CHAPTER 4

HEAT SHOCK PROTEIN AND INFLAMMATION

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Abstract: Heat Shock Proteins (HSP) are important modulators of both anti-inflammatory and pro-inflammatory responses. In this chapter, we address this apparent paradox by focusing on the effects of the highly heat inducible Hsp70 and its transcription machinery. This transcription machinery may exert important effects on inflammation through pathways, which are independent of heat shock proteins. We then discuss disease states where the balance between the anti-inflammatory and pro-inflammatory effectors is critical to disease outcome

Keywords: Cytokines; HSF-1; HSP; inflammatory response syndrome; heat stroke; inflammation; heat injury

Abbreviations: AP-1, activator protein 1; APC, antigen presenting cells; eHSP, extracellular HSP; GLN, glutamine; HMGB1, high-mobility group box 1 protein; HIV, human immunodeficiency virus; HSF-1, heat shock transcription factor 1; HSP, heat shock protein; HSR, heat shock response; IBD, inflammatory bowel diseases; IFN-γ, interferon-gamma; IKK, IkappaB kinase; IL-6, interleukin 6; JNK, c jun amino terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinases; MODS, multiple organ dysfunction syndrome; NF-κB, nuclear factor kappa B; NF-IL6, nuclear factor-IL6; NK, natural killer cells; SIRS, systemic inflammatory response syndrome; TLR, Toll like receptor; TNF-α, tumor necrosis factor alpha

INTRODUCTION

The heat shock response (HSR) is one of the most evolutionary conserved protective mechanisms in cells. It involves temporary modifications in gene expression and synthesis of different heat shock protein (HSP) family members to help the organism to cope with environmental and physiological stresses. In non-stressed conditions, HSP function as molecular chaperones, maintaining protein conformation and facilitating transport. In response to stress, HSP expression prevents protein aggregation, refolds

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damaged proteins, and promotes the degradation of irreversibly denatured proteins. While the cellular protein management functions of HSP are well described, this chapter focuses on the ability of the HSR to modulate the immune/inflammatory responses of multicellular organisms. In this regard, the components of the HSR, including HSP, especially the most highly heat inducible member, heat shock protein 70 (Hsp70), and heat shock transcription factor 1 (HSF-1) have evolved to act as both pro- and anti-inflammatory molecules in the production and release of inflammatory modulators. As an anti-inflammatory effector, the HSR modulates cytokine signal transduction and gene expression by inhibiting the translocation of the transcription factor nuclear factor-kappa B (NF-κB) to the nucleus and preventing the expression of inflammatory mediators. As pro-inflammatory mediators, necrotic and non-necrotic release of HSP into the extracellular environment produces a multifaceted immune/inflammatory response involving the activation of a variety of immune effector cells and cytokine release. Finally, the crucial indirect role of the HSR in maintaining gut epithelial barrier integrity has important anti-inflammatory effects on the movement of endotoxin into the circulation, and so helps to prevent the subsequent endotoxin-mediated inflammatory cascade. Therefore, the ability of the HSR to regulate inflammation is, in turn, an important aspect of the progression of a variety of pathophysiologic states such as the sepsis and heat stroke, which are characterized by dysregulated inflammatory response and multiorgan-dysfunction. In this chapter, we discuss the role of the HSR in activating both anti- and pro-inflammatory systems in the whole organism, the differences seen between human and non-human model systems, potential mechanisms by which the HSR could exert its effects on inflammation, and disease states where the HSR may alter pathophysiology. We have chosen to focus on a fairly linear model comprising the activation of HSF-1 and Hsp70. While other HSP such as Hsp27, the endoplasmic reticulum chaperone gp96, bacterial Hsp60, and others have been associated with immune and autoimmune disease states, the highly inducible Hsp70 provides a useful platform to discuss the role of these primitive and ubiquitous proteins in modulating human inflammatory disease.

HSP ARE ANTI-INFLAMMATORY

Initial observations in animals linked the HSR to an altered inflammatory response through the demonstration that heat conditioning conferred survival to an otherwise lethal endotoxin stress (Ryan et al. 1992). Subsequent studies demonstrated that this increased survival in endotoxin exposed animals was associated with elevated levels of intracellular Hsp70 in the liver as well as lower serum concentrations of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) but not interleukin 6 (IL-6; Kluger et al. 1997). Parallel data in cell culture demonstrated that heat conditioned peritoneal macrophages showed a decrease in endotoxin induced TNF-α transcription and secretion which was sustained for as long as the cells had elevated Hsp70 levels (Snyder et al. 1992, Ensor et al. 1994). Similar to the studies in the intact organism, endotoxin induction of IL-6 was unchanged in the heat conditioned cells. Interestingly, the decrease in cytokine production was also associated with a

decrease in cytokine mRNA suggesting an alteration on the transcription factor that regulates cytokine transcription genes.

The liver has been a convenient tissue to measure the HSR in animals because of the massive accumulation of Hsp70 following heat stress (Flanagan et al. 1995). While the large induction of the Hsp70 in liver has been a useful biomarker of stress, there is compelling data to support the direct link between liver Hsp70 accumulation and the altered survival and inflammatory cytokine profile seen in heat conditioned animals undergoing endotoxin stress. For example, when animals are treated with the liver protein synthesis inhibitor, D-galactosamine, to block liver HSP accumulation during the same heat conditioning regimen shown to induce endotoxin tolerance and decrease serum TNF-α, the result is an augmentation of endotoxin end organ damage and a marked increase in endotoxin induced TNF-α even though all organs but the liver showed the characteristic Hsp70 accumulation from the heat conditioning (Dokladny et al. 2001). These data demonstrate the importance of heat associated protein synthesis in the liver in conferring the altered inflammatory response and altered survival seen in endotoxin exposure in animals.

Heat is a pleotrophic stimulus, which activates numerous tissue responses altering not only the HSR but other protective systems important during endotoxin shock. The role of the HSR in modulating inflammation has been explored at several levels. Using a whole animal model, Xiao et al. (1999) demonstrated that mice deficient in HSF-1 are unable to generate a HSR, showing reduced survival and excessive production of TNF-α. At the protein level, studies of single gene over-expression have demonstrated that the protective effects of heat conditioning on subsequent endotoxin exposure can be conferred by Hsp70 expression alone in cells and in the whole organism. In this regard, Lau et al. (2000) used hearts of transgenic mice over-expressing Hsp70 to demonstrate a direct relationship between the level of Hsp70 expression and endotoxin tolerance. Ding et al. (2001) also reported that human peripheral blood monocyte-derived macrophages over-expressing Hsp70 inhibited Lipopolysaccharide (LPS)-induced production of TNF-α, IL-1β, IL-10 and IL-12. Recently, we have employed an adenovirus expression system to direct the expression of Hsp70 in the liver of rats. Liver Hsp70 expression duplicated the protective effects of whole body heat-conditioning by inhibiting cytokine production (TNF-α and IL-6) after endotoxin challenge (Dokladny et al. 2009). These data taken as a whole, demonstrate that Hsp70 expression is sufficient to alter pro-inflammatory cytokine production, increase endotoxin tolerance and survival in both cellular and intact organism and further suggest that specific organs such as the liver may be important for the effects of the HSR.

HSP Induction: How do HSP Directly Alter Cytokines?

Immune cells are able to detect low levels of LPS through its binding to toll-like receptors and initiate an inflammatory response by the activation of signaling pathways and transcription factors. The activation of various mitogen-activated protein kinases (MAPK) and IkappaB kinase (IKK) pathways directly or indirectly

phosphorylate/activate different transcription factors, including NFκB, nuclear factor interleukin 6 (NF-IL6), and activator protein 1 (AP-1). The NFκB transcription factors play a pivotal role in many cellular processes altering the expression of cytokines, chemokines, cell adhesion molecules, growth factors, anti-apoptotic proteins, and immunoreceptors (reviewed by Brasier 2006). Inactive NFκB is normally found in the cytoplasm bound to its inhibitory protein, IkB. NFκB is activated by a number of incoming signals from the cell surface, including ischemia, oxidative stress, and LPS. These signals lead to activation of IKK, which phosphorylates IkB, allowing NFκB to translocate into the nucleus and bind to its target gene (Zhang and Ghosh 2000). The targeted genes include those that activate inflammatory cytokines, including TNF-α, IL-1β, IL-6 and IL-12.

A number of potential explanations have been suggested for the HSP mediated NFκB repression. There are data from cell culture and intact animal studies supporting direct physical interactions between Hsp70 and the NFκB inhibitor protein, IkB-α, which appear to prevent NFκB dissociation (Wong et al. 1997, Malhotra and Wong 2002). Sun et al. (2005) demonstrated that LPS exposure – induced NFκB activation was suppressed by Hsp70 accumulation (induced by sodium arsenite) with a subsequent decrease in TNF-α production and mRNA expression. Additionally, Hsp70 blocked IκB-α degradation and up-regulated IκB-α mRNA expression. Using intact animals, Pritts et al. (2000) showed that either heat exposure or sodium arsenite infusion inhibited increases on NFκB activity and decrease in IκB-α after induction of the stress response in vivo decreases NFκB activity in jejunal mucosa of endotoxemic mice. Another possibly mechanism is Hsp70 indirect mechanisms, whereby repression of MAPK activation mediates the inhibition of NFκB cascade. In addition, Hsp70 related activation of Jun kinase (JNK) MAPK induces phosphorylation of c-JUN (combination with c-Fos) to activate the transcription factor AP-1. The AP-1 is involved in the up-regulation of the IL-18 pro-inflammatory cytokine, for which overproduction is related to severe inflammatory disorders. Wang et al. (2002) observed that HSR could suppress the expression of IL-18 in response to LPS through inhibition of the JNK MAPK signaling pathway. Recent identification of a novel inflammatory cytokine and late inflammatory mediator of endotoxin lethality, high-mobility group box 1 protein (HMGB1), has provided further insights into another potential site of action of Hsp70 in modulating the inflammatory response. It has been shown that HMGB1 can trigger the MAPK pathway and subsequent NFκB transcription factor also stimulating the synthesis and release of pro-inflammatory cytokines. Tang et al. (2005) observed that murine macrophage-like RAW264.7 cells, stimulated with LPS released HMGB1, induced the release of other pro-inflammatory mediators and mediated lethality. However, a heat shock pretreatment suppressed the release and translocation of HMGB1. Therefore, induction of Hsp70 by heat shock, sodium arsenite or over-expression can be an important factor in reducing mortality in experimental models of septic shock by down-regulating expression of inflammatory genes, such as TNF-α, IL-1, IL-12, and IL-18. These studies demonstrate a great degree of redundancy in Hsp70 cytokine interactions and are summarized in Figure 1.

