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REVIEW ARTICLE

Role of Heat Shock Proteins in Diseases and Their Therapeutic Potential

Gautam Kaul · Hitesh Thippeswamy

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Abstract Heat shock proteins are ubiquitously expressed intracellular proteins and act as molecular chaperones in processes like protein folding and protein trafficking between different intracellular compartments. They are induced during stress conditions like oxidative stress, nutritional deficiencies and radiation. They are released into extracellular compartment during necrosis. However, recent research findings highlights that, they are not solely present in cytoplasm, but also released into extracellular compartment during normal conditions and even in the absence of necrosis. When present in extracellular compartment, they have been shown to perform various functions like antigen presentation, intercellular signaling and induction of pro-inflammatory cytokines. Heat shock proteins represents as dominant microbial antigens during infection. The phylogenetic similarity between prokaryotic and eukaryotic heat shock proteins has led to proposition that, microbial heat shock proteins can induce self reactivity to host heat shock proteins and result in autoimmune diseases. The self-reactivity of heat shock proteins protects host against disease by controlling induction and release of pro-inflammatory cytokines. However, antibodies to self heat shock proteins haven been implicated in pathogenesis of autoimmune diseases like arthritis and atherosclerosis. Some heat shock proteins are potent inducers of innate and adaptive immunity. They activate dendritic cells and natural killer cells through toll-like receptors, CD14 and CD91. They play an important role in MHC-antigen processing and presentation. These immune effector functions of heat shock proteins are being exploited them as therapeutic agents as well as therapeutic targets for various infectious diseases and cancers.

Keywords Heat shock proteins · Chaperone · Infectious diseases · Autoimmune diseases

Introduction

Heat shock proteins were first discovered in 1962 by Ritossa and co-workers in overheated *Drosophila melanogaster* larvae [1]. Subsequent research work has demonstrated that heat shock proteins are present in all species. Heat shock proteins are phylogenetically conserved proteins and are categorized into several families on the basis of their approximate molecular weight. However the term heat shock proteins seems to be a misnomer, as they are not induced solely by heat shock. These proteins can be induced during wide range of cellular insults like oxidative stress, nutritional deficiencies, ultraviolet irradiation, and exposure to chemicals, bacterial infection, viral infection, and necrosis in mammalian hosts [2]. Whereas starvation, attack by toxic molecules like reactive oxygen species, changes in nutrients, changes in temperature, pH, and partial pressure of oxygen induces heat shock proteins in prokaryotes [3, 4]. Stressors that cause protein unfolding, misfolding and improper aggregation will also leads to stress response and induction of heat shock proteins, thereby re-establishing balance between protein synthesis, assembly and degradation.

Induction of heat shock protein synthesis is transcriptionally regulated both in prokaryotic and eukaryotic cells. In prokaryotic cells, $\sigma 32$ acts as positive transcription

G. Kaul (✉) · H. Thippeswamy
Biochemistry Department, National Dairy Research Institute, Karnal, Haryana, India
e-mail: gkndri@gmail.com

factor for heat shock protein expression [5]. During normal temperature (unstressed state) $\sigma 32$ has very short half life and is bound with intracellular hsp70. Hence $\sigma 32$ is unavailable to interact with promoter region of heat shock protein gene [6]. When temperature is elevated (stressed state), $\sigma 32$ stabilizes and accumulates in higher concentration due to decrease in the pool of free and available intracellular hsp70, hence $\sigma 32$ is free to interact with promoter region of heat shock protein gene. In eukaryotic cells, heat shock protein synthesis is regulated by interaction of the heat shock factor (HSF) transcription factors with heat shock elements (HSEs) present in the promoter regions of heat shock protein gene [7]. In the unstressed state, HSF1 (one of the principal heat shock factor transcription factor) is present in the cytoplasm as monomeric molecule and is unable to bind with HSE in promoter region of heat shock protein gene. Under stressed state, HSF1 is hyperphosphorylated in a ras-dependent manner by members of the mitogen activated protein kinase (MAPK) subfamilies. HSF1 is converted to phosphorylated trimers with an ability to bind with HSEs in promoter regions of heat shock protein gene [8, 9].

Heat shock proteins perform crucial functions in correct protein folding and unfolding, translocation of proteins as well as assembly and disassembly of protein complexes. Since heat shock proteins assist in these functions, they have been termed as molecular chaperones. However recent research progress in heat shock protein biology highlights the evidence that rather being solely intracellular, heat shock proteins are also present in and can be released into extracellular compartment during normal physiological conditions and even in absence of necrosis. Heat shock proteins in extracellular compartment elicit different functions like, antigen presentation, intercellular signaling and induction of pro-inflammatory cytokines, which eventually mediate both induction and regulation of immunity in mammalian species.

