ANTI-INFLAMMATION:
FINDING A NEW ROLE FOR HEAT SHOCK PROTEINS IN HUMAN HEALTH

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Heat shock proteins (Hsps) are highly conserved proteins and have been proven to protect prokaryotic and eukaryotic cells in most organisms against external stressors, whether environmental or pathophysiological. This defense mechanism probably depends on the roles of Hsps as molecular chaperones in governing proper protein assembly, folding and transport or as anti-apoptotic regulators of cell death pathways. Recent investigations on human inflammatory diseases indicate that Hsps may be involved in the process of inflammation. In this short review, we describe the general concept of heat shock proteins and inflammation and the new anti-inflammation role of heat shock proteins in various inflammatory diseases such as infection, autoimmune diseases, ischemia/reperfusion injury, cardiovascular diseases and chemical-induced diseases. The Hsps may act as autoantigens to stimulate the immune system and interact with inflammatory intracellular signaling pathways to regulate the inflammatory response. There are still unanswered questions about the heat shock response that require further investigation.

Les protéines de choc thermique (Hsps) sont des protéines hautement conservées qui protègent les cellules de la plupart des organismes procaryotes et eucaryotes contre les facteurs de stress externes, que ceux-ci soient d'origine environnementale ou pathophysiologique. Ce mécanisme de défense est probablement lié au rôle de molécule chaperonne des Hsps qui interviennent dans l'assemblage, le repliement et le transport des protéines ou qui agissent comme agents anti-apoptotiques (régulation de la mort cellulaire). Des recherches récentes sur les maladies inflammatoires humaines indiquent que les Hsps peuvent intervenir dans le processus d'inflammation. Dans le cadre de ce court résumé, nous décrivons le concept général de protéines de choc thermique et de l'inflammation ainsi que le nouveau rôle anti-inflammatoire des protéines de choc thermique dans diverses maladies inflammatoires telles que les infections, les maladies autoimmunes, les lésions d'ischémie/reperfusion, les maladies cardiovasculaires et les maladies d'origine chimique. Les Hsps peuvent agir à titre d'autoantigènes qui stimulent le système immunitaire et interviennent dans les voies de signalisation intracellulaire en cas d'inflammation pour réguler la réaction inflammatoire. Les questions qui restent toujours sans réponse à propos de la réaction au choc thermique doivent faire l'objet de recherches plus poussées.

HEAT SHOCK PROTEINS

More than forty years ago Ferruccio Ritossa (1962) made the first observation that elevated temperatures could trigger rapid and specific

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changes in chromosomal and metabolic activity in *Drosophila* salivary gland cells producing a "puffing" of chromosomes. Ritossa concluded that the new puffs were indicative of novel gene expression. Twelve years later, Tissieres et al. (1974) showed that, indeed, novel proteins were expressed in cells after brief elevation of temperature. These initial findings lead to the discovery of a large number of stress (heat shock) proteins in many prokaryotic and eukaryotic organisms, tissues, individual cells and subcellular structures. Although heat shock proteins (Hsps) may have different names according to species in which they have been discovered, their nucleotide and amino acid sequence are highly conserved. Based on their related functions and size, the Hsps are grouped into several families: the 110 kDa Hsps, the 90 kDa Hsps, the 70 kDa Hsps, the 60 kDa Hsps, small Hsps such as Hsp27 and ubiquitin (Currie & Plumier 1998). Hsp genes are regulated by heat shock transcription factors (HSFs). Under resting conditions, HSFs bind with Hsps in cytoplasm and are inactive. Under stressful conditions, Hsps are recruited to damaged or denatured proteins, leaving monomer HSFs free in the cytoplasm. HSF. is activated through a multi-step process, involving its phosphorylation by protein kinases such as protein kinase C (PKC), its trimerization and its translocation to the nucleus where it binds to the heat shock element (HSE) in the promoter region of Hsp genes and initiates transcription. Hsp mRNA is transcribed and leaves the nucleus for the cytoplasm where new Hsps are synthesized.

