J Med Microbiol Infec Dis, 2016, 4 (1-2): 1-7

Mini Review

HSP Roles as Biomarkers and Antigens in Bacterial and Viral Infections

Alireza Milani, Marzieh Basirnejad, Sepideh Shahbazi, Azam Bolhassani*

Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

Received Apr 24, 2017; Accepted Apr 29, 2017

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.

***Correspondence:** Azam Bolhassani

Department of Hepatitis and AIDS, Pasteur Institute of Iran, No. 69, Pasteur Ave, Tehran, Iran, 1316943551.

Email: azam.bolhassani@yahoo.com

Tel: +98 (21) 64112240 **Fax:** +98 (21) 66465132

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.

ACKNOWLEDGEMENT

This study is supported by Pasteur Institute of Iran.

CONFLICT OF INTEREST

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

REFERENCES

1. Bakthisaran R, Tangirala R, Rao ChM. Small heat shock proteins: role in cellular functions and pathology. Biochim Biophys Acta. 2015; 1854 (4): 291-319.

2. Studer S, Narberhaus F. Chaperone activity and homo-and hetero-oligomer formation of bacterial small heat shock proteins. J Biol Chem. 2000; 275 (47): 37212-8.

3. Franck E, Madsen O, van Rheede T, Ricard G, Huynen MA, de Jong WW. Evolutionary diversity of vertebrate small heat shock proteins. J Mol Evol. 2004; 59 (6): 792-805.

4. Kriehuber T, Rattei T, Weinmaier T, Bepperling A, Haslbeck M, Buchner J. Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J. 2010; 24 (10): 3633-42.

5. Kappé G, Franck E, Verschuure P, Boelens WC, Leunissen JA, de Jong WW. The human genome encodes 10 α-crystallin–related small heat shock proteins: HspB1–10. Cell Stress Chaperones. 2003; 8 (1): 53-61.

6. Pérez-Morales D, Espinoza B. The role of small heat shock proteins in parasites. Cell Stress Chaperones. 2015; 20 (5): 767-80.

7. Peeling RW, Kimani J, Plummer F, Maclean I, Cheang M, Bwayo J, Brunham RC. Antibody to chlamydial hsp60 predicts an increased risk for chlamydial pelvic inflammatory disease. J Infect Dis. 1997; 175 (5): 1153-8.

8. Shinnick TM. Heat shock proteins as antigens of bacterial and parasitic pathogens. Heat shock proteins and immune response. 1990; 145-60.

9. Polla BS. Heat shock proteins in host-parasite interactions. Immunol Today. 1991; 12 (3): A38-41.

10. Mustafa A, Lundin K, Oftung F. Human T cells recognize mycobacterial heat shock proteins in the context of multiple HLA-DR molecules: studies with healthy subjects vaccinated with Mycobacterium bovis BCG and Mycobacterium leprae. Infect Immun. 1993; 61 (12): 5294-301.

11. Young D, Lathigra R, Hendrix R, Sweetser D, Young RA. Stress proteins are immune targets in leprosy and tuberculosis. Proc Natl Acad Sci U S A. 1988; 85 (12): 4267-70.

12. Young RA. Stress proteins and immunology. Annu Rev Immunol. 1990; 8 (1): 401-20.

13. Lahaye X, Vidy A, Fouquet B, Blondel D. Hsp70 protein positively regulates rabies virus infection. J Virol. 2012; 86 (9): 4743-51.

14. Cooper AM. Cell-mediated immune responses in tuberculosis. Annu Rev Immunol. 2009; 27: 393-422.

15. Dheda K, Schwander SK, Zhu B, Van ZS, Richard N, ZHANG Y. The immunology of tuberculosis: from bench to bedside. Respirology. 2010; 15 (3): 433-50.

16. Britton WJ, Garsia RJ, Hellqvist L, Watson DJ, Basten A. The characterization and immunoreactivity of a 70 kD protein common to Mycobacterium leprae and Mycobacterium bovis (BCG). Lepr Rev. 1986; 57: 67-75.

17. Britton WJ, Hellqvist L, Basten A, Inglis AS. Immunoreactivity of a 70 kD protein purified from Mycobacterium bovis Bacillus Calmette-Guerin by monoclonal antibody affinity chromatography. J Exp Med. 1986; 164 (3): 695-708.

18. Wang Y, Kelly CG, Karttunen JT, Whittall T, Lehner PJ, Duncan L, MacAry P, Younson JS, Singh M, Oehlmann W, Cheng G, Bergmeier L, et al. CD40 is a cellular receptor mediating mycobacterial heat shock protein 70 stimulation of CC-chemokines. Immunity. 2001; 15 (6): 971-83.