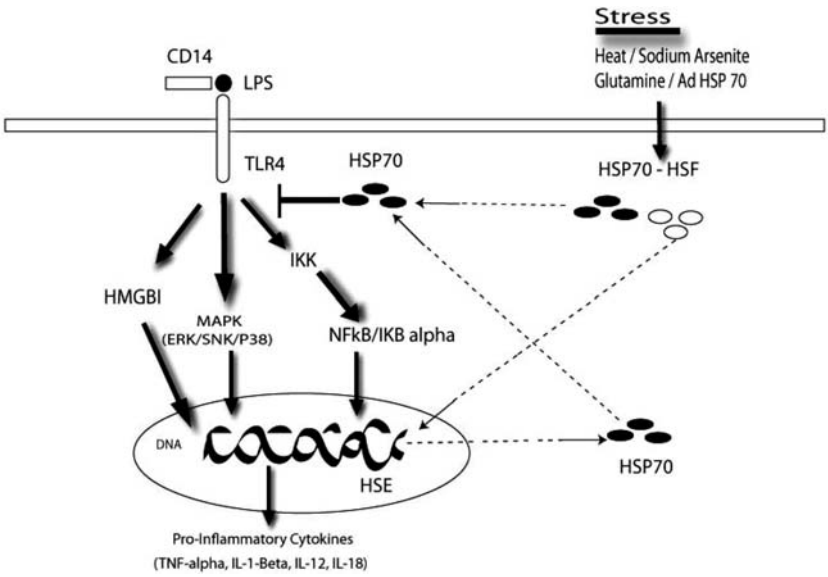

Figure 1. Interaction between HSP and cytokine signal transduction pathways

Are HSP Involved in Muscle IL-6 Production?

One of the interesting paradoxes of Hsp70 associated cytokine gene regulation is the concordant activation of both Hsp70 and IL-6 in exercising muscle. The large increase in serum IL-6 following exercise has been demonstrated to be of muscle origin (Pedersen et al. 2001). This IL-6 response appears to be distinct from endotoxin associated IL-6 production as described above. Further distinguishing the IL-6 response to exercise from that driven by endotoxin is its association with HSF-1 activation and cellular Hsp70 accumulation. As described above, cellular Hsp70 accumulation and HSF-1 activation both exert a potent inhibitory effect on NFκB mediated inflammatory signaling pathways. In contrast, HSF-1 activation and muscle cell Hsp70 accumulation precede muscle cell IL-6 production. Further, agents such as glutamine, which activate HSF-1, and which are associated with an HSP dependent inhibition of inflammatory IL-6 actually augment exercise induction of IL-6 (Hiscock et al. 2003). These data suggest a link between HSR and exercise associated IL-6 production in muscle, in contrast to the potent HSR inhibitory effects of inflammation.

HSF-1 as Non-HSP Transcription Factor Influences Inflammation

Hsp70 alone, whether induced as a part of the HSR or through the use of single gene expression systems, is sufficient to inhibit the production of cytokines in response to an inflammatory stimulus such as LPS. There is also data demonstrating that

the HSR mediates cytokine expression through direct actions of HSF-1 distinct and independent from cellular HSP accumulation. Cahill et al. (1996) first demonstrated that HSF-1 binds to the IL-1β promoter and represses its activity. Using HSF-1 knockout mice ($HSF^{1-/-}$), Wirth et al. (2004) also observed higher inflammatory mediators and severe lung damage compared to wild type mice after cadmium exposure. It was also reported that DNA binding of the transcription factor NFκB was higher in $HSF^{1-/-}$ mice than in wild-type mice after cadmium exposure. Previous study has also observed that over-expression of HSF-1 can increase the expression of anti-inflammatory IL-10 (Xiao et al. 2006). Specifically to TNF-α, Singh et al. (2002) identified a binding site in the TNF-α promoter for HSF-1 which represses the transcription of TNF-α. However, the anti-inflammatory activity of HSF-1 is not limited to its effects on cytokine transcription. In fact, recent data demonstrate that HSF-1 serves as a transcription factor for the gut tight junction protein, occludin-1 (Dokladny et al. 2008). The ability of HSF-1 to induce occludin-1 transcription may be an important part of the well-described ability of heat conditioning to preserve epithelial barrier integrity during heat stress (Moseley 1994). This increased epithelial barrier resistance may be a key factor in limiting gut associated endotoxin translocation during heat stress, and low perfusion states. Several inflammatory states are associated with loss of the intestinal barrier resulting in translocation of endotoxin, which enhances inflammation and leads to systemic inflammatory response syndrome (SIRS). Thus, in the intact organism, HSF-1 activation may limit endotoxin translocation through transcription of tight junction proteins, thereby limiting inflammation in an indirect fashion.

Finally, HSF-1 may also be linked with inflammatory pathway transduction and apoptotic cell signaling. Recently, Franceschelli et al. (2008) showed that Bag3, a protein with anti-apoptotic role, is regulated by HSF-1. Down-regulation of HSF-1 reduced the Bag3 protein levels, and increased the incidence of cell apoptosis. Additionally, Chen and Currie (2006) also demonstrated that knocking down HSF-1 gene caused significantly higher activation of NFκB and the pro-apoptotic protease activating factor 1 (Apaf-1), corroborating that HSF-1 has a more general role than specific cytokine regulation. Although these studies indicated that HSF-1 controlled these regulatory pathways we can not completely exclude the participation of HSP, since they also are known for the regulation of signal transduction and cellular survival pathways.

Glutamine (GLN) as HSF-1 Inducer

GLN provides an interesting opportunity to study the impact of nutrition and its potential role in activating the HSR as well as providing a model of the complex roles of HSR activators in altering the inflammatory response. There is evidence suggesting a link between GLN supplementation, activation of the HSR, and improved outcome of inflammatory conditions, such as those observed in sepsis, trauma, burns, and some critical illness (Griffiths et al. 1997, 2002, Morlion et al. 1998,

Novak et al. 2002, Wischmeyer et al. 2001c, Oudemans-van Straaten et al. 2001). In these investigations, GLN supplementation has been associated with lower mortality, decreased infection, and shortened hospital stay in acute and/or critically ill patients. Conversely, the state of GLN depletion was shown to be an independent predictor of outcome in acute and/or critically ill patients (Oudemans-van Straaten et al. 2001, Roth et al. 1982). Parallel studies in various animal models of sepsis and severe injury demonstrated that GLN supplementation reduced tissue injury, inhibited pro-inflammatory response, improved survival, and exerted an important benefit in preventing gut-origin sepsis after trauma (Wischmeyer et al. 2001a, b, Gianotti et al. 1995).

There are several potential mechanisms by which GLN could be linked to improved outcomes in critical illness, sepsis and multi-organ dysfunction syndrome (MODS). One simple mechanism is that during severe illness the circulating levels of GLN fall abruptly (Greig et al. 1996) and GLN replacement is necessary to maintain the metabolic needs of immune cells such as lymphocytes, macrophages, and enterocytes (Spittler et al. 1995, Exner et al. 2003). This fall is especially true in healthy humans exposed to bacterial endotoxin (Vesali et al. 2005) but is, interestingly, also associated with the infusion of recombinant human IL-6 (rhIL-6; Van Hall et al. 2008). In this human study, there was a profound fall in serum GLN with a concomitant flux of GLN from muscle. Another interesting facet of GLN effects on cytokines is data from exercising muscle where IL-6 serves as a component of the anti-inflammatory myokine group rather than an LPS induced pro-inflammatory cytokine (Hiscock et al. 2003). In contrast to the inhibition of GLN on inflammation associated IL-6, GLN supplemented during exercise further elevates plasma levels of IL-6 compared to exercise alone.

Another potential mechanism for GLN associated protection is its ability to induce the HSR (Ehrenfried et al. 1995). In cell culture experiments, cells deprived of GLN show a decrease in heat induced Hsp70 and increased susceptibility to apoptosis, as well as reduced responsiveness to pro-inflammatory stimuli (Eliasen et al. 2006). Conversely, intestinal epithelial cells supplemented with GLN showed enhanced Hsp70 expression and were more resistant to thermal and oxidant injury (Wischmeyer et al. 1997). In animal models, intravenous GLN supplementation induces Hsp70 in multiple organs such as lung, kidney, heart and colon, and reduces mortality as well as end-organ injury after endotoxemic shock (Wischmeyer et al. 2001a, b). Previous research reported that increases in Hsp70 with GLN supplementation were accompanied by suppressed NFκB activation and inhibition of p38 MAPK, and ERK phosphorylation. Further, lower pro-inflammatory cytokine release (TNF-α and IL-1β) and oxidative stress were observed in GLN supplemented animals exposed to endotoxin (Singleton et al. 2005a). More recent studies suggest that the HSR is necessary for the blunted cytokine response and protection afforded by GLN (Morrison et al. 2006, Singleton and Wischmeyer 2007). The initial study by Singleton et al. (2005b) observed that a single-dose of GLN post-sepsis initiation increased lung Hsp70 and HSF-1, and improved survival. Interestingly, the beneficial effects of GLN on survival were lost after the administration of the Hsp70

blocker quercetin. Similarly, Morrison et al. (2006) demonstrated that mouse fibroblasts lacking *hsp*70 genes (HSF-1$^{-/-}$) but supplemented with GLN had decreased survival compared to wild type cells supplemented with GLN. Moreover, the same group confirmed these initial results using an intact animal model (Singleton and Wischmeyer 2007). They reported that mice supplemented with GLN but also lacking the *hsp*70 genes (HSF-1$^{-/-}$) show greater mortality, higher pro-inflammatory cytokine expression and higher NFκB activation. The authors concluded that Hsp70 expression is required for GLN's effects on survival, tissue injury, and the inflammatory response after sepsis. These data strongly support a role for HSF-1 activation and perhaps Hsp70 in GLN associated protection in animal models.

In contrast to the relatively clear support for GLN activation of the HSR in cell culture and animal studies, clinical trials in humans are much less clear. Cellular Hsp70 accumulation has been shown to be impaired in granulocytes, and lymphocytes of patients with polytrauma (Weingartmann et al. 1999, Schroeder et al. 1999). However, a double-blind trial observed that patients in the intensive care unit receiving GLN showed enhanced serum Hsp70 (Ziegler et al. 2005). This serum Hsp70 increase also correlated with improved clinical outcome. However, the function of serum Hsp70 in this improved survival is unclear. It may provide a convenient and easily measurable marker of HSF-1 activation or Hsp70 expression in other tissues or may itself play a role in the observed outcome. Recently, we were unable to detect an effect of GLN infusion on either intracellular or serum Hsp70 in the LPS-induced cytokine response in healthy humans (Dokladny et al. 2009).

