[Frontiers in Bioscience S2, 916-938, June 1, 2010]

Role of endotoxin and cytokines in the systemic inflammatory response to heat injury

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1. ABSTRACT

Environmental heat exposure represents one of the most deadly natural hazards in the United States. Heat stroke is a life-threatening illness that affects all segments of society with few effective treatment strategies to mitigate the long-term debilitating consequences of this syndrome. Although the etiologies of heat stroke are not fully understood, the long-term sequelae are thought to be due to a systemic inflammatory response syndrome (SIRS) that ensues following heat-induced tissue injury. Endotoxin and cytokines have been implicated as key mediators of the heat-induced SIRS, based almost exclusively on correlative data that show high circulating concentrations of these substances in heat stroke patients and animal models. However, endotoxin and cytokine neutralization studies have not consistently supported this hypothesis indicating that the mechanisms of heat stroke morbidity / mortality remain poorly understood. This review discusses the current understanding of the role of endotoxin and cytokines in heat-induced SIRS. Insight is provided into genetic conditions that may predispose to heat stroke and potential therapeutic strategies that may be efficacious against the adverse consequences of this debilitating illness.

2. INTRODUCTION

Heat stroke is a life-threatening syndrome clinically associated with elevated core temperature (hyperthermia), collapse, and profound central nervous system (CNS) dysfunction that presents as delirium, seizures, agitation or coma. The sequelae of heat stroke include thermoregulatory, cardiovascular, hemodynamic and immune disturbances that present following organ (e.g., gut, liver, kidney) damage. The long-term consequences of heat stroke are thought to occur during a systemic inflammatory response syndrome (SIRS) that ensues following endotoxin leakage from the gut, which stimulates cytokine production (1). Although rapid cooling and fluid resuscitation are considered critical treatments for heat stroke, these therapies are inadequate to prevent tissue injury or death in many patients. For example, ~30% of patients succumbing to heat stroke during summer heat waves incur permanent neurological damage, despite cooling therapy (1-3).

In the past two decades, clinical and experimental studies have implicated heat-induced endotoxemia and "overproduction" of pro-inflammatory cytokines as key events for the heat-induced SIRS. These conclusions ar

Table 1. The heat illness continuum

Term	Characteristics
Heat Exhaustion	Mild to moderate illness characterized by inability to sustain cardiac output; Moderate (>38.5ºC; 101ºF) to high (>40.0ºC; 104ºF) T_c
Heat Injury	Moderate to severe illness with organ and/or tissue damage; High T_c, but usually not greater than 40ºC
Heat Stroke	Life-threatening illness with hyperthermia and profound encephalopathy (delirium, agitation, stupor, seizures, coma); Classic form occurs with passive exposure (very young or elderly); exertional form with intense exercise (young, healthy adults)

based primarily on correlative data that implicate pro- and anti-inflammatory cytokines, such as interleukin (IL)-6, interferon (IFN), and IL-10 as modulators of this syndrome. However, correlative data fall far short of proving a role for endogenous substances in the heat-induced SIRS and it is only through neutralization studies that our understanding of the role of endotoxin and cytokines in this syndrome has recently improved. This review will present an overview of our current understanding of the role of endotoxin and pro- / anti-inflammatory cytokines in the heat-induced SIRS and discuss future areas of research that may aid in the development of therapeutic strategies to mitigate the long-term consequences of this debilitating illness.

3. THE HEAT ILLNESS CONTINUUM

Heat illnesses are best viewed as existing along a continuum that transition from the fairly innocuous condition of heat cramps to heat exhaustion and heat stroke (Table 1). Heat exhaustion occurs during exercise in hot environments and is the most common heat illness syndrome that occasionally progresses to a moderately severe condition due to an inability to maintain adequate cardiac output; this condition often presents as elevated core temperature (T_c; moderate, >38.5˚C; high, >40˚C) and collapse (4). Although heat exhaustion may involve minor CNS dysfunction (e.g., headache, dizziness), these symptoms typically resolve rapidly following interventions, such as rest and hydration. Heat injury is a moderate to severe condition characterized by tissue (e.g., gut, skeletal muscle) and organ (e.g., renal, spleen, liver) injury and hyperthermia (4). Heat stroke is the most serious condition resulting from prolonged exposure to a hot environment and includes hyperthermia (T_c typically, but not always greater than 40˚C) collapse and profound encephalopathy that presents as delirium, agitation, stupor, seizures, or coma (4).

Classic (passive) heat stroke occurs at rest in very young or elderly populations that may be immunocompromised prior to heat exposure; these populations show enhanced mortality during heat waves (2,3,5,6). During the 2003 heat wave in France, ~15,000 individuals died from heat stroke, which was thought to be a consequence of the large aged population (~10,000 people over 100 years old) and a lack of air conditioning in homes and hospitals (2,6). Similarly, in 1995 a failure of air conditioning systems following power outages during Hurricane Katrina may have contributed to heat stroke deaths in aged individuals confined to nursing homes in New Orleans. Many aged individuals have pre-existing conditions, such as mental illness, prescription drug use (e.g., diuretics, anticholinergics) or infections that predispose to heat stroke (3,5). During the 1995 heat wave in Chicago, 57% of aged (>65 years old) heat stroke patients had evidence of infection upon clinical admission (3), suggesting that pre-existing inflammatory conditions compromise the body's ability to respond to a subsequent stressful event. A more extreme condition, known as exertional heat stroke (EHS) is typically observed in healthy, young individuals undergoing strenuous physical activity in hot environments. EHS is an occupational hazard for several populations, including athletes, military personnel, firefighters, race car drivers and agricultural workers, although heat acclimatization of these populations can reduce risk (7). Pre-existing infections can also predispose individuals to EHS risk in these populations, as shown in military recruits that present with prodromal viral illness (e.g., Epstein Barr and mononucleosis) and show enhanced EHS susceptibility (8). Unfortunately, EHS is a particularly complicated heat syndrome to study due to the difficulty in dissociating the direct effects of strenuous physical activity from that imposed by exposure to a hot environment and a multitude of other factors.

It is now documented that T_c of heat stroke patients varies widely, which may be a consequence of differences in heat acclimatization, time of T_c measurement (i.e., pre- or post-cooling), site of T_c measurement (e.g., inguinal vs. rectal) or other unidentified factors. T_c values as high as ~42˚C have been observed in clinical studies, as well as competitive runners with no adverse clinical signs indicating that reliance on a specific T_c criterion for heat stroke determination is rather tenuous (9). Although ranges of 41-42˚C are common and values as high as ~47˚C have been reported (8,10-14), a study of 100 heat stroke patients shows that ~10% of mortalities occur below ~41˚C (15). Recently, Bouchama and Knochel (1) proposed an alternative heat stroke definition as "a form of hyperthermia associated with SIRS leading to a syndrome of multi-organ dysfunction in which encephalopathy predominates". Note the lack of inclusion of a specific T_c value in this definition, which likely reflects consistent reports of wide variability in this measurement.

4.HEAT STROKE PATHOPHYSIOLOGY

4.1.Role of endotoxin in heat-induced SIRS

The primary cardiovascular response to heat exposure is an increase in skin blood flow, which facilitates dry heat loss to the environment and a reduction in total body heat storage. Increased skin blood flow is facilitated by a reduction in splanchnic blood flow, which is a compensatory response to maintain mean arterial pressure while blood is shunted to the skin surface for thermoregulatory control. Prolonged reductions in intestinal blood flow promote ischemia and nitrosative / oxidative stress, ultimately compromising GI permeability and causing leakage of endogenous bacteria (and its toxic cell wall component lipopolysaccharide, LPS) into the portal or systemic circulation (16,17). GI damage consisting of dilation of the central lacteals of the intestinal villi is commonly observed in clinical and experimental

