Chapter 6
The Role of Heat Shock Protein 70 in Infection and Immunity

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Abstract Heat shock protein 70 (HSP70) has been the subject of intense research concerned with infectious diseases and the immune response. HSP70 is found to be associated with both host and microbial cell surface membranes where it appears to assist in the attachment and colonization of host cells by pathogens. Following infection, HSP70 readily promotes microbial survival, although in certain circumstances such as during some viral infections, it inhibits microbial growth. Regarding immunity, HSP70 induces the activation of both innate and acquired immune responses. These unique immune capabilities of HSP70 are broadly employed for the design of novel vaccines against a variety of infectious diseases.

Keywords Infectious diseases • Parasites • Infection and immunity • Vaccines • Heat shock proteins and infection • HSP70 • Adjuvants

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Abbreviations

APCs	Antigen presenting cells
BCG	Bacillus Calmette–Guérin
CFA	Complete Freund's adjuvant
CMV	Cytomegalovirus
DnaK	Bacterial HSP70
DTH	Delayed type hypersensitivity
GroEL	Bacterial HSP60
H2O2	Hydrogen peroxide
HIV-1 and HIV-2	Human immunodeficiency virus 1 and 2
HIV-p24	Human immunodeficiency virus protein p24
HSP	Heat shock protein
HSV1	Herpes simplex 1
HTNV	Hantaan virus
KMP11	Kinetoplasmid membrane protein 11
LCMV	Choriomeningitis virus
LPS	Lipopolysaccharide
NO	Nitric oxide
NP	Nucleocapsid protein
Pf72	*Plamodium falciparum* HSP72
pfHop	*P. falciparum* organizing protein complex
PfHSP 70-1 and PfHSP 70-2	*P. falciparum* HSP70-1 and 2
PPD	Purified protein derivative
ROI	Reactive oxygen intermediates
RSV	Respiratory syncytial virus
SV40	Simian virus 40
TAP	Transporter associated with antigen processing
TC1	*T. cruzi* antigen 1
TLR	Toll-like receptor
VSV	Vesicular stomatitis virus

6.1 Introduction

Heat shock protein 70 (HSP70) is a family of ubiquitous molecules expressed by most organisms from microbes to mammals [1]. HSP70 is one of the most conserved proteins known. Analysis of the amino acid sequence demonstrates that human HSP70 is 72 % identical to *Leishmania amazonensis*, 73 % identical with *Drosophila* HSP70 and 47 % identical to *E. coli* dnaK (bacterial HSP70). Furthermore, *L. amazonensis* HSP70 is 95 % identical to *L. donovani* or *L. major* HSP70, and it is 85 % identical to the more distant HSP70 from *Trypanosoma cruzi*

[2, 3]. HSP70 proteins are expressed constitutively and further induced in response to a variety of stress conditions, including heat shock, oxidative stress, ischemia-reperfusion injury, radiation, chemicals,: nutrient deprivation and infections. The main function of HSP70, as well as other heat shock proteins (HSP), is to protect cells from injury by promoting the refolding of denatured proteins [4].

Studies of host pathogen interaction and expression of HSP at infection have led to hypothesize that HSP are important for the survival of intracellular pathogens such as *Plamodium*, *Leishmania*, *Mycobacteria*, and *Toxoplasma*. From the microbial viewpoint, host cells such as phagocytes represent a hostile environment due to the presence of toxic molecules including low pH, nutrient deprivation, proteases, nitric oxide (NO), reactive oxygen intermediates (ROI) and high temperatures. Experimental evidence indicates that HSP are important for microbes to survive within these toxic environments of host cells. With regard to HSP70, it promotes thermotolerance, a condition that allows microbes to withstand a secondary, more severe, heat shock treatment [5]. Indeed, *E. coli* strains lacking HSP70 are highly sensitive to heat shock, but become resistant to heat following transfection with HSP70 from *Plamodium falciparum* [6]. In the context of infection, experimental evidence also demonstrates that HSP70 contributes to intracellular survival of pathogens. Disruption of *P. falciparum* HSP70, as well as inhibition of the ATPase activity of HSP70, severely affects development of malarial parasites within erythrocytes [7, 8]. Of note, optimal function of HSP70 requires the presence of HSP70 partners including HSP40, HSP60 as well as HSP90 [9].

HSP in general are among the most immunogenic antigens found in nature, stimulating both innate and antigen-specific immunity. With respect to innate immunity, HSP70 is secreted from host cells into the extracellular milieu. Extracellular HSP70 triggers innate immunity via activation of antigen presenting cells (APCs) [10–12]. Regarding adaptive immunity, HSP70 is an abundant antigen of both B and T cells. *P. falciparum* HSP70 is expressed by most parasites within their life cycle and it is recognized by sera from malaria patients [13]. Sera from *S. mansoni*-infected individuals contain antibodies recognizing *S. mansoni* or *S. japonicum* HSP70 [14, 15]. Both antibodies and T cells recognize *M. tuberculosis* HSP70 as determined in patients with tuberculosis [16]. Interestingly, antibodies and T cells that recognize HSP70 have been identified also in apparently healthy individuals, suggesting that HSP70 may provoke autoimmunity due to molecular mimicry.

