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Review

Heat shock proteins in infection

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ABSTRACT

Heat shock proteins (HSPs) are constitutively expressed under physiological conditions in most organisms but their expression can significantly enhance in response to four types of stimuli including physical (*e.g.,* radiation or heat shock), chemical and microbial (*e.g.,* pathogenic bacteria, viruses, parasites and fungi) stimuli, and also dietary. These proteins were identified for their role in protecting cells from high temperature and other forms of stress. HSPs control physiological activities or virulence through interaction with various regulators of cellular signaling pathways. Several roles were determined for HSPs in the immune system including intracellular roles (*e.g.,* antigen presentation and expression of innate receptors) as well as extracellular roles (*e.g.,* tumor immunosurveillance and autoimmunity). It was observed that exogenously administered HSPs induced various immune responses in immunotherapy of cancer, infectious diseases, and autoimmunity. Moreover, virus interaction with HSPs as molecular chaperones showed important roles in regulating viral infections including cell entry and nuclear import, viral replication and gene expression, folding/assembly of viral protein, apoptosis regulation, and host immunity. Viruses could regulate host HSPs at different levels such as transcription, translation, post-translational modification and cellular localization. In this review, we attempt to overview the roles of HSPs in a variety of infectious diseases.

1. Introduction

Heat shock proteins (HSPs) were originally determined as a group of heat shock-inducible proteins, but it was soon recognized that HSPs can be stimulated by other agents including growth factors, infections and inflammation [1]. HSPs are highly conserved proteins that play a main role in survival of microorganisms under stress conditions. The modifications in environmental conditions affect the pathogenic properties of microorganisms leading to the synthesis of HSPs known as molecular chaperones [2]. Molecular chaperones are abundant within cellular environment which act as a defense mechanism against external media [3]. It was reported that HSPs biochemically alter the structure of inclusion bodies [4]. Generally, HSPs are categorized into six families based on their molecular weight including small HSPs (sHSPs), HSP40, HSP60, HSP70, HSP90 and large HSPs [5]. The range of HSPs varies from 10 to more than 100 kDa. Moreover, their specific sites and physiological roles change within the cell depending on their size [3]. Different types of HSP families along with their location and functions were briefly shown in Table 1.

The studies showed that some HSPs are effective inducers of innate and adaptive immunity. They activate dendritic cells (DCs) and natural killer cells (NK cells) through toll-like receptors (TLRs) as well as possess a major role in MHC-antigen processing and presentation. Thus, HSPs can be considered as therapeutic agents or therapeutic targets for a variety of infectious diseases and cancers [6]. Several studies indicated the role of host HSPs in the defense response against invasion by a pathogen [7]. Induction of host-specific HSPs occurred upon exposure to oxidants and in viral infections [8]. Moreover, heat shock proteins are important mediators of cellular homeostasis by maintaining protein stability and functionality, and activating potent immune responses. Various factors such as diet, microbial stimuli, environment and host immunity could affect HSP activity. It was observed that the overexpression and down-regulation of HSPs were associated with different disease phenotypes [5]. In this line, some researchers studied the role of HSPs as a biomarker. For example, the plasma concentrations of Hsp70 were increased with the progression of heart failure indicating its role as a potential screening biomarker for early diagnosis of disease [9] (Fig. 1).

Several studies showed that HSPs play a key role in modulating apoptosis and cell death. For example, Hsp27 (HSPB1) is a negative regulator of apoptosis which binds directly to cytochrome *c* released from mitochondria and prevents the binding of Apaf-1 to procaspase-9 suppressing its activation. Hsp27 interferes with mitochondria-mediated caspase-dependent cell death, as well. Hsp70 inhibits stress-

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Table 1
Different types of HSP families along with their location and functions [3].

Type	Location	Role
HSP10	Mitochondria	Protein folding
HSPb12	Plasma membrane	Liposomal membrane protection, and embryonic cardiac muscle development
HSP40	Cytosol	Chaperoning intermediate filament
HSP47	Endoplasmic reticulum (ER)	Caring protocollagen
HSP60	Mitochondria	Protein assembly by forming hetero-oligomeric protein complex
HSP70	Cytoplasm	Protein folding (assembly & refolding) in ER
HSP75	Mitochondria	Protection against apoptotic death
GRP78	ER	Enhancing the cellular resistance to apoptosis
HSP90	Cytoplasm	Myosin folding and sarcomere formation
HSP100	Cytoplasm	Refolding the aggregates
HSP110	Cytosol & nucleus	Inducing immune response

activated apoptosis by several different pathways. Under stress conditions, Hsp70 (HspA1A) prevents Bax activation which is required for release of proapoptotic factors from mitochondria [10]. In general, HSPs have been involved in different functions such as chaperone activity, protein folding, apoptosis, autophagy and immunity [11]. HSPs were suggested as main antigens in different infections because of two factors: a) these proteins are abundant especially under stress conditions, and b) immunologic memory is generated for cross-reactive determinants of conserved HSPs [12]. In this review, we described the important roles of HSPs in microbial infections especially bacterial and viral infections.

2. Relationship between HSPs and immune responses

Heat shock proteins are endogenous adjuvants that induce strong tumor- or pathogen-specific immunity. For instance, mammalian HSP60 and HSP70 could activate DCs and macrophages through TLR4 or TLR2 as observed for bacterial LPS. Thus, some researchers proposed that the immune activities of HSPs were due to bacterial contaminants co-purified with the recombinant (r) HSP60 or HSP70 proteins [1]. In contrast, other reports showed that mammalian HSP70 and GP96 purified from murine livers and kidneys could activate macrophages and DCs. On the other hand, highly purified whole HSP60 or a synthetic peptide of human HSP60 stimulated T cells through TLR2. Moreover, synthetic peptides of human HSP70 could activate NK cells. These data indicated that mammalian HSPs induce innate immunity in the lack of bacterial contaminants. In general, the endogenous HSPs were known as potential targets for efficient therapies in cancer, infection and autoimmunity [1]. The recent studies indicated that HSPs form a part of immune-inflammatory complex that responds to infectious agents, cellular damage and DNA breaks. HSP70 and HSP27 were especially related to base excision repair (BER) enzymes such as human AP endonuclease (APE1) and uracil DNA glycosylase (UDG) suggesting their major roles in DNA repair mechanisms and also presenting HSPs as modulators of certain DNA repair systems, and finally maintaining genome stability and integrity. In addition, HSPs exerted cytoprotection against inflammatory reactions through the modulation of inflammation cascades leading to the activation of pro-inflammatory cytokines such as TNF-α, thus attenuating chronic inflammation [13]. Pathogen-derived products could be presented by host cells and induce recognition of the infected cells by the immune system. Among them, HSPs act as important antigens in host protection and resistance against infectious agents especially due to their high conservation among various microbial pathogens as well as induction of strong humoral and cellular immune responses in a variety of infections [12].