GLN associated HSR mediated protection may not rely solely on Hsp70. Although the previous cell and animal studies have confirmed that HSR is necessary to GLN protection, it is less clear if Hsp70 is the effector or whether the requirement for HSF-1 activation mediates the GLN effects through non-Hsp70 pathways. As mentioned above, the importance of the HSR in mediating these effects is largely based on studies using engineered HSF-1$^{-/-}$ knockout systems (Peng et al. 2006, Singleton and Wischmeyer 2007). In these studies, the protective effect of GLN is lost without HSF-1 activation. While Hsp70 can suppress the activation and nuclear translocation of NFκB (Ran et al. 2004) and its related cytokines, attenuation of TNF-α occurs prior to an increase in Hsp70 protein detection, indicating that HSP translation might not be necessary to attenuate the TNF-α expression. In fact, Eliasen et al. (2006) showed that human monocytes deprived of GLN showed normal capacity of HSF-1 to bind to HSE and normal increases in Hsp70 mRNA, however the Hsp70 mRNA showed lowered stability compared to cells with GLN.

HSF-1 itself can attenuate the expression of pro-inflammatory cytokines by binding to HSE and blocking IL-1β (Cahill et al. 1996) and blocking NFκB activation or inducing transcription of non-HSP genes, such Occludin-1 (Dokladny et al. 2008). This induction of Occludin-1 provides a mechanism for improved epithelial barrier integrity following HSF-1 activation, which would not require Hsp70 expression. Thus, studies using HSF-1 knockout systems do not provide

evidence to exclude these other, non-HSP effects of HSF-1 in mediating GLN related alterations.

Another possible mechanism is that GLN can reduce the inflammatory response in whole-body by activating the HSR and increasing maintenance of the mucosal barrier following injury or illness (De-Souza and Greene 2005). LPS translocation affects the function and integrity of remote organs and tissues, causing SIRS and MODS. It has been shown that gut is affected by prolonged periods of physiologic stress characteristic of severe burns, sepsis, and trauma (Deitch 1992). GLN may play a protective role in the gut because of its role as a preferential substrate, maintaining the selective barrier permeability and function against unwarranted bacterial translocation. However, maintenance of the mucosal barrier with GLN supplementation may also occur through an up-regulated HSR. Gut Hsp70 up-regulation induced by GLN supplementation correlates with a decrease in intestinal permeability and plasma endotoxin (Singleton and Wischmeyer 2006). Hsp70 accumulation in epithelial cells maintains the intestinal permeability during heat stress (Moseley et al. 1994). As noted above, the preservation of epithelial barrier integrity associated with heat conditioning may also involve the up-regulation of occludin-1 (Dokladny et al. 2008). Therefore, GLN supplementation may preserve cell and organ function via the induction of the HSR decreasing intestinal permeability and plasma endotoxin, and consequently reducing inflammatory response.

Finally, GLN protective effects may be related in part to its role as a precursor of glutathione (GSH), and reducing oxidative stress. GSH is an antioxidant and plays a critical role in reducing free radicals and maintaining cellular redox status. Several clinical conditions are associated with reduced GSH levels, which can result a lowered cellular redox potential (Exner et al. 2000). Xue et al. 2008 observed that GLN supplementation reduced chemotherapy side effects in rats associated with increased in Hsp70 and lower oxidative stress, as measured by the ratio of rGSH:oxidized GSH in the colon. Moreover, epithelial cells over-expressing Hsp70 showed a greater ability to preserve GSH and consequently create an intracellular environment with increased reducing capacity in the face of hypoxia or ischemia (Guo et al. 2007). Thus, GLN may improve tolerance to oxidative stress, either by contributing to the HSR or by serving as a substrate for GHS. Conversely the increase in Hsp70 appears to preserve GSH and so could reduce the need for GLN consumption for GSH generation.

Taken as a whole, GLN provides an interesting probe for the study of the HSR in human inflammatory illnesses. Mechanistic studies in animals link GLN activation of HSF-1 as the critical component of its anti-inflammatory response, and GLN supplementation in admitted heterogeneous populations of critically ill humans has been associated with improved outcomes. At the same time, GLN has important non-HSP biologic effects; in addition, HSF-1's role as a transcription factor of non-heat shock genes provides alternative mechanisms for the potential role of GLN in altering the course of human inflammatory illnesses.

HSP ALTER CYTOKINE TOLERANCE

In addition to the ability to block cytokine production by immune effector cells, HSP also alters target cell tolerance to inflammatory cytokines exposure. Heat conditioning renders tumor cells resistant to TNF-α mediated cytotoxicity (Kusher et al. 1990, Gromkowski et al. 1989). Jaattela and Wissing (1993) confirmed that Hsp70, but not HSP27, protects highly TNF-sensitive murine fibrosarcoma cells from cytotoxicity mediated by TNF-α, using cells transfected with Hsp70. Additionally, Jaattela (1993) demonstrated that Hsp70 over-expression or inhibition of endogenous Hsp70 synthesis did not change the ability of tumor cells to bind TNF-α or internalize and degrade the receptor-bound TNF-α. However, the authors observed that both Hsp70-mediated and TNF-induced TNF resistance appears to be associated with impaired TNF-α induce phospholipase A2 activation.

The mechanisms by which the HSP confer protection from cytokines are not clear but may involve the interplay of intracellular signals related to cell survival, stress tolerance and inflammation. For example, it is well recognized that exposure of cells to TNF-α may induce apoptosis, through either the caspase cascade; or activation of two important transcription factors involved in the inflammatory response, AP-1 and NFκB (reviewed by Baud and Karin 2001). As described in the previous sections, the HSR has an array of protective properties, and thus can regulate the same apoptotic pathways and transcriptions factors associated with cytokine activation. Schett et al. (2003) reported that exposure of U937 macrophages to TNF-α caused transient downregulation of HSF-1 activation and Hsp70 synthesis, leading to cell apoptosis. Paradoxically, acute cytokine exposure (TNF-α or IL-1β) induced Hsp70 syntheses in cardiac myocytes and pancreatic islets (Sharma et al. 1996, Nakano et al. 1996, Helqvist et al. 1991). Moreover, Ran and Lu (2004) demonstrated that TNF-α triggered apoptosis was initiated when Hsp70 expression increased to high levels to disrupt NFκB signaling. Therefore, it seems that there is a tissue or cell specific response to cytokine exposure and a complex interaction among Hsp70, NFκB and apoptotic signals.

HSP70 AND GUT PERMEABILITY

In addition to nutrient digestion and absorption, the gut functions as a barrier to prevent the translocation of intraluminal bacteria endotoxin into the portal circulation and systemic circulation. However, under some clinical conditions such as burn, trauma and critical illness (Ziegler et al. 1988, Langkamp-Henken et al. 1995), the gut barrier integrity is compromised and may result in the translocation of bacterial endotoxin. The mechanisms underlying gut barrier dysfunction in these conditions are not completely understood, but are at least temporally related to splanchnic hypoperfusion, lack of enteral feeding and associated gut ischemia (Maynard et al. 1993). Although, increases in gut permeability, followed by endotoxin translocation to the systemic circulation are frequently observed in animal experimental models,

these are not consistently reported in clinical studies in humans (Moore et al. 1991). Newer evidence suggest that shock, trauma, or burn-induced gut injury can cause local gut and gut-associated lymphatic tissues to produce cytokines and other inflammatory mediators, which may lead to lung, kidney, and liver dysfunction associated with sepsis and SIRS (Deitch 2002). The authors also provide an alternative route for the inflammatory products whereby bacterial translocation across the gut cause local activation of the gut inflammatory response reaching the systemic circulation not via the portal circulation but via mesenteric lymph. Thus, it seems that the loss of intestinal permeability drives the systemic inflammatory response and the multi organ dysfunction which occur in injured and critically ill patients.

The ability to maintain gut barrier function during critical illness as well as during splanchnic hypofusion states, such as elevated temperature with extreme skeletal muscle work, may be important in preventing systemic inflammation (Moseley 1994, Dokladny et al. 2006). In this regard, the induction of the HSR clearly attenuates the loss of epithelial barrier and function, both through the cellular accumulation of Hsp70 and through the HSF-1 up-regulation of transmembrane tight junction proteins, occludin-1 (Dokladny et al. 2006, 2008). Studies using epithelial monolayers cells demonstrate that Hsp70 accumulation driven by a non-lethal heat challenge or through genetic modification prevent the loss of epithelial barrier integrity to a lethal heat shock (Moseley et al. 1994, Dokladny et al. 2006). Additionally, Eaves-Pyles et al. (2000) have demonstrated that mice treated with sodium arsenite to induce cellular Hsp70 accumulation show increased survival and decreased bacterial translocation in a mouse burn injury model. Although these previous studies link Hsp70 to improved epithelial barrier tolerance, the recent report that heat stress induces the expression of occludin-1 by HSF-1 (Dokladny et al. 2008) offers another mechanism by which the HSR preserves epithelial barrier integrity during heat stress. This increased epithelial barrier resistance may be a key factor in limiting gut associated endotoxin translocation during gut ischemia, heat stress, and low perfusion states such as the SIRS.

HSP ARE PRO-INFLAMMATORY

Intracellular HSP confer an anti-inflammatory state because they down regulate inflammatory cytokine production by immune effector cells, increase cell and tissue tolerance of cytokine mediated cytotoxicity, and attenuate epithelial barrier permeability changes. Paradoxically HSP, particularly Hsp70 and gp96, also orchestrate a vigorous and multifaceted inflammatory response when seen by immune effector cells in the local extracellular environment. The HSP are among the key molecules referred by Matzinger (2002) as “danger” signals for the immune system since they serve as co-stimulatory molecules for immune recognition. The release of Hsp70 into the extracellular environment is an area of current research interest, as there is evidence for active secretion as well as for their passive release from injured, necrotic, or virally infected cells. In this regard, a variety of cells, such as monocytes (Lancaster

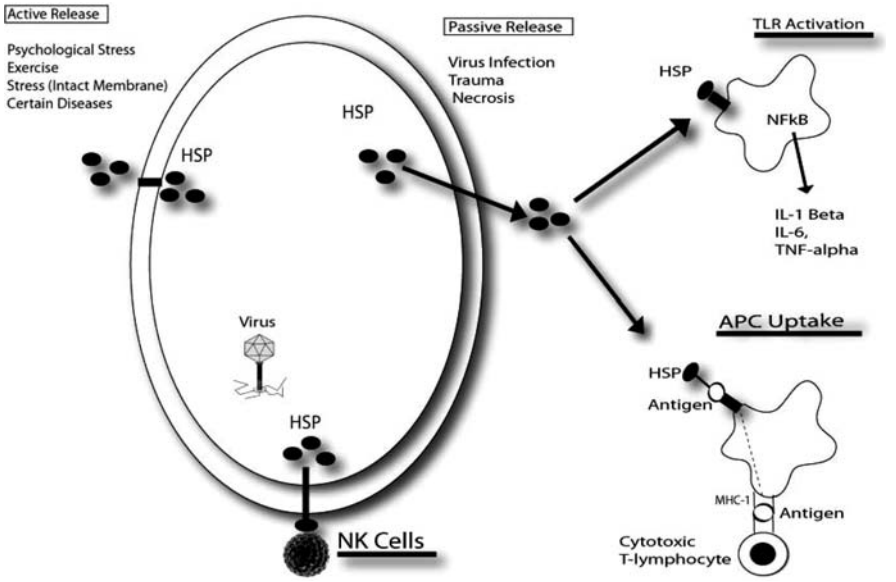

Figure 2. Mechanisms of HSP release into the extracellular environment

and Febbraio 2005), tumor (Broquet et al. 2003), glial (Guzhova et al. 2001), and B and T (Hunter-Lavin et al. 2004) cells actively release Hsp70 into the extracellular space. Additionally, extracellular Hsp70 (eHsp70) can interact with different peptides and microbial molecules to facilitate their detection to the immune system (Srivastava et al. 1994). Curiously, HSP have also been detected in the peripheral circulation of normal individuals with no signs of inflammatory or disease processes (Pockley et al. 1998). Thus the extracellular Hsp70 seems to be associated with both pathological and non-pathological conditions related to alerting the immune system to an altered cellular function. The model of pro-inflammatory HSP is described in Figure 2.