The immunogenicity of HSP70 and its capacity to activate antigen-specific immunity have made this protein an ideal candidate for vaccine development. Vaccines employing HSP70 have been tested against various infectious conditions. Protective immunity and resistance to infection have been observed to develop in *Salmonella* [17] and cytomegalovirus [18]. However, no protection has been experienced with vaccines against various fungal infections [19]. This review will examine current issues on the role that HSP70 plays in the infection process and its importance in immunity against microbial infection. It will also examine existing evidence suggesting that HSP70 can be a potential vaccine candidate adjuvant.

6.2 The Role of HSP70 in Infection

6.2.1 HSP70 and Bacterial Infections

Salmonella typhimurium is a common bacterium causing diarrhea in humans in many countries around the world. Evidence has shown that colonization of cells by bacteria requires the assistance of stress proteins. Treatment of *S. typhimurium* with the toxic H_2O_2 results in the induction of at least 30 proteins [20]. HSP70 (DnaK) is one of the 30 HSP proteins induced by H_2O_2, and is also induced by heat shock [21]. A study shows that S. *typhimurium* overexpresses both HSP60 (GroEL) and the HSP70 (DnaK) during infection of macrophages and the presence of these HSP is essential for survival of the pathogen within the infected cell [22, 23]. Furthermore, DnaK/DnaJ mutants of S. *typhimurium* could not survive or proliferate within macrophages, and the bacteria were unable to invade epithelial cells in vitro and could not secrete any of the invading proteins encoded within a Salmonella pathogenicity island 1 [24]. Interestingly, Monocytic cell line J774A.1 infected with virulent *S. choleraesuis* died spontaneously due to TNF-α production by the infected cell. Induction of HSP70, however, resulted in significant survival of the infected monocytes [25]. Thus S. *typhimurium* HSP70 (DnaK) and its co-chaperone DnaJ play a significant role in *Salmonella* infection [24].

Mycobacterium tuberculosis infection of human THP-1 cells induced expression of at least 16 proteins. Some of these proteins are also expressed by the bacteria extracellularly in culture medium in the presence of heat shock, H_2O_2 or low pH [26]. Furthermore, *M. tuberculosis* overexpressing HSP70 was shown to express full virulence at the initial stage of infection. The bacterium, however, was significantly impaired in its ability to persist during subsequent chronic phase of infection [27].

Helicobacter pylori is a gram-negative bacterium causing gastric ulcers. The microbial HSP70 as well as HSP60 is shown to associate with the microbial cell membrane and their presence mediates attachment of the bacteria to gastric epithelial cells. Interestingly, though, both HSP70 and HSP60 are increased following acid shock of cells in vitro and their expression correlates with increased inflammation of the gastric mucosa [28].

Chlamydia trachomatis is known for leading the burst of sexually transmitted infections globally causing pelvic inflammatory disease and infertility [29]. Chlamydial HSP70 has been found expressed on the cell surface of elementary bodies – the infectious forms of *C. trachomatis*. Expression of HSP70 on the cell membrane of bacteria, however, does not seem to help attachment of bacteria to host cells and it may be involved in host immune recognition [30, 31].

Yersinia enterocolitica is a facultative intracellular pathogen that invades epithelial cells of the intestine causing acute diarrhea in humans. At least 16 proteins, including *Y. enterocolitica* HSP70 (DnaK) were selectively induced in the macrophage-like J774-1 cells infected with *Y. enterocolitica*. This HSP70 (DnaK) was invariably induced by the bacteria in vitro in response to heat shock (HS) at

42 °C or following oxidative stresses. Thus, *Y. enterocolitica* HSP70 is expressed as a global stress response of the bacteria to the hostile environment of the macrophage [32].

Bordetella pertussis produces two toxins including adenylate cyclase-hemolysin and pertussis toxin. Expression of either adenylate cyclase-hemolysin or purified bacterial toxins reduces the expression of HSP70 in *B. pertussis* infected macrophages, suggesting that HSP70 may be involved in host protection against *B. pertussis* [33].

Staphylococcus aureus HSP70 plays a dual role in infection of host cells. From one side HSP70 is a receptor for *S. aureus* attachment and internalization by host monocytes [34]. From other side, HSP70 inhibits apoptosis of host monocytes induced by *S. aureus*. Indeed, human peripheral blood monocytes die by apoptosis following phagocytosis of *S. aureus*. However, induction of HSP70 expression renders monocytes resistant to *S. aureus*-induced apoptosis [35].

6.2.2 HSP70 and Parasitic Infections

HSP appear to play a major role in the survival of parasites following invasion of mammalian cells at 37 °C. In effect, parasites must adapt to the high temperatures of host cells and to the presence of stressing conditions of oxygen radicals, nitric oxide, lack of nutrients, toxic compounds, etc. In this regard, *Trypanosoma*, *Leishmania*, *Plasmodium* and *Schistosoma* have all been found to express constitutive or induced forms of HSP at high concentrations [36].