The studies showed that large HSPs are considered as homeostatic factors in maintaining cellular functions under physiological and stress conditions, and also are involved in inflammatory or immune-related diseases [14]. Recently, the large HSPs including Hsp110 and glucose-regulated protein 170 (Grp170) from HSP70 family received a special attention. The large sizes of Hsp110 and Grp170 were due to the presence of a loop structure leading to their high ability in binding to polypeptide substrates or non-protein ligands such as pathogen-associated molecules. The Hsp110 or Grp170 expression was stimulated by cytotoxic or proteotoxic stresses such as heat shock, chemical agents, hypoxia, oxidative stress, inflammation and viral infections. These HSPs cooperated with other chaperone molecules such as Hsp70 or Grp78 to restore protein folding and cellular function and promote cell survival under stress conditions [14]. Interestingly, members of the HSP70 family were highly expressed in both periapical granulomas and LPS-treated macrophages. A positive correlation between HspA6/HspA7 and DNAJC3 with IL-6 mRNA levels, DNAJC3, HspA6/HspA7 and HspB1 with IL-1b mRNA levels, and DNAJC3 and HspB1 with RANKL and TNF-α mRNA levels was observed for major role of HSPs as immune response activators in inducing the host immune defense against pathogens [15]. As known, innate immunity is the first line of defense against pathogens and is necessary for survival of the infected host. The heat shock transcription factor (Hsf) and also active viral replication were important for the induction of the response. A study showed that Hsf-deficient adult flies were hypersensitive to virus infection indicating a role of the heat shock response in antiviral defense [16]. Moreover, HSPs are intracellular molecules that can induce cytokine production and adhesion molecule expression as well as deliver maturation signals and peptides to antigen presenting cells (APCs) through receptor-mediated interactions. Indeed, HSPs are immunoregulatory agents with potent therapeutic uses [17]. In general, the immunogenicity of HSPs results from two different properties: a peptide-dependent function to chaperone and elicit adaptive cytotoxic T-lymphocyte (CTL) responses against antigenic peptides, and a peptide-independent immunomodulatory property [18].

3. HSPs and autoimmunity

The relationship between HSPs and autoimmunity is a complex subject because vaccination with HSPs could protect animals against autoimmune disease. It was observed that the recognition of HSP molecules by T cells reduced autoimmune disease. In this line, it was shown that HSP-reactive T cells have a regulatory function inducing IL-10 cytokine and suppressing autoimmunity. In contrast, HSPs especially Hsp70 induced the maturation signals in DCs leading to converting tolerogenic responses into activation of autoimmunity [19]. Indeed, high homology between human and microbial HSPs could stimulate autoimmune disorders through immune cross-reactivity. For instance, HSP-specific immune responses were involved in the pathogenesis of Behcet's disease (BD), arthritis, type 1 diabetes mellitus (T1DM) and systemic lupus erythematosus, but however, HSPs could suppress autoimmune diseases (*e.g.,* T1DM and arthritis). Recently, the regulatory activities of HSP60 were used to treat human T1DM. For example, the p277 peptide from human HSP60 (aa 437–460) arrested cell damage in newly diagnosed T1DM patients leading to the use of lower doses for exogenous insulin [1]. Moreover, several pathogenetic agents induced HSPs which react with host tissues and significantly elicited T-helper type 1 (Th1) cellular immune responses. High levels of anti-HSP antibodies were detected in inflammatory diseases (*e.g.,* ocular inflammatory disease) as well as neurological disorders. Moreover, high levels of HSPs and anti-HSP antibodies, and cellular immunity to HSPs (*e.g.,* excessive IFN-γ and IL-12) were observed in BD indicating its autoimmune nature. In this regard, high sequence homology and molecular pathway similarity between microbial and human HSPs (self-HSPs) could induce self-reactive T cells specific to the HSP peptides leading to positive selection of autoreactive T cells in BD. Indeed, overlapping the B-cell epitopes within mycobacterial Hsp65 or human Hsp60 with the T-cell epitopes could generate both IgG and IgA

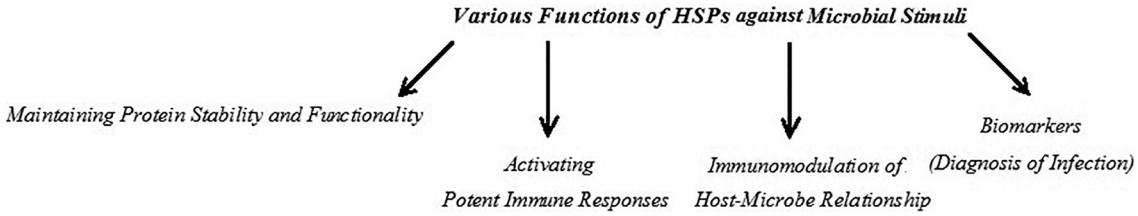

Fig. 1. Various functions of HSPs against microbial stimuli.

antibodies. Moreover, high levels of peripheral IL-2 and IFN-γ producing T cells supported a strong Th1 immune response *in vivo* [20].

4. HSPs in pathogenesis of fungi

Stress (biotic or abiotic) is a negative condition for fungi. To overcome stress, organism expresses molecular chaperons such as HSPs to perform biological functions. Hsp104, Hsp70, and Hsp40 were found to play an important role in replication of fungi, and Hsp90 was found to be effective in transcriptional and post-transcriptional processes of fungi. Hsp90 and Hsp70 alone or together could play a major role in morphogenesis and dimorphism [21]. Heat stress in fungi led to the induction of Hsp60, Hsp90, Hsp104, Hsp30 and Hsp10 proteins. In contrast, Hsp12 protein was induced in response to cold stress. On the other hand, Hsp30, Hsp70 and Hsp90 proteins were expressed against pH stress. It was observed that osmotic stress was controlled by small HSPs and Hsp60. Expression of Hsp104 was detected under high pressure conditions, as well. Among these HSPs, Hsp90 was predicted as a potential anti-fungal target due to its role in morphogenesis [21]. Small HSPs play important roles in microbial pathogenesis. Becherelli et al. demonstrated that HSPs were necessary to develop biofilms in *Candida albicans* during infection [7,22]. Moreover, the role of Hsp90 was identified in the stress response and pathogenesis of some fungi (*e.g., Saccharomyces cerevisiae*, *Aspergillus fumigates, Candida albicans*) [7,23]. On the other hand, the small *S. cerevisiae* HSP, Hsp12, was essential for the growth and survival of the fungus under different stress conditions or the small Hsp21 was involved in the adaptation of the fungus to specific environmental stresses, immune evasion and pathogenicity [7,24,25]. HSPs were known as molecular chaperones that interact with many molecules in different signaling pathways such as the calcium-calcineurin signaling pathway, the Ras1-cAMPPKA signaling pathway, MAPK signaling pathways, and cell cycle control pathways. These HSP-associated pathways were essential for controlling physiological activities and virulence of *Candida albicans*, and also conferring drug resistance to this fungus [26].

