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Role of Heat Shock Proteins in the initiation, elaboration and therapy of infectious diseases

Beatrice Gilea[1]*, Veronica Lazar[1]

ABSTRACT

The prokaryotic and eukaryotic cells respond to different types of lethal assaults by the synthesis of heat shock proteins (HSP), in order to protect themselves. HSPs are also synthesized constitutively and exhibit important housekeeping functions essential for cellular survival, by their participation in innate and adaptive immune responses, having important roles in peptides delivery. The understanding of these roles allowed the development of different practical applications for these proteins, as adjuvants in vaccination strategies, or in targeting or regulation of the HSP pathogenic mechanisms in new chemotherapeutic strategies. They have a wide distribution and a high homology among different species. This review provides an overview of the roles of HSP in immunity with a focus on the pathogenesis of infectious diseases.

Keywords: *Heat shock proteins, stress response, immunotherapy, vaccination*

1. INTRODUCTION

The pathogenic bacteria life cycle comprises two stages: the first stage is carried out in their ecological niche, and the second one in the infected hosts. In nature, they have to adapt to different environmental conditions. For instance, aquatic bacteria must adapt to physico-chemical changes, while in the host organism, they have to face, in addition to degradative action of proteases, a low pH and higher temperatures than in their natural environment [1].

Environmental parameters changes lead to changes in gene expression, and during the transition in a different phase, bacteria have developed a number of adaptive mechanisms to protect themselves from lethal assaults. Temperature is a major factor in the growth and cell survival, and therefore bacteria have developed the ability to produce specialized proteins, with increased expression when cells are briefly exposed to temperatures above their normal growth temperature, generically called heat shock proteins (HSP - Heat Shock Proteins).

The synthesis of HSPs is a universal phenomenon, occurring in all plant and animal species which have been investigated, including humans. Because HSPs can also be induced by oxidants, toxins, heavy metals, free radicals, viruses, and other stressors, they are also named 'stress proteins' [2].

However, HSPs are not produced only by stressed cells; some HSP are synthesized constitutively and perform important housekeeping functions [3]. Heat shock proteins function mainly as molecular chaperones. They are ubiquitous in the cells, showing a high genetic conservation between species and are essential to ensure proper folding, stabilization and protein transport in any conditions [4]. Their increased synthesis in stress conditions o (such as thermal shock) is explained

[1]Department of Microbiology, Faculty of Biology, University of Bucharest, Ale. Portocalelor 1-3, 60101, Bucharest, Romania

*Corresponding author e-mail address: ***beatrice_gilea@yahoo.com***

by the greater need for prevention of unfolded proteins and newly synthesized polypeptide chains aggregation, as well as of degradation by proteases. In addition to their chaperone role, HSPs are involved in a number of different specialized activities such as transcriptional control [5], diminishing the consequences of mutations [6] and apoptosis control [7]. Heat shock proteins have an important role in regulating the immune response activation, HSP recognition by the immune system providing a mechanism by which it can detect stressed or damaged cells and also can respond to the presence of invasive pathogens. In addition, it was shown that the immune system is able to exploit the molecular chaperone activity of HSP to facilitate the collection of peptides for antigen presentation [8].

An increase in heat shock protein synthesis is associated with an increased virulence of pathogenic bacteria, a process that requires activation of the determinants required for bacterial survival in extreme conditions of the host organism causing the infection, and subsequently triggering the infectious process.

2. ROLE OF HEAT SHOCK PROTEINS IN THE IMMUNE RESPONSE

2.1. Implication of heat shock proteins in initiation of immune response. Heat shock proteins are ubiquitous and homologous in different species, and thus, they represent major antigenic targets of the immune response. It has been demonstrated that bacterial HSPs are immunogenic molecules that stimulate both T and B cells [9, 10]. GroEl proteins, and to a lesser extent, bacterial DnaK may also become major antigens, as their expression is strongly increased under stress. Heat shock proteins are antigenic molecules and the recognition of specific epitopes of so highly conserved antigens can contribute to the immune protection or they can have autoimmune pathological consequences [11-15]. Molecular mimicry between bacterial and human HSPs has been well-documented and may allow micro-organisms to avoid the host's mechanisms of defense [11]. A humoral response against microbial HSPs may be destructive for the host, leading to an autoimmune response. Three models have been proposed to link microbial infections to subsequent autoimmune reactions involving HSPs [12]. These models are based on (i) molecular mimicry between microbial HSPs and HSPs or constitutive proteins from the host, (ii) inflammation-induced exposure of cryptic cell epitopes that could be a target for immune reactions, and (iii) antigen persistence in infected sites leading to chronic immunological reactions. For a long time, HSP autoimmune responses were thought to be the result of cross-reactivity between bacterial and host HSPs. Srivastava *et al.* discovered that HSPs could act as carriers of antigenic peptides derived from tumors or virus-infected cells. These HSP-peptide complexes "shuttle" antigenic peptides to the MHC class I presentation pathway of antigen-presenting cells.

