Mini Review

HSP Roles as Biomarkers and Antigens in Bacterial and Viral Infections

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Diagnosis of infectious diseases remains an important issue in medical science. Identification of biomarkers can be used to predict early infections. Recently, heat shock proteins (HSPs) have been known as the conserved compounds expressed under stress conditions in both prokaryotic and eukaryotic systems. These proteins act as molecular chaperones. Several studies showed the increased levels of HSPs in patients suffering from infectious diseases suggesting the role of HSPs as promising biomarkers. Also, Hsps possess significant roles in antigen presentation, the maturation of dendritic cells and the activation of lymphocytes. Thus, these proteins can be utilized to develop vaccines in bacterial and viral infections. In this mini-review, we will briefly describe the important roles of HSPs in diagnosis and immunity in bacterial and viral infections. *J Med Microbiol Infec Dis, 2016, 4 (1-2): 1-7.*

Keywords: Heat shock protein, Infection, Biomarker, Immunity.

Heat Shock Proteins

Both prokaryotes and eukaryotes tolerate different stress conditions (*e.g.,* metabolic, environmental and pathophysiological stress) by up-regulating the expression of heat shock proteins (HSPs). These proteins are divided into six main families such as Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small heat shock proteins (sHsps) based on their molecular weights (MW) and sequence homology [1, 2]. Among them, sHsps (MW: 12-43 kDa) including Hsp27, αA- and αB-crystallin are distinguished by the presence of a highly conserved sequence (80-100 amino acids) called as the "α-crystallin domain" (ACD) [3-5]. The studies showed that Hsps are targets of the host immune responses (*e.g.,* Hsp60 in bacterial infections) [6] and play a significant role as a biomarker in infectious diseases. For example, women with pre-existing antibody responses to chlamydial Hsp60 (CHsp60) indicated a high risk of developing pelvic inflammatory disease (PID) during a new chlamydial infection as compared to women without CHsp60 antibody [7]. Hsps can elicit strong humoral and cellular immune responses in various infectious diseases [8], [9]. For instance, antibody and T cell responses specific for Hsp60 were induced in leprosy and tuberculosis (TB) patients as well as in individuals vaccinated with *Mycobacterium bovis* BCG [8, 10-12]. In contrast, Hsp70 has an important role in viral infections such as rabies virus infection [13].

The Role of Hsps as a Biomarker in Bacterial and Viral Infections

Bacterial Infections. Determination of valid biomarkers against host protection is necessary for the diagnosis of the pathogens [14, 15]. Because the Hsps are expressed by both prokaryotic and eukaryotic organisms, bacterial Hsps could play a significant role in antibacterial immunity. Generation of humoral and cellular immune responses during leprosy, TB, malaria and trypanosomiasis showed that bacterial Hsps (MW: 65 kDa and 70 kDa) are primary immune targets which can be considered as immunodominant antigens [11, 16, 17]. *Mycobacterium tuberculosis* (MTB) Hsp70 could enhance the levels of IL-12 and RANTES [18], and stimulate $CD8^+$ T cell responses infusion form of Hsp70 protein [19-22]. The studies indicated that TB caused by MTB is still a significant health problem in the world [23] [24]. Thus, it is necessary to determine the potential biomarkers, *e.g.,* Hsps. For instance, the host and MTB Hsps were notably enhanced in the sera and CSF samples of pulmonary TB patients. The data showed that alteration in immune response led to a change in both levels of host (*i.e.,* Hsp70, Hsp60, Hsp90 and Hsp25) and MTB Hsps (*i.e.,* Hsp16, Hsp65 and Hsp71), suggesting them as possible biomarkers for these infections [25]. A study indicated that the environmental factors might act through the modified stability of Hsps especially MTB Hsp65 during autoimmune diseases [26]. Several years ago, a Hsp was characterized in some strains of *Helicobacter pylori* named as HspB [27, 28]. This protein was shown to enhance the risk of gastric carcinoma in patients with the *H. pylori*-positive strain [29]. The studies indicated that co-expression of *H. pylori's* proteins CagA and HspB in AGS cells generates an increased level of c-jun protein, E2F transcription factor, cyclin D3, and phosphorylated retinoblastoma protein, involved in the transition from G_1 to S phase. Also, an increase in cell proliferation was observed due to a high accumulation of the cells in the S-G2-M phase of the cell cycle.