Heat shock protein synthesis is increased to protect mammalian hosts from various insults caused by infection, inflammation, or similar events. Heat shock proteins represent as prominent antigens in several infectious diseases and autoimmune diseases, wherein they mediate humoral and cellular immune response. Even altered chaperone function of heat shock proteins has been associated with development of diseases like ischemic heart disease, and neurodegeneration. Therefore, ability of the heat shock proteins to induce and regulate immunity, and modulation of chaperone activities became emerging field of therapeutics development. In this review, we give an overview of the role of heat shock proteins in various diseases both in experimental animal models and humans, their therapeutic potential value to combat various infectious diseases and cancer.

Chaperone Function of Heat Shock Proteins

Heat shock proteins are expressed both constitutively and during stressful conditions. These are present in cytoplasm and nucleus of eukaryotic cells, with the exception of hsp60, which is present in mitochondria. The major function of heat shock proteins appears to act as molecular chaperones. The molecular chaperones are the proteins which recognize and bind with nascent polypeptide and partially folded protein intermediates, preventing their aggregation and misfolding. They are also involved in protein trafficking between different intracellular compartments. Heat shock proteins are classified on the basis of their molecular weight. Major classes of heat shock proteins include small hsps, hsp40, 60, 70, 90 and hsp110 families [10, 11]. Members of the hsp60 and hsp70 families are primarily involved in molecular chaperone function. In mammalian species, hsp60 family consists of mitochondrial hsp60 and cytosolic hsp60. The cytosolic hsp60 have been shown to assist in folding of cytoskeletal proteins like actin and tubulin [12]. Hsp70 family includes constitutive cytosolic hsp73, stress induced hsp70, and endoplasmic reticulum (ER) Bip (Binding protein or also called as grp78) [2, 10, 11]. Hsp70 and its cognate proteins present in the cytosol are involved in the protein trafficking processes between different intracellular compartments [13]. Bip (grp78, glucose regulated protein 78) present in ER binds with multimeric protein complexes like immunoglobulin, T cell receptor, and major histocompatibility complex (MHC) molecules and thereby aid in proper assembly of these multimeric protein complexes [14–16]. Hsp90 family includes cytosolic hsp90 (alpha and beta) and ER form, gp96 (also called as grp74). The gp96 have been shown to be involved in assembly of antibody molecules [17].

Heat Shock Proteins in Intercellular Signaling and Antigen Presentation

Heat shock proteins are not solely present in the cytoplasm, but also released into extra cellular compartment during normal physiological conditions, stressful conditions and even in the absence of necrosis. However the mechanism by which heat shock proteins are released into extracellular environment is not yet fully understood. Heat shock proteins in extracellular environment have been shown to have number of immunological effects. Bacterial hsp60, and mycobacterial hsp65 and hsp70 have been shown to induce pro-inflammatory cytokine expression [18–21]. Bacterial hsp60, DnaK and GroEL of *E. coli* have been shown to induce intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression

on vascular endothelial cells. Chlamydial hsp60 and human hsp60 can induce expression of E-selectin, ICAM-1, VCAM-1 on vascular endothelial cells, and also activate vascular endothelial cells, smooth muscle cells and macrophages to secrete interleukin-6 (IL-6) [22]. Hsp60 also induces a release of tumor necrosis factor alpha (TNF-alpha), nitric oxide, IL-12 and IL-15 from macrophages [23]. Ability of heat shock proteins to elicit a wide range of immunological effects has instigated scientific community in finding cell surface receptors for these molecules. It has been shown that the heat shock proteins mediate these immunological effects by binding with specific receptors like CD14, Toll like receptor 2 and 4 and CD91 [24, 25].

Several lines of research findings demonstrate that heat shock proteins play an important role in MHC-antigen processing and presentation [14, 16, 26]. Members of hsp70 family are principally involved in the processing and presentation of antigens [14, 26–28]. Bip and another endoplasmic chaperone, calnexin have been shown to promote proper assembly of MHC class I and MHC class II molecule in the ER [16, 29–32].

Hsp70 and gp96 chaperoned peptides are taken up by dendritic cells by CD91 receptor mediated endocytosis [33]. These chaperoned peptides are presented to MHC class I molecules and re-presented on the cell surface for recognition of CD8 positive T-cells [34]. This will leads to maturation signals to dendritic cells, induction of MHC-antigens and co-stimulatory molecule like B7, ICAM-1, and induction of cytokines which eventually generate immune response to chaperoned peptides. However it is unclear whether heat shock proteins directly interact with antigenic peptides and MHC molecules for antigen presentation or whether hsp-peptide complexes are recognized by alternative antigen presentation pathways [35].