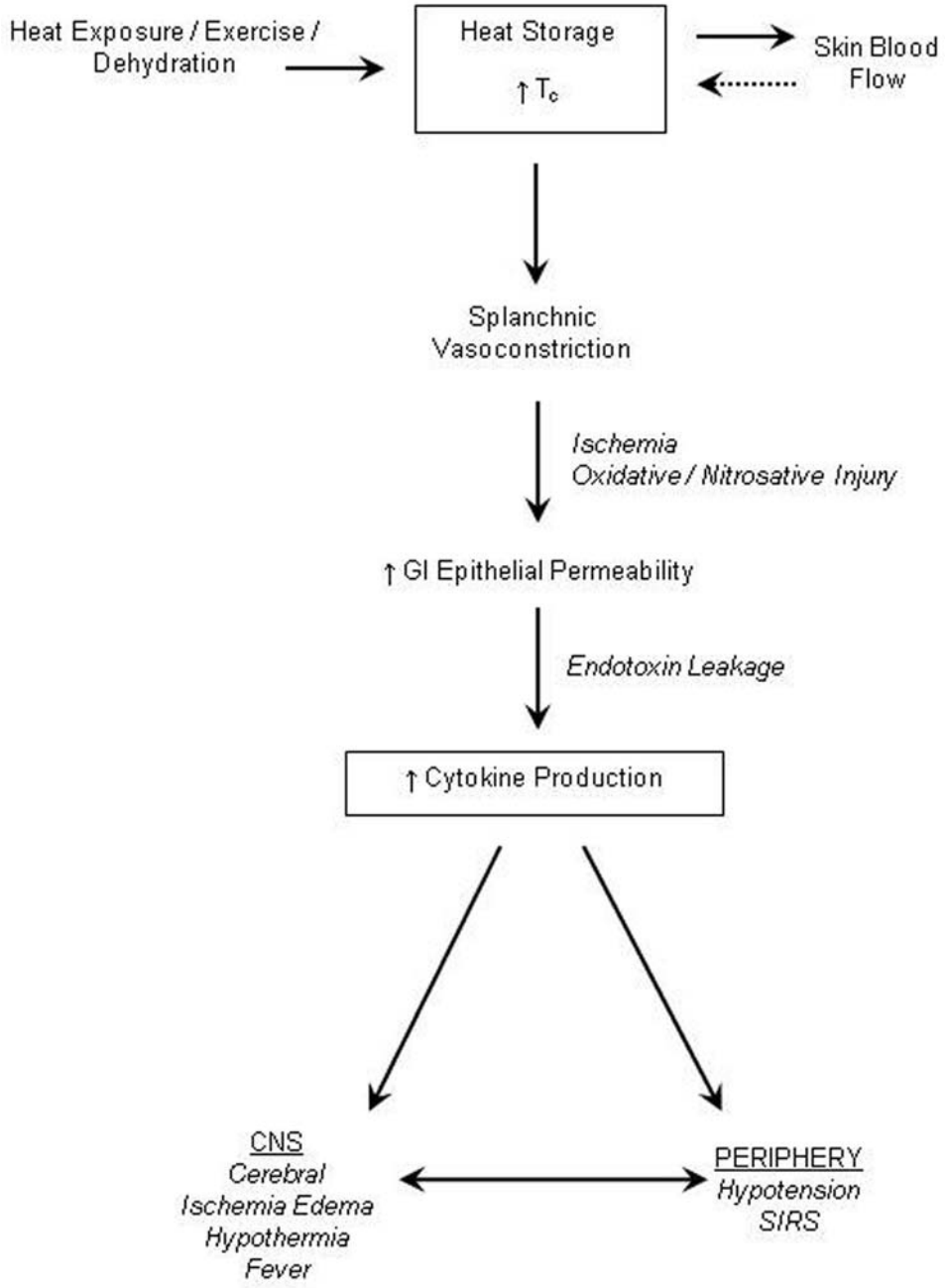

Figure 1. Effect of prolonged heat exposure, exercise and/or dehydration on skin blood flow, GI permeability, and cytokine actions. A rise in core temperature (T_c) stimulates reflexive increases in cutaneous blood flow and decreases in splanchnic blood flow to facilitate heat dissipation to the environment. Gut ischemia causes increased epithelial permeability and leakage of endotoxin into the systemic and portal circulation, which stimulates pro- and anti-inflammatory cytokine production. Peripheral and CNS actions of cytokines are thought to mediate many of the adverse consequences of the heat stroke syndrome.

heat stroke studies. Elevations in circulating endotoxin concentrations are thought to be responsible for the stimulation of cytokine production under heat stroke conditions (Figure 1).

GI epithelial barrier disruption is thought to be the precipitating event for the heat-induced SIRS, as the breakdown of this barrier facilitates endotoxin leakage from the GI lumen into the systemic circulation. Although the mechanisms by which heat exposure alters gut epithelial membrane permeability are not completely known, heat shock proteins (HSPs) are thought to mediate thermotolerance at the cellular level and provide protection against GI damage in the heat stroke syndrome. HSPs

range in size from 27 to 110 kDa, but the 70 kDa family is the most heat responsive. As such, the protective functions of HSP70 against heat-induced disruptions of epithelial barriers have been examined. Using an *in vitro* model system composed of Madin-Darby canine kidney epithelial monolayers, Moseley *et al.* (18) showed that a conditioning heat stress of 42°C for 90 minutes was sufficient to maintain epithelial barrier permeability and this response correlated with increased HSP70 expression levels. In follow-on studies, the exposure of intestinal epithelial cells (Caco-2) to 39º or 41ºC was associated with a linear increase in HSP27, HSP40, HSP70, and HSP90 expression (19). The expression of occludin, a plasma-membrane protein located at tight junctions, was also increased with heat exposure to 41°C. When Caco-2 cells were treated with quercetin, an inhibitor of heat shock transcription factor-dependent transcriptional activity, HSP and occludin expression was inhibited and the protective effect of heat preconditioning on Caco-2 monolayer permeability was effectively blocked. Although future studies are needed to validate these findings in animal models of heat stress, these data suggest an integral role of HSPs and occludin in the maintenance of epithelial integrity following heat exposure (19).

Although it is generally believed that endotoxin leakage from the GI tract is the precipitating event that stimulates increased production of pro- and anti-inflammatory cytokines for initiation of the SIRS, there are few studies that have directly tested this hypothesis or shown a strong correlation between these two events. Bouchama *et al.* (10) reported increased circulating LPS concentrations in human heat stroke patients that collapsed during the annual pilgrimage to Hajj, but were unable to show a correlation of this response with circulating cytokine levels. Although IL-1α and TNFα are detectable at clinical admission and show a significant decrease following the cessation of cooling, changes in LPS concentrations occur independently from decreases in T_c and cytokine production (10). In some studies, the inability to measure elevated endotoxin concentrations may simply be due to an inadequate level of heat stress used experimentally to test for GI damage. For example, Chung *et al.* (20) did not detect plasma endotoxin in former heat stroke patients exposed to a 60 minute heat stress presumably because T_c was induced to a level less than 39.5°C in these patients, which was probably not a robust enough hyperthermia to compromise GI membrane integrity. It is noteworthy that splanchnic blood flow initially decreases at T_c~40°C in rats (16) whereas in primates, elevations in circulating endotoxin are undetectable below T_c~41.5°C and only show more robust increases at T_c~43.0°C (21).

Given the known interaction of HSPs and cytokines, it is not unreasonable to hypothesize that cytokines may influence epithelial barrier integrity in heat exposed individuals. Although the role of cytokines in heat-induced increases in GI permeability has not been directly examined, several studies show a protective effect of the anti-inflammatory cytokine IL-10 on vascular permeability during sepsis. For example, microvascular permeability of cremasteric post-capillary venules in IL-10 knockout mice is significantly increased 4 hours following a peripheral LPS injection when compared to wild-type controls (22). This effect is thought to be mediated through the inhibition of leukocyte recruitment to the vascular wall as E- and P-selectin blockade reduces leukocyte rolling to control levels in LPS-injected IL-10 knockout mice (22). Although these results suggest a direct effect of endogenous IL-10 on vascular permeability changes, IL-10 is known to inhibit the production of several cytokines (IL-1beta, IL-6, TNFalpha) that could be mediating these effects. It is reasonable to assume that a complex network of cytokine interactions is responsible for vascular permeability changes induced by sepsis (and heat exposure). For example, IFN-gamma mediates endothelial barrier dysfunction by enhancing the expression of cell adhesion molecules via stimulation of IL-1 beta and TNFalpha production while IFNgamma and IL-10 reciprocally modulate intestinal microvascular leakage by controlling the expression of endothelial tight junction proteins (23). In a similar manner, IL-6 knockout mice are protected against cecal ligation and puncture-induced increases in intestinal permeability, which correlates with ~20-fold higher mucosal levels of IL-10 (24). These studies illustrate the complex network of intestinal permeability modulators and the importance of understanding the ratio of pro- and anti-inflammatory cytokines during an inflammatory event, such as that induced by sepsis or heat stroke.