Trypanosoma is a unicellular parasite transmitted by the bite of an insect vector causing Chagas disease in Central and South America. *Trypanosoma* Also causes sleeping sickness in Africa. The epimastigote developmental stage of *Trypanosoma* is transmitted by the insect vector upon feeding on host blood. Within the mammalian host, epimastigotes differentiate into flagellated trypomastigotes. A stress response may play a role during parasite transition from insect vector to mammalian host and may trigger expression parasite HSP. Indeed, mRNAs of *T. brucei* HSP70 and HSP83 are augmented 100-fold in trypomastigotes exposed at 37 °C as compared with forms found in the insect vector at approximately 24 °C [37]. At least six *hsp70* genes, which are transcribed as long polycistronic molecules, have been described in *T. brucei* [36]. Furthermore, epimastigotes of *T cruzi* express 10 major proteins ranging from 60 to 83 kDa upon heat shock at 41 °C [38]. Approximately 11 genes encoding HSP70 proteins have been identified in the *T. cruzi* proteome. Some of these are highly expressed in epimastigotes whereas some others are expressed in trypomastigotes [39].

Leishamnia is an intracellular protozoan parasite transmitted by the bite of sandflies. *Leishmania* causes a wide spectrum of diseases including cutaneous, mucocutaneous and visceral leishmaniasis. The parasite life cycle includes two forms: a flagellated promastigote surviving within the alimentary tract of the insect vector and the amastigote (without flagellum) living within the parasitophorous

vacuole of infected macrophages. *Leishmania* contains multiple copies of *hsp70* genes, with absolute copy number varying among strains with *L. major* containing at least 14-associated *hsp70* genes [40].

Leishmania has to survive within host macrophages and requires adaptation to this new environment following infection. Both *L. donovani* HSP70 and HSP60 were found expressed in murine macrophages following infection [41]. Heat shock (HS) treatment of *L. chagasi* promastigotes makes leismanial parasites resistant to macrophage-induced oxidative stress [42]. Similarly, HS treatment of *L. tarentolae* promastigotes causes the parasite to develop resistance to the leishmanicidal effects of pentavalent antimonials [43]. Interestingly, Balb/c mice inoculated with *Leishmania infantum* lacking *hsp70* genes did not develop *Leishmania*-associated pathology or disease. Instead, these mice develop a Th1 immunity and resistance to *L. infantum* infection suggesting that *L. infantum* HSP70 may be associated with leishmanial pathogenesis [44, 45].

Malarial disease is caused by intracellular parasites of the genus *Plasmodium*. Within this genus, *Plamodium falciparum* is the most common species identified, causing nearly 75 % of all malaria cases. *P. falciparum* is transmitted by a female mosquito of the genus *Anopheles*. Most HSP70 studied in malaria are referred to the intracellular erythrocytic stage of the parasite. At least six HSP70 (pfHSP70) isoforms have been described in *P. falciparum* [46, 47]. The proteins encoded by these genes are constitutively expressed at all blood stages of *P. falciparum*. A member of the HSP70 family of 75 kDa is expressed on the surface of merozoites, and it is recognized by the immune response [48]. Another *P. falciparum* HSP70-1 (Pf72/HSP70-1) is a major immunogen expressed in infected erythrocytes and found experimentally to protect Saimiri monkeys against malarial infection [49].

P. falciparum HSP70 functions to promote parasite survival within host cells. Disruption of *P. falciparum* HSP70 and HSP90 complexes and inhibition of the ATPase activity of HSP70 inhibits development of parasites in infected erythrocytes. Exposure of parasites at 41 °C, the equivalent to malaria-induced febrile disease in the host, promotes parasite development in human erythrocytes [7, 8]. Furthermore, transfection of *E coli* lacking HSP70 (DnaK) with *P. falciparum* HSP70 causes thermosensitive *E coli* to become thermoresistant [6]. Together these observations suggest that HSP70 promote parasite survival possibly by inducing thermotolerance.

It is becoming clear that, *P. falciparum* HSP70 works together with other molecular chaperones during malarial infection. Within erythrocytes *P. falciparum* HSP70 associates with HSP90 and form the HSP70-HSP90 organizing protein (pfHop) complex. This protein conglomerate promotes parasite survival via chaperoning signal transduction pathways [50]. Furthermore, gene analysis demonstrated that within erythrocytes, HSP70 and HSP90 associate with co-chaperone HSP40 [51]. In addition, HSP70 was found exported from the parasite to the erythrocyte cytosol where it associates with HSP40 [52], and both HSP70 and HSP40 form the *P. falciparum* virulence factor (pfEMP1) complex. The pfEMP1 complex provides adherence to the infected erythrocyte [52, 53]. Thus, *P. falciparum* HSP70 and its associated molecular chaperones work in concert to promote parasite survival and transmission.

Toxoplasmosis gondii is a protozoan parasitic that invades mammalian cells causing neurologic diseases. It can cross the placenta and infect the fetus, causing abortion [54]. At least five HSP70 isoforms have been identified in *T. gondii* [40]. The role played by *T. gondii* HSP70 in parasite survival has not been completely defined. One study shows that *T. gondii* HSP70 assists the conversion of parasite from the bradyzoite to the tachyzoite stage, and that this effect occurs primarily during reactivation of chronic toxoplasmosis [55]. Another study shows that *T. gondii* HSP70 induce maturation of dendritic cells, and that maturation involves Toll-like receptor 4 (TLR4)-mediated signalling pathway. These mature DCs are able to prime Th1 T cell responses and promote resistance against *T. gondii* infection [56]. Interestingly, *T gondii*-infected mice develop antibodies against *T. gondii* HSP70, which cross-react with human HSP70 suggesting autoimmune recognition [57].