THE MECHANISM OF HSP RELEASE

Necrotic cell death, as consequence of mechanical injury or bacterial and viral infection, is the most evident mechanism to a cell release HSP. Experiments performed by Basu et al. (2000) demonstrated that necrosis induced by freeze and thaw result in the release of intracellular content, including Hsp70, into the extracellular environment. Interestingly, the authors also observed that during apoptotic stimulus induced by irradiation, the cell content seems to be packed into apoptotic bodies and the presence of eHsp70 is not detected even 24 h after death. Moreover, exposure of dendritic cells to necrotic but not apoptotic cell content elicited expression of maturation markers of

dendritic cells. Similar results were observed by Melcher et al. (1998) who observed that necrosis of tumor cells was associated with high immunogenicity and the presence of eHsp70. In contrast, induction of apoptosis showed low levels of eHsp70 and less immunogenicity. In humans, the presence of Hsp70 in the serum has been reported in patients after severe trauma or major surgical interventions such as coronary artery bypass (Pittet et al. 2002, Dybdahl et al. 2004). Dybdahl et al. (2005) also demonstrated that patients with acute myocardial infarction showed increased levels of serum Hsp70, and these values correlated significantly with important markers of cell necrosis (creatine kinase and cardiac troponin T). The presence of Hsp70 in the circulation would act as a danger signal or message alerting the antigen presenting cells to cellular damage of the surround or distant tissues and also activating the immune cells to a possible bacterial invasion.

Recent studies have also demonstrated active secretion of Hsp70 into the extracellular milieu by pathways other than cell damage. Under conditions of non-detectable cell death, eHsp70 has been shown to be released in glial cells (Guzhova et al. 2001), B cells (Clayton et al. 2005), and human peripheral blood mononuclear cells (Hunter-Lavin et al. 2004, Lancaster and Febbraio 2005). Although Hsp70 do not have a peptide leader sequence targeting secretion, it is suggested that the release occur via a non-classical pathway. In this regard, Bausero et al. (2005) demonstrated a possible mechanism by which HSP could be actively released from viable cells. The authors observed that Hsp70 could be released through exosomes when stimulated with certain inflammatory cytokines (IFN-y, IL-10). Additionally, Lancaster and Febbraio (2005) confirmed that exosome are the major pathway for secretory vesicular release of Hsp70. However, when the lipid rafts structure was disrupted through heat stress, the PBMCs were able to continue secreting Hsp70, suggesting additional pathways. Parallel with the active mechanism of release of Hsp70 in the extracellular milieu, secretory vesicles may fuse with the plasma membrane resulting in the expression of the Hsp70 in the surface of certain cells. In fact, different human tumors cells express Hsp70 in their plasma membrane (Multhoff et al. 1995, Melendez et al. 2006).

In whole organisms, serum Hsp70 has been detected in peripheral circulation of apparently healthy individuals (Pockley et al. 1998) and increased in response to different stressors, including certain diseases (Wright et al. 2000, Pockley et al. 2003), exercise (Walsh et al. 2001) and even psychological stress (Campisi et al. 2003). The source and mechanism of appearance of Hsp70 in the serum is not clear. It is possible that during disease states a combination of necrotic and active release by stressed tissue are responsible for Hsp70 in the serum. However, in conditions where cell necrosis or injury is not detectable, such as psychological stress and certain exercises, serum Hsp70 is possibly dependent on active release.

HOW DOES HSP70 INDUCE INFLAMMATION?

HSP are primarily considered a primitive intracellular molecule. The presence of HSP in the extracellular milieu of multicellular organism with immune surveillance

has been seen as an inducer of a highly immunogenic response. However, the immune effects of HSP released by necrotic are possibly different from the immune effects of HSP released during psychological stress. Additionally, different cells actively release HSP which can have different significance/interaction with immune effector cells. Here we will briefly discuss a direct LPS-like inflammatory response of extracellular HSP through Toll-like receptor (TLR) activation, as well as a role for HSP in antigen uptake of HSP-peptide complexes by antigen presenting cells and facilitated antigen presentation to cytotoxic T cells.

Cytokine Response Through TLR Activation

eHsp70 can bind to different cell surface receptors such as TLRs 2 and 4, resulting in signaling events and activation of antigen presenting cells. This activation results in a signaling cascade including stimulation of the inflammatory myeloid differentiation primary response, downstream IL-1 receptor associated kinase 4 (IRAK) and the NFκB signal transduction pathway. Asea (2007), for example, demonstrated that recombinant Hsp70 could signal through TLR-2 and TLR-4 with the involvement of CD14 of human monocytes stimulating rapid intracellular calcium efflux, activating NFκB and up-regulating pro-inflammatory cytokine secretion (IL-1β, IL-6 and TNF-α).

Enhanced Antigen Uptake and Presentation of HSP-Peptide Complexes

Extracellular HSP, particularly Hsp70, Hsp90, and gp96, serve as antigen carriers and facilitate antigen uptake by dendritic or antigen presenting cells (APC; Srivastava et al. 1994). Uptake appears to be through several mechanisms, including the alpha2-macroglobulin receptor (Binder et al. 2000). The HSP-peptide complex is more efficiently taken up by APCs than antigen alone. In addition to facilitated uptake, HSP appears to drive APC maturation and activate NFκB signal pathways (Basu et al. 2000). Finally, HSP facilitates antigen processing and transfer to a major histocompability complex I class molecule for presentation to cytotoxic T-lymphocytes.

NK Cell Activation

Hsp70 is expressed on the surface of tumors cells in culture (Multhoff et al. 1997) as well as in cells infected with several viruses, including HIV. The Hsp70 expression on tumour cells correlates with direct natural killer (NK) cell induced cytotoxicity and can be blocked by incubating target cells with antibodies directed against Hsp70 prior to NK cell exposure (Roigas et al. 1998).

UNDERSTANDING THE PARADOX OF THE PRO- AND ANTI-INFLAMMATORY ACTIONS OF HSP: THE HSP-VIRUS CONNECTION

Given the potency of intracellular HSP in blunting cytokine production and in increasing cell resistance to these same cytokines, it seems paradoxical, on first glance, that eHSP would have similar potency in activating the same cytokine pathways. It would seem even more paradoxical that these eHSP drive both NK cell killing and facilitate antigen uptake. We propose that this paradox represents an evolutionary adaptation of multicelled organisms to the threat of viral infection. It is beyond the scope of this chapter to detail the viral HSP connection, but this subject has been well reviewed by Sullivan and Pipas (2001). Most viral infections, whether bacteriophages, plant viruses, or animal viruses, are associated with an HSR. While this HSR was seen as a cellular response to stress or the appearance of new proteins within the cell, data from adenovirus studies reveal that, in fact, the HSP are essential for viral replication and so represent a viral rather than host cell adaptation (Glotzer et al. 2000). The HSP are employed by viruses as anti-apoptotic factors, as a means to move viral proteins to the cell nucleus, and in virion assembly. One consequence of the virus's requirement for HSP is that both free HSP as well as HSP-viral peptide complexes are released during virion release and/or virally-induced cell death. Since the HSP requirement and release by viruses occurs in both single and multicellular organisms, the immune systems of multicelled organisms evolved in a setting of HSP release. We propose that immune system evolution incorporated HSP and HSP-viral peptide release as a signal for activation. This sort of immune signaling is not unique. The immune/inflammatory activation of other primitive signals, such as CpG nucleotides, can also be considered under this paradigm.

DISEASE AND PATHOLOGIC STATES

Sepsis

Sepsis is a spectrum of diseases that encompasses infection, the SIRS, the development of organ dysfunction (severe sepsis), cardiovascular collapse (septic shock), and death (Hotchkiss and Karl 2003). Experimental and clinical data indicate that pro-inflammatory cytokines play a major role in sepsis-inducing systemic inflammatory response (Christman et al. 1995). To date previous studies have demonstrated that induction of the HSR protects animals in an experimentally induced sepsis model possibly by a reduction of the pro-inflammatory cytokines. As reviewed above, the transcription factor NFκB is an important regulator of inflammatory cytokines. Patients with sepsis show elevated levels of NFκB and it correlates with mortality rates (Bohrer et al. 1997). Interestingly, Schroeder et al. (1999) showed that peripheral blood mononuclear cells of patients with severe sepsis stimulated with LPS showed significantly less Hsp70 than healthy donors control subjects. Although these are two different studies, they indicate that the low Hsp70 observed

might be associated with a greater activation of the NFκB transcription factors, and consequently pro-inflammatory cytokines.

Heat Stroke-Associated Inflammation

Heat stroke is a life threatening condition where hyperthermia and its associated alterations in perfusion to critical tissues induce cellular dysfunction of critical tissues and the SIRS (Moseley 1997, Bouchama and Knochel 2002). While the mechanism by which heat activates this systemic inflammatory response is not completely clear, evidence is accumulating that hyperthermic stress drives inflammation through local tissue injury from ischemia/reperfusion as well as increased intestinal permeability, which facilitates endotoxin translocation across the intestinal lumen into the portal and splanchnic circulation. For example, it has been shown that levels of endotoxin in heat stroke patients are more than 1000-fold higher than normal healthy subjects (Bouchama et al. 1991). Additionally, patients suffering from heat stroke frequently present with pro-inflammatory mediators, such as TNF-α, IL-1β, IL-6 and nitric oxide synthase, in the systemic circulation (Bouchama et al. 1991). The serum concentrations of IL-6, IL-1β, and INF-γ are inversely correlated with the severity of illness (Bouchama et al. 1993).