Although GI barrier disruption occurs in direct response to heat exposure (i.e., early in the heat stroke syndrome), there is an increase in tissue damage during progression of the heat stroke syndrome, as injury to the liver, kidney, spleen, heart, lung, small intestine, brain and skeletal muscle (rhabdomyolysis) is observed during the hours, days, weeks and years of recovery (3,25-29). The severity of heat stroke is purported to be primarily related to the extent of damage incurred in the CNS, liver and kidneys (29,30) and is most readily assessed by analysis of serum enzyme levels, such as creatine phosphokinase (CPK; skeletal muscle), biliary urea nitrogen (BUN; kidney), alanine aminotransferase (ALT; liver) and aspartate aminotransferase (AST; liver). However, circulatory measures of organ function do not always accurately reflect the extent of tissue injury as many patients succumb to heat stroke during the weeks, months and years following hospitalization (31). Renal failure is almost a universal finding in heat stroke patients and animal models and is characterized by glomerular ischemia, tubular necrosis and hemorrhage (25,27,29,32,33). Protein clumping in tubular epithelial cells of the kidney is thought to be a consequence of direct thermal injury, rhabdomyolysis or disseminated intravascular coagulation (DIC; (13,26,30,32,34-36)). In the spleen, cytoplasmic protein clumping is a common observation that is thought to be a direct result of hyperthermia (32). Interestingly, the time course of liver damage differs from that of the other organs as it is typically seen in long-term survivors. The later time course of liver damage suggests it may be a consequence of the inflammatory response that ensues during recovery, rather than representing an acute

(immediate) response to hyperthermia (25). A breakdown in liver function may also contribute to endotoxemia, as this organ is one of the major sites of endotoxin clearance (37,38). Similarly, renal failure has been suggested as a potential mechanism for increased plasma cytokine concentrations, as cytokine clearance is a reported function of this organ (39). Ultimately, multi-organ system dysfunction is the cause of heat stroke mortality and is revealed at autopsy as edema and micro-hemorrhages in several organs of the periphery as well as specific brain regions (25,32).

Prolonged heat exposure can induce immune dysfunction, observed as disturbances in the distribution of several peripheral lymphocyte subpopulations (40,41). The degree of hyperthermia is directly correlated with increased lymphocytes and T suppressor-cytotoxic cells (40). Changes in regional blood flow, increased catecholamine and cortisol release and direct effects of exercise, cytokines and endotoxin are proposed mechanisms of increases in these cell types with heat stroke (9,40-47). In a baboon model of heatstroke, leukocyte patterns differed depending on the level of heat stroke severity (29). That is, whereas leukocytosis was observed from 3 to 12 hours following moderate heat stroke, a significant leucopenia was evident at the onset of severe heat stroke, but returned to baseline within an hour (48). These responses correlated with increased IL-6 production, which is a key cytokine that modulates leukocytosis and endothelial cell activation, the latter of which represents a primary target for coagulopathies.

DIC is a systemic intravascular disorder resulting from uncontrolled activation of coagulation and/or impairment of fibrinolyis or anticoagulation (1,27). Increased thrombomodulin levels are indicative of endothelial injury and are elevated in the most severe incidences of heat stroke (29). However, the pathophysiological mechanisms mediating coagulopathies are not well defined, and studies of the sepsis syndrome have provided the majority of data regarding DIC pathway components that are affected by cytokines. Cytokine modulation of DIC is supported by several lines of evidence including: (1) increased plasma levels of IL-1beta, IL-6 and TNF in patients with DIC, with high IL-6 correlating with organ failure (49-51), (2) alteration of coagulation following IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, or TNF injection (52-56), and (3) attenuation of coagulation following injection of cytokine neutralizing antibodies (57,58). There are several different components of the coagulation, fibrinolytic and anticoagulation pathways that are affected by cytokines and endotoxin in the sepsis syndrome. Tissue factor (TF) is a cell surface receptor expressed by monocytes and vascular endothelial cells and increased expression of TF results in initiation of the coagulation cascade via the extrinsic pathway. TF expression is induced following exposure to blood; thus major trauma, burns, hereditary vascular or chronic inflammatory disorders increase an individual's susceptibility to DIC via increased TF expression (59,60). The expression of TF is regulated by endotoxin, as well as pro- and anti-inflammatory cytokines with TNF, IL-1alpha, IL-1beta, IL-6, IL-8, leukemia inhibitory factor, IFNgamma and monocyte chemoattractant protein (MCP)-1 stimulating TF and TGFbeta, IL-4, IL-10 and IL-13 inhibiting its expression (53,61-64). In addition to affecting the extrinsic pathway of coagulation, endotoxin and cytokines modulate the fibrinolytic and anticoagulation pathways. The fibrinolytic pathway is a natural anticoagulant pathway that is important for host protection against excessive clotting. Data from human and animal experiments suggest that cytokines mediate LPS-induced increases in plasminogen activator inhibitor (PAI)-1 in several organs, including liver, kidney, and lung (65). PAI-1 is a negative inhibitor of tissue plasminogen activator (tPA), an essential enzyme involved in fibrin clot degradation. High PAI-1 levels have been shown to precede DIC incidence and correlate with poor outcome; PAI-1 knockout mice are resistant to LPS-induced kidney thrombosis (65,66). Protein C has an important role in the response to inflammation and concomitant coagulopathy. The main effect of protein C is to reduce the production of thrombin, by inactivating factors Va and VIII and inhibit the influence of TF. Cytokines modulate the protein C-protein S anticoagulation pathway at several levels, preventing proteolytic cleavage of several factors (Va and VIIIa) involved in the coagulation pathway (67,68). The reader is referred to reviews that discuss the intricacies of cytokine modulation of these pathways with DIC (52,69).

4.2. Role of cytokines in heat-induced SIRS: friend or foe?

Cytokines are intercellular messengers released by several cell types (macrophages, T and B cells, endothelial cells, astrocytes) that exhibit pleiotropic, redundant actions in a variety of disease and injury states (70-73). Several cytokines are elevated in the circulation of heat stroke patients and animal models, although the time course of pro- and anti-inflammatory cytokine production varies throughout progression of this syndrome (8,10-12,28,74-82). Pro-inflammatory cytokines are thought to mediate heat stroke morbidity / mortality, but these conclusions are based primarily on correlative data, which provide little or no information regarding the endogenous actions of these proteins.

4.2.1. Elevated circulating cytokine levels in heat stroke patients and animal models

The majority of data showing increased circulating concentrations of cytokines were obtained in the 1980s to early 1990s from heat stroke patients that collapsed during the annual pilgrimage to Makkah (the Hajj) in Saudi Arabia. There are several obvious factors inherent to this religious ritual that predispose individuals to heat stroke, including hot desert conditions (T_a ~38-50˚C), strenuous physical activity, wearing of heavy clothing that impedes heat loss, a large aged population, pre-existing medical conditions (e.g., diabetes and cardiovascular disease) and lack of acclimatization in participants from adjacent regions (14,83). Several studies show elevations in circulating levels of IL-1alpha, IL-1beta, IL-1ra, IL-6, soluble IL-6 receptor (sIL-6R), IL-10, IFNgamma, TNFalpha and soluble TNF receptor (sTNFR) concentrations in these patients (10,11,14,39,74).

Interestingly, IL-6 is the only cytokine that is typically elevated in 100% of patient cohorts and consistently correlates with morbidity / mortality (11,14,39). Based on these studies, IL-6 is implicated as a key mediator of the adverse consequences of heat stroke. However, two important aspects of clinical studies warrant consideration: (1) cytokine measurements are usually obtained at only one or two time points, which correspond to the time of clinical admission or shortly after cooling, and (2) only a few cytokines are measured in each individual. (This is also true of most animal studies, which are described in more detail below.) Based on these studies, our understanding of the time course of changes in the balance of pro- and anti-inflammatory cytokine production during long-term progression of this syndrome remains poorly understood. Furthermore, it is unclear how cytokines interact *in vivo* to mediate heat stroke responses, making it difficult to rely on correlative data for the development of therapeutic strategies to target specific heat stroke mechanisms. For example, few studies examine the relationship of cytokines to one another or their naturally occurring receptor antagonists (e.g., IL-1:IL-1ra) or soluble receptors (e.g., IL-6:sIL-6R). Hammami *et al* (39) showed a direct correlation of sTNFR60 and sTNFR80 levels with morbidity in human heat stroke patients that collapsed during the Hajj, but was unable to detect TNFalpha or TNFbeta in the circulation. This represents a common problem with peripheral measurements and makes it difficult to discern the role of a cytokine in the heat stroke syndrome. That is, negative findings may indicate that (1) the cytokine does not have endogenous actions in the syndrome, (2) soluble receptors may be masking assay detection of the cytokines (84), or (3) local production of the cytokine and/or its soluble receptor is more important than circulating levels in the observed physiological responses. More confounding is the ability of soluble receptors to act as either agonists or antagonists of endogenous cytokine actions. Soluble TNF receptors are produced by the shedding of extracellular domains of the TNF type I (p60) and II (p80) receptors and bind TNFalpha and TNFbeta with similar affinity as membrane bound receptors, allowing them to function as natural antagonists of TNF binding / activity or inhibitors of TNF proteolytic degradation (85). Conversely, a unique relationship exists between IL-6 and its soluble receptor in that injection of the sIL-6R into rats potentiates, rather than inhibits endogenous IL-6 actions *in vivo* (86). Whether IL-6:sIL-6R complexes or other cytokine-receptor relationships alter endogenous cytokine functions *in vivo* in heat stroke patients is currently unknown, but represents an area of investigation that may be integral to our understanding of the role of these complex modulators in this syndrome.