5. HSPs in pathogenesis of parasites

HSPs were generated by a variety of parasitic organisms. Some members of HSPs such as Hsp86, Hsp70, Hsp60, Hsp58 and Hsp27 were detected in *Schistosoma mansoni*. Moreover, *Chlamydia trachomatis* Hsp60 was recognized as a potential extracellular stimulus of oncogenesis in pre-neoplastic lesions. The chlamydial Hsp60 could bind TLRs and induce a cascade of signaling leading to neoangiogenesis, macrophage activation and anti-apoptosis mediated by complexing with Bax and Bak [27]. On the other hand, the differential expression of HSPs was tested in murine macrophage-like cell line J774.G8 following infection with *Leishmania donovani (L. donovani)*. The data showed the up-regulation of host Hsp70 and Hsp90 and down-regulation of host Hsp60 in response to infection with virulent promastigotes. The maximum changes in expression of HSPs were observed at 18 h post-infection, *i.e.,* time period for the parasite transformation from promastigote to the amastigote form. Thus, host HSPs can play a key role in parasite differentiation or survival during infection with L. *donovani* [28].

HSPs exert various functions in parasites such as: a) HSPs play important roles in the correction of cell protein functions under stress conditions and in some immunological processes of innate and adaptive immunity. Their expression was also induced against apoptosis especially in intracellular protozoan; b) HSP70 was used as a vaccine candidate in *Schistosomiasis japonicum* as well as in the serological diagnosis of *Schistosomiasis mansoni*; c) HSP70 and HSP90 of adult *Schistosoma japonicum* were suggested in immunomodulation of host-parasite relationship. Moreover, Hsp70, Hsp86 and Hsp60 detected in the secretory products of *Schistosoma mansoni* were effective in parasite development [29]; d) In hydatid disease, Hsp70 of *Echinococcus granulosus* (*E. granulosus*) as an immunogenic protein could induce both humoral and cellular immune responses. The Hsp20 of *Echinococcus multilocularis (E. multilocularis)* was used as a vaccine candidate, as well; e) Small HSP (Hsp10) and Hsp60 were observed in all the stages of *Streptococcus ratti* (*S. ratti*). Other small HSPs such as Hsp17 were detected in the parasitic excreted/secreted (E/S) products leading to the secretion of IL-10 in host cells. In contrast, Hsp70 of this parasite could induce Th1 response suggesting its protective effects against infection *in vivo*; f) Several HSPs were increased during tissue injuries in trichinosis such as Hsp25, Hsp60, Hsp90 and Hsp70 from *Trichinella spiralis* (*T. spiralis*); Moreover, *T. spiralis* Hsp70 generated strong immunogenic responses in mice against parasite infection [29]; g) Hsp70 of both *Wuchereria bancrofti* (*W. bancrofti*) and *Brugia malayi* (*Brugia spp*.) were recognized as immunogenic proteins leading to the generation of high IgG3 levels in human. Furthermore, *B. malayi* Hsp12.6 was used in a trivalent fusion vaccine (rBmHATαc) along with AL007 or AL019 adjuvants stimulating a significant protection in mice; and h) other reports focused on HSPs in other diseases caused by helminthes. For example, Hsp60 of *Onchocerca volvulus* (*O. volvulus*) was detected in all life-cycle stages. Hsp35.6 in *Taenia solium* reacted with the majority of sera from patients with active neurocysticercosis. Moreover, IgA response was induced by Hsp70 in different *Echinostoma* spp. On the other hand, Hsp70 showed high potency as a biochemical marker in infection with *Fasciola gigantica* (*F. gigantica*). In clonorchiasis, high expression of Hsp27 and Hsp90 led to the secretion of inflammatory cytokines, as well [29].

6. HSPs in viral infections

Unlike eukaryotes and bacteria, viruses do not have heat shock proteins and rely on host HSPs for viral protein folding. Thus, processes that regulate host stress proteins are likely targets of strategic manipulation by both viruses and infected hosts [30]. Induction of HSP synthesis was critical for pathogen survival under different conditions [2]. Some viruses could induce the overexpression of Hsps in the infected cells. Moreover, several HSPs were associated with some viral particles. For example, Hsp70 was related to *vesicular stomatitis virus*, *vaccinia virus*, *adenovirus*, *Sindbis virus*, *rabies virus*, and *the human immunodeficiency virus type 1* (HIV-1) Gp160 during its transportation and oligomerization [31]. It was observed that Hsp70 expression was significantly higher in the lymphocytes of HIV-positive individuals as compared to the HIV- negative controls. It seems that the concentration of Hsp60 in the HIV particles was lower than that of the Hsp70. The presence of a Hsp60-like molecule was found in the envelope of infectious HIV strain IIIB virions grown in H9, MT4 and U937 cell lines

which can specifically interact with the transmembrane glycoprotein 41 (Gp41) [31]. On the other hand, natural antibodies against HSP60 and HSP70 families and cholesterol were detected in most healthy individuals. HIV infection significantly increased anti-HSP70 and anti-cholesterol antibodies in human sera without change in the level of anti-HSP60 antibodies. Highly active antiretroviral therapy (HAART) led to the normalization of anti-HSP70 and anti-cholesterol antibodies in sera [31].

Hepatitis C virus (HCV) was able to interact with different chaperones and thus target the various molecular systems and pathways that it requires for its propagation in hepatocytes. The HCV life cycle was divided into six steps: binding and internalization; cytoplasmic release and uncoating; viral polyprotein translation and processing; RNA genome replication; encapsidation (packaging) and assembly; and virus morphogenesis (maturation) and secretion. All phases of the HCV life cycle need the interaction of viral proteins with chaperones especially HSPs [32]. Among HCV proteins, non-structural protein 5A (NS5A) was involved in regulation of viral genome replication, translation from an internal ribosome entry site (IRES), and viral packaging [33,34]. A report showed that the ribosomal protein L22 (RPL22), a RNA-binding protein could facilitate synthesis and translation of viral RNA. Indeed, human RPL22 protein interacted with HCV-NS5A for increasing viral RNA translation [33]. Moreover, treatment of infected cells such as HepG2 cells with HSPs led to up-regulation of long RNA-Alu molecule (or stimulation of the Alu non-coding element) which inhibited the expression of RPL22 gene, and subsequently decreased HCV replication in these cells. Thus, treatment with heat shock proteins could be considered as a novel therapeutic strategy against HCV infection without cytotoxic effects. In addition, treatment of infected cells with heat shock proteins (45 °C for 5 min) could reduce virus replication through low expression of viral NS5A up to 30% compared to 70% in the infected cells as a control [33]. On the other hand, treatment with an inhibitor of HSP synthesis (*i.e.,* Hsp40 and Hsp70), Quercetin, significantly decreased baseline IRES activity as well as inhibited infectious particle production [34].