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These data suggested that CagA and HspB, independent from the bacterial infection, have a direct effect on the growth of the gastric cells [30]. T lymphocytes specific for recombinant *Mycobacterium bovis* bacille Calmette-Guerin (BCG) Hsp65 isolated from joints of rheumatoid arthritis (RA) patients were found to modulate the development of arthritis in rats [31-33]. Indeed, in the rats pretreated with Hsp65 as an antigen, arthritis induction could be suppressed against streptococcal cell wall [34], MTB [35], pristane [36] and collagen II [37] challenge. Thompson *et al*. [36] showed that mice injected with *Escherichia coli* equivalent GroEL have no effect on pristane-induced arthritis. Thus, further experiments are needed to determine whether the *E. coli* Hsps (GroEL, 60 kD, and DnaK or 70 kD) can mediate similar effects as the mycobacterial homologs on rat arthritis [38]. On the other hand, during persistent infections, *Chlamydia trachomatis* (CT) also produced a significant amount of Hsp60 (CT-Hsp60) [39], involved in the pathogenesis of autoimmune disorders (*e.g.,* arthritis) [33, 40]. CT-Hsp60 could induce the generation of pro-inflammatory cytokines in endothelial and smooth-muscle cells, and macrophages [41], and also activate the specific immune cells via a Toll-like receptor [42]. During cell stress or carcinogenesis, the chaperonin (Hsp60) is exposed on the cell surface or is secreted from cells into the extracellular space and circulation. The secreted Hsp60 can develop diseases such as autoimmune arthritis, multiple sclerosis, atherosclerosis (ATS), vasculitis, diabetes, and thyroiditis [43]. Moreover, humoral immune responses to bacterial Hsp60 (*e.g., Chlamydia pneumonia* and *E. coli* Hsp60) were indicated to be involved in vascular endothelial injury during ATS pathogenesis [44, 45]. The *Porphyromonas gingivalis* [46-48] and *H. pylori* [49] infections were correlated with a higher risk of coronary ATS because of high cross-reactivity of anti-microbial Hsp60 antibodies with human chaperonin. For the same reasons, *E. coli* Hsp60 was also used in the pathogenesis of autoimmune rheumatic [50] and pancreatic [51] diseases, and Sjogren syndrome [52]. Moreover, high levels of autoantibodies against endogenous Hsp60 could promote the onset of diabetes in cystic fibrosis patients [53]. Women with pre-existing antibody responses to the recombinant chlamydial Hsp60 (CHsp60) demonstrated an increased risk of developing CT pelvic inflammatory disease (PID) during a new chlamydial infection as compared to the risk in women without CHsp60 antibody. The reports showed that antibody levels to CHsp60 predict a 2- to 3-fold increased risk for CT PID [7]. *Streptococcus pneumoniae* is a major bacterial cause of pediatric pleural infection in adults [54]. The presence of extracellular Hsp70 may have broad biological significance in pediatric pleural infection. Hsp70 is present in different body fluids such as normal serum [55], cerebrospinal [56, 57], synovial [58] and bronchoalveolar lavage fluid [59]. The authors showed that pleural mesothelial cells could release Hsp72 in response to bacterial infection and levels were raised in infectious pleural effusions [60]. Table 1 lists the roles of Hsps as biomarkers in infectious diseases.

Table 1. HSPs as biomarkers in infectious diseases

Hsp	Pathogen	Strain	Disease	Reference (s)
Hsp65	Bacteria	*Mycobacterium leprae*	Autoimmune Disease	[26]
HspB	Bacteria	*H. pylori*	Gastric cancer	[30]
Hsp71-Hsp65	Bacteria	MTB	Arthritis	[35, 38]
Hsp16, Hsp65, Hsp71	Bacteria	MTB	Pulmonary and extrapulmonary tuberculosis	[25]
Hsp60	Bacteria	*E. coli*	Autoimmune rheumatic and pancreatic diseases, Sjogren syndrome	[50-52]
Hsp60	Bacteria	*Chlamydia trachomatis pelvic*	Tubal infertility and ectopic pregnancy	[7]
Hsp70	Viral	Rabies virus	Fatal disease	[13]
Hsp70, Hsp90	Viral	Dengue	Dengue fever to a hemorrhagic fever (DHF)	[66]
Hsp72	Viral	HCV	Hepatitis	[67]
Hsp90, Hsp60	Viral	HBV	Hepatitis	[68, 72]
Hsp90	Viral	Ebola virus (EBOV)	Hemorrhagic fever	[81]
Hsp40	Viral	HIV	Acquired immune deficiency syndrome	[83]