4.2.2. Danger signals

Danger associated molecular patterns (DAMPs) are activated in response to pathogens and trauma and represent the initial trigger for a systemic inflammatory response. DAMPs may originate from endogenous (e.g., injured cells) or exogenous (e.g., microbes) sources and include pathogen-associated molecular patterns (PAMPs) and endogenous danger signals referred to as alarmins (87). Pattern-recognition receptors (PRRs), including the toll-like receptor (TLR) family, recognize exogenous and endogenous DAMPs resulting in modulation of systemic inflammation via immune system stimulation (i.e. activation of macrophages, lymphocytes, and neutrophils) and NF-kB transcriptional activation of cytokine synthesis. For example, TLR-2 and TLR-4 mediated expression of IL-1beta and TNFalpha are increased for seven days post-thermal injury when compared to sham animals (88). Increased TLR expression occurs in both immune and non-immune cells (89,90), providing evidence that both cell types are involved in mediating tissue injury following sepsis. In addition to exogenous LPS, murine sepsis and inflammatory studies implicate neutrophil elastase as a DAMP that may augment chemokine release in tissues that express elevated levels of TLR-4 (89).

Alarmins are endogenous DAMPs that restore cellular homeostasis. DAMPs are immune system targets and products, up-regulated in necrotic cells but not apoptotic cells, that restore homeostasis within the cell (87). Alarmins participate in immune-cell migration to sites of injury and modulate immune system responses (91). High mobility group box 1 (HMGB1) is considered the classic alarmin with the ability to bind a wide range of molecules which define the resulting immune response in shock, trauma, and sepsis. For instance upon binding LPS, HMGB1 induces IL-6 secretion (87) and when associated with IL-1beta produces a substantial inflammatory response (92). Acting as a danger signal, HMGB1 binding to TLR-2 and TLR-4 appears to be dependent upon ligand binding that promotes a pro-inflammatory response similar to LPS. However it is not clear if HMGB1 itself can stimulate the secretion of pro-inflammatory cytokines, although *in vivo* HMGB1 can be manipulated by immune effectors into simulating immune responses characteristically associated with trauma (93).

Additional proteins including IL-1alpha, S100 family members, uric acid, defensins, and annexins also display properties characteristic of alarmins. Interestingly HSPs are classified as alarmins due to their rapid expression and ability to modulate a wide range of immune responses to heat and associated stressors. The responses to danger signals appear to be organ specific and it has been suggested that alarmin responses are influenced by genetics. However no research exists addressing the specific role, if any, of these factors in heat-induced SIRS.

4.2.3. Neutralization studies in animal models

The IL-1ra is a naturally occurring receptor antagonist that induces steric hindrance of the IL-1 receptor, preventing IL-1 binding and cellular signaling. The IL-1ra is the most widely used cytokine receptor antagonist, presumably due to its wide commercial availability and detailed understanding of IL-1ra concentrations that are required for inhibition of endogenous IL-1 actions (~100 to 1000-fold higher than IL-1; (94). Peripheral and central injections of IL-1ra are protective against fever, hypotension, shock and heat stroke mortality in animal heat stroke models and these effects do not appear to be time-dependent, as efficacy is observed if IL-1ra injections are given prior to, during or following

heat stroke collapse (76,77,79). The attenuating effect of peripheral IL-1ra injections on hyperthermia may represent an indirect response to reductions in cardiovascular strain or a direct consequence of the inhibitory action(s) of IL-1ra on hypothalamic T_c mechanisms; to-date, the efficacy of IL-1ra on heat stroke responses following direct injection into the hypothalamus has not been tested. The ability of IL-1ra to regulate CNS levels of serotonin and protect against cerebral edema / ischemia suggests a neuroprotective role for this cytokine (77). Although the IL-1ra significantly prolongs heat stroke survival time (by ~100 minutes) in all studies in which it has been tested, it is important to note that these studies were conducted in anesthetized animals (i.e., anesthesia compromises autonomic and behavioral thermoregulatory mechanisms; (95,96)) and the animals eventually succumbed to heat stroke despite the treatment; thus, it remains unclear if IL-1ra is protective against long-term heat stroke mortality, such as days, weeks, months or years following collapse.

Heat stroke studies using IL-6 and TNF double receptor (TNFR) knockout mice have shed new light on the complex nature of these cytokines' actions in heat-induced SIRS. As previously discussed, most correlation studies suggest a detrimental effect of high circulating IL-6 levels with heat stroke outcome, suggesting that neutralization of this cytokine may be beneficial for heat stroke protection or recovery. However, contrary to this hypothesis, IL-6 knockout mice (i.e., mice that are unable to produce endogenous IL-6) show enhanced heat stroke mortality compared to their wild-type controls (97). Enhanced heat stroke susceptibility is also observed in TNFR knockout mice (i.e., mice that produce endogenous TNFalpha/beta, but cannot respond with a cellular signal due to the absence of signaling receptors), despite a more rapid rate of heating and cooling (i.e., both protective responses) compared to wild-type controls (97). These data are particularly intriguing since circulating TNFalpha levels were undetectable in wild-type heat stroked mice tested in this laboratory (97). Thus, TNFalpha appears to exhibit local tissue actions which currently remain unidentified in the heat stroke syndrome. Although the cellular mechanisms for enhanced heat stroke susceptibility in gene knockout mouse models are not fully understood, these studies indicate that correlation studies have mistakenly implicated endogenous IL-6 and TNF actions as harmful in this syndrome, when they may have permissive (basal) actions that are required for survival (97).

5. GENETIC MUTATIONS THAT MAY PREDISPOSE TO HEAT STROKE

5.1. Malignant Hyperthermia.

Malignant hyperthermia (MH) is a pharmocogenetic disorder characterized by clinical symptoms of hypercapnia, tachycardia, metabolic acidosis, muscle rigidity and late onset of hyperthermia (98). The MH syndrome is triggered in most individuals by exposure to volatile anesthetics (e.g., halothane, isoflurane) or succinylcholine, which is a depolarizing skeletal muscle relaxant. However, a small subset of MH susceptible patients (~5-10%) experience reactions to exercise, heat exposure or emotional stress, which has led to the hypothesis that MH and EHS may have a common etiology (98,99). Biochemical studies on skeletal muscle from MH patients and a porcine MH model indicate that inhalational anesthetics induce the massive release of calcium from the type I ryanodine receptor (RyR1) of the sarcoplasmic reticulum, which overwhelms the cellular mechanisms of calcium homeostasis, causing muscle rigidity and additional pathophysiological responses associated with the MH syndrome (100). RyR1 is the most common mutation in skeletal muscle, but additional isoforms have been identified in B and T cells, CNS (thalamus, hippocampus) and heart (101-103). RyR1 can be activated by a variety of pharmacological compounds (caffeine, halothane, 4-chloro-m-cresol), which has aided in the development of an *in vitro* contracture test that can be applied to skeletal muscle biopsies to identify MH individuals (104,105). Dantrolene inhibits calcium release from the sarcoplasmic reticulum and is a common treatment strategy for MH episodes along with cessation of anesthesia exposure and rapid cooling. In the absence of these treatments, mortality rates may exceed 70% but improved monitoring standards, early detection and the use of dantrolene has decreased mortality to less than 5% (106). In rats, pretreatment with dantrolene delays heat stroke development and facilitates more rapid cooling during recovery, indicating its potential as a prophylactic agent (107). The effects of dantrolene are specific to passively heated rats, although effects on exercise endurance were noted (107). Interestingly, dantrolene has also shown efficacy in animal models of septic shock by decreasing circulating and tissue concentrations of inflammatory cytokines (108-110).