19. Huang Q, Richmond JF, Suzue K, Eisen HN, Young RA. In vivo cytotoxic T lymphocyte elicitation by mycobacterial heat shock protein 70 fusion proteins maps to a discrete domain and is CD4+ T cell independent. J Exp Med. 2000; 191 (2): 403-8.

20. Cho BK, Palliser D, Guillen E, Wisniewski J, Young RA, Chen J, Eisen HN. A proposed mechanism for the induction of cytotoxic T lymphocyte production by heat shock fusion proteins. Immunity. 2000; 12 (3): 263-72.

21. Harmala LA, Ingulli EG, Curtsinger JM, Lucido MM, Schmidt CS, Weigel BJ, Blazar BR, Mescher MF, Pennell CA. The adjuvant effects of Mycobacterium tuberculosis heat shock protein 70 result from the rapid and prolonged activation of antigen-specific CD8+ T cells *in vivo*. J Immunol. 2002; 169 (10): 5622-9.

22. Suzue K, Zhou X, Eisen HN, Young RA. Heat shock fusion proteins as vehicles for antigen delivery into the major histocompatibility complex class I presentation pathway. Proc Natl Acad Sci. 1997; 94 (24): 13146-51.

23. India T. RNTCP Status report. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi-110001. 2010.

24. Katti MK. Pathogenesis, diagnosis, treatment, and outcome aspects of cerebral tuberculosis. Med Sci Monit. 2004; 10 (9): RA215-29.

25. Shekhawat SD, Jain RK, Gaherwar HM, Purohit HJ, Taori GM, Daginawala HF, Kashyap RS. Heat shock proteins: possible biomarkers in pulmonary and extrapulmonary tuberculosis. Hum Immunol. 2014; 75 (2): 151-8.

26. Parada CA, Portaro F, Marengo EB, Klitzke CF, Vicente EJ, Faria M, Sant'Anna OA, Fernandes BL. Autolytic Mycobacterium leprae Hsp65 fragments may act as biological markers for autoimmune diseases. Microb Pathog. 2011; 51 (4): 268-76.

27. Macchia G, Massone A, Burroni D, Covacci A, Censini S, Rappuoli R. The Hsp60 protein of *Helicobacter pylori*: structure and immune response in patients with gastroduodenal diseases. Mol Microbiol. 1993; 9 (3): 645-52.

28. Dunn BE, Roop RM, Sung CC, Sharma SA, Perez-Perez GI, Blaser MJ. Identification and purification of a cpn60 heat shock protein homolog from *Helicobacter pylori*. Infect Immun. 1992; 60 (5): 1946-51.

29. Iaquinto G, Todisco A, Giardullo N, D'Onofrio V, Pasquale L, De Luca A, Andriulli A, Perri F, Rega C, De Chiara G, Landi M, Taccone W, et al. Antibody response to *Helicobacter pylori* CagA and heat-shock proteins in determining the risk of gastric cancer development. Dig Liver Dis. 2000; 32 (5): 378-83.

30. De Luca A, Baldi A, Russo P, Todisco A, Altucci L, Giardullo N, Pasquale L, Iaquinto S, D'Onofrio V, Parodi MC, Paggi MG, Iaquinto G. Coexpression of *Helicobacter pylori's* proteins CagA and HspB induces cell proliferation in AGS gastric epithelial cells, independently from the bacterial infection. Cancer Res. 2003; 63 (19): 6350-6.

31. Breedveld F, Van Embden JA, Schaar C, Van Eden W, Cohen I, De Vries RP. Synovial fluid T cell reactivity against 65 kD heat shock protein of mycobacteria in early chronic arthritis. Lancet. 1988; 332 (8609): 478-80.

32. Gaston JH, Life PF, Jenner PJ, Colston M, Bacon PA. Recognition of a mycobacteria-specific epitope in the 65-kD heat-shock protein by synovial fluid-derived T cell clones. J Exp Med. 1990; 171 (3): 831-41.

33. van Eden W, Tholet JE, van der Zee R, Noordzij A, van Embden JD, Hensen EJ, Cohen IR. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. 1988; 331 (6152): 171-3.

34. van den Broek MF, Hogervorst E, Van Bruggen M, Van Eden W, van der Zee R, Van den Berg W. Protection against streptococcal cell wall-induced arthritis by pretreatment with the 65-kD mycobacterial heat shock protein. J Exp Med. 1989; 170 (2): 449-66.