Schistosomiasis is a human disease caused by helminths of the genus Platyhelminths and highly prevalent in Africa, the Middle East and Asia. Children with schistosomiasis develop anemia, malnutrition and learning difficulties [58]. Four genes corresponding to HSP70 have been cloned from schistosomes. The HSP70 proteins are expressed in larvae and in adult organisms. At least one of these *hsp70* genes is inducible in *S. mansoni* [59]. Interestingly, fully-differentiated schistosomula – the stage found in humans – can induce expression of the *hsp70* gene. Furthermore, the *S. mansoni hsp70* gene is expressed constitutively in miracidia, the parasite stage in snails but not in cercaria (the developmental form that infects humans). In cercaria, however, the gene is induced at 42 °C [36, 59]. These observations suggest that the expression of *Schistosoma* HSP70 may help parasite transmission from low temperatures to high host temperature (37 °C).

6.2.3 HSP70 and Fungal Infections

Histoplasma capsulatum is a dimorphic fungus that survives as a multicellular filamentous stage (mycelia) at temperatures close to 25 °C, and as unicellular (yeast) at 37 °C. The transition between mycelia and yeast can be reversibly induced in the laboratory by shifting these temperatures. Interestingly, mRNA studies have shown that *H. capsulatum* maintains mRNA processing at high temperatures when phase transition from mycelia to yeast is induced in a culture at 42 °C. Since HSP are abundant in mycelial cells at normal temperatures and during phase transition [36], it is suggested that HSP, particularly HSP70, may promote normal mRNA processing and normal cell function at 37 °C by protecting the splicesome [60, 61]. In the yeast *Candida albicans* optimal expression of HSP appears to vary between strains with different degree of virulence. Non-virulent strains express maximum transmission of *hsp70* and *hsp82* genes at 34 °C. On the contrary, virulent organisms expressed maximal transcription of *hsp* genes at 37 °C [62]. Of note, in *Cryptococcus neoformans* HSP70 is associated with the fungal cell surface, where it may have a role in interaction of the yeast and host cells [63].

6.2.4 HSP70 and Viral Infections

HSP70 is one of the most studied HSP with respect to the role of chaperones in the biology of viruses. HSP70 induces replication of various DNA viruses including herpes virus (HSV1) [64, 65], vaccinia virus [66], adenovirus [67, 68], simian virus 40 (SV40) and others. HSP70 also assist positive- and negative-stranded RNA viruses in infection of host cells. In measles (negative-strand RNA virus), HSP70 interacts with virus nucleocapsid N protein and assists viral capsid formation and optimal viral replication [69]. Furthermore, transgenic mice overexpressing *hsp72* gene in neurons showed augmented measles viral burden in the brain of mice following viral infection [70].

HSP70 assists virus replication at various levels. In human cytomegalovirus (CMV)-infected cells, HSP70 localizes to the nucleus early in infection and then translocate to the cytoplasm late after infection [71]. In the Hantaan virus (HTNV) infection of Vero E6 cells, HSP70 is also shuttled to the cell nucleus and then to the cytoplasm. Within the cytoplasm HSP70 associates with HTNV nucleocapsid protein (NP) resulting in control of expression levels of viral structural proteins and virus assembly [72].

Virus infection, replication and assembly may require the assistance of various molecular chaperones. In polyomavirus, HSP70 interacts with capsid proteins VP1, VP2 and VP3 in an ATP-sensitive manner within the cytoplasm of various host cells. When bound to VP1, HSP70 inhibits the assembly of viral capsids. However, in the presence of ATP and DnaJ and GrpE chaperones, VP1 assembles into complete uniform capsids [73].

In simian virus 40 (SV40), infection of mouse cells by SV40 results in the induced expression of HSP70 and HSP90 [74]. In mouse keratinocytes, HSP70, HSP60, as well as HSP90 were found induced following SV40 infection. However, induction of these chaperones was accompanied by down-regulation of small HSP27 [9]. Thus, diverse chaperone families take part in virus replication but their activation and/or inhibitory activity on viruses may depend on the infected host cell and the conditions of infection.

Remarkably, HSP70 has been identified as a virion component in various RNA viruses, including influenza A virus, vesicular stomatitis virus, rabies virions, and HIV-1 particles [9]. RNA viruses have developed a unique adaptation pathway for multiplication, and HSP70 may be directly involved in this replication pathway.

Rotavirus causes gastroenteritis and watery diarrhea and children around the world are most susceptible. Rotavirus infection of epithelial cells lining the gastrointestinal tract is assisted by host HSP70. HSP70 is part of a receptor complex that binds rotaviruses [75]. Attachment of viral particles to HSP70 occurs via rotaviral structural protein VP5 [9]. Following rotavirus infection, levels of intracellular HSP70 are subsequently induced, resulting in augmented production of rotavirus structural proteins VP2, VP4, and VP6. Interestingly, inhibition of intracellular HSP70 in rotavirus-infected cells results in significant reduction of viral particles produced [76]. Thus, HSP70 has a double role in rotavirus infection, a viral receptor component, and as a promoter of viral replication.