Viral Infections. During viral infections, a lot of viral proteins are generated and folded by HSPs molecular chaperones [13]. The studies indicated that rabies virus infection induces the cellular expression of Hsp70, which accumulates in Negri body-like (NBL) structures, *i.e.,* the sites of viral transcription and replication [61, 62]. Hsp70 protein was located in purified nucleocapsids from infected cells and also in purified virions. It can interact with N-nucleoprotein. The data indicated that down-regulation of Hsp70 using specific chaperone inhibitors led to a significant decrease in the levels of viral mRNAs, proteins and virus particles [13]. Other chaperones could play a role in rabies viral RNA syntheses such as Hsp60 [63] and Hsp40 [64]. The recent studies represented that Hsp70 protein was associated with complexes formed between Hsp40 and the viral protein Nef in HIV-infected cells suggesting their role in viral gene expression and replication [65]. Also, Hsp90 and Hsp70 proteins from human blood monocytes could interact with Dengue (DEN) virus E protein and participate in virus entry as a receptor complex in human cell lines (*e.g.,* neuroblastoma cells) as well as in monocytes/macrophages. Both Hsps were associated with membrane lipid rafts in response to dengue virus infection [66]. On the other hand, Hsp72 could play a positive regulatory role in the hepatitis C virus (HCV) RNA replication by increasing levels of the replicase complex. This function was correlated with the enhanced stability of the viral proteins in the replicase complex and/or to the

increased translational activity of the internal ribosome entry site of HCV [67]. Regarding the published data, Hsp90 can enhance HBV capsid stability by interacting with HBV core protein *in vitro* and *in vivo*. Indeed, down-regulation of Hsp90 decreased HBV production in HepG2.2.15 cells [68]. Some results showed that a decrease in HBV replication could reduce the frequency of Treg cells in patients with chronic hepatitis B [69-71]. A recent study indicated the correlation of HBcAg-specific IL-10-secreting Treg cells and the serum level of Hsp60 in patients with chronic hepatitis B. As observed, the serum level of Hsp60 in patients with chronic HBV was significantly higher than that in patients with chronic HCV. Moreover, pre-incubation of CD4+CD25+cells with the recombinant Hsp60 significantly enhanced the frequency of HBcAg-specific IL10-secreting Treg cells [72]. In general, Hsp90 was shown to be an important host factor for the replication of negative-strand viruses [73]. For instance, the inhibition of Hsp90 blocked the replication of *vaccinia* virus by interaction with the viral core protein 4a in the cytoplasm [74]. In the HCV life cycle, Hsp90 was necessary for proper cleavage of newly synthesized HCV NSP2/3 protein [75, 76] and also the activity of HBV reverse transcriptase [77-79]. In *polio virus*, Hsp90 was needed for proper folding of the viral capsid protein, and Hsp90 inhibitors showed antiviral activity [80]. On the other hand, the data demonstrated that inhibition of Hsp90 significantly decreased the replication of Ebola virus (EBOV) [81]. Recently, a report indicated that HIV-1 Nef protein could interact with Hsp40 as well as it can induce the expression of Hsp40 in HIV-1-infected cells leading to enhancement of viral gene expression and virus replication through modulating the activity of positive transcription elongation factor b (P-TEFb). The similar result was obtained in avian *adenovirus* CELO which the viral protein Gam1 induced Hsp40 expression leading to the development of viral replication [82, 83]. The evaluation of human *papillomavirus* (HPV)-related cervical cancer patients' seroreactivities against three recombinant proteins such as HPV E7, the N-terminal of gp96 (NT-gp96) and C-terminal of gp96 (CT-gp96) showed significantly higher levels of these markers in squamous cell carcinoma (SCC), but not in adenocarcinoma and control groups. It should be noted that glycoprotein 96 (gp96), an endoplasmic reticulum (ER) molecular chaperone, has been known as a potent adjuvant for inducing immune responses in vaccine development [84].