Viruses are one of the inducers of the stress response in the infected cells [35]. HSPs especially the major inducible 70 kDa heat shock protein (Hsp70) were stimulated by viral infections that this event led to an increase in viral gene expression [36]. Moreover, when Hsp70 was released from cells, it induced innate immune response through TLR-2 and TLR-4. *In vitro* studies showed that the increased virus gene expression using Hsp70 could stimulate innate immunity (*e.g.,* the expression of type 1 interferon) [36]. A study showed that Moscow strain of *ectromelia (mousepox) virus* (ECTV-MOS)-infected RAW 264.7 cells up-regulated Hsp70 and Hsp90 expression during the phase of intensive virus replication. Intensive virus replication in the spleen and lymph nodes of BALB/c (H-2d) mice was associated with high expression of Hsp27, Hsp70 and Hsp90. Furthermore, $CD11b^+$ cells (or Mac-1 is a β2 integrin expressed on macrophages, mast cells and NK cells which plays a main role in elimination of infectious agents) showed the up-regulation of Hsp27 (in spleen & lymph nodes) and Hsp70 (in spleen) at the peak of virus replication (primary stage), and the up-regulation of Hsp90 during later stages of infection (15–20 days after infection) [37]. Another study indicated that HSP may bind to human T-cell leukemia virus type 1 (HTLV-1) proteins leading to its accumulation, as well as to stabilize and facilitate the transport of viral proteins between different regions of cytosol [35,37]. *Baculovirus* infection also induces a heat shock response which is critical for successful infection of insect cells as a host. However, the viral proteins or factors that trigger this response are unknown. Previously, IE2, an early gene product of *baculovirus* could form unique nuclear bodies for the strong trans-activation of various promoters in mammalian cells [38]. Heat shock proteins were found to be one of the major IE2-associated proteins. Indeed, HSPs were significantly induced by IE2 and protected IE2 from proteasome degradation. Thus, HSPs can be used to determine IE2 protein levels in insect cells infected with *baculovirus*. Inhibition of HSP expression led to suppression of *baculovirus* IE2 and subsequently viral replication [38].

Heat shock protein 70 (Hsp70) was identified as a cellular interaction partner of the influenza virus ribonucleoprotein (RNP) complex [39]. Moreover, it was shown that *Influenza virus* matrix protein 1 (M1) plays a major role in the virus replication, assembly and budding. Heat shock cognate protein 70 (Hsc70) was known as a M1 binding protein. The C-terminal domain of M1 interacts with Hsc70. It was observed that Hsc70 did not correlate with the transport of M1 to the nucleus, but it could suppress the nuclear export of M1 and the nucleoprotein (NP) leading to the inhibition of virus production. Hsc70 was directly associated with M1 and thus it was required for virus production [40]. Another study indicated that Hsp70 was involved in the regulation of influenza A transcription and replication. Hsp70 could interact with the PB1 and PB2 subunits of viral ribonucleoprotein (*RNP*), interfere with the integrity of RNP, and suppress the virus replication *in vitro* and *in vivo* likely through disruption of the binding of viral polymerase with viral RNA [39]. The influenza viral protein NS1 also inhibited the processing of Hsp70 pre-mRNA blocking Hsp70 protein expression. Furthermore, NS1 bound to Hsp90 could promote an association of Apaf-1 with cytochrome C and activate the cytotoxic caspase cascade [10]. The up-regulation of Hsp70 limited apoptotic effects by binding to Apaf-1, inhibiting apoptosome formation and caspase-9 recruitment. Indeed, the initial high Hsp levels could reduce cell death and increase viral replication; but however, Hsp70 levels were decreased with time, and another viral protein M1 binds to Hsp70 leading to caspase induction of apoptosis, cell lysis and virus release [10]. Another study also demonstrated that prostaglandin A1 (*PGA1*) inhibited the replication of *Mayaro virus* (MAYV) in Hep-2 cells at early stages of viral replication prior to generation of virus structural proteins likely through Hsp70 induction [41].

As known, HBcAg-specific regulatory T (Treg) cells play an important role in the pathogenesis of chronic hepatitis B. The function of HBcAg-specific Treg cells was enhanced by soluble Hsp60 (sHsp60) secreted from HBV-infected hepatocytes through TLRs. Entecavir treatment suppressed the frequency and function of Treg cells leading to the persistence of HBV infection [42]. Moreover, down-regulation of Hsp70 or Hsp90 by small interfering RNA significantly inhibited HBV production but did not influence cellular proliferation or apoptosis. On the other hand, a significant reduction of HBV secretion in HepG2.2.15 cells was observed by using the Hsp90 inhibitor, *e.g.,* 17-allylamino-17-demethoxygeldanamycin. Indeed, the interaction of Hsp90 with Hsp70/Hsp60 formed a multi-chaperone machine that may represent therapeutic targets for HBV-associated diseases [43]. Many studies showed that the increased expression of HSPs was used as biomarkers for several viral infections. For example, Zhu et al. showed that high expression of Grp94 was significantly accompanied by the progression of HBV infection and could be used as a prognostic or diagnostic biomarker for HBV-induced diseases. Similarly, the expression of Grp78/BiP and Hsp90 was increased in HBV-related hepatocellular carcinomas suggesting their role as important prognostic biomarkers for HBV-induced hepatic cancer [44,45].

HIV-1 viral protein R (Vpr) contributes to the nuclear translocation of the viral pre-integration complex, thus facilitating HIV-1 replication in macrophages. Hsp70 was induced shortly after HIV-1 infection in macrophages and could suppress nuclear translocation of HIV-1 Vpr as an innate antiviral factor. It was interesting that Hsp70 stimulated nuclear import and replication in macrophages of the Vpr-deficient HIV-1 construct suggesting that Hsp70 and Vpr act in the same approach when were expressed separately, but they neutralized each other's activity when were present together [46]. Another study was designed to evaluate the effect of acute HIV-1 infection on host intracellular expression of the HSPs. It was observed that Hsp27 and Hsp70 mRNA transcription was detected at early times, 3–8 h, following viral infection. Indeed, Hsp27 and Hsp70 mRNA transcripts were down-regulated by 24 h, related to the first appearance of the full-length genomic HIV-1 mRNA. Then, Hsp27 and Hsp70 mRNA

transcripts appeared again at the end stages of the viral replicative cycle, directed to virion release and CD4 cell death. The CEM.NKR, Jurkat, H9 and MT-2 cells showed similar patterns of virus-associated modulation of host Hsp27 and Hsp70 protein and RNA expression in the HIV-1 viral life cycle [47]. On the other hand, latency led to the *persistence* of HIV-1 in long-term cellular reservoirs preventing virus eradication. The Hsp90 was required to express HIV-1 genes and mediate HIV-1 replication in hyperthermia. Specific inhibitors of Hsp90 (*e.g.,* 17-N-allylamino-17-demethoxygeldanamycin or AUY922) suppressed HIV-1 reactivation in $CD4^+$ T cells. The effect of Hsp90 was studied on several pathways involved in HIV-1 reactivation from latency including protein kinase C (PKC), mitogen activated protein kinase (MAPK) and NF-κB. It was reported that Hsp90 was required for PKC activity but not for MAPK activity [48]. Also, inhibition of Hsp90 reduced degradation of IkBα, blocked nuclear translocation of transcription factor p65/p50 and subsequently suppressed the NF-κB pathway. Indeed, Hsp90 could interact with inhibitor of nuclear factor kappa-B kinase (IKK) along with cochaperone Cdc37 required for the activity of several kinases. Targeting of Hsp90 by AUY922 inhibitor dissociated Cdc37 from the complex. Thus, Hsp90 could control HIV-1 reactivation from latency by the IKK complex activation, and connect T-cell activation with HIV-1 replication. AUY922 along with a PKC-θ inhibitor completely suppressed HIV-1 reactivation without cytotoxicity in phase II clinical trial. Generally, targeting of the Hsp90/Cdc37 interaction may prevent HIV-1 reactivation from latency [48].