Human immunodeficiency virus 1 and 2 (HIV-1 and HIV-2) target and kill $CD4^+$ T helper cells. Evidence shows that HSP70 plays a role in HIV infection and replication. Increased levels of HSP70 are observed in human lymphoma cells chronically infected with HIV-1 as well as in lymphocytes from HIV-1 infected patients [77, 78]. Within infected cells, HSP70 facilitates the import of HIV-1 pre-integration complexes into the cell nucleus, leading to virus integration in host chromosomes [79]. Interestingly enough, in macrophages, the presence of recombinant HSP70 significantly diminished replication of HIV-1 [80]. However, the presence of co-chaperone HSP40 induces viral gene replication, suggesting that coordination between HSP70 and its co-chaperone HSP40 decides either inhibition or activation of HIV-1 replication [81].

Regarding replication inhibition, various studies showed that in some circumstances HSP70 is involved reduction of viral infections. For example, increased expression of HSP70 by heat treatment significantly reduced virus replication in neurons infected with vesicular stomatitis virus (VSV) [82]. Furthermore, constitutive expression of *hsp70* genes in neurons led to the clearance of VSV particles from mice brain, resulting in reduced mice mortality. Interestingly, this effect correlated with the secretion of HSP70 by VSV-infected neurons and with the enhanced expression of type I interferons [82]. Another study showed that expression of HSP70 correlated with protection against influenza virus. This mechanism of protection involves the polymerase activity, which negatively regulates viral transcription [83, 84]. Negative effects of HSP70 in viral replication have also been reported in rotaviruses [76] as well as in respiratory syncytial viruses [85]. The mechanisms associated with HSP70 down-regulation of viral replication are not well understood. They may be explained, in part, in the context of a global heat shock response. Heat treatment of cells down-regulates the NF-kB signalling pathway, leading to replication inhibition of some viruses such as HIV-1 [9, 65].

6.3 HSP70 and the Host Immune Response to Infection

6.3.1 HSP70 as Antigen

HSP in general are among the most immunogenic antigens found. It is suggested that the immunogenicity of these proteins is a direct consequence of their abundance, which by virtue of mass action leads to the processing and presentation by antigen-presenting cells [13]. It is also suggested that the immunogenicity of at least some HSP may be related to functional association with the MHC-processing machinery [86]. Furthermore, invading microbes undergo stress due to primary host defence mechanisms, which causes up-regulation of microbial HSP, making them targets of immunity [87].

In any case, HSP appear to be highly immunogenic in their own right. Antibodies and T cells that recognize HSP have been identified in a variety of infections, and also in apparently healthy individuals. The later findings have led to the suggestion

that HSP may play an important role in immune surveillance. Thus, anti-HSP immune responses appear to be regularly induced as a result of frequent contact with low virulence organisms. Repeated contact with low virulence pathogens impels the immune system to focus on regions of HSP conserved in the microbial world. This may provide a mechanism for rapid and specific responses to eventual encounters with more highly virulent microbes [86].

With respect to HSP as immunogens of infection, evidence indicates that HSP70 and in some cases HSP90 are major targets of the immune response in parasitic infections. For example, *P. falciparum* HSP70-1 and 2 (PfHSP 70-1 and PfHSP 70-2) are two abundant antigens of *P. falciparum* HSP70 that are expressed at all stages of the parasite life cycle. Although they share 64 % of amino acid identity, antibodies raised against either of them do not show cross-reactivity indicating that the common sequences are non-immunogenic [13]. The PfHSP 70-1 antigen is expressed on the surface of infected hepatocytes, where it is the target of antibody-dependent cell-mediated cytotoxicity.

As mentioned, a major HSP70-related immunogen Pf72/HSP70-1, which is present in blood stages of *P. falciparum*, has been found to protect *Saimiri* monkeys against infection. Fifty-two percent of individuals living in an endemic zone in West Africa have antibodies to this antigen. Furthermore, T cells specific for epitopes within the C-terminus of this protein are found in individuals continuously exposed to the parasite. The same epitopes are not recognized by T cells of non-exposed Europeans. However, since some of these T cell epitopes are also present in the homologous human HSP70, the use of this antigen in vaccine development against malaria remains controversial [49]. An antigen of *P. falciparum* which shares 55 % of amino acid identity with PfHSP 70–1 and 72 % identity with grp78 is also recognized by sera from infected patients [88].

In *T. cruzi*, the antigen TC1 that belongs to the HSP70 family has been cloned from the λ gt11 expression library. Antibodies against this antigen do not cross react with human HSP70, despite 73 % of amino acid homology between the proteins [89]

A HSP70 molecule from *S. mansoni* is recognized by sera from *S. manosni*-infected individuals. The same molecule is not recognized by sera from patients infected with *S. japonicum*, indicating that the two groups of sera recognize different epitopes within the two HSP70s [14, 15]. HSP70 is also a major B cell antigen in patients infected with either *Brugia mamayi* or *Onchocerca volvulus*. The immunogenic domain in both these cases has been localized to the HSP70 carboxy-terminus [90].