35. Billingham ME, Carney S, Butler R, Colston MJ. A mycobacterial 65-kD heat shock protein induces antigen-specific suppression of adjuvant arthritis, but is not itself arthritogenic. J Exp Med. 1990; 171 (1): 339-44.

36. Thompson SJ, Rook GA, Brealey RJ, Zee RVD, Elson CJ. Autoimmune reactions to heat shock proteins in pristine induced arthritis. Eur J Immunol. 1990; 20 (11): 2479-84.

37. Ito J, Krco C, Yu D, Luthra H, David C. Preadministration of a 65 kDa heat-shock protein, groEL, inhibits collagen induced arthritis in mice. J Cell Biochem A. 1991; 15: 284.

38. Kingston A, Hicks C, Colston M, Billingham M. A 71kD heat shock protein (hsp) from Mycobacterium tuberculosis has modulatory effects on experimental rat arthritis. Clin Exp Immunol. 1996; 103 (1): 77-82.

39. Bavoil P, Stephens R, Falkow S. A soluble 60 kilo Dalton antigen of Chlamydia spp. is a homologue of Escherichia coli GroEL. Mol Microbiol. 1990; 4 (3): 461-9.

40. Anderton SM, Van Der Zee R, Prakken B, Noordzij A, Van Eden W. Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. J Exp Med. 1995; 181 (3): 943-52.

41. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. J Clin Invest. 1999; 103 (4): 571-7.

42. Stephen RS. The cellular paradigm of Chlamydia pathogenesis. Trends Microbiol. 2003; 11 (1): 44-51.

43. Cappello F, de Macario EC, Di Felice V, Zummo G, Macario AJ. Chlamydia trachomatis infection and anti-Hsp60 immunity: the two sides of the coin. PLoS Pathog. 2009; 5 (8): e1000552.

44. Mayr M, Metzler B, Kiechl S, Willeit J, Schett G, Xu Q, Wick G. Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of *Escherichia coli* and *Chlamydia pneumoniae*. Circulation. 1999; 99 (12): 1560-6.

45. Knoflach M, Mayrl B, Mayerl C, Sedivy R, Wick G. Atherosclerosis as a paradigmatic disease of the elderly: role of the immune system. Immunol Allergy Clin North Am. 2003; 23 (1): 117-32.

46. Choi JI, Chung SW, Kang HS, Rhim B, Kim SJ, Kim SJ. Establishment of Porphyromonas gingivalis heat-shock-protein-specific T-cell lines from atherosclerosis patients. J Dent Res. 2002; 81 (5): 344-8.

47. Chung SW, Kang HS, Park HR, Kim SJ, Kim SJ, Choi JI. Immune responses to heat shock protein in Porphyromonas gingivalis infected periodontitis and atherosclerosis patients. J Periodontal Res. 2003; 38 (4): 388-93.

48. Yamazaki K, Ohsawa Y, Itoh H, Ueki K, Tabeta K, Oda T, Nakajima T, Yoshie H, Saito S, Oguma F, Kodama M, Aizawa Y, et al. T cell clonality to Porphyromonas gingivalis and human heat shock protein 60s in patients with atherosclerosis and periodontitis. Oral Microbiol Immunol. 2004; 19 (3): 160-7.

49. Okada T, Ayada K, Usui S, Yokota K, Cui J, Kawahara Y, Inaba T, Hirohata S, Mizuno M, Yamamoto D, Kusachi S, Matsuura E, et al. Antibodies against heat shock protein 60 derived from Helicobacter pylori: diagnostic implications in cardiovascular disease. J Autoimmun. 2007; 29 (2-3): 106-15.

50. Yokota SI, Hirata D, Minota S, Higashiyama T, Kurimoto M, Yanagi H, Yura T, Kubota H. Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases: comparison with antibodies against other Hsp60 family proteins. Cell Stress Chaperones. 2000; 5 (4): 337-46.

51. Szebeni A, Schloot N, Kecskemeti V, Hosszufalusi N, Panczel P, Prohászka Z, Füst G, Uray K, Hudecz F, Meierhoff G. Th1 and Th2 cell responses of type 1 diabetes patients and healthy controls to human heat-shock protein 60 peptides AA437-460 and AA394-408. Inflamm Res. 2005; 54 (10): 415-9.

52. Shovman O, Sherer Y, Gliburd B, Gerli R, Bocci E, Monache Fd, Luccioli F, Shoenfeld Y. Low levels of heat shock proteins-60 and-65 autoantibodies in Sjogren's syndrome. Isr Med Assoc J. 2005; 7 (12):778-80.