2.2. Role of heat shock proteins in autoimmunity. The immune response to HSP may be directed against autologous molecules and involves T lymphocytes when epitopes are presented by MHC class II molecules [16]. Yamazaki et al. have shown that T cells reactive to HSP60 accumulate in the gingival tissue of patients with periodontitis, and this response is inhibited by class II anti-MHC antibodies [17]. Also, Lo et al. have shown that class Ib MHC molecules are involved in autoimmune infections with Gram-negative bacteria, and identified an immunodominant epitope (GMQFDRGYL) derived from HSP60 family present in *Salmonella typhimurium*, *S. typhi*, *E. coli*, *Y. enterolitica*, *Klebsiella pneumoniae* and *H. pylori* [18]. There are well documented studies on molecular similarity between bacterial heat shock protein and human heat shock proteins. This fact could enable a microorganism to avoid host defense mechanisms [11]. In order to test if the presence of cross-reactive antibodies is correlated with autoimmunity, Qazi et al. analyzed the ability of sera

from immunized and also untreated mice, to bind to a number of antigens to detect auto-antibodies. The results revealed that the presence of cross-reactive antibodies is not necessarily correlated with autoimmunity. This type of antibodies are commonly detected in sera from healthy individuals and they are induced in normal primary immune responses shortly after antigens action. Moreover, the concept of heat shock protein as a cause of autoimmunity is not fully elucidated. For example, it was demonstrated that preventing certain autoimmune conditions can be achieved by pre-treating animals with mycobacterial HSP70 or with a series of epitopes defined in HSP sequences [19]. Immune responses by cross action cannot be explained only by an association between HSP and a range of inflammatory conditions. The HSP effects on the innate immune system should not be overlooked. Heat shock proteins activates inflammatory immune network. GroEl protein has been shown to stimulate the production of interleukin-6 (IL-6) and interleukin-8 (IL-8) by human gingival fibroblasts without affecting their viability [20], and also stimulate the production of IL-6 by a confluent monolayer of gingival epithelial cells, being cytotoxic at high concentrations. HSP are potent molecules that signal damaging tissue and cellular stress to immune system [21]. HSP60 is probably the best activator of human monocytes and dendritic cells [22].

2.3. Heat shock proteins in immunotherapy. Immunogenic properties of DnaK and GroEL proteins were studied in infections with *Flavobacterium columnare*, a Gram-negative bacterium that causes columnaris disease in freshwater fish worldwide. It has been shown that these proteins may serve as important candidate molecule for the development of vaccines against columnaris disease [23]. In addition to their chaperone activity, HSP70 molecules can function as adjuvants in vaccination [24, 25]. HSP70 derived from tumor cells or virus infected cells can generate responses of $CD8^+$ CTL (cytotoxic T lymphocytes) *in vitro* and *in vivo* against a large range of antigens expressed in the cells from which this immunogenic proteins have been purified [8]. Bacterial extracellular HSP70 proteins can bind to antigenic peptides and can activate in the same time a cascade of events, including the presentation of chaperoned peptides to MHC I and MHC II, proinflammatory cytokine secretion and also phenotypic and functional maturation of dendritic cells [24]. These properties make the HSP70 a potent adjuvant integrating the innate immune response and the adaptive one. HSP70 contains epitopes for T cells, serving as carrier molecules for antigens, and induces specific activation of B cells as well as T cells $CD4^+$ and $CD8^+$, without the need for an adjuvant [26]. The effectiveness of inducing a protective immune response against tuberculosis by the protein complex between the fusion protein consisting of MTB ESAT-6 (early secreted antigenic target, 6kDa) as potent immunogenic protein and the C-terminal of HSP70 MTB (HSP70 (359-610)), as carrier protein and adjuvant to induce an effective immune response *in vivo* in mouse models, was also studied [27].

Using heat shock proteins as carrier protein is a particularly interesting fenomenon for the development of vaccination strategies, since all individuals have come into contact with microbial HSPs through natural contact with bacterial agents and anti-tuberculosis vaccines. The C-terminal domain of HSP70 seems to be quite safe and immunogenic, and its function in association with small and light antigens, has led researchers to consider this protein a suitable adjuvant for vaccines [26]. HSP represent dominant antigens in numerous microbial infections, and therefore a potential use of pathogen-derived HSP for vaccination has been suggested. It has been demonstrated that different vaccination strategies using pathogens HSP in various infectious disease models, have induced significant protection. For example, vaccination with GroES and GroEL homologue of *H. pylori* protects mice against infection and gastro-duodenal disease [28]. Vaccination with hsp70 of *Histoplasma capsulatum* enhances host resistance against infection [29] and vaccination with hsp60 from the same pathogen protects mice against pulmonary histoplasmosis [30]. Adoptive transfer of