HSP have been also been found to be major targets of the immune response in bacterial infections. Mice immunized with either *M. tuberculosis* or *M. leprae* produce antibody responses to a limited set of proteins. Amongst these are represented at least four stress protein groups: HSP70, HSP60, HSP18 and HSP12 [13]. HSP70 was initially identified by a mAb raised against an extract of *M. leprae*. HSP70 was then found to be recognized by both T cells and antibodies in patients with leprosy [91]. Similarly, HSP70 from *M. tuberculosis* was also identified with a mAb raised against *M. tuberculosis*. This HSP70 was recognized by antibodies and T cells from patients with tuberculosis [16]. Furthermore, $CD8^+$ T-cell clones

isolated from patients with tuberculosis proliferate in response to HSP70 from mycobacteria, *E. coli*, and human. Interestingly, mice infected with *M. tuberculosis* develop a strong antibody response to mycobacterial HSP70 and little or no response to murine HSP70. However, immunization of mice with the mycobacterial HSP70 induces antibodies that cross-react with self HSP70 [92].

6.3.2 The HSP70 as Chaperone of Antigenic Peptides and Proteins

A method for immunization against cancer exploiting the peptide-binding capabilities of HSP was explored in the 1990s. Mice immunized with HSP70 purified from Balb/c Meth A sarcoma cells were found to be protected against an otherwise lethal challenge with tumor cells. Protection was specific since it was not protective when mice were immunized with either (i) HSP70 from normal tissue, or (ii) HSP70 treated with ATP which removed HSP70-bound immunogenic peptides. Thus, protection appeared to require a combination of HSP70 and co-purifying bound peptides. Further analysis indicated that HSP70 bound peptides were in the range of 1,000–5,000 Da [93]. It was suggested that HSP-chaperoned peptides were efficiently processed endogenously by antigen-presenting cells (APCs) and presented in the context of MHC class I molecules.

Further evidence indicated that the chaperoning capacity of HSP is not limited to immunopeptides, but also to entire immunogenic proteins attached to HSP. HSP70 from *M. tuberculosis* fused to the human immunodeficiency virus protein p24 (HIV p24) elicited both humoral and cellular immune responses against p24 following immunization of mice with the HSP70-p24 complex in the absence of adjuvant [94]. Another experiment showed that mice immunized with HSP70 fused to the Hantaan virus nucleocapsid protein (NP) elicited significantly higher levels of NP-specific antibodies, IFN-gamma-producing cells and cytotoxic T lymphocytes than mice immunized with NP protein alone [95]. In a series of experiments with various chaperones involved in chaperoning antigens including gp96, HSP90, and HSP70, it was proposed that the complex of chaperone-peptides are internalized via the CD91 receptor into endosomal compartments, where they are targeted for presentation. Furthermore, some peptides HSP-peptide complexes were found to enter an acidic compartment and loaded onto MHC class II where peptides are presented to $CD4^+$ T cells [96].

6.3.3 HSP70 and Cross-Presentation of Antigens

More recent evidence demonstrated that HSP preferentially enter the MHC class I processing pathway via cross-presentation [97–99]. In this pathway, antigens are taken up by dendritic cells (DCs), and following internalization, they are processed and loaded onto MHC class I molecules and presented to $CD8^+$ T cells, which

destroyed pathogen-infected cells [100]. The mechanisms of cross-presentation by HSP70 or by HSP in general have not been completely defined. Interaction of the HSP-peptide complex with CD91 results in the internalization of the complex into a non-acidic compartment. Transfer of the complex to the cytosol allows peptides to be processed by the proteosome and transported into the ER by the transporter associated with antigen processing (TAP), which assist peptide loading onto MHC I molecules [101, 102]. This cross-presentation model has been tested in *M. tuberculosis* HSP70 and OVA peptide (OVA257-264). Cross-presentation of OVA peptide occurred via MHC-I in B cells. Processing was dependent on linkage of OVA peptide to HSP70 and was a CD91-dependent process [98].

6.3.4 HSP70 and Activation of Innate Immunity

In addition to chaperoning peptides and proteins for antigen presentation, HSP in general and HSP70 in particular possess intrinsic mechanisms that trigger innate immunity. When conjugated to poorly immunogenic peptides or oligosaccharides, HSP enhance the immune response to these relatively weak antigens. For example, immunization of mice with the polypeptide (NANP 40) [*P. falciparum* circumsporozoite protein] conjugated to the mycobacterial HSP70 or HSP60, resulted in a strong antipeptide IgG antibody response. This response was similar to the response observed when a purified protein derivative (PPD) is used as a carrier, in spite of the fact that no conventional adjuvant is used in the case of the HSP70-conjugated peptides [103]. Furthermore, priming with Bacillus Calmette–Guérin (BCG) prior to immunization was required in cases when HSP60 was used as carrier. However, priming with BCG was not required when HSP70 was employed [103].

The mechanism by which HSP70 provides adjuvant effects has been the focus of intense research. Recent studies showed that HSP70 possesses intrinsic adjuvant capabilities and that this protein can trigger activation of innate immunity. The following findings support the idea that HSP70 activates innate immunity: (i) HSP70 has been found localized on the cell surface membrane; (ii) HSP70 is secreted from the cell into the surrounding environment of cells [104–107]; (iii) secretion of HSP70 occurs in response to cytokines IFN-γ and IL-10 treatment [108]; (iv) HSP70 activates APCs following TLR4 and TLR2 engagement. Activation of APCs by HSP70 is NF-kB-dependent leading to proinflammatory cytokine production [12]; (v) extracellular HSP70 is internalized by APCs via cell surface receptors including CD40, CD91, LOX-1 and CD94 [109]. Together, these and previous observations demonstrate that HSP70 is not only a chaperone but also an inducer of cytokine production by APCs. HSP70 is a chaperokine [10, 11].