It has been demonstrated that elevations of core temperature result in early peripheral vasodilation with an associated fall in blood flow in the splanchnic circulation (Kregel et al. 1988). In this study, splanchnic dilation preceded terminal hypotension and death, implicating early splanchnic ischemia in the pathogenesis of heat stroke. Later studies using organ specific Hsp70 accumulation as a biomarker of stress demonstrate that the intestine and liver are the tissues most affected by hyperthermic stress (Flanagan et al. 1995, Beck et al. 1995). In vitro epithelial models also support early epithelial permeability as a key component of hyperthermic stress. These early changes occur at relatively mild temperatures (>38.3ºC) and are completely reversible (Moseley et al. 1994, Dokladny et al. 2006). Altogether, these data suggest that hyperthermia impairs the ability of cells to maintain critical cell-to-cell interaction probably affecting overall gut function and allowing LPS to leak into the portal circulation and eventually in the systemic circulation.

In addition to the inflammatory cascade unleashed by the leak of endotoxin as well as direct thermal and ischemic challenges to the gut, Hsp70 in the extracellular environment may also be involved in propagating the inflammatory response observed during heat stroke. Cells, especially from the comprising critical target tissues, undergoing the multiple stresses of hyperthermia activate the HSR, up-regulating the intracellular content of HSP (Flanagan et al. 1995). In cases of cell necrosis, HSP are released into the extracellular milieu, enhancing the immune/inflammatory response. Thus, there is a balance between intracellular HSP in inflammatory cells blunting inflammation and the release of HSP from injured cells activating inflammation. A model of extracellular Hsp70 related inflammation has been proposed for the maternal pre-eclampsia and the syndrome of hemolysis, elevated liver enzymes, and low

platelet count (HELLP, Molvarec et al. 2007, Madach et al. 2008). It is reported that patients with HELLP present an excessive maternal inflammatory response to pregnancy with cytokine-mediated endothelial damage in the liver, platelet activation and hepatocellular necrosis (Baxter and Weinstein 2004). In these diseases, extracellular Hsp70 might function like endotoxin, binding to specific receptors (CD14, CD91 and Toll-like receptors) on APC's, resulting in NFκB activation and the production of pro-inflammatory cytokines (TNF-, IL-1β and IL-6). Similarly in heat stroke patients, liver damage frequently is reported in heat stroke patients (Giercksky et al. 1999, Kew et al. 1970). Thus, extracellular Hsp70 might be important to drive to the systemic inflammatory response observed in heat stroke patients.

Aging Susceptibility to Heat Injury/SIRS

The aging process involves a progressive decline in physiological function, also accompanied by a reduction in stress tolerance. Several studies demonstrate that animals with advancing age have lower tolerance to a variety of physiological challenges, including heat stress (Hall et al. 2000, Zhang et al. 2003). In humans, an example of age-associated loss of stress tolerance is a higher occurrence of morbidity and mortality during periods of heat waves in older than younger individuals (Levine 1969, Semenza et al. 1996). Although the mechanism underlying the lack of thermotolerance in aging animals is not clear, previous studies have indicated that the HSR is impaired in older organisms (Liu et al. 1989, Fargnoli et al. 1990, Blake et al. 1991). The initial work done by Kregel et al. (1995) reported that the increases in liver and myocardial Hsp70 accumulation in response to non-exertional heat stress were attenuated in old rats. In a subsequent study, it was reported that the lower expression of Hsp70 was followed by extensive liver injury and higher mortality in older animals after two consecutives heat exposures (Hall et al. 2000). Interestingly, Hsp70 expression after the first heat stress was moderately reduced compared to the younger rats but by 48 h Hsp70 expression was dramatically lower. One interpretation of these results is that this lower Hsp70 response in the aged organism allowed tissue necrosis and release of Hsp70 in the extracellular milieu. The eHsp70, in turn, supported the inflammatory state which is similar to that reported in an aged organism (Dobbs et al. 1999). Thus, the HSR, through Hsp70 leak may be a driver of the greater susceptibility of systemic inflammatory response in aging.

Although this lower cellular Hsp70 accumulation seen in the severe heat stress model could be interpreted as an age- associated impairment of Hsp70 accumulation, curiously the aged organism is actually able to generate a similar Hsp70 response compared to the young organism when exercise is used as a stimulus (Kregel and Moseley 1996). Additionally, Nitta et al. (1994) observed reduced Hsp70-mRNA after 10 min of ischemia in aged rat hearts but, with longer period of ischemia, Hsp70 level was similar to young hearts. Thus, the data suggest that the aging process does not represent a loss of HSR but alterations in the quality or intensity needed for Hsp70 accumulation.

Inflammatory Bowel Diseases (IBD)

The inflammatory bowel diseases (IBD), which include the Crohn's disease and ulcerative colitis, are chronic relapsing idiopathic inflammatory disorders of the gastrointestinal tract. It is suggested that defects in both the barrier function of the intestinal epithelium and the local mucosal immune system promotes an inappropriate and ongoing inflammatory response to the presence of normal gut luminal flora (Podolsky 2002). Activation of immune cells by luminal antigens promotes the production of inflammatory mediators, including cytokines, chemokines and growth factors, resulting in tissue damage. Accumulated evidence suggests that TNF-α secreted by intestinal monocytes cells may play a role in the development of inflammatory bowel disease (Van Deventer 1997). It is reported that high levels of TNF-α are secreted by cells isolated from mucosal biopsy specimens of patients with IBD (Breese et al. 1994). In a cellular model of epithelial barrier, TNF-α caused an increase in intestinal tight junction permeability, shown by an increase in epithelial permeability (Ma et al. 2004). Furthermore, clinical and animal studies have shown that application of anti-TNF antibody decreased disease activity and caused transient remission confirming that TNF-α is an important mediator of IBD. The mechanism of the TNF-α induce an increase in intestinal epithelial tight junction permeability seems to be regulated in part by NFκB (Ma et al. 2004, Ye et al. 2006). Curiously, biopsies of patients suffering from Crohn's disease and Ulcerative Colitis show enhanced expression of Hsp70 (Ludwig et al. 1999). An interesting question is if the up regulation observed in patients with IBD is just a marker of cellular injury or if the cells are becoming more resistant to the inflammatory stress.

Additionally it has been hypothesized that Hsp70 may participate in the process of inflammatory bowel diseases when it is present in the surface of epithelial gut cells or exported to the local gut environment. As mentioned above, it is reported that biopsies of patients with inflammatory bowel disease show an enhanced epithelial expression of HSP (Ludwig et al. 1999). This enhanced epithelial Hsp70 expression may be involved in the surface presentation of altered self-protein to the immune system, activating immune cells or through molecular mimicry or homology to bacterial Hsp70 (Petrof et al. 2004). A more recent alternative hypothesis involving eHSP and bowel disease has been proposed by Fleshner et al. (2005). The authors suggest that eHSP released after exposure to stress binds to TLR4 expressed in epithelial cells, triggering endogenous bacterial translocation, increased phagocytosis, and an up-regulation of co-stimulatory molecules, resulting in an exaggerated inflammatory response. In this regard, Whittall et al. (2006) observed that both mucosal cells and circulating monocyte-derived dendritic cells from Crohns diseases patient showed an increased TNF-α production compared to control samples.

THE IMMUNO-PHYSIOLOGY OF ADAPTATION

As described above, the HSR modulate the immune/inflammatory response in both cultured cells and in animals. Numerous human and non-human studies demonstrate

that the physiologic stresses of exercise and whole body hyperthermia, as well as supplementation with agents such as GLN result in cellular Hsp70 accumulation. Early studies have shown that Hsp70 levels correlate with the environmental temperature of the ecological niche of a given species (Ulmasov et al. 1992). However, there remains considerable uncertainty whether the magnitude of the HSR in humans under these stresses is sufficient to alter tolerance to high core temperatures and inflammatory response.

Heat Acclimation and Adaptation

Heat adaptation can be divided into thermotolerance and acclimatization (Moseley 1997). The thermotolerant state is defined as the organism's ability to survive an otherwise lethal stress, including heat or endotoxin, from a prior heat exposure causing cellular accumulation of Hsp70. Some characteristics of the thermotolerant state are the ability of whole organism to tolerate a higher core temperature and endotoxin shock. The mechanism by which thermotolerant animals can tolerate higher core temperature may be due in part to the changes seen in cell culture studies, but there are also other important differences. Animal survival in thermotolerance depends more upon maintenance of tissue and organ function than on individual cell survival. When animals are subjected to severe heat stress, there is a marked fall in splanchnic perfusion (Kregel et al. 1988), which precedes terminal hypotension. This type of severe stress also results in the animals activation of a robust stress response in the gut and liver (Flanagan et al. 1995), suggesting an important role of gut and liver injury from thermal stress. The maintenance of intestinal epithelial tight junction barriers, for example, may block the passage of gut-associated endotoxin translocation. Thermotolerant animals may also maintain the liver Kupfer cells function during period of heat stress degrading endotoxin present in the portal circulation. Finally, thermotolerant animals may have a lower inflammatory response due to lower cytokine release from immune blood cells (Snyder et al. 1992, Ensor et al. 1994). Thus, it seems that cellular accumulation of Hsp70 is responsible for this thermotolerant state.

Heat acclimatized state is defined as an organism's ability to perform increased work in the heat because of improvements in heat dissipation brought on by repeated mild elevations in core temperature (Moseley 1997). Additionally, it is suspected that humans working in the heat, for example athletes, also demonstrate the ability to achieve higher core temperatures, in combination with the ability to sustain work at these higher core temperatures (Pugh 1967). This ability may not be the result of physiological changes observed in heat dissipation with heat acclimation but appears to result from cellular adaptations that allow continued function at higher temperatures, resembling the thermotolerant state. Studies using a heat acclimation protocol in humans induced the synthesis of Hsp70 in peripheral blood mononuclear cells (PBMC), suggesting a possible link between these forms of thermal adaptation (Yamada et al. 2007, McClung et al. 2008). More recently, we observed that heat

acclimation, which also resulted in cellular Hsp70 accumulation, had no effect on reducing PBMC injury after heat shock and did not alter anti- and pro-inflammatory cytokine release after LPS exposure (unpublished data). In contrast, *ex vivo* heat shock of 42.5ºC induced a much greater increase in PBMC Hsp72 compared to an acute bout of exercise in the heat on days 1 and 10 of heat acclimation. Thus, heat acclimation can induce changes in Hsp70 but the magnitude of induction may not able to induce a state of thermotolerance or modify the cytokine response to endotoxin.