Regarding different studies, Hsp70 was shown to be an infection factor for many viruses. The role of Hsp70 was investigated in the *Zika virus* (ZIKV) infection. Inducing and suppressing Hsp70 expression increased and decreased the production of infectious ZIKV particles, respectively. Finding the interactions between Hsp70 and ZIKV may lead to the design of novel therapeutics against ZIKV infection especially for pregnant women and fetuses [49]. Moreover, the Hsp70 chaperone plays a key role in different processes such as protein translation, folding, intracellular trafficking and degradation as well as in the replication of a variety of viruses. It was shown that infection with *rabies virus* induced the cellular expression of Hsp70 in Negri body-like structures (a place for viral transcription and replication). Furthermore, Hsp70 could interact with the nucleoprotein N. The down-regulation of Hsp70 using quercetin or RNA interference led to a significant decrease of the level of viral mRNAs (transcription), viral proteins (translation) and viral particles (production). These results indicated that Hsp70 was involved in at least one stage of the viral life cycle [3]. Another report indicated that HSPs play a major role in several intracellular processes including apoptosis, and protein delivery to intracellular compartments. Small HSPs were shown to induce IL-8 cytokine. Specifically, activation and translocation of transcription factor NF-κB-p65 occurs in a p38-dependent fashion. The data indicated that Hsp27, p38 and NF-κB-p65 formed a signalosome in virus-infected cells and influenced downstream expression of pro-inflammatory mediators [50].

The role of HSPs was studied in *paramyxovirus* infection, as well. Paramyxoviral RNAs were synthesized by a viral RNA-dependent RNA polymerase (RdRp) containing the large (L) protein, and its cofactor phosphoprotein (P protein). The L protein was a multifunctional protein that catalyzes RNA synthesis, mRNA capping and mRNA polyadenylation. The stability of several *paramyxovirus* L proteins was regulated by Hsp90. Moreover, Hsp90 activity was important for replication of *mumps virus* (MuV) [51]. The Hsp90 activity was necessary for the stability of L-protein and replication of *mumps virus* (MuV). The Hsp90-specific inhibitor (17-AAG) led to the degradation of the MuV L protein through the CHIP (C-terminus of Hsp70-interacting protein)-mediated proteasomal pathway. However, high doses of 17-AAG showed strong cytotoxicity in the cells, thus the combination of an Hsp70 inhibitor (VER155008) with 17-AAG could increase the degradation of the L protein and reduce the concentration of 17-AAG to suppress MuV replication with low cytotoxicity. This event (*i.e.,* regulation of the L protein by Hsp90 and Hsp70 chaperones) was observed for the measles virus [51].

Several findings suggested that the viral infection suppressed Hsp70 levels, thereby promoting caspase activation and apoptosis. For instance, *West Nile virus* (WNV) capsid protein linked to Hsp70, prevented its functional role in protein folding, and mediated disease severity in the host. Furthermore, WNV capsid induced caspase-dependent apoptosis and mitochondrial dysfunction after binding to Hsp70 and attenuating Hsp70 function [10,52,53]. On the other hand, Hsp70 was bound to nuclear protein antigen of *Epstein-Barr virus* (EBV) leading to a damage in the stress response induced by Hsp70, and also immortalization of B lymphocytes directed to malignant transformation and the development of Burkett's lymphoma [54]. Low levels of Hsp70 in placental homogenates were related to high caspase-3 levels and apoptotic nuclei, and subsequently high *herpes simplex virus* (HSV) antibody titers, as well [55]. Moreover, the virulence of NDV strains was inversely correlated with the levels of chaperone-mediated autophagy (CMA). Low induction of Hsp25/27 by virulent strains of NDV increased tumor necrosis, and also enhanced antigen presentation and activity of cytotoxic $CD8^+$ T cells [56].

Our recent study evaluated the efficiency of the potential serologic markers including human papillomavirus (HPV) E7 oncoprotein, small Hsp20, small Hsp27 proteins and Hp91 peptide in Iranian HPV-exposed women. Our data showed that the HPV E7 and small Hsp27 proteins had higher efficiency than small Hsp20 and Hp91 for detection of individuals exposed to HPV infections [57]. On the other hand, the evaluation of cervical cancer patients' seroreactivities associated with HPV infection against three recombinant proteins (rE7, rNT-gp96 and rCT-gp96) showed significantly higher levels of these markers in squamous cell carcinoma (SCC), but not in adenocarcinoma and control groups. Indeed, patients with high antibody response to HPV16 E7 had significant seroreactivity to CT-gp96 fragment. The NT-gp96 and CT-gp96 are the N-terminal and the C-terminal fragments of Gp96, a member of HSP90 family located in ER, respectively [58].

Generally, some viruses such as HPV, adenovirus, polyomavirus and dengue virus use an activated cellular stress response in host, and increase their replication using high levels of HSPs. In contrast, the replication of other viruses such as HIV and influenza A was reduced when host HSPs were high. For example, Hsp70 inhibited viral gene expression and replication [39,59]. Thus, it is clear that some viruses (*e.g.,* HSV, HCV, influenza A and West Nile virus) induce chronic fatigue and malaise to limit the host stress response. On the other hand, hosts could respond to viral infections through several protective plans such as fever, and immunological defense (*e.g.,* production of IFN-gamma, and reduction of Hsp synthesis) [10]. A study demonstrated that infection by different strains of NDV including avirulent B1-Hitchner and avirulent NJ-LaSota significantly enhanced the levels of HSPs (Hsp90, Hsp70, Hsp23 and Grp78), while other strain of NDV including the virulent AV importantly reduced the levels of Hsp70 and Hsp23, and nearly abolish Hsp90 and Grp78 levels indicating the effects of different strains of viruses in down- or up-regulation of a variety of host HSPs [10,60]. On the other hand, certain viral infections that induced HSPs may promote oncogenesis (*e.g.,* EBV and Burkitt's lymphoma; or HPV and cervical cancer). Indeed, viruses act as oncolytic agents by stimulating abnormal high levels of HSPs. HPV16 E7 oncogene increased Hsp70 expression, and viral replication in the infected keratinocytes. In cervical cancer lesions, the levels of Hsp70 were correlated with lesion severity [61–63]. Several roles of HSPs in viral infections [64–84] were summarized in Table 2.