Role of Heat Shock Proteins in Infectious Diseases

Infectious diseases occur as an eventual, when microbial pathogens encounter with their mammalian hosts. To establish themselves in mammalian hosts, microbial pathogens express various virulence factors, toxins, and other defenses to evade exaggerated host defense mechanisms. Although such components of microbial pathogens are often directly involved in disease, in many cases immune mechanisms are involved in pathogenesis of a disease. Microbial pathogens also express increased levels of heat shock proteins as another defense component to survive in their hosts during infection. Immune mechanisms directed towards these heat shock proteins are responsible for immunopathogenesis of a disease. Thus pathogen entering the host rapidly faces with altered pO_2, pH, and temperature levels, and at the later stage of infection it will be attacked by host defense mechanisms including reactive oxygen molecules. Fields et al. [36] have been shown that direct evidence for a role of heat shock proteins in bacterial defense against the host. Increased levels of pathogen derived heat shock proteins are rapidly degraded by host processing machinery. Pathogen derived heat shock proteins act as immunodominant antigens and elicit immune responses to various infectious diseases caused bacteria, protozoa, fungi, and nematodes (Table 1), as well as various experimental infection models.

These heat shock proteins are highly conserved among various microbial pathogens. Many of the members of hsp60 or hsp70 family have high degree of sequence homology among various bacteria [37]. For example, hsp60 of mycobacteria is homologous to *Pseudomonas aeruginosa* hsp60 and to hsp60 of other gram-negative bacteria [38]. Hence heat shock proteins have been shown to act as major antigens and very strong inducers of humoral and cellular immune responses to various infectious diseases. The evidence for the heat shock proteins as major antigens during these infectious diseases have been confirmed by experiments revealing increased levels anti heat shock protein antibodies and activation of specific cellular immune response by activation of γS T cells. Moreover, in vitro experiments have demonstrated that pathogen derived heat shock protein-peptides especially hsp60 induces pro-inflammatory cytokines [21] which are responsible for tissue destruction and immunopathology at the site of infection.

Increased reactivity to hsp60 as an immunodominant target for antibody and T cell response during mycobacterial infections in mice and humans have been demonstrated [39]. Hsp60 specific antibodies have been detected in patients suffering from tuberculosis, leprosy, typhoid, and also in mice, after infection with *Mycobacterium tuberculosis* [40, 41]. The direct role of hsp60 specific T cells in anti-pathogenic immune response of mice infected with *Yersinia enterocolitica* has been demonstrated [40], where in CD4 T cells specific for heat shock proteins were increased in infected mice and mediated significant protection against infection when adaptively transferred. Increased antibody levels to hsp70 have been demonstrated in sera of patients with malaria, leishmaniaisis, schistosomiasis, and candidiasis [37]. Hsp60 reactive γS T cells and hsp70 reactive γS T cells are specifically activated in experimental listeriosis of mice and conferred protection against *Listeria monocytogenes* infection. It has been shown that, depletion of $\gamma\delta$ T cells with monoclonal antibodies has led to increased listerial multiplication in mice [42].

The impact of heat shock protein expression by microbes in mammalian hosts varies with different infections. Heat shock protein expression by microbe especially

Table 1 Pathogen derived heat shock proteins as immunodominant antigens during various infectious diseases

Pathogen	Disease	Heat shock protein (hsp) family
Bacteria		
Mycobacterium tuberculosis	Tuberculosis	hsp60, hsp70
Mycobacterium leprae	Leprosy	hsp10, hsp60, hsp70
Chlamydia trochomatis	Trochoma	hsp60, hsp70
Borrelia burgdoferi	Lyme disease	hsp60, hsp70
Helicobacter pylori	Gastritis	hsp60
Yersinia enterocolitica	Yersiniosis	hsp60
Bordetella pertussis	Pertussis	hsp60
Listeria monocytogenes	Listeriosis	hsp60
Salmonella typhimurium	Typhoid	hsp60, hsp70
Protozoa		
Plasmodium falciparum	Malaria	hsp70, hsp90
Trypanosoma cruzi	Chaga's disease	hsp70, hsp90
Leishmania donovani	Leishmanasis	hsp70
Toxoplasma gondii	Toxoplasmosis	hsp60
Helminths		
Schistosoma mansoni	Schistosomiasis	hsp70, hsp90
Onchocerca volvulus	Onchocercosis	hsp70
Fungi		
Candida albicans	Candidiasis	hsp90
Histoplasma capsulatum	Histoplasmosis	hsp60, hsp70

intracellular pathogens is essential for their survival in host cells like macrophages. For example, hsp60 have been shown to expressed in *Salmonella typhimurium* infection. Mutants of *S. typhimurium* which over-expresses hsp60 have been shown to be resistant to variety of oxidizing agents and killing by activated macrophages. Conversely, deletion mutants of *S. typhimurium* defective in hsp gene expression have been found to have less resistance to intracellular killing by macrophages [36, 43, 44].