All living organisms respond to a wide variety of stresses such as heat shock (HS), heavy metals, ischemia, sodium arsenite, microbial infections, nitric oxide, and antibiotics by synthesizing Hsps. Hsps are present in cytoplasm, mitochondria, endoplasmic reticulum and nuclei. Some Hsps are constitutively expressed at high levels (eg, Hsp90, Hsc70), whereas other Hsps are rapidly inducible to high levels in response to cellular stress (eg, Hsp70, Hsp27). Hsps primarily function as molecular chaperones and facilitate the refolding, assembling and stabilization of denatured proteins (Hartl 1996, Rogalla et al. 1999). Molecular chaperones have been defined as a class of proteins that mediate the correct folding of other proteins, but do not take part in the final assembly of the new structures (Ellis & van der Vies 1991). Hsp chaperoning activity occurs in both nonstressed and stressed conditions, and maintains cytoskeletal function and cellular homeostasis. In addition, several Hsps have anti-apoptotic roles, regulating the activity of caspases, c-Jun NH_2-terminal kinase (JNK), and the nuclear factor κB (NF-κB) pathway. Interestingly, the NF-κB pathway is regulated by inflammation and it is also regulating the inflammatory response (Beere 2001, Polla et al. 1998). This may provide the missing link between the heat shock proteins and inflammation. Here, we review the roles of Hsps in various inflammatory diseases and propose a new role for Hsps in regulating inflammation.

INFLAMMATION: PHYSIOLOGICAL AND PATHOLOGICAL

Inflammation is defined as "a fundamental, stereotyped complex of cytologic and chemical reactions that occur in affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent" in Stedman's concise medical dictionary (Dirckx 2001). The inflammatory response is triggered whenever body tissues are injured, whether by infectious agents (bacteria, virus, parasites), physical agents (burns, trauma, radiation), chemical agents (toxins, heavy metals, reactive oxygen species (ROS)), immunological agents such as certain antigens causing allergy and autoimmunity, or any other phenomenon, and it is essentially a vascular reaction. The main purpose of inflammation is the quick death and clearance of foreign pathogen or antigen as well as injury repair. The immune system is responsible for regulating inflammation by producing substances such as histamines, prostaglandins, cytokines, chemokines, and adhesion molecules. Cell types such as neutrophils, eosinophils, basophils, lymphocytes, macrophages, and mast cells and platelets are involved in the inflammatory process, releasing specific inflammatory mediators and initiating the intricate chemical cascade. In the early phase of inflammation, pro-inflammatory mediators cause a marked increase in vascular permeability, local blood flow, and cellular infiltration into the injured site. This early process is hallmarked by five cardinal signs: redness, swelling, heat, pain and loss of function. In the late phase of inflammation, mediators contribute to angiogenesis, tissue remodeling and regeneration, connective tissue formation, wound healing, phagocytosis, and apoptosis. While we often attempt to regulate or suppress inflammation, it is an essential physiological process, which begins following a sub-lethal injury and ends with complete healing (Florey 1970).

Under normal conditions, the inflammatory process is turned off as soon as possible to avoid its deleterious effects. Mechanisms involve activation of regulatory T cells and secretion of anti-inflammatory cytokines in the late phase of inflammation, helping the organism to suppress the process. CD4+ T cell can be divided into at least two different subsets that counterbalance each other (Mosmann & Sad 1996). T-helper type 1 (Th1) lymphocytes are the most prevalent type of CD4+ T cells, and they promote inflammation by activation of macrophages and production of pro-inflammatory cytokines such as interleukin (IL)-2, IL-6, IL-8, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ. Counteracting the Th1 cells, the Th2 cells suppress inflammation and dampen macrophage activity by producing anti-inflammatory cytokines, IL-5, IL-10, and IL-13. To maintain body homeostasis, the immune system has to diagnose the need for inflammation, control the exact mix of inflammatory molecules, and regulate the process spatially, often at multiple sites, temporally and in

magnitude depending on the injury. If the inflammatory process is inappropriately regulated, spatially, temporally, or in magnitude of response, or fails to terminate, then the inflammation response itself can be pathological and become the cause of significant damage (Cohen & Efroni 2003). Severe infection such as sepsis and non-infectious systemic inflammation response syndrome (SIRS; eg, surgery, trauma, burns, cardiac arrest and resuscitation, acute allograft rejection, etc) can cause acute and over-activated inflammatory response. Sustained inappropriate inflammatory response causes chronic inflammation and plays a role in autoimmune diseases such as type 1 diabetes, rheumatoid arthritis and atherosclerosis (Wick et al. 2001). Pathological inflammation is a feature of many diseases and the regulation of the inflammatory response and the suppression of pathological inflammation is the focus of extensive research efforts.