MH has been identified in several animal species, including dogs (111), boars (112), cats (113), and horses (114), but the most common experimental animal used to study MH is a porcine model that possesses a single mutation in the RyR1 gene of skeletal muscle. These animals develop the MH syndrome in response to inhalational anesthetics as well as exercise, heat and other stressors. Interestingly, this pig model exposed to mild exercise prior to anesthesia shows accelerated progression of the syndrome and a shortened latency to death, suggesting inflammatory mediators released by skeletal muscle may predispose to MH (115). The ability of skeletal muscle to locally produce IL-1beta, IL-6 and IL-10 suggests these cytokine may modulate EHS susceptibility in MH patients, although this has not been tested *in vivo*. However, when immortalized B cells isolated from MH patients with the RyR1 mutation are stimulated with caffeine and 4-chloro-m-cresol, IL-1beta is overexpressed ~5-fold compared to control cells (116). The ability of dantrolene to prevent IL-1beta stimulation suggests a direct link between cytokine production and the clinical MH syndrome. The recent development of a RyR1 knockin mouse has proven useful to study the MH / EHS link and could shed light on the role that inflammatory cytokine production from skeletal muscle (and other organs) has in this syndrome (117). In addition, the correlation of RyR1 mutations with EHS incidence suggests that the screening of young, healthy individuals (i.e., athletes, military personnel) for the MH mutation could be a powerful tool to

prevent heat stroke mortalities in this otherwise heat-tolerant population.

5.2. Cytokine polymorphisms

One of the most prominent aspects of the pathophysiological response of patients to heat stroke is the wide inter-individual variability in cytokine expression profiles. While this is typically regarded as a consequence of variations in presentation times or clinical treatment regimens following collapse, cytokine polymorphisms are a factor to consider as well. There are several cytokine (IL-1, IL-2, TNF, IFNgamma, IL-10), cytokine antagonist (IL-1ra) and TLR-2 and TLR-4 genes that contain polymorphisms within their promoter regions, affecting gene transcription and possibly clinical outcome to infectious or inflammatory diseases (for a complete list of cytokine polymorphisms, see http://www.nanea.dk/cytokinesnps. Cytokine polymorphisms exist as single nucleotide polymorphisms (SNPs) or microsatellites (i.e., variable number tandem repeats) and have been implicated in a variety of diseases, including sepsis, type I diabetes, arthritis, inflammatory bowel disease and rheumatic fever (118-121). To our knowledge, cytokine polymorphisms have not been identified in heat stroke victims, but may be an important predisposing factor that has been overlooked.

Given that pre-existing inflammatory states involving cytokine production have been associated with heat stroke susceptibility in military and civilian populations (3,8), it is intriguing to speculate that cytokine polymorphisms may be responsible for mortality in a subset of heat stroke cases. It has been suggested that even for those cytokine polymorphisms that are located distal to a critical promoter region and do not directly affect gene transcription rates, co-inheritance of multiple immune responsive genes by a process known as linkage disequilibrium may be responsible for altered immune function. For example, some TNFalpha polymorphisms exist in linkage disequilibrium with HLA haplotypes and the co-inheritance of these genes may ultimately be responsible for poor sepsis outcome (121,122). It is likely that resistance to heat stroke is at least partly a heritable trait that is controlled by multiple genetic and environmental factors. Wide variability in heat stroke responsiveness between experimental mouse and rat strains provides evidence in support of this hypothesis (Leon *et al.*, unpublished observations; (123)).

It is important to recognize that not all cytokine polymorphisms result in enhanced expression profiles or altered disease mortality rates. For example, Mockenhaupt *et al.* (124) reported increased risk for severe malaria in African children with a TLR-4 polymorphism (Aps299Gly), yet mortality rates were similar to controls. Therefore, the presence of certain genetic variants in a population may increase the risk yet positively influence the outcome of a disease, which would then be selected for in a given population (124,125). That is, several studies show an association of disease risk with the presence of certain cytokine polymorphisms (http://www.nanea.dk/cytokinesnps. The natural extension of these findings is that perhaps clinicians will be able to one day pre-screen an individual for genetic variants that will predict risk to heat stroke (and other diseases), which will facilitate development of prevention / treatment strategies that optimize protection (126,127).

6. POTENTIAL THERAPEUTIC TARGETS FOR HEAT INJURY PREVENTION

6.1. Induced hypothermia

Rapid core cooling and fluid resuscitation are currently the most effective heat stroke treatments, but despite these efforts up to ~30% of heat stroke survivors experience permanent neurological impairments or death within 1-year of hospitalization (2,3). A recent epidemiological study of U.S. Army heat stroke hospitalization cases shows ~2-fold increase in the risk of death from cardiovascular disease and ischemic heart disease within 30 years of hospitalization compared to individuals hospitalized for a non-heat related illness (31). These data suggest that more effective treatment strategies are required to limit organ damage that will culminate in health complications or death in ensuing years of recovery. While several of the clinical responses (hyperthermia, dehydration, renal and liver damage) occurring during progression or shortly after heat stroke collapse are clinically recognized and treated, those occurring during the months and years following hospitalization are less known, making the development of appropriate treatments more complicated.

Hypothermia is a heat stroke recovery response that may impart a survival advantage for heat injury prevention and recovery (25,128). Recurrent, intermittent hypothermia is occasionally observed in heat stroke patients, but is typically regarded as an unregulated response that occurs in response to ischemia-induced damage to the hypothalamic center; however, post-mortem analysis of 125 heat stroke cases failed to detect hypothalamic damage (25). In experimental animal models, hypothermia is commonly displayed $\leq$12h following removal from the heat and the prevention of this hypothermia response causes increased heat stroke mortality, suggesting a protective role against tissue injury (129-131). In mice, the depth and duration of hypothermia are directly related to heat severity and these characteristics of the T_c profile serve as sensitive biomarkers of tissue injury (130,132). Hypothermia develops naturally in a variety of laboratory test species exposed to severe environmental insults that induce CNS injury, such as hypoglycemia (133), dehydration (134), hypoxia (135), and heat stroke (129-131). This raises the question as to the potential therapeutic benefit of induced (e.g., forced) hypothermia for the mitigation of multi-organ injury in heat stroke patients. Induced hypothermia mitigates CNS injury in patients and animal models of cerebral ischemia, traumatic brain injury and other disorders (136,137), presumably due to (1) a reduction of CNS oxygen requirements through a decrease in metabolic rate (138,139), and (2) a decrease of tissue injury as the production of damaging ROS is suppressed (140-142). Currently, clinical cooling therapy dictates that heat stroke

patients are cooled to <38.8˚C rather than baseline or hypothermic levels. Whether induction of hypothermia in heat stroke patients would be beneficial is currently unknown.

To maximize hypothermia efficacy, the timing of its application is critically important; for example, inducing hypothermia within 1-2 hours after an ischemic event will retain its efficacy whereas a delay past ~3 hours is thought to significantly decrease its clinical benefit (143). In some cases, hypothermia imparts a permissive action onto therapeutic treatments that would otherwise show no neuroprotective effects in a normothermic animal. For example, the protective effect of IL-10 treatment against brain ischemia is increased ~42% when applied in combination with modest hypothermia (33-34˚C) in a rat model of vessel occlusion (144). It is also important to recognize that the protective effects of many treatment strategies (e.g., diazepam, clomethiazole) are due to the induction of hypothermia to a depth and duration that naturally imparts a neuroprotective effect (145).