Yersinia hsp60-reactive CD4 αβ T cells mediates protection against lethal infection with *Y. enterocolitica* in mice [31] and also, immunization of mice with Yersinia hsp60 induces protection against *Y. enterocolitica* [32]. Adoptive transfer of γδ T cells specific for hsp60 of *Plasmodium yoelii* confers partial protection against infection with *P. yoelii* in mice [33]. Adoptive transfer of CD8 T cells specific for mycobacterial hsp60 confers partial protection against *M. bovis* infection in mice [34]. *Mycobacterium tuberculosis* HSP were studied intensively and the results revealed that vaccination with transgenic cell line expressing mycobacterial hsp60 protects mice against infection with *M. tuberculosis* [35] and the adoptive transfer of αβ T cells or γδ T cells specific for mycobacterial hsp60 confers protection against infection with *M. tuberculosis* [36]. Moreover, treatment of mice with DNA encoding the mycobacterial hsp60 and hsp70 protects mice against *M. tuberculosis* infection [37, 38]. The immunization with GroEl and DnaK from *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa* induces protection of holoxenic mice against infectious processes [39, 40].

3. REGULATION OF HEAT SHOCK PROTEINS DURING THE INFECTIOUS PROCESS

The infectious process subjects both bacterial cells and host cells to a series of potentially harmful stress stimuli, such as exposure to extreme pH values, reactive free radicals and degradation enzymes. It was shown that during infection there is an increase of HSP expression in response to these conditions [41-46]. Currently, using the expression profiles obtained using microtests, HSP response can be followed the context of the entire genome. For example, it was noted that immediately after the action of macrophages on *Salmonella enterica*, expression of genes for heat shock regulator *rpoH* (σ32), for homologous protein Hsp70 (yegD) and for heat shock protease HtrA-heat shock and chaperone family members Hsp20 crystalline-like (ibpA, ibpB) is strongly increased with a ratio of induction five times than in normal conditions [46].

Similarly, the gene encoding for a chaperone protein from Hsp20 family (hsp/acr2), in *Mycobacterium tuberculosis*, wich is strongly induced by heat shock [47] is, at the same time, the gene which is induced in the highest amount immediately after phagocytosis [48]. HSP70 (dnaK) and Hsp100 (clpB) chaperones synthesis is also strongly stimulated in intracellular *M. tuberculosis*. Therefore, if *in vivo* expression of HSPs is impressive then HSP induction amplitude can be truly dramatic. For example, it was shown that transcription of *acr* into Hsp20 chaperone of *M. tuberculosis* increases 800 times in mice, compared to *in vitro* culture [48] and expression of GroEL (HSP60) from *Rickettsia prowazekii* increased 50 times in host cells [49]. Bacteria can use various systems of induction and repression to control the heat shock proteins expression [50]. The importance of regulatory mechanisms for pathogenesis is illustrated in the case of *M. tuberculosis*, where chaperones overexpression due to regulator HspR deletion leads to an increased immune response and lower the survival rate of bacteria in experimental models [51]. It was also demonstrated that *Helicobacter pylori* controls the expression of the major heat shock chaperones using HspR mediated repression [52], but in addition, this bacteria uses a post-transcriptional control mechanism through CsrA [53]. In this case, regulation of stress response by CsrA deletion attenuates bacteria during infection [53]. These studies highlight an issue for the pathogen: to survive the initial stages of infection requires a large number of HSP, and in the same time, these proteins provide a strong signal to the host immune response.

4.CONCLUSIONS

Heat shock proteins have an important role in generating an immune response to pathogenic bacteria. Bacteria express these molecules for protection against stress conditions encountered *in vivo*, but however, hosts exploit HSP recognition mechanisms for signaling the presence of pathogens. This represents the ability of the hosts to use the existing recognition systems to detect self-heat shock proteins in infected or destroyed cells, or it may be evolution of specific receptors for pathogens and of signaling mechanisms. In addition, the host can use both its own HSPs and pathogen proteins to signal bacterial antigens through an efficient path for antigen-presenting molecules CMH I. Thus, there is considerable pressure on the pathogen to carefully regulate their HSP expression in order to be effective in survival as well as to minimize the immune system stimulation. There is a significant potential for the use of HSP-dependent immune mechanisms in the delivery of peptides, as well as in acting as an adjuvant in vaccination strategies, or in targeting or regulation of the HSP pathogenic mechanisms in new chemotherapeutic strategies. However, studies about the role of HSPs in autoimmunity have revealed the complexity of HSP involvement in immunity and much is still to understand for a good use of these systems.

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