HSPS PROTECT AGAINST INFLAMMATORY DISEASES

Infection diseases

Infection represents a stress for both the host and the pathogen. The host cell stress response is differentially regulated according to the type of infected cell and the type of pathogen, the microenvironment and the specific intercellular or intracellular communication (Polla et al. 1998). Pathogen toxins damage the host by triggering inappropriate inflammation, and the host is made sick by its own inflammatory reaction to pathogenic stimuli that trigger TNF-α, IFN-γ and other pro-inflammatory mediators (Calandra et al. 2002). Hsps are involved in cytoprotection against various pathogen infections. Hsp70 protects macrophages infected with *Salmonella choleraesuis* against TNF-α-induced cell death and induces immunoregulatory CD4+ T cells during the course of infection with *Listeria monocytogenes* (Yoshikai 1998). Hsp70 also protects rat pancreas from *Cerulein*-induced pancreatitis-like injury (Bhagat et al. 2000). Over-expression of Hsp70 inhibits bacterial lipopolysaccharide (LPS)-induced production of cytokines in human monocyte-derived macrophages, LPS-induced lung inflammation, septic shock and accelerates mouse recovery after endotoxic challenge (Ding et al. 2001, Vreugdenhil et al. 2003, Eaves-Pyles et al. 2000, Paidas et al. 2002). In addition, HSF-1 deficient (HSF-/-) mice produced greater amounts of pro-inflammatory cytokines and were more susceptible to endotoxin-mediated lethality compared with wild-type mice (Xiao et al. 1999). During viral infection, elevated Hsps inhibit specific events in the viral cycle leading to inhibition of viral replication (Santoro 1994), and in fact, Hsp70 may be the cellular mediator interfering with viral protein synthesis (Santoro 1997). Sodium arsenite, cadmium, azetidine, and HS, all induce the expression of Hsps and inhibit the synthesis of Sendai virus protein synthesis as long as Hsp synthesis is occurring in the

infected human cells (Santoro 1997).

Autoimmune diseases

Autoimmune diseases are the classic example of inappropriate inflammation. Recent experiments in the adjuvant-induced arthritis (AIA) rat model of rheumatoid arthritis (RA) have shown promising results using Hsp peptides to suppress the inflammatory injury. Nasal administration of Hsp60 180-188 peptides induces highly effective protection against AIA through generation of regulatory T cells that produce anti-inflammatory cytokines such as IL-4, TGF-β and mainly IL-10 (Prakken et al. 2002, 2003). Mycobacterial Hsp10 (Agnello et al. 2002) and Hsp70 (Wendling et al. 2000) have also been found to suppress AIA. A phase I clinical trial was performed treating early RA patients with dnaJp1 peptide derived from the *E. coli* Hsp (Puga Yung et al. 2003). Interestingly, oral treatment with dnaJp1 reduces the T cell proliferative response, IL-2, IFN-γ and TNF-α, and stimulates the T cells to produce IL-4 and replaces the majority of TNF-α-producing cells with IL-10 and IL-4 producing cells. Such treatment with dnaJp1 suggests that Hsps or peptides derived from Hsps will be suitable candidates for immune therapy in chronic arthritis. In addition, treatment with Hsp60 or its peptides can modulate the progression of autoimmune diabetes. Vaccination of non-obese diabetic (NOD) mice with peptides of Hsp60 successfully inhibits the development of spontaneous diabetes (Elias et al. 1997, Bockova et al. 1997). A double-blind, phase II clinical trial found that type 1 diabetes in humans is also susceptible to immunomodulation by p277 peptide of Hsp60 (Raz et al. 2001). The administration of p277 preserves the endogenous levels of C-peptide and is associated with lower requirements of exogenous insulin, revealing the suppression of β-cell destruction. Inflammatory bowel diseases (IBDs), such as ulcerative colitis and Crohn's disease, are characterized by chronic relapsing inflammation. Hsps induced by hyperthermia prevent leukotriene B4 production, subsequent neutrophil infiltration, epithelial damage, and alterations in tachykinergic control of smooth muscle in an experimental IBD (Goldhill et al. 1999).