6.2. Glucocorticoid therapy

Glucocorticoid hormones (cortisol in humans, corticosterone in rodents) are an integral component of the hypothalamic-pituitary-adrenal (HPS) axis, whose main actions are mobilization of energy stores and inhibition of cytokine production in response to stress (146-148). In human volunteers, marked increases in cortisol levels occur in response to exercise and/or heat exposure and are considered a sensitive index of heat intolerance (149,150). Heat acclimatization improves thermal tolerance and normalizes cortisol levels in trained individuals, but inconsistencies between studies suggest multi-variable influence on these responses (149-152). Glucocorticoids are used prophylactically or their production is inhibited by adrenalectomy (ADX) to examine heat stroke tolerance in experimental animal models. Rats treated with the synthetic glucocorticoid dexamethasone show improved heat stroke tolerance, as illustrated by attenuation of hypotension, cerebral ischemia, and neuronal damage and a prolongation to time of death; interestingly, these protective effects are unrelated to changes in the hyperthermic profile (153). In ADX rats, the elimination of endogenous glucocorticoid levels (basal and stress-induced) enhances susceptibility to passive heat exposure, which is an effect that is readily reversible with dexamethasone replacement therapy (153). Whether permissive actions of glucocorticoids (i.e., basal levels) are sufficient for cytokine regulation and heat stroke protection or stress-induced levels are required is currently unknown, but the mechanism of protection of these steroids appear to be at least partially mediated through the inhibition of IL-1 (and perhaps other cytokine) actions (153). More recently, dexamethasone efficacy was tested in a baboon model of heat stroke – surprisingly, despite dexamethasone treatment prior to heat stress and during cooling, protection against the lethal effects of heat stroke were not realized. Rather, dexamethasone caused sustained elevation of plasma IL-6 levels, decreased complement system activation and aggravated multi-organ system dysfunction with similar mortality rates between control and treated animals (154). Perhaps not surprisingly, glucocorticoid efficacy is thought to be at least partially dependent on heat severity, as metyrapone (an inhibitor of corticosterone synthesis) is without effect on cytokine mRNA expression, except at high heat loads in which it induces increased TNFalpha mRNA expression (155). Although correlative data suggest that enhanced TNFalpha mRNA expression may enhance mortality rates, this effect of metyrapone was not directly determined in this study; however, it provides food for thought regarding the sensitivity of the HPA axis-cytokine negative feedback loop with respect to glucocorticoid efficacy for heat stroke prevention and / or treatment. Clearly, more studies are required in this area to determine the potential clinical benefit of glucocorticoid therapy as a heat stroke prevention and / or treatment strategy.

6.3. Activated Protein C therapies

There is now compelling evidence that the exogenous administration of activated protein C (APC) to severe sepsis patients improves outcome (156). The use of this therapy is based on the premise that part of the pathophysiology of sepsis is caused by inappropriate coagulation in the microcirculation. In large multicenter clinical trials, treatment with APC was associated with reduced 28-day mortality of sepsis patients, although severe bleeding was identified as a risk factor of this treatment (156). The efficacy of APC appears to be dependent on a variety of patient conditions, including age (most effective in patients older than 50 years of age), extent of organ dysfunction (benefit not apparent if failure of only one organ) and the presence of shock at the time of infusion (correlated with improved efficacy; (157). In heat stroke, APC has also shown protection against a myriad of pathophysiological responses, although its efficacy differs depending on the animal model. In rats, a single dose of recombinant human APC provided at the onset of heat stroke inhibited inflammation, coagulopathy, prevented organ failure, and improved survival; however, if treatment was delayed for 40 minutes following the onset of heat stroke, no beneficial effect on survival time was evident (158). The efficacy of APC was less obvious in a baboon heat stroke model as infusion for 12 hours following heat stroke onset attenuated plasma IL-6, thrombomodulin and procoagulant components, but had no effect on mortality (159). Based on these interspecies differences, caution is warranted in extrapolating from animal models of heat stroke to the human condition regarding the efficacy of APC or other therapies. Similarly, the risk of bleeding following APC treatment is a serious concern regarding its efficacy for sepsis and/or heat stroke treatment, despite extensive knowledge regarding the mechanisms of action of this drug. APC is the first biologic agent approved in the United States for the treatment of severe sepsis due to two decades of research in this area (157). Although there is not sufficient evidence to justify the use of this treatment in heat stroke patients at this time, this area of research deserves further consideration.

6.4. Anti-cytokine therapies

As previously described, attenuations in splanchnic blood flow during heat stroke contribute to

increased GI permeability and an associated rise in circulating endotoxin (16,160). This series of events is hypothesized to stimulate elevations in plasma IL-1alpha, IL-1beta, IL-1ra, IL-6, sIL-6R, IL-10, IFNgamma, TNFalpha and sTNFR levels, as seen in human heat stroke patients and animal models (10,11,14,33,39,74-77,79-81,153). Based on these data the question arises: Will anti-cytokine therapies be efficacious for heat stroke prevention / intervention?

Although high circulating cytokine levels have been strongly implicated in the adverse consequences of heat stroke, there are considerable discrepancies between studies. For example, the report of high circulating TNFalpha levels in clinical and experimental studies are conflicting (10,39). Although TNFalpha levels were significantly elevated in human heat stroke patients following cooling, studies using non-human primates and murine models have been unable to detect circulating TNFalpha levels following elevation of T_c to levels as high as 42.4^0C or during 24h of recovery (33,48). These discrepancies are thought to reflect differences in heating protocols, species, sampling time points or methodologies used to detect the cytokine, but it is likely that the inconsistencies are a consequence of the transient nature of cytokine production throughout progression of the heat-induced SIRS. As discussed earlier, neutralization studies conducted in TNFR knockout mice suggest a more complicated nature of cytokine responses. Specifically, what is easily measurable in the circulation may misrepresent the changes occurring at the tissue level, the latter of which is most important in terms of multi-organ system failure and death. To further complicate the issue, cytokines exhibit pleiotropic, overlapping actions *in vivo* that are difficult to discern from one another. For example, TNFalpha has synergistic actions with IL-1 as the two cytokines signal through a common transduction pathway. This brings into question the mechanism(s) by which the IL-1ra has shown protection in animal models of heat stroke – are the cardio- and/or neuro-protective actions of IL-1ra a function of the inhibition of IL-1 actions *per se* or an indirect effect that is manifest through alterations in the cytokine milieu? Results from human sepsis studies indicate that the results obtained with anti-cytokine therapies in rodent models need to be viewed with cautious optimism. For example, although human patients with sepsis display high circulating levels of IL-1 that appear to be directly correlated with morbidity and mortality (161), IL-1ra treatment has been unsuccessful in reducing mortality in these populations (162).

The lack of success in human sepsis patients suggests that a short therapeutic time window exists for efficacy of anti-cytokine therapies to be realized. In Phase III clinical trials, it is thought that IL-1ra was ineffective in large part because at the time of administration circulating levels of IL-1beta were detectable in less than 5% of the patient population (163). Furthermore, kinetic studies have shown the half-life of IL-1ra to be ~20 minutes (164). These are only a few of the timing issues that complicate the use of anti-cytokine therapies in the clinical setting. For instance, administration of anti-cytokine therapies before sepsis onset may increase the rate of infection-related complications by suppressing the immune response in a non-specific manner. On the other hand, anti-cytokine therapies given too late in sepsis progression may have no effect due to the overwhelming nature of the septic event (165). The transient nature of cytokine production / clearance and lack of correlation between serum levels and disease severity further complicate treatment scenarios. Given the short half-life of TNFalpha (~6-7 minutes; (166) and biphasic clearance patterns of IL-1beta and IL-6 (rapid disappearance in the first 3 minutes followed by an attenuated clearance over the next 1 – 4 hours; (167)), it is clear that the narrow protective window in which anti-cytokine therapies may be effective is a difficult obstacle to overcome.

Interleukin-10 is a potent anti-inflammatory cytokine that suppresses the production of several pro-inflammatory cytokines, including IL-1beta, IL-6 and TNFalpha (168) which is an important negative feedback loop during infection / disease (169). Experimental heat stroke studies conducted in baboons and mice show high circulating IL-10 levels following heat stroke collapse (33,48). In rats, 30 days of heat acclimation induced elevations in plasma IL-10 levels that correlated with protection against traumatic brain injury (170). Although not well understood, it is hypothesized that the beneficial effects of IL-10 are related to its inhibitory effects on IL-1beta, IL-6 or TNFalpha production. Few if any studies have investigated the efficacy of IL-10 treatment for heat stroke protection / recovery, although the cytokine has been commonly used in the treatment of multiple auto-immune diseases including rheumatoid arthritis, Crohn's disease, multiple sclerosis, and sepsis (171,172). IL-10 works mainly as an immunosuppressant but has numerous biological roles which highlight the complications of using IL-10 based therapies (171). For example, in bone marrow transplanted mice, high doses of recombinant IL-10 augmented graft versus host disease but low doses protected the same population (173). Furthermore in mice IL-10 administered prior to heart transplantation is protective but little protection is afforded if the cytokine is given at the time of or after the allograft (174). As with other cytokines the timing of IL-10 administration must be carefully considered. IL-10 decreases IFNgamma production by natural killer and Th1 cells; this is an important consideration, as IL-10 administration provided early in infection has been shown to suppress clearance of infectious organisms via reductions in IFNgamma (175,176). Consistent with anti-cytokine therapies, these data demonstrate that the beneficial effects of exogenous IL-10 administration are dependent upon multiple factors including time of administration, route, and the site to which the cytokine is targeted (171,177). These considerations along with the immune suppressive and unpredictable nature of IL-10-based therapies have resulted in diminished enthusiasm for the cytokine as a possible treatment modality for sepsis.