53. Jensen P, Johansen HK, Carmi P, Høiby N, Cohen IR. Autoantibodies to pancreatic hsp60 precede the development of glucose intolerance in patients with cystic fibrosis. J Autoimmun. 2001; 17 (2): 165-72.

54. Tobin CL, Lee YG. Pleural infection: what we need to know but don't. Curr Opin Pulm Med. 2012; 18 (4): 321-5.

55. Pockley A, Shepherd J, Corton J. Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. Immunol Invest. 1998; 27 (6): 367-77.

56. Hecker JG, Sundram H, Zou S, Praestgaard A, Bavaria JE, Ramchandren S, McGarvey M. Heat shock proteins HSP70 and HSP27 in the cerebral spinal fluid of patients undergoing thoracic aneurysm repair correlate with the probability of postoperative paralysis. Cell Stress Chaperones. 2008; 13 (4): 435-46.

57. Kang R, Cao LZ, Tang DL, Zhang GY, Yu Y, Xiao XZ. Significance of heat shock protein 70 in cerebrospinal fluid in differential diagnosis of central nervous system infection in children. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2007; 19 (6): 346-8 [In Chinese].

58. Suzuki T, Segami N, Nishimura M, Hattori H, Nojima T. Analysis of 70Kd heat shock protein expression in patients with internal derangement of the temporomandibular joint. Int J Oral Maxillofac Surg. 2000; 29 (4): 301-4.

59. Wood KL, Nunley DR, Moffatt-Bruce S, Pope-Harman A, Huang Q, Shamo EN, Phillips GS, Baran C, Batra S, Marsh CB, Doseff AI. The role of heat shock protein 27 in bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant. 2010; 29 (7): 786-91.

60. Varano Della Vergiliana JF, Lansley SM, Porcel JM, Bielsa S, Brown JS, Creaney J, Temple SE, Waterer GW, Lee YC. Bacterial infection elicits heat shock protein 72 release from pleural mesothelial cells. PloS one. 2013; 8 (5): e63873.

61. Lahaye X, Vidy A, Pomier C, Obiang L, Harper F, Gaudin Y, Blondel D. Functional characterization of Negri bodies (NBs) in rabies virus-infected cells: Evidence that NBs are sites of viral transcription and replication. J Virol. 2009; 83 (16): 7948-58.

62. Sagara J, Kawai A. Identification of heat shock protein 70 in the rabies virion. Virology. 1992; 190 (2): 845-8.

63. Qanungo KR, Shaji D, Mathur M, Banerjee AK. Two RNA polymerase complexes from vesicular stomatitis virus-infected cells that carry out transcription and replication of genome RNA. Proc Natl Acad Sci U S A. 2004; 101 (16): 5952-7.

64. Couturier M, Buccellato M, Costanzo S, Bourhis JM, Shu Y, Nicaise M, Desmadril M, Flaudrops C, Longhi S, Oglesbee M. High affinity binding between Hsp70 and the C terminal domain of the measles virus nucleoprotein requires an Hsp40 co chaperone. J Mol Recognit. 2010; 23 (3): 301-15.

65. Kumar M, Rawat P, Khan SZ, Dhamija N, Chaudhary P, Ravi DS, Mitra D. Reciprocal regulation of human immunodeficiency virus-1 gene expression and replication by heat shock proteins 40 and 70. J Mol Biol. 2011; 410 (5): 944-58.

66. Reyes-del Valle J, Chávez-Salinas S, Medina F, del Angel RM. Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. J Virol. 2005; 79 (8): 4557-67.

67. Chen YJ, Chen YH, Chow LP, Tsai YH, Chen PH, Huang CY, Chen WT, Hwang LH. Heat shock protein 72 is associated with the hepatitis C virus replicase complex and enhances viral RNA replication. J Biol Chem. 2010; 285 (36): 28183-90.

68. Shim HY, Quan X, Yi YS, Jung G. Heat shock protein 90 facilitates formation of the HBV capsid via interacting with the HBV core protein dimers. Virology. 2011; 410 (1): 161-9.

69. Peng G, Li S, Wu W, Sun Z, Chen Y, Chen Z. Circulating CD4+ CD25+ regulatory T cells correlate with chronic hepatitis B infection. Immunology. 2008; 123 (1): 57-65.