Ischemia/reperfusion injury

Ischemia/reperfusion (I/R) injury is widely recognized as a significant source of morbidity and mortality in a number of clinical situations such as transplantation, trauma, liver, lung, or bowel resection and haemorrhagic shock. A hallmark of I/R is the production of reactive oxygen species (ROS) during the reperfusion phase and it is thought that the production of ROS mediates much of the post-ischemic tissue injury by causing direct cellular damage and/or acting as second messengers in the activation of cellular responses controlling cell death and inflammation. Inflammatory response such as lymphocyte activation, neutrophil infiltration, cytokine release and fibrosis exists in both the

acute and chronic phases of I/R injuries. Hsps are highly inducible by I/R injury through the activation of HSFs by ischemia (Tacchini et al. 1997). Heme oxygenases (HO)-1 or Hsp32 is upregulated with I/R and is thought to be not just anti-oxidative, but also a complex immunomodulator protecting against inflammation in I/R injury (Katori et al. 2002a, 2002b). Interestingly, the expression of Hsp40 and Hsp70 increases during lung rejection in human pulmonary allografts (Rizzo et al. 1998), and elevated levels of Hsp70 correlates with the improved pulmonary function after I/R in an isolated rabbit lung model (Long et al. 2003). The cytoprotective effects of Hsps have also been found in I/R injury in brain, liver, kidney and surgical flap (Plumier et al. 1997, Currie et al. 2000, Uchinami et al. 2002, Wagner et al. 2003, Rucker et al. 2001).

Cardiovascular diseases

Heat shock proteins are expressed in the cardiovascular system and appear to have a beneficial role. Currie et al. (1988) reported that whole body heat shock in rats is associated with improvement of cardiac functional recovery after global ischemia and these findings are correlated with significantly increased cardiac Hsp70. Transgenic mice expressing high level constitutive expression of the human inducible Hsp70 demonstrate the direct role of Hsp70 in the protection of the myocardium from ischemia and reperfusion injury (Plumier et al. 1995). In addition, heat shock treatment and the concommitant high levels of Hsp70 and Hsp27 and their phosphorylated isoforms protect against angiotensin (Ang) II-induced hypertension and inflammation in heart and aorta (Chen et al. 2004a, 2004b). Heat shock proteins have a role in the treatment of atherosclerosis. Using mucosal administration of Hsp65 Maron et al. (2002) vaccinated low-density lipoprotein receptor deficient (LDL-/-) mice to reduce the inflammatory process, hence provided a new immunologic approach for the treatment of atherosclerosis.

Other inflammatory diseases

There is also evidence for Hsps having a role in various other models of inflammation. Hsp25, Hsp32, Hsp47, and Hsp70 have been demonstrated to play an anti-inflammation role in some chemical-induced diseases such as acetic acid-induced gastric ulcer (Guo et al. 2002), thioacetamide-induced liver disease (Zborek et al. 2002) and carrageenin-induced pleurisy (Ianaro et al. 2001). Upregulation of Hsp70 also reduces inflammation in asthma and the acute respiratory distress syndrome (Bertorelli et al. 1998, Weiss et al. 2002). Thus Hsps are associated with inflammation.