It was interesting that some viral proteins act as molecular chaperones such as HSPs. For instance, the R1 subunit of HSV ribonucleotide reductase protecting the cells against apoptosis showed chaperone-like activity similar to Hsp27 and contributed to the viral infection [85]. HSV type 2 was also reported to encode a homologue (ICP10PK) to small Hsp11 and modulate virus-induced apoptosis through activation of the extracellular-signal-regulated kinase (ERK) signaling pathway, stabilization of Bcl-2, and up-regulation of other

Table 2
Functions of HSPs in different viral diseases.

HSP Family	Member	Localization	Function	Reference
HSP70	Grp75 (HspA9)	Mitochondria	Interaction with HCV NS5A protein	[32]
HSP70	Grp78 (HspA5)	ER	In HCV infection: Regulation of viral protein homeostasis; Maintenance of a balance between viral and cellular translation to prevent viral protein overload	[32]
HSP70	Hsc70 (HspA8)	Cytoplasm	In HCV infection: Virion assembly; Viral entry through clathrin-mediated endocytosis	[32]
HSP70	Hsp70 (HspA1A)	Cytoplasm	In HCV infection: IRES-mediated translation of viral genome	[32]
HSP70	Hsp70B (HspA6)	Cytoplasm	Associated with 3′-non-coding region (3' NCR) of HCV genome	[32]
HSP40	DNAJA1; DNAJA2	Cytoplasm	Associated with HCV NS3-NS4A; IRES-mediated translation of viral genome	[32]
HSP40	DNAJA3	Mitochondria	HCV-induced mitochondrial dysfunction	[32]
HSP40	DNAJB1	Cytoplasm	In HCV infection: Regulation of apoptosis	[32]
HSP40	DNAJB6	Cytoplasm	Viral RNA replication; Interaction with HCV NS5B protein	[32]
HSP40	DNAJB9	ER	In HCV infection: Regulation of apoptosis	[32]
HSP40	DNAJC1	ER	Interaction with HCV E1 and E2 proteins	[32]
HSP40	DNAJC7	Cytoplasm	Regulation of apoptosis; Associated with HCV NS3-NS4A	[32]
HSP40	DNAJC8	Cytoplasm	Upregulated in HCV infection	[32]
HSP40	DNAJC10	ER	In HCV infection: ER protein homeostasis promoting virus production; Proper folding of low-density lipoprotein receptor (LDLR; viral entry)	[32]
HSP40	DNAJC14	ER	In HCV infection: Viral RNA replication	[32]
HSP110	Hsp105 (HspH1)	Cytoplasm	Overexpressed in HCV infection	[32]
HSP110	Hsp70RY (HspA4)	Cytoplasm	Overexpressed in HCV infection; Knockdown decreases viral RNA replication	[32]
HSP110	Hip (HspBP1)	Cytoplasm	Knockdown decreases virus production	[32]
HSP90	Grp94 (Hsp90B1)	ER	Regulation of viral protein homeostasis; Maintenance of a balance between viral and cellular translation to prevent viral protein overload; Suppression of HCV-induced apoptosis; HCV-induced liver fibrosis and autoimmune disease	[32]
HSP90	Hsp90 (Hsp90AA1/Hsp90AB1)	Cytoplasm	HCV RNA replication; Maturation and stability of HCV proteins; IRES-mediated translation of viral genome; Preventing IFN-β response in peripheral B cells; Regulation of miRNA levels along with GW182 (a crucial protein for *miRNA*-mediated *downregulation*); Interaction with HCV NS5A and NS5B	[32]
HSP60 (chaperonins)	Hsp60 (HspD1/HspE1)	Mitochondria	Regulation of *reactive oxygen species* (*ROS*) production and apoptosis; Interaction with HCV core, NS3-NS4A and viral genome	[32]
HSP60	TRiC/CCT (TCP1/CCT2-8)	Cytoplasm	Viral RNA replication by assisting in *replication* complex (*RC*) assembly; Increased TCP1, CCT2, and CCT5 expression; Decreased CCT4 expression; CCT4 associated with HCV NS3-NS4A; Knockdown of CCT5 decreases viral RNA replication	[32]
Small HSPs	Hsp22 (HspB8)	Cytoplasm	In HCV infection: Blocking apoptosis	[32]
Small HSPs	Hsp27 (HspB1)	Cytoplasm	Binds HCV NS5A; Decreases apoptosis	[32]
HSP70	Hsp70	Cytoplasm	Interaction with HPV E1 and E2; Increased genome replication	[64]
HSP70	Hsp70	Cytoplasm	Interaction with HSV-1 UL9; Increased genome replication	[64]
HSP70	Hsp70	Cytoplasm	Interaction with nucleocapsid in canine distemper virus or measles virus; Increased nucleocapsid transcriptional activity and genome replication	[64]
HSP70	Hsp70	Cytoplasm	Interaction with nucleocapsid in respiratory syncytial virus; Increased nucleocapsid transcriptional activity	[64]
HSP70	Hsp70	Cytoplasm	Interaction with nucleocapsid in rabies virus; Supporting formation of replication factories (Negri bodies)	[64]
HSP70	Hsp70	Cytoplasm	Interaction with P33 in tomato bush stunt virus; Supporting replicase function	[64]
HSP70	Hsp70	Cytoplasm	Interaction with Simian virus 40 Tag (harboring J Domain of Hsp40 for host Hsp70 recruitment in viral assembly); Interaction of SV40 Tag with host Hsc70 in viral replication; Genome replication	[64]
HSP70	Hsp70	Cytoplasm	Interaction with human cytomegalovirus Hap46; Stimulation of viral transcriptional activity	[64]
HSP70	Heat shock cognate protein 70 (Hsc70)	Cytoplasm	Rotavirus entry into the cytoplasm; Conformational change of the viral capsid to facilitate its entry into the cytoplasm	[65]
HSP70/ HSP90/GRP78	Hsp70, Hsp90, Grp78	Cytoplasm, ER	Viral entry into the host cells; Chaperone proteins as viral receptors such as Hsp90 and Hsp70 for dengue virus, Hsp70 for Japanese encephalitis virus, and Grp78 for coxsackievirus A9	[66–68]
HSP70	Hsc70	Cytoplasm	Nuclear import in polyomavirus. Retrovirus and the influenza virus through its association with viral capsid proteins	[69]
HSP70	Hsp70	Cytoplasm	HIV-1 nuclear import; Hsp70 acts similar to the HIV-1 viral protein R (Vpr)	[70]
HSP90	Hsp90	Cytoplasm	Hsp90 as an essential factor for the maturation and activity of the HCV NS2/3 protease (Viral replication); Forming a chaperone complex with NS5A in HCV RNA replication	[71]
HSP90	Hsp90, Grp94	Cytoplasm ER	Hsp90 was involved in the reverse transcription of HBV genome; The glucose-regulated chaperone protein GRP94 was a critical regulator in the stabilization and activation of HBV RNA polymerase	[72]
HSP60	Hsp60	Mitochondria	The activation of HBV polymerase prior to its encapsidation into the core particle required for initiating HBV replication in newly infected cells	[73,74]
HSP90	Hsp90	Cytoplasm	Interaction with the viral RNA-dependent RNA Polymerase; A role in the assembly and nuclear transport of viral RNA polymerase subunits during influenza virus infection	[75,76]
HSP70 or HSP40	Hsp70 and Hsp40	Cytoplasm	Binding of the papillomavirus replication initiator E1 helicase to the origin of DNA replication, indirectly enhancing viral replication	[77]
HSP40	Hsp40	Cytoplasm	Viral RNA replication; Regulation of viral gene expression; interacting HIV-1 Nef protein with Hsp40 promoting its nuclear translocation and association with the cyclin-dependent kinase 9 transcription complex (this complex regulates long terminal repeat-mediated gene expression)	[78]