CONCLUSION

The heat shock response has clear and potent effects on inflammation. While it is tempting to construct simple linear models of cause and effect, the reality is one of complex interactions, paradoxes, and balances. Using the highly stress-inducible Hsp70 as an example, we have seen that it can modulate inflammation by interrupting NFκB signaling as well as creating cytokine resistance in target cells. Hsp70 also directly enhances the functional resistance of multicellular tissues, such as epithelial barriers, to different stresses. This enhanced epithelial barrier resistance, in turn, may decrease endotoxin translocation into the circulation and so may indirectly alter cytokine production by decreasing the stimulus for cytokine production. Further, while the role of HSF-1 has been seen as limited to HSP transcription, more recent data suggests that it, too, directly modulates cytokine production. The recent observations that HSF-1 directly alters transcription of the tight junction protein, occludin 1, also suggest a much broader role for the HSR transcription machinery.

At the same time, paradoxes abound. Hsp70, which has been demonstrated to inhibit endotoxin induced IL-6, and has been correllated to GLN associated decreases in IL-6 in critically ill patients and animal studies is temporally linked to exercise induced IL-6 production in exercise. In addition, GLN exposure in exercise enhances muscle IL-6 production, suggesting a role for the HSR in this increase. Secondly, we have briefly reviewed the potent pro-inflammatory effects of the HSP, again seen through the Hsp70 model. This response is quite broad based. In that it involves inflammatory cytokine production, NK cell targeting, and marked enhancement of antigen presentation resulting in a specific cytotoxic T cell response. We have speculated that this pro-inflammatory response is an evolutionary adaptation to the role of HSP in the replication of viruses. This requirement extends across viruses infecting eukaryotic and prokaryotic life forms, demonstrating its ubiquity and high degree of conservation. Finally, studies using acute exercise or acclimation protocols in humans, and which have demonstrated Hsp70 alterations have largely failed to alter cytokine levels from stimulated cells. This failure may reflect an insufficient level of Hsp70 induction compared to the robust inflammatory stimulation. Clearly, there are a number of disease states where the ability to activate Hsp70 in cells, limit eHsp70, or create a specifically targeted eHsp70 response offers the opportunity to address a wide variety of disease states.

REFERENCES

Asea A. Mechanisms of HSP72 release. J Biosci. 2007 Apr;32(3):579–84.

Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int Immunol. 2000 Nov;12(11):1539–46.

Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol. 2001 Sep;11(9):372–7.

Bausero MA, Gastpar R, Multhoff G, Asea A. Alternative mechanism by which IFN-gamma enhances tumor recognition: active release of heat shock protein 72. J Immunol. 2005 Sep 1;175(5):2900–12.

Baxter JK, Weinstein L. HELLP syndrome: the state of the art. Obstet Gynecol Surv. 2004;59:838–45.

Beck SC, Paidas CN, Tan H, Yang J, De Maio A. Depressed expression of the inducible form of HSP 70 (HSP 72) in brain and heart after in vivo heat shock. Am J Physiol. 1995;269:R608–R13.

Binder RJ, Harris ML, Ménoret A, Srivastava PK. Saturation, competition, and specificity in interaction of heat shock proteins (hsp) gp96, hsp90, and hsp70 with CD11b+ cells. J Immunol. 2000 Sep 1;165(5):2582–7.

Blake MJ, Fargnoli J, Gershon D, Holbrook NJ. Concomitant decline in heat-induced hyperthermia and HSP70 mRNA expression in aged rats. Am J Physiol. 1991 Apr;260(4 Pt 2):R663–R7.

Bouchama A, al-Sedairy S, Siddiqui S, Shail E, Rezeig M. Elevated pyrogenic cytokines in heatstroke. Chest. 1993 Nov;104(5):1498–502.

Bouchama A, Knochel JP. Heat stroke. N Engl J Med. 2002 Jun 20;346(25):1978–88.

Bouchama A, Parhar RS, el-Yazigi A, Sheth K, al-Sedairy S. Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. J Appl Physiol. 1991 Jun;70(6):2640–4.

Brasier AR. The NF-kappaB regulatory network. Cardiovasc Toxicol. 2006;6(2):111–30.

Breese EJ, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio P, Walker-Smith JA, MacDonald TT. Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology. 1994 Jun;106(6):1455–66.

Broquet AH, Thomas G, Masliah J, Trugnan G, Bachelet M. Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. J Biol Chem. 2003 Jun 13;278(24):21601–6.

Böhrer H, Qiu F, Zimmermann T, Zhang Y, Jllmer T, Männel D, Böttiger BW, Stern DM, Waldherr R, Saeger HD, Ziegler R, Bierhaus A, Martin E, Nawroth PP. Role of NFkappaB in the mortality of sepsis. J Clin Invest. 1997 Sep 1;100(5):972–85.

Cahill CM, Waterman WR, Xie Y, Auron PE, Calderwood SK. Transcriptional repression of the prointerleukin 1beta gene by heat shock factor 1. J Biol Chem. 1996 Oct 4;271(40):24874–9.

Campisi J, Leem TH, Fleshner M. Stress-induced extracellular HSP72 is a functionally significant danger signal to the immune system. Cell Stress Chaperones. 2003 Fall;8(3):272–86.

Chen Y, Currie RW. Small interfering RNA knocks down heat shock factor-1 (HSF-1) and exacerbates pro-inflammatory activation of NF-kappaB and AP-1 in vascular smooth muscle cells. Cardiovasc Res. 2006 Jan;69(1):66–75.

Christman JW, Holden EP, Blackwell TS. Strategies for blocking the systemic effects of cytokines in the sepsis syndrome. Crit Care Med. 1995 May;23(5):955–63.

Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z. Induction of heat shock proteins in B-cell exosomes. J Cell Sci. 2005 Aug 15;118(Pt 16):3631–8. Epub 2005 Jul 26.

Deitch EA. Multiple organ failure: pathophysiology and potential future therapies. Ann Surg. 1992;216:117–34.

Deitch EA. Bacterial translocation or lymphatic drainage of toxic products from the gut: what is important in human beings? Surgery. 2002 Mar;131(3):241–4.

De-Souza DA, Greene LJ. Intestinal permeability and systemic infections in critically ill patients: effect of glutamine. Crit Care Med. 2005 May;33(5):1125–35.

Ding XZ, Fernandez-Prada CM, Bhattacharjee AK, Hoover DL. Over-expression of HSP-70 inhibits bacterial lipopolysaccharide-induced production of cytokines in human monocyte-derived macrophages. Cytokine. 2001 Dec 21;16(6):210–9.

Dobbs RJ, Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson DW. Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. Acta Neurol Scand. 1999;100:34–41.

Dokladny K, Kozak A, Wachulec M, Wallen ES, Menache MG, Kozak W, Kluger MJ, Moseley PL. Effect of heat stress on LPS-induced febrile response in D-galactosamine-sensitized rats. Am J Physiol Regul Integr Comp Physiol. 2001 Feb;280(2):R338–R44.

Dokladny K, Wharton W, Lobb R, Ma TY, Moseley PL. Induction of physiological thermotolerance in MDCK monolayers: contribution of heat shock protein 70. Cell Stress Chaperones. 2006 Autumn;11(3):268–75.

Dokladny K, Ye D, Kennedy JC, Moseley PL, Ma TY. Cellular and molecular mechanisms of heat stress-induced up-regulation of occludin protein expression: regulatory role of heat shock factor-1. Am J Pathol. 2008 Mar;172(3):659–70. Epub 2008 Feb 14.

Dybdahl B, Slørdahl SA, Waage A, Kierulf P, Espevik T, Sundan A. Myocardial ischaemia and the inflammatory response: release of heat shock protein 70 after myocardial infarction. Heart. 2005 Mar;91(3):299–304.

Dybdahl B, Wahba A, Haaverstad R, Kirkeby-Garstad I, Kierulf P, Espevik T, Sundan A. On-pump versus off-pump coronary artery bypass grafting: more heat-shock protein 70 is released after on-pump surgery. Eur J Cardiothorac Surg. 2004 Jun;25(6):985–92.

Eaves-Pyles T, Wong HR, Alexander JW. Sodium arsenite induces the stress response in the gut and decreases bacterial translocation in a burned mouse model with gut-derived sepsis. Shock. 2000;13(4):314–9.

Ehrenfried JA, Chen J, Li J, Evers BM. Glutamine-mediated regulation of heat shock protein expression in intestinal cells. Surgery. 1995 Aug;118(2):352–6.

Eliasen MM, Winkler W, Jordan V, Pokar M, Marchetti M, Roth E, Allmaier G, Oehler R. Adaptive cellular mechanisms in response to glutamine-starvation. Front Biosci. 2006 Sep;1(11):3199–211.

Ensor JE, Wiener SM, McCrea KA, Viscardi RM, Crawford EK, Hasday JD. Differential effects of hyperthermia on macrophage interleukin-6 and tumor necrosis factor-alpha expression. Am J Physiol. 1994 Apr;266(4 Pt 1):C967–C74.

Exner R, Tamandl D, Goetzinger P, Mittlboeck M, Fuegger R, Sautner T, Spittler A, Roth E. Perioperative GLY–GLN infusion diminishes the surgery-induced period of immunosuppression: accelerated restoration of the lipopolysaccharide-stimulated tumor necrosis factor-alpha response. Ann Surg. 2003 Jan;237(1):110–15.

Exner R, Wessner B, Manhart N, Roth E. Therapeutic potential of glutathione. Wien Klin Wochenschr. 2000 Jul 28;112(14):610–16.

Fargnoli J, Kunisada T, Fornace AJ Jr, Schneider EL, Holbrook NJ. Decreased expression of heat shock protein 70 mRNA and protein after heat treatment in cells of aged rats. Proc Natl Acad Sci U S A. 1990 Jan;87(2):846–50.

Flanagan SW, Ryan AJ, Gisolfi CV, Moseley PL. Tissue-specific HSP70 response in animals undergoing heat stress. Am J Physiol. 1995;268:R28–R32.

Fleshner M, Johnson JD. Endogenous extra-cellular heat shock protein 72: releasing signal(s) and function. Int J Hyperthermia. 2005 Aug;21(5):457–71.

Franceschelli S, Rosati A, Lerose R, De Nicola S, Turco MC, Pascale M. Bag3 gene expression is regulated by heat shock factor 1. J Cell Physiol. 2008 Jun;215(3):575–7.

Gianotti L, Alexander JW, Gennari R, Pyles T, Babcock GF. Oral glutamine decreases bacterial translocation and improves survival in experimental gut-origin sepsis. JPEN J Parenter Enteral Nutr. 1995 Jan–Feb;19(1):69–74.

Giercksky T, Boberg KM, Farstad IN, Halvorsen S, Schrumpf E. Severe liver failure in exertional heat stroke. Scand J Gastroenterol. 1999;34:824–7.