MECHANISMS OF ANTI-INFLAMMATION REGULATION OF HSPS

Extra-cellular mechanisms: autoantigen role of Hsps

Hsps are now known to be strongly immunogenic despite their high level of evolutionary conservation (van Eden et al. 2003). While Hsps are intracellular proteins, they are also expressed on the cell surface (van Eden 2000). Most self-hsp cross-reactive T cells are also IL-10 producing regulatory T cells (van Eden et al. 2003). Three mechanisms of anti-inflammatory T cell induction by Hsps have been proposed (Van Eden et al. 2003): (1) *Mucosal tolerance*: Hsp reactive T cells recognize microbial Hsp epitopes in the gut lymphoid tissue and develop a tolerance when confronted with self-Hsps expressed elsewhere in the body. (2) *Altered peptide ligands regulation*: Microbial-Hsp reactive T cells perceive self-Hsp homologs as partial agonists or altered peptide ligands and redirect pro-inflammatory (Th1) T cell response to regulatory Th2 response. (3) *Anergy*: The ubiquitous low level of Hsp is also expressed on non-professional antigen presenting cells that lack the co-stimulatory molecules needed to induce a T cell response. This constitutive Hsp expression in non-professional antigen presenting cells in the absence of proper co-stimulation drives self-Hsp recognizing T cells into a state of anergy. Such "anergic" T cells inhibit the proliferation and activation of other T cells when confronted with professional or activated antigen presenting cells.

Intra-cellular mechanisms: crosstalk between Hsps and cell signaling transduction pathways

Recently, several studies show that Hsps interact with some intra-cellular signaling transduction pathways involved in inflammation. NF-κB is a pivotal transcription factor that functions to control the transcription of a variety of pro-inflammatory genes, including cytokines, growth factors, adhesion molecules, immunoreceptors and acute-phase proteins. The HS response and NF-κB signaling pathway are two fundamental cellular processes. The interaction between Hsps and HS response, and NF-κB pathway effect various levels of the pathway (Figure 1). Our laboratory (Chen et al. 2004a, 2004b) and others (Wong et al. 1997, Pritts et al. 2000, Yoo et al. 2000) have found that heat shock suppresses NF-κB activation by inhibiting the phosphorylation and degradation of its inhibitor, IκB-α. In fact Hsp70 through its molecular chaperone activity interacts and forms complexes with IκB-α and is thought to stabilize IκB-α and attenuate NF-κB activation (Shimizu et al. 2002). Hsp70 may also directly interact or chaperone NF-κB, as a partial substitute for IκB (Guzhova et al. 1997). Alternatively, the human IκB-α gene promoter region contains a contiguous 20 bp segment that matches with the heat shock element in the promoter region of Hsp genes (Wong et al. 1997). After heat shock (HS), both IκB-α and another

NF-κB inhibitor IκB-β are expressed and, thus, can be regarded as heat shock proteins (Wong et al. 1997, 1999, Stasiolek et al. 2000). HS inhibits radiation-induced activation of IκB kinase (IKK) (Curry et al. 1999, Yoo et al. 2000) and appears to increase intracellular phosphatase activity (Malhotra & Wong 2002). Consistent with this, HS decreases the cytoplasmic level of IKK-α (Chen et al. 2004b) and is correlated with the higher expression of Hsp70, Hsp27 and their phosphorylated isoforms. Interestingly, Park et al. (2003) found that Hsp27 interacts directly with IKK-α and IKK-β in down-regulation of TNF-α induced NF-κB activation via its phosphorylation. Hsp70/HS have also been found to suppress other proinflammatory pathways such as JNK and p38 (He et al. 2000, Wang et al. 2002) and it is likely that HS and Hsps will suppress pro-inflammatory and increase anti-inflammatory transcription factors.
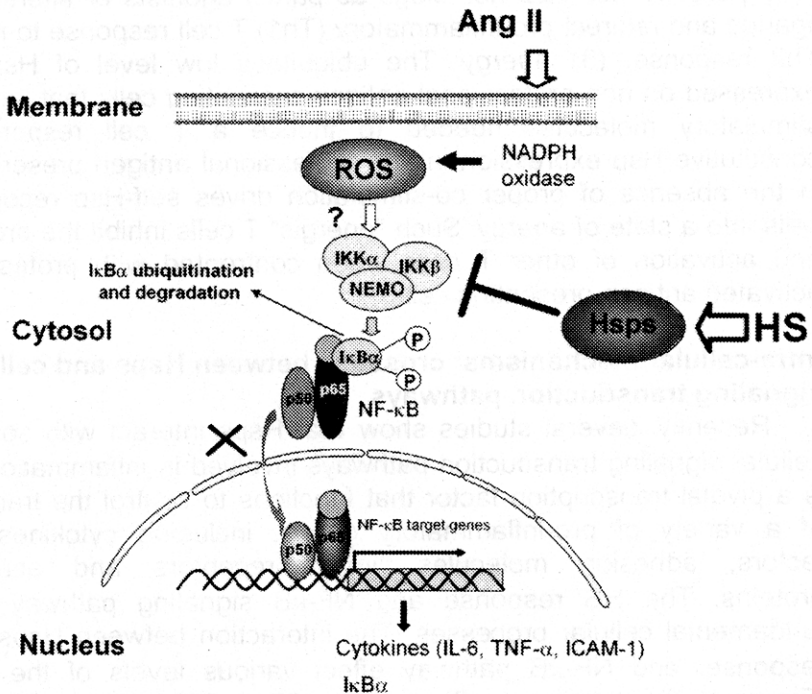