70. Barboza L, Salmen S, Goncalves L, Colmenares M, Peterson D, Montes H, Cartagirone R, Gutiérrez Mdel C, Berrueta L. Antigen-induced regulatory T cells in HBV chronically infected patients. Virology. 2007; 368 (1): 41-9.

71. Manigold T, Racanelli V. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. Lancet Infect Dis. 2007; 7 (12): 804-13.

72. Kondo Y, Ueno Y, Kobayashi K, Kakazu E, Shiina M, Inoue J, Tamai K, Wakui Y, Tanaka Y, Ninomiya M, Obara N, Fukushima K, et al. Hepatitis B virus replication could enhance regulatory T cell activity by producing soluble heat shock protein 60 from hepatocytes. J Infect Dis. 2010; 202 (2): 202-13.

73. Connor JH, McKenzie MO, Parks GD, Lyles DS. Antiviral activity and RNA polymerase degradation following Hsp90 inhibition in a range of negative strand viruses. Virology. 2007; 362 (1): 109-19.

74. Hung JJ, Chung CS, Chang W. Molecular chaperone Hsp90 is important for vaccinia virus growth in cells. J Virol. 2002; 76 (3): 1379-90.

75. Ujino S, Yamaguchi S, Shimotohno K, Takaku H. Heat-shock protein 90 is essential for stabilization of the hepatitis C virus nonstructural protein NS3. J Biol Chem. 2009; 284 (11): 6841-6.

76. Waxman L, Whitney M, Pollok BA, Kuo LC, Darke PL. Host cell factor requirement for hepatitis C virus enzyme maturation. Proc Natl Acad Sci. 2001; 98 (24): 13931-5.

77. Hu J, Seeger C. Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. Proc Natl Acad Sci U S A. 1996; 93 (3): 1060-4.

78. Hu J, Toft DO, Seeger C. Hepadnavirus assembly and reverse transcription require a multi component chaperone complex which is incorporated into nucleocapsids. EMBO J. 1997; 16 (1): 59-68.

79. Stahl M, Retzlaff M, Nassal M, Beck J. Chaperone activation of the hepadnaviral reverse transcriptase for template RNA binding is established by the Hsp70 and stimulated by the Hsp90 system. Nucleic Acids Res. 2007; 35 (18): 6124-36.

80. Geller R, Vignuzzi M, Andino R, Frydman J. Evolutionary constraints on chaperone-mediated folding provide an antiviral approach refractory to development of drug resistance. Genes Dev. 2007; 21 (2): 195-205.

81. Smith DR, McCarthy S, Chrovian A, Olinger G, Stossel A, Geisbert TW, Hensley LE, Connor JH. Inhibition of heat-shock protein 90 reduces Ebola virus replication. Antiviral Res. 2010; 87 (2): 187-94.

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

83. Kumar M, Mitra D. Heat shock protein 40 is necessary for human immunodeficiency virus-1 Nef-mediated enhancement of viral gene expression and replication. J Biol Chem. 2005; 280 (48): 40041-50.

84. Bolhassani A, Zahedifard F, Taslimi Y, Taghikhani M, Nahavandian B, Rafati S. Antibody detection against HPV16 E7 & GP96 fragments as biomarkers in cervical cancer patients. Indian J Med Res. 2009; 130 (5): 533-41.

85. Corigliano MG, Fenoy I, Sander V, Maglioco A, Goldman A, Clemente M. Plant heat shock protein 90 as carrier-adjuvant for immunization against a reporter antigen. Vaccine. 2013; 31 (49): 5872-8.

86. McNulty S, Colaco CA, Blandford LE, Bailey CR, Baschieri S, Todryk S. Heat shock proteins as dendritic cell targeting vaccines–getting warmer. Immunology. 2013; 139 (4): 407-15.

87. Sanchez GI, Sedegah M, Rogers WO, Jones TR, Sacci J, Witney A, Carucci DJ, Kumar N, Hoffman SL. Immunogenicity and Protective Efficacy of aPlasmodium yoelii Hsp60 DNA Vaccine in BALB/c Mice. Infect Immun. 2001; 69 (6): 3897-905.

88. Dakshinamoorthy G, Samykutty AK, Munirathinam G, Shinde GB, Nutman T, Reddy MV, Kalyanasundaram R. Biochemical characterization and evaluation of a Brugia malayi small heat shock protein as a vaccine against lymphatic filariasis. PloS one. 2012; 7 (4): e34077.

89. Kaur T, Sobti R, Kaur S. Cocktail of gp63 and Hsp70 induces protection against *Leishmania donovani* in BALB/c mice. Parasite Immunol. 2011; 33 (2): 95-103.