(*continued on next page*)

Table 2 (*continued*)

HSP Family	Member	Localization	Function	Reference
HSP70	Grp78/BiP	ER	To assist the folding and assembly of viral proteins and virions into functional conformations; Grp78/BiP and calreticulin interact with misfolded aggregates containing HCV viral glycoproteins involved in their repair process	[79]
HSP70	Grp78/BiP	ER	Assembly of viral particles; The ER chaperones Grp78/BiP, calnexin and calreticulin are involved in the maturation of the oligosaccharide chains of the non-structural viral protein NSP4, oxidative folding of VP7 and formation of disulfide bonds of VP7 in rotavirus and cytomegalovirus	[80]
HSP70	Grp78/BiP	ER	GRP78/BiP binds to early folding intermediates of vesicular stomatitis virus glycoprotein; Similarly, the unfolded glycoprotein of rabies virus was associated first with GRP78/BiP and subsequently with calnexin, as part of a folding mechanism	[81,82]
HSP90	Hsp90	Cytoplasm	Hsp90 binds in a p53-independent and ATP-dependent manner to immature conformations of the SV40 large tumor antigen (T-Ag) required for formation of a functional structure; Hsp90 helps in the process of viral capsid protein folding and assembly of various *picornaviruses* including *poliovirus*, *rhinovirus* and *coxsackievirus*	[83,84]

apoptosis regulators such as Hsp70 and Hsp27 [86]. The rotavirus non-structural glycoprotein NSP4 was shown to act as an ER chaperone to regulate the folding of structural protein VP4, and facilitate the transport systems through the ER membrane during virion assembly, as well [87].

7. HSPs in bacterial infections

Bacterial HSPs have variable degrees of homology to their eukaryotic counterparts but are highly conserved among pathogens [88]. For this reason, bacterial HSPs could act as antigens leading to increased levels of anti-HSP antibodies and induction of humoral and cellular immune responses [89]. Bacterial HSPs possess both protective and pathogenic activities in the human host depending on the infection [90]. Three of the best studied bacterial HSPs in human health and disease were HtpG, DnaK and GroEL proteins [5]. Bacterial HSPs were highly immunogenic to induce antibody production and T-cell activation. Huang et al. showed that host Hsp25 and Hsp70 have a major role in thermotolerance and the prevention of apoptosis [91]. Oura et al. and Macario et al. indicated the roles of host Hsp70 and Hsp90 in chaperoning and pro-inflammatory responses [92,93]. Chen et al. reported the role of host Hsp60 in modulating immunity and inflammation by activating macrophages and monocytes. Different HSP cognate proteins including Hsp60 or Hsp70 showed a high degree of sequence homology among various pathogenic or non-pathogenic bacteria. Moreover, the reports indicated a major role for Hsp60-specific T cells in mycobacterial infection [12,94].

Various functions of host HSPs were studied to control bacterial infections. Many studies were performed to determine novel biomarkers that can distinguish active *tuberculosis* (TB) and *Latent tuberculosis* infection (LTBI). A study showed that the HSPs can discriminate between LTBI and active TB, and identify individuals who are at the highest risk of developing active TB. Hsp65 and Hsp71 were used for the diagnosis of TB [95]. Moreover, *Mycobacterium tuberculosis* (MTB) Hsp16 was necessary for the survival of bacteria in the stationary phase. Hsp16 was more specific to latency and was used as a diagnostic marker for LTBI. The levels of the host HSPs (*i.e.,* Hsp25, Hsp60, Hsp70 and Hsp90) and *Mycobacterium tuberculosis* (MTB) Hsp16 were significantly elevated in the active TB group compared to the high-risk exposure group, the low-risk exposure group and the control group. Importantly, the levels of these HSPs were increased in the high-risk exposure group compared to the low-risk exposure group [95]. On the other hand, the association between *Helicobacter pylori* (*H. pylori*) infection and role of HSP was focused as an important issue because some reports showed that HSPs led to the induction of immunity and defense against *H. pylori* infection, while other studies indicated that HSPs led to the progression of *H. pylori*-associated gastric carcinogenesis [30].

Since HSPs are immunodominant antigens in many human pathogens, some studies have recently focused on the potential roles of HSPs in oral diseases. The cytotoxicity of some bacterial HSPs was likely related to tissue damage, whereas the presence of common epitopes in host and microbial HSPs was associated to autoimmune responses [2]. The immune response to a cross-reactive epitope in *H. pylori* Hsp60 was lower in infected individuals than in healthy controls. In contrast, the infected patients had a higher antibody response to whole *H. pylori* Hsp60. Indeed, humoral responses to specific HSP epitopes were different from responses to the whole molecule. Moreover, this result was not observed for *E. coli* Hsp60 suggesting that the humoral response was not induced by other bacteria [2]. Another study indicated that HSPs are diagnostic markers for *H. pylori* infection. In fact, Hsp27, Hsp60, Hsp70 and Hsp90 proteins were reduced in gastric epithelial cells, but they were significantly increased in DCs and macrophages. Moreover, inflammation-associated proteins such as iNOS-2 and COX-2 could play a key role in the expression of HSPs for defensing the host cells against *H. pylori* infection [96]. *H. pylori* Hsp60 was also reported to induce IL-6 production in monocytes/macrophages and in chronically inflamed

gastric tissues. *H. pylori* Hsp60-induced IL-6 mRNA expression and NF-κB activation in RAW 264.7 cells was abrogated in the presence of a proteasome inhibitor (MG-132). In contrast, inhibitors of protein kinase A or C, mitogen-activated protein kinase kinase, and phosphoinositide 3-kinase had no effect on IL-6 mRNA levels. This study demonstrated the induction of innate immune responses by highly conserved *H. pylori* Hsp60, indicating the role of this protein in the pathophysiology of chronic gastritis [97]. On the other hand, HSPs were considered as important diagnostic markers in the pathogenesis of several infections. For example, Mycobacterial GroEL (Hsp65) had a high specificity as a marker for Behçet's disease. Moreover, the saliva of patients with gingivitis included higher levels of anti-mycobacterial GroEL antibodies (mostly IgA isotype) than healthy subjects suggesting the diagnosis of gingivitis [2]. The studied showed that interaction between *M. tuberculosis* and various TLRs is complex and it seems that distinct mycobacterial components may interact with different members of the TLR family. The recombinant purified mycobacterial Hsp65 and Hsp70 could induce NF-κB activity in a dose-dependent manner in human endothelial cells. Furthermore, while mycobacterial Hsp65 signals through TLR-4, Hsp70 signals through TLR-2. Mycobacterial Hsp65-induced NF-κB activation was MyD88-, TIRAP-, TRIF-, and TRAM-dependent and required the presence of MD-2 [98].