Chlamydial hsp60 has been found to be pathogenic antigen in ocular and urogenital tract infections caused by chlamydia sp. Experimental infection of guinea pig with *Chlamyida psittaci* which causes trachoma revealed delayed type hypersensitive reaction against hsp60 [45]. Hsp60 have been shown to act as major antigen in diseases like pertuissis, gastritis caused by *Helicobacter pylori*, histoplasmosis, toxoplasmosis and lyme disease [46–49]. In onchocercosis caused by *Onchocerca volvulus*, hsp70 represent as major immunogen during infection [50].

Role of Heat Shock Proteins in Autoimmune Diseases

The process of infection is bimodal, which is determined by the host and pathogen. During infection, not only pathogen but also host increases heat shock protein synthesis as it faces several cellular insults from pathogens. Induction of heat shock proteins in response to pathogen encounter is certainly to attack infected host cells by immune effector mechanisms. These immune effector functions are mediated by host heat shock protein induced activation of natural killer cells which causes lysis of infected cells, and induction of Th_2 type of regulatory cytokines from macrophages, which subsequently helps to control production of pathogen induced pro-inflammatory cytokines. This regulatory immune function minimizes tissue destruction and other immunopathologies.

However, several lines of studies evidenced high degree of sequence homology between mammalian and pathogenic heat shock protein cognates. Nearly 50–60% sequence identity was found in case of hsp60 family. This phylogenetically conserved nature of heat shock proteins between mammalians and prokaryotes has led to proposition that, whether the immune system recognizes heat shock proteins as dominant pathogenic antigens or potentially harmful self antigens. Conserved epitopes of heat shock proteins among mammalian cells and prokaryotes leads to cross reactivity and induces immune reactivity to self heat shock proteins which eventually results in autoimmune diseases [51]. The influence of microbial and host induced heat shock proteins in pathogenesis of autoimmune diseases have been best studied in experimental arthritis models, patients with rheumatoid arthritis, and vascular diseases.

Several research investigations conducted on arthritis models like adjuvant arthritis, streptococcal cell wall induced arthritis in Lewis rats, and rheumatoid arthritis in humans implicated direct role of hsp60 in autoimmunity. Protection against adjuvant arthritis in Lewis rats induced by immunization with self hsp60 peptide [52], protection against adjuvant arthritis by vaccination with mycobacterial hsp60 peptide cross reactive to self hsp60 [53] have confirmed the involvement of hsp60 in adjuvant arthritis. Similarly immunization with mycobacterial hsp60 conferred protection against streptococcal cell wall induced arthritis in Lewis rats [54]. The hsp60 also been implicated in rheumatoid arthritis (RA) of humans. Increased expression of hsp60 in synovial tissue of RA patients and raised levels of antibodies against self hsp60 [55], binding of hsp60 specific antibodies to synovial tissue of RA patients [56], ability of antibodies (obtained from synovial tissue of RA patients) to bind with mycobacterial hsp60 [57] and T cells (from synovial fluid and peripheral blood) reactivity to mycobacterial hsp60 have been clearly highlighted the role of hsp60 in RA.

Vascular diseases comprise atherosclerosis and its complications like coronary artery disease and peripheral vascular diseases. Atherosclerosis is characterized by lipid laden macrophages, increased levels of localised CD4 T cells, monocytes, pro-inflammatory cytokines and other inflammatory mediators at atherosclerotic lesion [58, 59]. Several research findings also propose that, atherosclerosis involves immune component during its pathogenesis which is mediated by expression and reactivity to heat shock proteins. However precise molecular mechanisms of heat shock protein during pathogenesis of atherosclerosis is still unclear. Despite, there is positive relationship between heat shock protein expression and severity of atherosclerosis. Furthermore concomitant infections by some microbes can aggravate atherosclerosis by induction of cross reactive antibodies and reactive T cells to self-hsp60. Evidence for the involvement of heat shock proteins in atherosclerosis have been demonstrated by increased levels of antibodies to self hsp60, ability of anti hsp60 antibodies of Mycobacteria and Chlamydia to react with self hsp60 and, localised enrichment of γS T cells at atherosclerotic lesion [60]. Haemodynamic risk factors like increased blood pressure and infection with Mycobacteria and Chlamydia leads to production of cross reactive antibodies and $\gamma\delta$ T cells reactivity to self hsp60 expressed on vascular endothelial cells and monocytes. This consequently leads to induction of pro-inflammatory cytokines, ICAM, VCAM and E-selectin on endothelial cells and macrophages at atherosclerotic site. Eventually there will be migration, proliferation and extracellular matrix production, local tissue destruction and hence development of plaques which constitutes atherosclerotic lesion.