90. Li J, Li KN, Gao J, Cui JH, Liu YF, Yang SJ. Heat shock protein 70 fused to or complexed with hantavirus nucleocapsid protein significantly enhances specific humoral and cellular immune responses in C57BL/6 mice. Vaccine. 2008; 26 (25): 3175-87.

91. Colaco CA, Bailey CR, Walker KB, Keeble J. Heat shock proteins: stimulators of innate and acquired immunity. BioMed Research International. 2013; 2013.

92. Bolhassani A, Rafati S. Heat-shock proteins as powerful weapons in vaccine development. Expert Rev Vaccines. 2008; 7 (8): 1185-99.

93. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. Lancet. 1995; 346 (8986): 1339-45.

94. Changhong S, Hai Z, Limei W, Jiaze A, Li X, Tingfen Z, Zhikai X, Yong Z. Therapeutic efficacy of a tuberculosis DNA vaccine encoding heat shock protein 65 of Mycobacterium tuberculosis and the human interleukin 2 fusion gene. Tuberculosis (Edinb). 2009; 89 (1): 54-61.

95. Chionh YT, Arulmuruganar A, Venditti E, Ng GZ, Han J-X, Entwisle C, Ang CS, Colaco CA, McNulty S, Sutton P. Heat shock protein complex vaccination induces protection against *Helicobacter pylori* without exogenous adjuvant. Vaccine. 2014; 32 (20): 2350-8.

96. Van Crevel R, Ottenhoff TH, van der Meer JW. Innate immunity to Mycobacterium tuberculosis. Clin Microbiol Rev. 2002; 15 (2): 294-309.

97. Bulut Y, Michelsen KS, Hayrapetian L, Naiki Y, Spallek R, Singh M, Arditi M. Mycobacterium tuberculosis heat shock proteins use diverse Toll-like receptor pathways to activate pro-inflammatory signals. J Biol Chem. 2005; 280 (22): 20961-7.

98. Bolhassani A, Rafati S. Mini-chaperones: potential immuno-stimulators in vaccine design. Hum Vaccin Immunother. 2013; 9 (1): 153-61.

99. Daemi A, Bolhassani A, Rafati S, Zahedifard F, Hosseinzadeh S, Doustdari F. Different domains of glycoprotein 96 influence HPV16 E7 DNA vaccine potency via electroporation mediated delivery in tumor mice model. Immunol Lett. 2012; 148 (2): 117-25.

100. Homer MJ, Aguilar-Delfin I, Telford SR, Krause PJ, Persing DH. Babesiosis. Clin Microbiol Rev. 2000; 13 (3): 451-69.

101. Kjemtrup A, Conrad PA. Human babesiosis: an emerging tick-borne disease. Int J Parasitol. 2000; 30 (12-13): 1323-37.

102. He L, Yu Q, Zhang WJ, Zhang QL, Fan LZ, Miao XY, Khan MK, Hu M, Zhou YQ, Zhao JL. Molecular cloning and characterization of a novel heat shock protein 20 of Babesia orientalis. Veterinary Parasitology. 2014; 204 (3-4): 177-83.

103. Hübel A, Krobitsch S, Hörauf A, Clos J. *Leishmania major* Hsp100 is required chiefly in the mammalian stage of the parasite. Mol Cell Biol. 1997; 17 (10): 5987-95.

104. Hombach A, Ommen G, MacDonald A, Clos J. A small heat shock protein is essential for thermotolerance and intracellular survival of *Leishmania donovani*. J Cell Sci. 2014; 127 (21): 4762-73.

105. Montalvo-Álvarez AM, Folgueira C, Carrión J, Monzote-Fidalgo L, Cañavate C, Requena JM. The Leishmania HSP20 is antigenic during natural infections, but, as DNA vaccine, it does not protect BALB/c mice against experimental *L. amazonensis* infection. J Biomed Biotechnol. 2008; 2008: 695432.

106. Campbell K, Diao H, Ji J, Soong L. DNA immunization with the gene encoding P4 nuclease of *Leishmania amazonensis* protects mice against cutaneous leishmaniasis. Infect Immun. 2003; 71 (11): 6270-8.

107. Mohamed RM, Aosai F, Chen M, Mun H-S, Norose K, Belal US, Piao LX, Yano A. Induction of protective immunity by DNA vaccination with *Toxoplasma gondii* HSP70, HSP30 and SAG1 genes. Vaccine. 2003; 21 (21-22): 2852-61.