Glotzer JB, Saltik M, Chiocca S, Michou AI, Moseley P, Cotten M. Activation of heat-shock response by an adenovirus is essential for virus replication. Nature. 2000 Sep 14;407(6801):207–11.

Greig JE, Keast D, Garcia-Webb P, Crawford P. Inter-relationships between glutamine and other biochemical and immunological changes after major vascular surgery. Br J Biomed Sci. 1996 Jun;53(2):116–21.

Griffiths RD, Allen KD, Andrews FJ, Jones C. Infection, multiple organ failure, and survival in the intensive care unit: influence of glutamine-supplemented parenteral nutrition on acquired infection. Nutrition. 2002 Jul–Aug;18(7–8):546–52.

Griffiths RD, Jones C, Palmer TE. Six-month outcome of critically ill patients given glutamine-supplemented parenteral nutrition. Nutrition. 1997 Apr;13(4):295–302.

Gromkowski SH, Yagi J, Janeway CA Jr. Elevated temperature regulates tumor necrosis factor-mediated immune killing. Eur J Immunol. 1989 Sep;19(9):1709–14.

Guo S, Wharton W, Moseley P, Shi H. Heat shock protein 70 regulates cellular redox status by modulating glutathione-related enzyme activities. Cell Stress Chaperones. 2007 Autumn;12(3):245–54.

Guzhova I, Kislyakova K, Moskaliova O, Fridlanskaya I, Tytell M, Cheetham M, Margulis B. In vitro studies show that HSP70 can be released by glia and that exogenous HSP70 can enhance neuronal stress tolerance. Brain Res. 2001 Sep 28;914(1–2):66–73.

Hall DM, Xu L, Drake VJ, Oberley LW, Oberley TD, Moseley PL, Kregel KC. Aging reduces adaptive capacity and stress protein expression in the liver after heat stress. J Appl Physiol. 2000 Aug;89(2): 749–59.

Helqvist S, Polla BS, Johannesen J, Nerup J. Heat shock protein induction in rat pancreatic islets by recombinant human interleukin 1 beta. Diabetologia. 1991 Mar;34(3):150–6.

Hiscock N, Petersen EW, Krzywkowski K, Boza J, Halkjaer-Kristensen J, Pedersen BK. Glutamine supplementation further enhances exercise-induced plasma IL-6. J Appl Physiol. 2003 Jul;95(1):145–8. Epub 2003 Feb 28.

Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med. 2003 Jan 9;348(2):138–50.

Hunter-Lavin C, Davies EL, Bacelar MM, Marshall MJ, Andrew SM, Williams JH. HSP70 release from peripheral blood mononuclear cells. Biochem Biophys Res Commun. 2004 Nov 12;324(2):511–7.

Jäättelä M. Overexpression of major heat shock protein HSP70 inhibits tumor necrosis factor-induced activation of phospholipase A2. J Immunol. 1993 Oct 15;151(8):4286–94.

Jäättelä M, Wissing D. Heat-shock proteins protect cells from monocyte cytotoxicity: possible mechanism of self-protection. J Exp Med. 1993 Jan 1;177(1):231–6.

Kew M, Bersohn I, Seftel H, Kent G. Liver damage in heatstroke. Am J Med. 1970;49:192–202.

Kluger MJ, Rudolph K, Soszynski D, Conn CA, Leon LR, Kozak W, Wallen ES, Moseley PL. Effect of heat stress on LPS-induced fever and tumor necrosis factor. Am J Physiol. 1997 Sep;273(3 Pt 2): R858–R63.

Kregel KC, Moseley PL. Differential effects of exercise and heat stress on liver HSP70 accumulation with aging. J Appl Physiol. 1996 Feb;80(2):547–51.

Kregel KC, Moseley PL, Skidmore R, Gutierrez JA, Guerriero V Jr.. HSP70 accumulation in tissues of heat-stressed rats is blunted with advancing age. J Appl Physiol. 1995 Nov;79(5):1673–8.

Kregel KC, Wall PT, Gisolfi CV. Peripheral vascular responses to hyperthermia in the rat. J Appl Physiol. 1988 Jun;64(6):2582–8.

Kusher DI, Ware CF, Gooding LR. Induction of the heat shock response protects cells from lysis by tumor necrosis factor. J Immunol. 1990 Nov 1;145(9):2925–31.

Lancaster GI, Febbraio MA. Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. J Biol Chem. 2005 Jun 17;280(24):23349–55. Epub 2005 Apr 12.

Langkamp-Henken B, Donovan TB, Pate LM, Maull CD, Kudsk KA. Increased intestinal permeability following blunt and penetrating trauma. Crit Care Med. 1995 Apr;23(4):660–4.

Lau SS, Griffin TM, Mestril R. Protection against endotoxemia by HSP70 in rodent cardiomyocytes. Am J Physiol Heart Circ Physiol. 2000 May;278(5):H1439–H45.

Levine JA. Heat stroke in the aged. Am J Med. 1969 Aug;47(2):251–8.

Liu A, Lin Z, Choi H, Sorhage F, Li B. Attenuated induction of heat shock gene expression in aging diploid fibroblasts. J Biol Chem. 1989;264:12037–45.

Ludwig D, Stahl M, Ibrahim ET, Wenzel BE, Drabicki D, Wecke A, Fellermann K, Stange EF. Enhanced intestinal expression of heat shock protein 70 in patients with inflammatory bowel diseases. Dig Dis Sci. 1999 Jul;44(7):1440–7.

Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA, Said HM. TNFalpha-induced increase in intestinal epithelial tight junction permeability requires NFkappa B activation. Am J Physiol Gastrointest Liver Physiol. 2004;286:G367–G76.

Madách K, Molvarec A, Rigó J Jr, Nagy B, Pénzes I, Karádi I, Prohászka Z. Elevated serum 70 kDa heat shock protein level reflects tissue damage and disease severity in the syndrome of hemolysis, elevated liver enzymes, and low platelet count. Eur J Obstet Gynecol Reprod Biol. 2008 Feb 2;139(2):133–8.

Malhotra V, Wong HR. Interactions between the heat shock response and the nuclear factor-kappa B signaling pathway. Crit Care Med. 2002 Jan;30(1 Suppl):S89–S95.

Matzinger P. The danger model: a renewed sense of self. Science. 2002 Apr 12;296(5566):301–5.

Maynard N, Bihari D, Beale R, Smithies M, Baldock G, Mason R, McColl I. Assessment of splanchnic oxygenation by gastric tonometry in patients with acute circulatory failure. JAMA. 1993 Sep 8;270(10):1203–10.

McClung JP, Hasday JD, He JR, Montain SJ, Cheuvront SN, Sawka MN, Singh IS. Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. Am J Physiol Regul Integr Comp Physiol. 2008 Jan;294(1):R185–91.

Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nat Med. 1998 May;4(5):581–7.

Melendez K, Wallen ES, Edwards BS, Mobarak CD, Bear DG, Moseley PL. Heat shock protein 70 and glycoprotein 96 are differentially expressed on the surface of malignant and nonmalignant breast cells. Cell Stress Chaperones. 2006 Winter;11(4):334–42.

Molvarec A, Prohászka Z, Nagy B, Kalabay L, Szalay J, Füst G, Karádi I, Rigó J Jr.. Association of increased serum heat shock protein 70 and C-reactive protein concentrations and decreased serum alpha (2)-HS glycoprotein concentration with the syndrome of hemolysis, elevated liver enzymes, and low platelet count. J Reprod Immunol. 2007 Apr;73(2):172–9.

Moore FA, Moore EE, Poggetti R, McAnena OJ, Peterson VM, Abernathy CM, Parsons PE. Gut bacterial translocation via the portal vein: a clinical perspective with major torso trauma. J Trauma. 1991 May;31(5):629–36, discussion 636–8.

Morlion BJ, Stehle P, Wachtler P, Siedhoff HP, Köller M, König W, Fürst P, Puchstein C. Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: a randomized, double-blind, controlled study. Ann Surg. 1998 Feb;227(2):302–8.

Morrison AL, Dinges M, Singleton KD, Odoms K, Wong HR, Wischmeyer PE. Glutamine's protection against cellular injury is dependent on heat shock factor-1. Am J Physiol Cell Physiol. 2006 Jun;290(6):C1625–C32.

Moseley PL. Heat shock proteins and heat adaptation of the whole organism. J Appl Physiol. 1997 Nov;83(5):1413–7.

Moseley PL, Gapen C, Wallen ES, Walter ME, Peterson MW. Thermal stress induces epithelial permeability. Am J Physiol. 1994 Aug;267(2 Pt 1):C425–C34.

Multhoff G, Botzler C, Jennen L, Schmidt J, Ellwart J, Issels R. Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells. J Immunol. 1997 May 1;158(9):4341–50.

Multhoff G, Botzler C, Wiesnet M, Müller E, Meier T, Wilmanns W, Issels RD. A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. Int J Cancer. 1995 Apr 10;61(2):272–9.

Nakano M, Knowlton AA, Yokoyama T, Lesslauer W, Mann DL. Tumor necrosis factor-alpha-induced expression of heat shock protein 72 in adult feline cardiac myocytes. Am J Physiol. 1996 Apr;270(4 Pt 2):H1231–H9.

Nitta Y, Abe K, Aoki M, Ohno I, Isoyama S. Diminished heat shock protein 70 mRNA induction in aged rat hearts after ischemia. Am J Physiol. 1994 Nov;267(5 Pt 2):H1795–H803.

Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: a systematic review of the evidence. Crit Care Med. 2002 Sep;30(9):2022–9.

Oudemans-van Straaten HM, Bosman RJ, Treskes M, van der Spoel HJ, Zandstra DF. Plasma glutamine depletion and patient outcome in acute ICU admissions. Intensive Care Med. 2001 Jan;27(1):84–90.

Pedersen BK, Steensberg A, Fischer C, Keller C, Ostrowski K, Schjerling P. Exercise and cytokines with particular focus on muscle-derived IL-6. Exerc Immunol Rev. 2001;7:18–31.

Peng ZY, Hamiel CR, Banerjee A, Wischmeyer PE, Friese RS, Wischmeyer P. Glutamine attenuation of cell death and inducible nitric oxide synthase expression following inflammatory cytokine-induced injury is dependent on heat shock factor-1 expression. JPEN J Parenter Enteral Nutr. 2006 Sep–Oct;30(5):400–6.

Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, De Simone C, Chang EB. Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology. 2004 Nov;127(5):1474–87.

Pittet JF, Lee H, Morabito D, Howard MB, Welch WJ, Mackersie RC. Serum levels of HSP 72 measured early after trauma correlate with survival. J Trauma. 2002 Apr;52(4):611–7.

Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegård J. Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. Hypertension. 2003 Sep;42(3):235–8. Epub 2003 Aug 4.

Pockley AG, Shepherd J, Corton JM. Detection of heat shock protein 70 (HSP70) and anti-HSP70 antibodies in the serum of normal individuals. Immunol Invest. 1998 Dec;27(6):367–77.

Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002 Aug 8;347(6):417–29.

Pugh LG, Corbett JL, Johnson RH. Rectal temperatures, weight losses, and sweat rates in marathon running. J Appl Physiol. 1967 Sep;23(3):347–52.

Pritts TA, Wang Q, Sun X, Moon MR, Fischer DR, Fischer JE, Wong HR, Hasselgren PO. Induction of the stress response in vivo decreases nuclear factor-kappa B activity in jejunal mucosa of endotoxemic mice. Arch Surg. 2000 Jul;135(7):860–6.

Ran R, Lu A, Zhang L, Tang Y, Zhu H, Xu H, Feng Y, Han C, Zhou G, Rigby AC, Sharp FR. Hsp70 promotes TNF-mediated apoptosis by binding IKK gamma and impairing NF-kappa B survival signaling. Genes Dev. 2004 Jun 15;18(12):1466–81.

Roigas J, Wallen ES, Loening SA, Moseley PL. Heat shock protein (HSP72) surface expression enhances the lysis of a human renal cell carcinoma by IL-2 stimulated NK cells. Adv Exp Med Biol. 1998;451:225–9.

Roth E, Funovics J, Mühlbacher F, Schemper M, Mauritz W, Sporn P, Fritsch A. Metabolic disorders in severe abdominal sepsis: Glutamine deficiency in skeletal muscle. Clin Nutr. 1982 Mar;1(1): 25–41.

Ryan AJ, Flanagan SW, Moseley PL, Gisolfi CV. Acute heat stress protects rats against endotoxin shock. J Appl Physiol. 1992 Oct;73(4):1517–22.

Schett G, Steiner CW, Xu Q, Smolen JS, Steiner G. TNF-α mediates susceptibility to heat-induced apoptosis by protein phosphatase-mediated inhibition of the HSF1/HSP70 stress response. Cell Death Differ. 2003 Oct;10(10):1126–36.

Schroeder S, Lindemann C, Hoeft A, Putensen C, Decker D, von Ruecker AA, Stüber F. Impaired inducibility of heat shock protein 70 in peripheral blood lymphocytes of patients with severe sepsis. Crit Care Med. 1999 Jun;27(6):1080–4.

Semenza JC, Rubin CH, Falter KH, Selanikio JD, Flanders WD, Howe HL, Wilhelm JL. Heat-related deaths during the July 1995 heat wave in Chicago. N Engl J Med. 1996 Jul 11;335(2):84–90.

Sharma HS, Weisensee D, Löw-Friedrich I. Tumor necrosis factor-alpha-induced cytoprotective mechanisms in cardiomyocytes. Analysis by mRNA phenotyping. Ann N Y Acad Sci. 1996 Sep;30(793): 267–81.

Singh IS, He JR, Calderwood S, Hasday JD. A high affinity HSF-1 binding site in the 5′-untranslated region of the murine tumor necrosis factor-alpha gene is a transcriptional repressor. J Biol Chem. 2002 Feb 15;277(7):4981–8.

Singleton KD, Beckey VE, Wischmeyer PE. Glutamine prevents activation of NF-kappab and stress kinase pathways, attenuates inflammatory cytokine release, and prevents acute respiratory distress syndrome (ARDS) following sepsis. Shock. 2005a Dec;24(6):583–9.

Singleton KD, Serkova N, Beckey VE, Wischmeyer PE. Glutamine attenuates lung injury and improves survival after sepsis: role of enhanced heat shock protein expression. Crit Care Med. 2005b Jun;33(6):1206–13.

Singleton KD, Wischmeyer PE. Oral glutamine enhances heat shock protein expression and improves survival following hyperthermia. Shock. 2006 Mar;25(3):295–9.

Singleton KD, Wischmeyer PE. Glutamine's protection against sepsis and lung injury is dependent on heat shock protein 70 expression. Am J Physiol Regul Integr Comp Physiol. 2007 May;292(5):R1839–R45.

Snyder YM, Guthrie L, Evans GF, Zuckerman SH. Transcriptional inhibition of endotoxin-induced monokine synthesis following heat shock in murine peritoneal macrophages. J Leukoc Biol. 1992 Feb;51(2):181–7.

Spittler A, Winkler S, Götzinger P, Oehler R, Willheim M, Tempfer C, Weigel G, Függer R, Boltz-Nitulescu G, Roth E. Influence of glutamine on the phenotype and function of human monocytes. Blood. 1995 Aug 15;86(4):1564–9.

Srivastava PK, Udono H, Blachere NE, Li Z. Heat shock proteins transfer peptides during antigen processing and CTL priming. Immunogenetics. 1994;39(2):93–8.

Sullivan CS, Pipas JM. The virus-chaperone connection. Virology. 2001 Aug 15;287(1):1–8.

Sun D, Chen D, Du B, Pan J. Heat shock response inhibits NF-kappaB activation and cytokine production in murine Kupffer cells. J Surg Res. 2005 Nov;129(1):114–21.

Tang D, Shi Y, Jang L, Wang K, Xiao W, Xiao X. Heat shock response inhibits release of high mobility group box 1 protein induced by endotoxin in murine macrophages. Shock. 2005 May;23(5):434–40.

Ulmasov KA, Shammakov S, Karaev K, Evgen'ev MB. Heat shock proteins and thermoresistance in lizards. Proc Natl Acad Sci U S A. 1992 Mar 1;89(5):1666–70.

Van Deventer SJ. Tumour necrosis factor and Crohn's disease. Gut. 1997 Apr;40(4):443–8.

Van Hall G, Steensberg A, Fischer C, Keller C, Møller K, Moseley P, Pedersen BK. Interleukin-6 markedly decreases skeletal muscle protein turnover and increases non-muscle amino acid utilization in healthy individuals. J Clin Endocrinol Metab. 2008 Apr 22;93(7):2851–8.

Vesali RF, Klaude M, Rooyackers O, Wernerman J. Amino acid metabolism in leg muscle after an endotoxin injection in healthy volunteers. Am J Physiol Endocrinol Metab. 2005 Feb;288(2):E360–4. Epub 2004 Sep 14.

Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA. Exercise increases serum HSP72 in humans. Cell Stress Chaperones. 2001 Oct;6(4):386–93.

Wang Y, Li C, Wang X, Zhang J, Chang Z. Heat shock response inhibits IL-18 expression through the JNK pathway in murine peritoneal macrophages. Biochem Biophys Res Commun. 2002 Aug 23;296(3):742–8.

Whittall T, Wang Y, Kelly CG, Thompson R, Sanderson J, Lomer M, Soon SY, Bergmeier LA, Singh M, Lehner T. Tumour necrosis factor-alpha production stimulated by heat shock protein 70 and its inhibition in circulating dendritic cells and cells eluted from mucosal tissues in Crohn's disease. Clin Exp Immunol. 2006 Mar;143(3):550–9.

Weingartmann G, Oehler R, Derkits S, Oismüller C, Függer R, Roth E. HSP70 expression in granulocytes and lymphocytes of patients with polytrauma: comparison with plasma glutamine. Clin Nutr. 1999 Apr;18(2):121–4.

Wirth D, Bureau F, Melotte D, Christians E, Gustin P. Evidence for a role of heat shock factor 1 in inhibition of NF-kappaB pathway during heat shock response-mediated lung protection. Am J Physiol Lung Cell Mol Physiol. 2004 Nov;287(5):L953–61. Epub 2004 Jun 25.

Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine induces heat shock protein and protects against endotoxin shock in the rat. J Appl Physiol. 2001a Jun;90(6):2403–10.

Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. Shock. 2001b Nov;16(5):398–402.

Wischmeyer PE, Lynch J, Liedel J, Wolfson R, Riehm J, Gottlieb L, Kahana M. Glutamine administration reduces Gram-negative bacteremia in severely burned patients: a prospective, randomized, double-blind trial versus isonitrogenous control. Crit Care Med. 2001c Nov;29(11):2075–80.

Wischmeyer PE, Musch MW, Madonna MB, Thisted R, Chang EB. Glutamine protects intestinal epithelial cells: role of inducible HSP70. Am J Physiol. 1997 Apr;272(4 Pt 1):G879–84.

Wong HR, Ryan M, Wispé JR. Stress response decreases NF-kappaB nuclear translocation and increases I-kappaBalpha expression in A549 cells. J Clin Invest. 1997 May 15;99(10):2423–8.

Wright BH, Corton JM, El-Nahas AM, Wood RF, Pockley AG. Elevated levels of circulating heat shock protein 70 (HSP70) in peripheral and renal vascular disease. Heart Vessels. 2000;15(1):18–22.

Xiao X, Zhang H, Tang D, Shi Y. Gene expression regulation of cytokines by heat shock factor 1 (hsf1) and HSP70 during endotoxemia. Shock. 2006;25(6):63–4.

Xiao X, Zuo X, Davis AA, McMillan DR, Curry BB, Richardson JA, Benjamin IJ. HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. EMBO J. 1999 Nov 1;18(21):5943–52.

Xue H, Sawyer MB, Field CJ, Dieleman LA, Murray D, Baracos VE. Bolus oral glutamine protects rats against CPT-11-induced diarrhea and differentially activates cytoprotective mechanisms in host intestine but not tumor. J Nutr. 2008 Apr;138(4):740–6.

Yamada PM, Amorim FT, Moseley P, Robergs R, Schneider SM. Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. J Appl Physiol. 2007 Oct;103(4):1196–204

Ye D, Ma I, Ma TY. Molecular mechanism of tumor necrosis factor-alpha modulation of intestinal epithelial tight junction barrier. Am J Physiol Gastrointest Liver Physiol. 2006;290:G496–504.

Zhang G, Ghosh S. Molecular mechanisms of NF-kappaB activation induced by bacterial lipopolysaccharide through Toll-like receptors. J Endotoxin Res. 2000;6(6):453–7.

Zhang HJ, Xu L, Drake VJ, Xie L, Oberley LW, Kregel KC. Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation. FASEB J. 2003 Dec;17(15):2293–5.

Ziegler TR, Ogden LG, Singleton KD, Luo M, Fernandez-Estivariz C, Griffith DP, Galloway JR, Wischmeyer PE. Parenteral glutamine increases serum heat shock protein 70 in critically ill patients. Intensive Care Med. 2005 Aug;31(8):1079–86.

Ziegler TR, Smith RJ, O'Dwyer ST, Demling RH, Wilmore DW. Increased intestinal permeability associated with infection in burn patients. Arch Surg. 1988 Nov;123(11):1313–9.