Data from numerous cytokine-based therapy studies demonstrate that the *in vivo* balance between pro- and anti-inflammatory cytokines is delicate and the use of

exogenous cytokine therapies is likely to be complicated. Drawing from sepsis (161) and auto-immune disease studies, some of the major issues that need to be addressed in future research include how to target a cytokine in a specific tissue (i.e., targeted therapeutics), effects of cytokine therapies on the naturally occurring cytokine mileu *in vivo*, the timing of cytokine therapy administration, and the possible side-effects from administration of pleiotropic cytokines, whose wide-ranging effects are not completely understood.

6.5. Prostaglandin inhibitors / heat shock protein inducers

Prostaglandins (PGs) are metabolic end products of arachidonic acid metabolism that are synthesized in response to a variety of external stimuli, including bacterial infection and heat shock (178-183). PGs regulate a variety of physiological responses, such as fever, cell proliferation and differentiation, inflammation, and cytokine production (184,185). PGs are cytoprotective against liver injury induced by sepsis and ischemia (186,187), suggesting a potential protective role for these substances in heat-induced hyperthermia and / or the SIRS.

The common occurrence of fever in heat stroke patients and animal models suggest that alterations in the temperature setpoint during heat exposure and / or recovery may have a confounding influence on thermoregulatory responses to heat. Non-steroidal anti-inflammatory drugs (NSAIDs), such as salicylate (i.e., aspirin) are inhibitors of cyclooxygenase (enzymatically converts arachidonic acid to PGs and thromboxane) that exhibit potent anti-inflammatory, analgesic, and antipyretic properties. High doses of salicylate induce dose-dependent hypothermia in afebrile rats (~1.0˚C to 5.0˚C) during exposure to cold temperatures through increases in tail and foot vasodilation, whereas T_c is unaffected at a mildly elevated ambient temperature of 29˚C (188). In anesthetized monkeys (*Macaca cyclopis*), thermal tolerance was improved following intracerebroventricular (third ventricle) injection of salicylate, which correlated with stimulation of the appropriate heat dissipating mechanisms, such as evaporative heat loss and tail vasodilation (189). Unfortunately, the effect of salicylate on baseline T_c homeostasis was not reported in this study so it is difficult to discern if the observed effects were specific to thermal exposure. Indomethacin, a potent prostaglandin pathway inhibitor, improved heat tolerance of sedentary sheep and rabbits (190). However, contrary to studies performed in monkeys, sheep and rabbits, a high dose of salicylate potentiated hyperthermia in human volunteers performing work in a desert or humid environment (191). Whether discrepancies between the above mentioned studies is due to species differences in unclear, but does suggest that the influence of fever on heat tolerance and/or heat stroke recovery has not yet been clearly delineated.

In a human monocytic cell line, NSAIDs repress IL-1beta, IL-6, IL-8, IL-10, and TNFalpha production in response to LPS stimulation (191). Whether the effects of NSAIDs on cytokine expression will provide protection from the long-term sequelae of heat stroke *in vivo* remains undetermined, but is an important area of investigation given the high use of NSAIDs as an over-the-counter medication for pain and / or fever relief. In human heat stroke victims, fever is a common symptom that presents intermittently during the days and weeks of recovery (25). In mice, fever is observed following hypothermia re-warming and the magnitude and duration of this response is unrelated to heat stress severity (130). The fever response observed during heat stroke recovery has several similar characteristics to that observed in animal sepsis models, as it is correlated with endogenous IL-6 production (33), occurs in a biphasic T_c profile similar to that induced by sepsis or endotoxin injection (192,193), and is noted only during the day (inactive period) as is seen following LPS injections (192). The fever-like state observed in heat stroke patients and animal models is presumed to be a PG-mediated event that occurs in response to endotoxin leakage and cytokine stimulation. Although the adaptive value of fever in bacterial infections is recognized, the effect of NSAID treatment on this T_c response and its effects on long-term heat stroke recovery remain unknown.

An additional mechanism of PG-related thermotolerance modulation may be through its effects on HSP expression. HSP gene expression is regulated by heat shock factors (HSF) interacting with the heat shock element (HSE) promoter (194,195). HSF-1 is the major stress responsive transcription factor in mammalian cells, which is constitutively present in a non-DNA binding state and rapidly transformed to a nuclear form following exposure to heat or other stresses. Acting as a cellular-based defense mechanism, HSP expression is induced by damaged proteins and serves a protective function by ensuring proper folding of target proteins within the cell, which facilitates rapid adaptation to noxious stimuli. *In vivo* preconditioning studies that increase basal HSP70 expression protects against future bouts of severe heat stress (196,197), suggesting that exogenous administration of pharmaceutical agents, such as NSAIDs that modulate HSP70 expression may have effects on heat stroke outcome.

Interestingly, consistent with results of PG studies, Bouchama (154) demonstrated that administration of dexamethansone (i.e. glucocorticoid) before and during recovery from heatstroke, *increased* tissue injury and organ dysfunction in a baboon model of experimental heat stroke. Dexamethasone blocks the function of phospholipase A_2 suggesting that the bioavailability of arachidonic acid for PG synthesis was reduced (Figure 2), resulting in the inhibition of HSP70 co-induction. Co-inducers target transcription factors (e.g. HSF-1) thereby enhancing the body's natural ability to synthesize HSP70. Structurally, co-inducers are similar to aspirin and stabilize the HSF and HSE interaction thereby enhancing HSP70 synthesis. However, co-inducers do not stimulate *de novo* expression of HSP70. Herbal remedies and experimental drugs have been identified that, similar to PG, activate HSF-1 resulting in transcription of HSP70. These co-inducers include the herbal extract Paeoniflorin (198) and the drug Bimoclomol which if given prior to heat stress induces HSP expression at higher levels than in cells subjected to heat stress alone

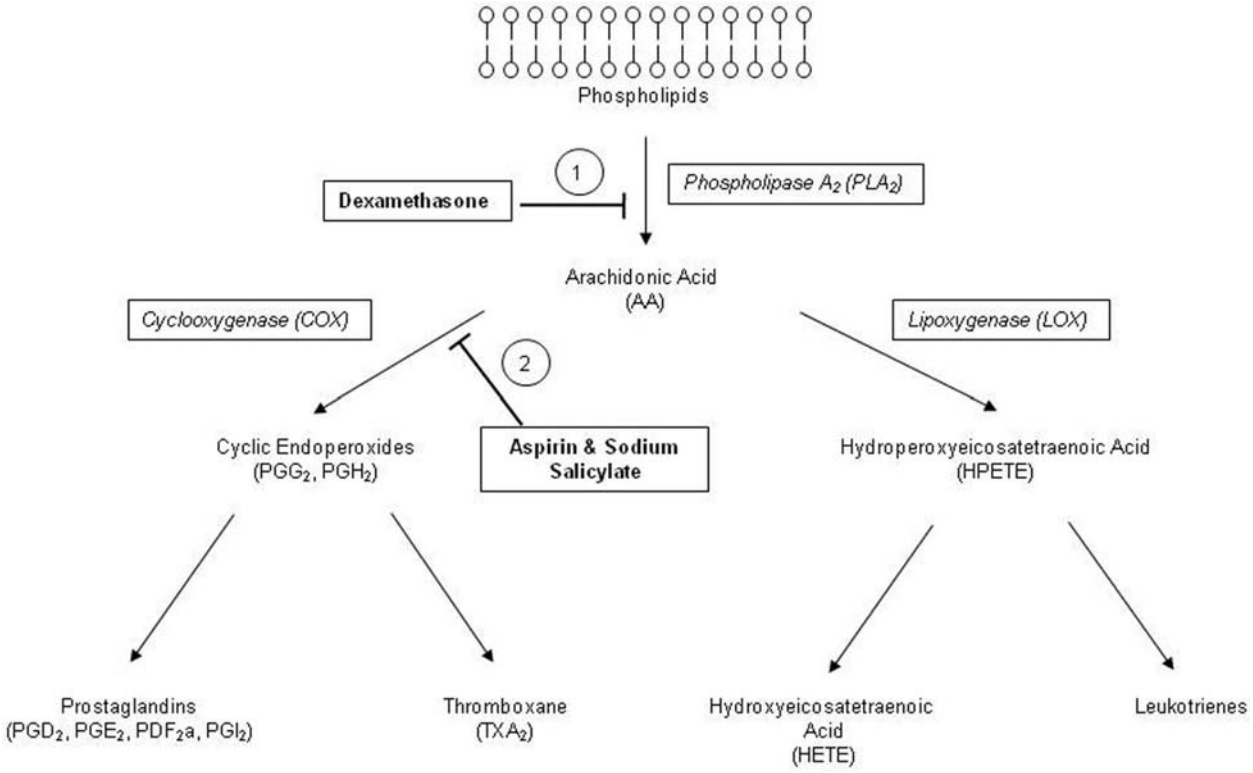

Figure 2. Eicosanoid metabolism is initiated when phospholipids are converted to arachidonic acid (AA) by the enzymatic actions of phospholipase A_2 (PLA_2). Cyclooxygenase (COX) converts AA to cyclic endoperoxides which serve as precursors for the formation of prostaglandins and thromboxane. Alternatively AA serves as a precursor for leukotriene synthesis utilizing the enzyme lipoxygenase (LOX). (1) The steroid dexamethasone inhibits the actions of PLA_2 thereby blocking eicosanoid metabolism. (2) Non-steroidal anti-inflammatory drugs (NSAIDs; aspirin, sodium salicylate) block the action of COX enzymes preventing the production of cyclic endoperoxides and the associated inflammation. COX inhibitors may also block formation of the cytoprotective molecule HSP70 resulting in questions regarding its use in treatment of heat stroke.

