstream of TNF receptor signaling (Micheau and Tschopp 2003; Walczak 2013). An important component of the heat shock response induced by fever is to promote stress responses that will enable a cell to survive during an inflammatory response. Janeway and colleagues showed that heat shock of fibrosacoma cells reduced sensitivity to TNFα-mediated lysis (Gromkowski, Yagi and Janeway 1989). Furthermore, incubation of TNFα secreting T lymphocytes at elevated temperatures reduced their secretion of TNFα and their cytolytic capabilities (Gromkowski, Yagi and Janeway 1989). Consistent with this, incubation of HeLa cells at a mild hyperthermic temperature of 40°C promoted thermotolerance and conferred protection from apoptosis induced by lethal hyperthermia (42°C –45°C). Protection was associated with the increased expression of various HSPs including HSP 27, 32, 60, 72 and 90 (Bettaieb and Averill-Bates 2008). In a mouse model of non-cerebral malaria, mice deficient for HSP32 (*Hmox1*$^{-/-}$) succumbed to infection due to hepatic failure despite having equivalent levels of plasmodium as infected wild-type mice. The heme detoxification conferred by HO-1 was required to prevent sensitization of hepatocytes to TNF-mediated apoptosis (Seixas *et al.* 2009). Thus, inhibition of apoptosis by HO-1 promotes tolerance of malaria by preventing hepatic failure and death. In addition to controlling sensitivity to TNF, HSPs have been shown to influence downstream signaling upon activation of the death receptor (Meng *et al.* 1999; Hasday and Singh 2000; Johnson and Fleshner 2006). TNFα can also induce necrosis in some cell types (Morgan, Kim and Liu 2008). HSP90 has been reported to determine whether a cell will face an apoptotic or necrotic death fate (Fulda *et al.* 2010).

HSPs also influence the intrinsic apoptosis pathway involving the release of cytochrome c from the mitochondria in response to cell death signals. Cytochrome c binds to Apaf-1, inducing oligomerization and recruitment of procaspase-9 resulting in the formation of the apoptosome and activation of caspase-9, which then activates caspase-3. This results in freeing of caspase-9 from the apoptosome and is then replaced with another caspase-9 molecule. HSP70 and HSP90 have also been observed to inhibit apoptosome formation (Beere *et al.* 2000; Pandey *et al.* 2000; Saleh *et al.* 2000). HSP-60 primarily resides in the mitochondrial matrix and has been shown to exert its antiapoptotic effects by influencing caspase-3 activation (Xanthoudakis *et al.* 1999). Various HSPs have also been demonstrated to block apoptosis by manipulating processes downstream of caspase activation (Creagh, Sheehan and Cotter 2000). During anaerobic glycolysis, the resulting acidosis leads to the influx of Ca^{2+} into cells and an inhibition of membrane-bound pumps and channels that are normally responsible for maintaining intracellular calcium homeostasis. Mitochondrial dysfunction may result from the excess intracellular Ca^{2+} leading to cell death via the intrinsic apoptotic pathway. Hypothermia has been shown to alleviate pump dysregulation and help maintain intracellular Ca^{2+} homeostasis and may prevent cell death (Siesjo *et al.* 1989; Hall 1997). This effect is likely indirect as studies have shown that, while not affecting apoptotic caspase activation, cold temperatures delay apoptosis in response to stress by delaying cytochrome c release (Goldstein *et al.* 2000).

Heat and response proteins associated with heat have also been shown to promote various types of cell death. Permeabilization of the mitochondrial membrane is dependent on the activation and oligomerization of multidomain Bcl2-family proteins Bax and Bak. Heat can directly activate Bax and Bak to induce cytochrome c release (Pagliari *et al.* 2005). Necroptosis, a programmed form of necrosis type cell death, has important roles in host defense against viral infections and inflammation. Emerging evidence suggests that HSP90 is required for the induction of necroptosis (Jacobsen *et al.* 2016; Zhao *et al.* 2016). Thus, body temperature changes during infection likely directly or indirectly orchestrates the optimal balance of pro- and anti-cell death regulator mechanisms that likely influence host tolerance.

OPEN QUESTIONS AND FUTURE PERSPECTIVES

It is clear from experimental and clinical studies that thermoregulation has broad beneficial effects for host defense against infectious diseases and beyond (Table 1, Fig. 2). However, we know very little about the mechanisms by which thermoregulation—both hypothermia and hypothermia influence host defenses. The field of thermoregulation in general and in the context of host defense needs to be reignited. We propose an integration of the conceptual framework of resistance and tolerance into these studies will shed light on our understanding of the

function of fever and hypothermic responses in surviving infectious diseases and beyond.

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