The induction of innate immunity by HSP70 has been observed in various models of host microbial interaction. For example, purified HSP70 from *T. gondii* or *T. cruzi* induced maturation of DCs. These DCs increased the expression of costimulatory molecules CD40, CD80, CD86, and activated DCs produced increased amounts of proinflammatory cytokines IL-12 and TNF-α two key cytokines involved in Th1 priming [56, 110].

Of note, it has been argued that the inflammatory properties of HSP70 are not due to HSP70 itself, but to lipopolysaccharide (LPS) contamination, which remains bound to the protein following purification from *E. coli*. In fact, experiments have shown that LPS-free HSP70 is immunosuppressive and that rather than inducing stimulation, HSP70 inhibits T cells and reduces the capacity of DCs to produce inflammatory cytokines [111]. Furthermore, a recent observation shows that *Francisella tularensis* HSP70 inhibits alkaline phosphatase in mice lungs infected with *Francisella*, suggesting that *Francisella* HSP70 could down-regulate host immunity by interfering with host cell signalling pathways [112].

Challenging the argument of inhibitory effect of HSP70, a recent observation shows that LPS-free HSP70, expressed in baculovirus expression vector system containing no LPS, invariably induced activation of mouse splenocytes and enhanced production of proinflammatory cytokines [113]. The apparent contradiction of HSP70 as an immunostimulatory or as an immunosuppressive molecule is still a matter of controversy. However, it is clear that LPS-contamination alone does not explain the multiple observations associated with the activation of the immune response by HSP70.

In addition to activating APCs, HSP70 also activates natural killer cells (NK cells) as shown in various cancer models. Little is known, however, on the role of HSP70-mediated activation of NK cells during infection. In malaria, HSP70 is recruited to the surface of *P. falciparum*-infected erythrocytes [114]. The infected erythrocytes become targets of NK cell-mediated cytotoxicity via granzyme B [115]. It should be noted that NK cells, which produce IFN-γ are important in the control of intracellular infections. IFN-γ is a primary cytokines involved in development of Th1 type immunity.

6.4 The Potential of HSP70 in Vaccine Development

The ability of HSP70 to chaperone antigenic peptides and proteins as well as its unique adjuvant capabilities are attractive features for vaccine development. In addition, HSP70 non-conserved amino acid sequences are potential vaccine candidate antigens. In this context, both host and pathogen HSP70 has been tested in various vaccine infection models.

6.4.1 HSP70 as Antigen

Bacterial HSP70 as a Vaccine Antigen HSP70 has been employed in vaccine preparations tested in vaccines against various bacterial infections. Mice immunized with *Salmonella typhi* HSP70 and complete Freund's adjuvant (CFA) developed significant increased levels of antibodies and a mixed Th1/Th2 response against *S. typhi*. Immunized mice displayed 70–90 % protection against *S. typhi* [17]. In leprosy, immunization with the C-terminal fragment of *M. leprae* HSP70 resulted

in increased delayed type hypersensitivity (DTH) against both C-terminus and the whole HSP70 molecule. In vitro, lymph node cells from the immunized mice recognized and proliferated in response to both C-terminus and whole the HSP70, suggesting protective immunity [116]. In *Helicobacter pylori*, immunization with DNA from *H. pylori* HSP70 triggered Th1 immunity against *H. pylori*. Immunized mice showed less microbial load and less gastric mucosal inflammation than non-immunized control mice [117].

Protozoal HSP70 as a Vaccine Antigen Antigenic capabilities of HSP70 has also been tested in vaccines against various protozoal infections. In toxoplasmosis, mice immunized with DNA containing *T. gondii hsp70* gene developed Th1 type immunity as determined by cytokine responses in vitro. As compared to controls, immunized mice showed a significant reduction of parasite loads in the brain following infection challenge with *T gondii* infecting doses [118].

In the *L. donovani* infection model, Balb/c mice immunized with *L. donovani* HSP70 and HSP83 proteins in the presence of adjuvant monophosphoryl lipid A (MPLA) developed significant levels of Th1 type immunity. Vaccinated mice were resistant to *L. donovani* infection [119]. Similar Th1 protective results were observed in mice immunized with *L. donovani* HSP70 and the major leishmanial surface glycoprotein gp63 as antigen [120]. However, immunization of BALB/c or C57BL/6 mice with *L. major* HSP70 resulted in a mixed Th1/Th2 development and no protection against *L. major* infection was observed [121]. In Chagas disease, however, mice immunized with *T cruzi* HSP70 developed HSP70-specific $CD4^+$ T cells producing IFN-γ, IL-2 and TNF-α suggesting Th1 type protective immunity [122]. Interestingly, $CD8^+$ cytotoxic T cells recognizing T cruzi HSP70 epitopes were identified in Chagas disease patients indicating presence of protective immunity [123].