Hsps in Vaccine Development against Bacterial and Viral Infections

The Hsps have recently been reported to play significant roles in antigen presentation, the activation of lymphocytes, and the maturation of dendritic cells [85]. Thus, the immuno-stimulatory properties of Hsps were used to develop prophylactic vaccines against infectious diseases especially viral and bacterial diseases. These Hsp-based vaccines were generated as Hsp-antigen conjugates and/or recombinant Hsp combined with selected antigens against challenging diseases [86]. The protective efficiency of Hsps has been reported against different infections including *Plasmodium yoelii* [87], *Brugia malayi* [88], *Leishmania donovani* [89], and *Hantaan virus* [90]. Among Hsps, Hsp70 is an immunodominant antigen during infections caused by various pathogens [86, 91, 92]. The BCG vaccine is only available vaccine used against TB in the world [93]. The recent studies showed that injection of the Hsp65-IL2 DNA vaccine in mice enhanced Th1-type cellular responses by producing greater amounts of IFN-γ and IL-2 with a higher titer of antigen-specific anti-Hsp65 IgG2a. This DNA vaccine was able to induce both CD4 and CD8 T-cell responses with a high activity of antigen-specific cytotoxicity against target cells as compared to the BCG vaccine. Moreover, after treatment with the DNA vaccine, the bacterial numbers in TB-infected mice were significantly reduced. The protective and therapeutic effects of the IL-2 and Hsp65 fusion DNA vaccine were superior to that of the Hsp65 DNA vaccine against TB in mice by improving the Th1 response [94]. Chiohn *et al.* showed that vaccination with *H. pylori* Hsp60 (GroEL/S) as subcutaneous or respiratory mucosal route stimulated a high antibody response and gastric cytokine levels. The level of protection induced by non-adjuvanted Hsp60 vaccine against live challenge with *H. pylori* was similar to vaccination with adjuvanted vaccines [95]. Furthermore, GroEL was known as an effective immunomodulator against *Bacillus anthracis* infection. Indeed, anti-GroEL IgG antibody could enhance spore uptake by phagocytes and the next killing of the spores. GroEL could increase nitric oxide (NO) release from lymphocytes and decrease bacterial load from the organs, likely via the activation of macrophages and over-expression of certain innate immunity receptors [81]. Recent immunological studies suggested that innate immunity plays an important role in host defense against MTB infection [96]. One study showed that the recombinant mycobacterial Hsp65 and Hsp70 proteins induce NF-κB activity through TLR-4 and TLR-2 signaling receptors in human endothelial cells, respectively [97]. Up to now, many different therapeutic vaccines were designed using Hsps as an antigen or adjuvant against viral infections. Some studies indicated that immune response reside within N- or C-terminal fragments of Hsps [98]. For example, a report showed that subcutaneous injection of E7 DNA linked to the C-terminal of gp96 (CT-gp96) fragment could significantly increase the potency of DNA vaccines against *human papillomavirus* (HPV) infections [99].

Hsps in Parasites

Babesial parasites infect many mammalian species such as cattle, dogs, horses, and humans [100, 101]. The studies showed that Hsp20 of *Babesia orientalis* (BoHSP20) was an immunodominant antigen and a useful diagnostic reagent to detect antibodies against this parasite in water buffalo [102]. A 100 kDa Hsp, Hsp100, is abundant in the intracellular amastigote stage of *Leishmania major* which persists in the mammalian host. In experimental infections of BALB/c mice, the lack of Hsp100 in the gene replacement mutants led to a delayed lesion development as compared to that in infections with wild-type *L. major* [103]. Moreover, Hsp23 expression is a prerequisite for *L. donovani* survival at mammalian host temperatures and a

crucial virulence factor [104]. A report showed that a new Hsp in *Leishmania amazonensis* belonging to the sHSP family, Hsp20, possesses antigenic properties during *Leishmania* infection [105]. Another study indicated that a combined vaccine including DNA encoding P4 and Hsp70 stimulated a significant protection in mice against *L. amazonensis*, but no protection was observed after injection of these genes alone [106]. Recently, it has been reported that vaccination with *Toxoplasma gondii* Hsp30 gene, a member of the small HSP family, elicited protection in mice against a challenge with this parasite [107].

This mini-review has attempted to summarize the roles of HSPs as biomarkers in bacterial and viral infections. It has also shown some data about their role as an antigen in infectious diseases. Generally, the presence of extracellular HSPs may have an extensive biological significance in infections. The Hsps can induce potent immune responses, thus promoting anti-infectious or auto-aggressive immune responses directed against unique pathogen- or disease-associated antigens, respectively. However, there are some debates about the roles of Hsps in infectious diseases and immunity which should be further determined in future studies.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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