(199). Administration of Arimoclomol (i.e. an analog of Bimoclomol) 1 hour prior to heat stress effectively enhances HSP70 production in the liver and skeletal muscle in rats (200). Like Bimoclomol, Arimoclomol requires heat exposure to induce HSP70 synthesis and is one of the few co-inducers tested for efficacy as a heat stroke treatment (200). As with most co-inducers, Arimoclomol and Bimoclomol were originally developed and are currently in clinical trials as a potential treatment for CNS disorders. Paeoniflorin has not been extensively studied for its ability to induce HSP70, likely because of its herbal nature. Although the drug Arimoclomol is currently in Phase II clinical trials and appears to hold therapeutic promise, none of the compounds discussed in this section have enough data to support their current use in treatment / prevention of heat stroke.

Compounds that induce *de novo* synthesis of HSP70, such as Geranylgeranlyacetone (originally developed to treat gastric ulcer and gastritis (201,202)) have been shown to induce increased expression of HSP70 in several species and cell types including guinea pig gastric mucosal cells (201), a rat cerebral ischemia model (203) and rat hepatocytes (204). Interestingly, not all tissue types are affected as mesangial, renal tubular, glioma, hepatoma, neuroblastoma, alveolar macrophage and synovial cells are unaffected by this compound (201,204). The tissue-specific effects of compounds such as Gerenaylgeranlyacetone are important to consider as they may provide an advantage over more "global" strategies.

6.6. Erythropoietin injury protection

Erythropoietin (EPO) is the primary hematopoietic hormone in the human body. Renal tissue hypoxia stimulates accumulation of the transcription factor hypoxia-inducible factor-1 (HIF-1) which induces EPO production and secretion. This initial event is followed by binding of EPO to the EPO receptor (EPOR), homodimerization of the receptor and activation of a signaling cascade that promotes red blood cell maturation (205). The adult kidney is a major site of EPO production, however expression of EPO and EPOR mRNA has been identified in endothelial, vascular smooth muscle, Kupffer, uterine, placental, gastric mucosal, pancreatic beta, cardiac, and neural cells (206).

Complimentary to these localization studies, novel physiological functions of EPO have been discovered that suggest the hormone may provide tissue protection via receptors associated with non-hematopoietic cells. For

example, Brines *et al.* (207) demonstrated that protection against organ injury is critically dependent upon binding of the hormone to a heterodimer receptor isoform comprised of EPOR and the common beta receptor (betacR) subunit. Utilizing a 30-day heat acclimatization (HA) model, Shein *et al.* (208) demonstrated significantly reduced edema following closed head injury in HA animals compared to controls. These findings correlated with significantly increased frontal cortex expression of HIF-1alpha and EPOR in HA mice 4 hours post-CHI; 3-days post CHI, significant improvements in cognitive function and preservation of CNS function were observed, which correlated with activated EPO, EPOR, and HIF-1alpha pathways. EPO and EPOR are present in a variety of neural tissues across a wide range of species (209-211). In response to hypoxia increases in EPO and EPOR stimulate tissue-specific growth factors which are protective against neuronal cell death in both humans and rodents (212). Interestingly, although EPO is a heavily glycosylated molecule, the hormone is transported across the BBB (213) although it remains unknown if the concentration that crosses the BBB is sufficient to provide protection against ischemia.

EPO-mediated protection has been demonstrated in several peripheral tissues as well. Maloyan (214) demonstrated that elevated EPO mRNA expression in the kidneys of heat acclimatized rats was protective against a subsequent ischemia-reperfusion infarct when compared to control rats. These responses appeared to be mediated through HIF-1alpha expression, which was hypothesized as a mechanism of cross-tolerance in the heart of acclimatized rats (208,214). The effectiveness of EPO-related therapies for cardiac protection against heat stress is largely unknown. However, seminal work by Brines and others (215-219) developing EPO-derivatives that provide tissue protection without the adverse consequence of erythropoiesis suggests that the potential protective effect(s) of these compounds spare cardiac tissue from injury. A lack of current data indicates that future studies should determine the combined effects of heat stress and heat acclimatization on EPOR homo- ($EPOR_2$) and heterodimer (EPOR+betacR) expression.

Unfortunately, tissue protection via EPO administration requires a much higher dose of the hormone than the dose needed to induce erythropoiesis. Studies in rats have shown that tissue protective doses raise blood pressure and increase the risk of coagulation in the blood (215-217,217). Such coagulation-related issues have led to the recent and continued development of modified EPO-like molecules that afford tissue protection without inducing erythropoiesis via targeting of the EPOR-betacR heterodimer. Among these new compounds asialoEPO has been shown, *in vivo*, to have a reduced plasma half-life resulting in substantially increased tissue protective effects (217,220) without the erythropoietic properties that are dependent upon sustained plasma EPO half-life. Recently a carbamylated EPO (CEPO) has been designed that lacks affinity for the EPOR homodimer and thus prevents coagulation while retaining strong tissue protective properties via interaction with the EPOR-betacR heterodimer (216). Collectively the results of initial EPO and CEPO studies indicate that non-erythropoietic forms of the EPO molecule may hold promise as therapeutic and prophylactic modalities against heat stroke.

7. PERSPECTIVE

Environmental heat exposure is one of the most deadly natural hazards in the United States with ~200 deaths per year (221). Between 1979 and 2003, extreme heat exposure claimed more American lives than the combined effects of hurricanes, lightning, earthquakes, floods and tornadoes (222). Potential explanations for the dire outcome following prolonged heat exposure are several-fold: (1) strategic planning to prevent prolonged heat exposure in the most vulnerable populations (e.g., the young and elderly) is inadequate or ignored, (2) there are currently few guidelines to identify those individuals at greatest risk for heat stroke, (3) the contribution of underlying pathophysiological conditions to heat tolerance are not well understood, and (4) there are currently no effective therapeutic treatments to prevent heat injury in patients that present clinically with the syndrome. Unfortunately, advances in our understanding of heat injury mechanisms has not yet culminated in the development of effective treatment strategies to mitigate the long-term consequences of this syndrome. Given the current upward trend in heat wave incidence and climate change toward global warming, we may expect mortality rates from this deadly syndrome to continue to increase over the ensuing decades unless medical advances in the development of heat stroke treatments are soon realized.

8. ACKNOWLEDGMENTS

The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

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Abbreviations: ADX, adrenalectomy; BBB, blood brain barrier; βcR common beta receptor; CEPO, carbamylated erythropoietin; CHI, closed head injury; CNS, central nervous system; EHS, exertional heat stroke; EPO, erythropoietin; EPOR, erythropoietin receptor; GI, gastrointestinal; HA, heat acclimatization; HIF, hypoxia inducible factor; HPA, hypothalamic-pituitary-adrenal axis; HSE, heat shock element; HSF, heat shock transcription factor; HSP, heat shock protein; IFN, interferon; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; LPS, lipopolysaccharide; MH, malignant hyperthermia; NSAID, non-steroidal anti-inflammatory; PG, prostaglandin; RyR1, type I ryanodine receptor; SIRS, systemic inflammatory response syndrome; Tc, core temperature; TLR, toll-like receptor; TNF, tumor necrosis factor.

Key Words: Exertional Heat Stroke, Cytokines, Sepsis, Hyperthermia, Hypothermia, Heat Injury, Review

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