Fungi HSP70 as a Vaccine Antigen In fungi models of infection, immunization with fungal HSP70 was not found to induce resistance against these infection types. Mice immunized with *C. albicans* HSP70 showed high levels of IgG antibodies and cell-mediated immune responses against HSP70. However, no protection against *C. albicans* infection was detected [19]. Similar results were observed following immunizations with *Histoplasma capsulatum* HSP70 [124]. Interestingly, immunization of mice with *H. capsulatum* HSP60 did confer significant immune protection and resistance to infection against *H. capsulatum* [125]. These results demonstrate that different HSP may be recognized differently by the immune response and this results in particular immune protection capabilities to fungal infections.

6.4.2 HSP70 as Adjuvant

HSP70 as Adjuvant in Vaccines Against Bacteria The ability of HSP70 to chaperone antigenic molecules and its capacity to trigger activation of the immune

response has been employed in various experimental vaccines. Mice immunized with *L. monocytogenes* HSP70 loaded with *Listeria* antigenic peptides were found to develop $CD8^+$ T cells producing IFN-γ requiring the help of $CD4^+$ T cells for expansion [126]. In tuberculosis HSP70 as well as HSP60, in combination with Bacillus Calmette–Guérin (BCG) as an antigen, induced more robust immunity and conferred greater protection to immunized mice than BCG alone [127]. Furthermore, *M. tuberculosis* HSP70 specifically stimulates antigen-primed cells to produce proinflammatory cytokines in vitro [128]. However, this effect was not observed with human HSP70, suggesting that adjuvanticity of HSP70 may be selective to some, but not to all HSP70s [129]. An expression system based on HSP70 fused to diverse antigen-encoding sequences, were developed recently. Mice immunized with the HSP70/antigen complexes efficiently elicited antigen-specific $CD8^+$ T cell responses without the need of adjuvant [130].

HSP70 as Adjuvant in Vaccines Against Protozoal Parasites A parasitic DNA vaccine containing *L. amazonensis* HSP70 and *L. amazonensis* gene encoding P4 nuclease were tested in a vaccine in BALB/c mice. Mice immunized with P4 and HSP70 vaccine developed modest protective immunity and little resistance to infection against *L. amazonensis* [131]. In Chagas disease, mice immunized with *T. cruzi* HSP70 fused to the kinetoplastid membrane protein 11 (KMP11) antigen from *T cruzi* developed $CD8^+$ T cell cytotoxic responses against cells expressing KMP11 antigen [132]. Another experiment showed that C57BL/6 mice immunized with *P. falciparum* EB200 antigen and both Cholera toxin (CT) and *T. cruzi* HSP70 as adjuvants develop high antibody levels against EB200 and enhanced secretion of IFN-γ by splenocytes in vitro, suggesting that CT and HSP70 can work synergistically to improve immunogenicity [133, 134].

HSP70 as an Adjuvant in Vaccines Against Viruses *M. tuberculosis* HSP70 and the HIV-1 p24 protein antigen were tested in a vaccine against HIV-1. Immunized mice develop antibodies against P24 protein and their immune cells responded to P24 antigen in vitro [94]. Another study showed that mice immunized with *M. tuberculosis* HSP70 non-covalently bound to MHC class II influenza A peptide responded by increasing T cell responses against influenza A peptide [135]. In choriomeningitis virus (LCMV), mice were immunized with an epitope from LCMV and recombinant HSP70 as adjuvant. Immunized mice developed high levels of memory $CD8^+$ T cells. Infection challenge with LCMV resulted in Virus titres reduced by 10–100 fold as compared to control non-immunized mice groups [18]. Regarding respiratory syncytial virus (RSV), RSV antigen G1F/M2 was chemically linked to HSP70. Mice immunized with G1F/M2-HSP70 conjugate developed significantly higher levels of antibodies against G1F/M2 and $CD8^+$ cytotoxic T cells than mice immunized without HSP70 [85]. Together, these results demonstrate that HSP70 is an adjuvant that significantly enhances both humoral and $CD8^+$ T cells against chaperoned peptides derived from virus.

6.5 Conclusion

Studies concerned with the role of HSP70 in microbial infections revised here demonstrate that: (i) microbial HSP70 can associate with microbial cell surface where it may assist pathogen invasion of host cells; (ii) HSP70 can play dual roles, as a host receptor of microbes and as a chaperone for microbial survival within host cells; (iii) HSP70 promotes microbial survival by helping microbes cope with the toxic environment of host cells; (iv) HSP70 assists in microbial invasion and survival, and HSP70 works in concert with other associated molecular chaperones, including HSP90 and HSP40; (v) HSP70 is immunogenic, and is recognized by both antibodies and T cells in infected as well as in apparently healthy individuals; (vi) HSP70 induces the activation of acquired immunity and (vii) HSP70 is an efficient adjuvant that enhances both humoral and cell-mediated responses against various intracellular infections. Nevertheless, in certain conditions HSP70 cannot trigger the immune response, but on the contrary, down regulates immunity. Furthermore, HSP70 has the potential to provoke autoimmune reactions due to molecular mimicry between host and microbial HSP70s. Future research will be required to unquestionably clarify the potential of HSP70 as a vaccine adjuvant and as an antigen.

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