Therapeutic Potential of Heat Shock Proteins

In addition to chaperone functions during physiological and stress condition, heat shock proteins have been shown to induce both humoral and cellular immunity. Under different circumstances, they activate or suppress innate and adaptive immunity by several mechanisms. The microbial heat shock proteins act as dominant pathogenic antigens during several infectious diseases. Heat shock proteins stimulates cell surface receptors like CD91 [61] on antigen presenting cells and results in MHC class I mediated CD8+ cell meditated immunity [62]. They also stimulate innate immunity and activate dendritic cells and natural killer cells by binding with CD40, TLR-2, TLR-4, CD14 and, scavenging receptor-A [63–66]. These immune effector functions mediated by the heat shock proteins and modulation of chaperone activities have been exploited heat shock proteins as therapeutic agents as wall as therapeutic targets in several infectious diseases, cancers and neurodegenerative disorders. The potential therapeutic value of heat shock protein lies in their capacity to induce pro-inflammatory responses at low concentrations and induce regulatory immune responses at high doses. Induction of pro-inflammatory response is to protect against infections and cancer, and regulatory immunity to protect against autoimmune diseases.

Pathogen derived heat shock proteins have been utilized as antigen in making vaccines against some infectious diseases like histoplasmosis, tuberculosis, toxoplasmosis, and yersiniosis. Vaccination with hsp60 of *H. capsulatum* [48] and vaccination with transgenic cell line expressing mycobacterial hsp60 [67] have been shown to protect mice against histoplasmosis and tuberculosis respectively. Immunization of mice with yersinia hsp60 conferred protection against *Y. enterocolitica* [40]. Similarly vaccination with hsp70 of *T. gondii* [68] conferred protection against toxoplasmosis.

Research studies conducted by Srivastava and coworkers [69, 70] have been shown that, exogenous administration of tumor derived heat shock protein gp96 and hsp70 peptides conferred protection against tumors in mice. Further investigations revealed that the tumor immunity was triggered not by heat shock protein themselves but by the peptides chaperoned by heat shock proteins indicating presence of heat shock proteins serves as adjuvant and boost immunity to tumor associated antigens [34, 71]. An autologous heat shock protein vaccine, Onchophage^{R} containing gp96 tumor derived peptides has been passed phase III clinical trails and has been shown to effective against patients with different cancers [72].

Heat shock protein 90 is over-expressed in mammalian tumors. It acts as key antiapoptotic regulator conferring protection of tumor cell against apoptosis. This hsp90

interacts and stabilizes various kinases like Src kinase, Met tyrosine kinase, Raf kinase which are involved in malignant transformation. Therefore employing hsp90 inhibitors have shown to induce tumor suppression. Hsp90 inhibitors like Geldanamycin, 17AAG (17-allyl amino 17-dimethoxy geldanamycin) [73], Dihydroxy-phenylpyrazole and, Trichostatin-A [74] have entered clinical trials and are effective in suppressing tumors.

Conclusion

This review has attempted to illuminate the role of heat shock proteins in different infectious and autoimmune diseases. Heat shock proteins are ubiquitously expressed and act as dominant antigens of microbes during infection. Together with phylogenetic similarity between prokaryotic and eukaryotic heat shock proteins, self-reactivity to host heat shock proteins can lead to autoimmune responses. However, the molecular mechanisms by which heat shock proteins are released into extracellular compartment during infection are still unclear. Further investigations are required to evaluate induction and regulation of heat shock proteins mediated immune effector functions during several infections and autoimmune diseases. There is a need to define the specificity of heat shock protein response to distinguish self and non-self reactivity so that the contribution of infective agents to pathogenesis of autoimmune diseases can be truly attributed. The unique ability of heat shock proteins to activate or suppress both innate and adaptive immune responses to its associated antigens under different circumstances made them to exploit as therapeutic agents as well as therapeutic targets in many infections and cancers.

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