Figure 1. NF-κB pro-inflammatory signaling pathway. NF-κB consisting of p50/p65 subunits is normally resident in the cytosol and is maintained in an inactive form by its inhibitor IκBα. Angiotensin II (Ang II) stimulates NADPH oxidase to release reactive oxygen species (ROS) that are thought to activate the IκB kinase (IKKα, IKKβ, and their regulator NEMO) complex. The activated IKK complex phosphorylates IκBα and initiates its ubiquitination and degradation, exposing the nuclear localization signal on NF-κB. Freed NF-κB is translocated to the nucleus and binds to the promoter region of genes expressing pro-inflammatory cytokines such as Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), intercellular adhesion molecule-1 (ICAM-1) and IκBα. Heat shock (HS) strongly elevates the expression of several heat shock proteins that interact with the IKK complex to suppress its activation.

PARADOXES OF HSPS AND PERSPECTIVES

As an alternative to controlling inflammation by using anti-cytokine treatments that are currently less than fully effective at controlling the many cytokines, regulation of Hsps may be a novel and more effective approach to anti-inflammatory therapy. In fact, many of the current widely used nonsteroid anti-inflammatory drugs (NSAIDs) induce HSF activation and increase the expression of Hsps (Polla et al. 1998). Paradoxically, not all aspects of the heat shock response are protective. Firstly, some Hsps, and especially the Hsp60 family have been found to induce macrophages to secrete pro-inflammatory mediators such as TNF-α, IL-6, IL-12, and nitric oxide (Ohashi et al. 2000, Flohe et al. 2003). Contradictory pro-inflammatory and anti-inflammatory effects of Hsp60 in various cell types may be because Hsp60 works as a ligand both for toll-like receptor-4 (TLR-4) and TLR-2 in T cells and B cells. Hsp60 and its fragments can regulate the physiology of inflammation itself by acting as ligands for TLR-2 (Zanin-Zhorov et al. 2003), while Hsp60 might elicit a potent pro-inflammatory response through TLR-4 signaling (Ohashi et al. 2000). Whether Hsp60 is a "smart" molecular switch in the regulation of inflammation requires further investigation. Secondly, HS and expression of Hsps can have contradictory effects such as being benefical *before* a pro-inflammatory stimulus (e.g., NF-κB activation), compared to cytotoxic effects *after* a pro-inflammatory stimulus (DeMeester et al. 2001). Such paradoxical effects in HS and NF-κB regulation may be involved in critical illnesses such as multiple organ dysfunction. Thirdly, sustained inflammation in some chronic diseases may be caused by the undesired prolonged survival of pro-inflammatory cells. The cytoprotective effects of Hsps during apoptosis, although beneficial in other pathologic conditions, may contribute to chronic inflammation. The increase in HSF activity and subsequent Hsp production may differentially influence three mitogen-activated protein kinase (MAPK) pathways under various stressful conditions (Malago et al. 2002). Hsps protect against apoptosis via ERK1/2 MAPK pathway, while they suppress inflammation through JNK and p38 MAPK activity. However, all three MAPK pathways can be activated at one time. A better understanding of interactions between Hsps and regulators of apoptosis and inflammation and how these interactions can be modulated by the levels of the Hsps needs to be investigated as an alternative approach for controlling inflammatory diseases.

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