It was interesting that the dose of microbes in a specific region in body affects HSP expression. It was observed that the expression of inducible HSPs (*e.g.,* Hsp27 or Hsp70) in the intestine was retained through interaction with commensal microbes. Hsp27 and Hsp70 in human or Hsp25 in mice were significantly expressed in colonic epithelium as compared to the small intestine which was exposed to relatively smaller amounts of microbes [5,99,100]. Another study indicated high levels of anti-*Helicobacter pylori* Hsp60 IgG at secondary progressive multiple sclerosis (SPMS) patients. Moreover, a positive correlation was found between *Helicobacter pylori* Hsp60 and age of patients as well as duration of disease indicating the role of *H. pylori* Hsp60 as a useful biomarker for progression of MS [101].

The researchers indicated that HSP70 was found in body fluids during infection. For example, mesothelial release of Hsp72 was significantly enhanced when cells were treated with live and heat-killed *Streptococcus pneumoniae*. In mice, intraperitoneal injection of *Streptococcus pneumoniae* increased the levels of Hsp72 in peritoneal lavage. Extracellular Hsp72 inhibited mediator release from the cultured mesothelial cells. Moreover, Hsp72 levels were significantly higher in effusions of infectious origin as compared to non-infectious effusions. The data showed that pleural mesothelial cells could release Hsp72 against bacterial infections, and these levels were enhanced in infectious pleural effusions [102]. Other studies represented that serum antibodies against Hsp65/60 from *Chlamydophila pneumoniae* cross-react with human Hsp60, cHsp60 and GroEL, and mediate endothelial cytotoxicity. These findings suggested that humoral immune reactions to bacterial HSPs (*e.g.,* cHsp60 and GroEL) may play a major role in vascular endothelial injury as a key event in the pathogenesis of atherosclerosis [103]. On the other hand, small Hsps (sHsps) were molecular chaperones that inhibit the aggregation of non-native proteins. The sHsp system of the bacterium *Deinococcus radiodurans* (*e.g.,* Hsp17.7 and Hsp20.2) led to the resistance against various stress conditions. While Hsp20.2 cooperated with the ATP-dependent bacterial chaperones in their refolding, Hsp17.7 kept substrates in a refolding-competent state by transient interactions. Thus, these two sHsps were significantly different in chaperone activities [104].

8. HSPs in antiviral therapy

Due to the interactions between viruses and HSPs, therapeutic strategies against viral infections were designed to target HSPs. In this line, HSP inhibitors were developed in antiviral strategies. For example, Hsp90 inhibitors were suggested as therapeutic agents for *picornavirus* infection. Moreover, inhibition of Hsp90 by geldanamycin, a blocker of its ATPase activity, could damage the replication of *poliovirus*, *rhinovirus* and *coxsackievirus in vitro* as well as significantly reduced viral load in *poliovirus*-infected mice. The mechanism of action of geldanamycin on *picornaviral* infection was likely the inhibition of viral capsid protein folding and assembly [105]. The studies indicated that geldanamycin or its derivative 17-AAG could delay the growth of *influenza virus* using inhibition of nuclear import and assembly of viral RNA polymerase complex [106]. In addition, geldanamycin prevented the replication of HCV through destabilizing non-structural protein NS3 [107], and inhibited HSV-1 replication by promoting aberrant folding, mislocalization and proteasomal degradation of the viral polymerase [108]. In HBV infection, the multichaperone machine formed by Hsp90 and Hsp70/Hsp60 as a potential target was proposed to develop antiviral therapeutic strategies [109]. Some host signaling molecules (*e.g.,* phosphatidylinositol 3-kinase (PI3K) and AKT proteins) were also determined as the client proteins of the Hsp90. Hsp90 inhibitors could disrupt the PI3K/Akt pathway. For example, a therapeutic strategy was proposed to target the PI3K/Akt pathway in *Epstein-Barr virus,* and control the EBV-associated natural killer/Tcell lymphoma [110]. Geldanamycin was also reported to suppress cytomegalovirus (CMV) replication through disruption of the PI3K/Akt signaling pathway [111]. In addition to the ability of HSPs to interact with viral proteins, their inherent adjuvant and immunogenic properties made them as attractive candidates for development of anti-viral vaccines [112,113]. For example, immunization with Hsp70 linked to antigen generated both adaptive and innate immunity in *simian immunodeficiency virus* (SIV) infection [114], and/or the *Mycobacterium tuberculosis* Hsp70 was used as a potential adjuvant for development of prophylactic and therapeutic vaccines in chronic HBV infection. Hsp70 was also proposed to be useful in the design of vaccines for HSV and HIV-1 [115–117]. Our researches also showed that the use of small Hsp27 as an adjuvant represents promising applications for improvement of HPV therapeutic vaccines [118]. Moreover, the comparable regions of Grp94 or Gp96 (N-/C-terminal fragments of Gp96) showed qualitatively different immunological effects in vaccine development against HPV infection [119].

9. Relationship of dietary stimuli and HSPs

Food-derived nutrients and bioactive substances protect cells through mechanisms that induce HSPs against stress or fasting [5]. The studies showed that fasting increased Hsp27 and Hsp90, but not Hsp70, in the gastrointestinal tract of piglets. In contrast, glutamine as a major substrate for intestinal cells protected the cells against cellular stress by inducing Hsp70 expression. Plant-derived products were reported to possess different effects on HSP abundance in the gut. For instance, plant lectins reduced the levels of Hsp70, Hsp72 and Hsp90 in the jejunum of rats. Down-regulation of HSPs in response to lectin led to severe disruptions in epithelial layer integrity [5,120–124].

10. Conclusion

This review has attempted to describe the role of heat shock proteins in a variety of infectious diseases. Some HSPs are effective inducers of innate and adaptive immunity which can activate dendritic cells and natural killer cells through toll-like receptors as well as possess a major role in MHC-antigen processing and presentation. Moreover, several HSPs are considered as therapeutic agents or therapeutic targets for a variety of infectious diseases. Indeed, heat shock proteins are important mediators of cellular homeostasis by maintaining protein stability and functionality, and activating potent immune responses. Various factors such as diet, microbial stimuli, environment and host immunity influence HSP activity. Thus, the overexpression and down-regulation of HSPs are associated with different disease phenotypes. However, further studies are needed to evaluate induction and regulation of heat shock proteins during different infections and also

autoimmunity. In addition, it is important for determination of the specificity of HSP responses in a variety of infectious diseases and their use as biomarker.

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Declaration of Competing Interest

The authors declare no competing financial interests.

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