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Hsp70 in parasites: as an inducible protective protein and as an antigen

B. Maresca* and G. S. Kobayashi[a]

International Institute of Genetics and Biophysics, CNR, Via Marconi 12, I-80125 Naples (Italy) and [a]Washington University, School of Medicine, Department of Internal Medicine, Division of Infectious Diseases, Campus Box 8051, St.Louis (Missouri 63110, USA)

Abstract. The heat shock (HS) response is a general homeostatic mechanism that protects cells and the entire organism from the deleterious effects of environmental stresses. It has been demonstrated that heat shock proteins (HSP) play major roles in many cellular processes, and have a unique role in several areas of cell biology, from chronic degenerative diseases to immunology, from cancer research to interaction between host and parasites. This review deals with the *hsp70* gene family and with its protein product, hsp70, as an antigen when pathogens infect humans. Members of HSP have been shown to be major antigens of many pathogenic organisms when they experience a major temperature shift upwards at the onset of infection and become targets for host B and T cells.

Key words. Parasite; *Candida*; histoplasma; salmonella; *Leishmania*; macrophage; antigen; hsc70; hsp70; HIS-62; HIS-80.

Introduction

The physiological response of cells or entire organisms to abrupt increases in environmental temperatures, characterized by a rapid induction of a specific set of genes due to transcriptional activation, is termed the heat shock (HS) response[27,28]. The response is universal and, in addition to temperature increases, a wide variety of other stresses, such as ethanol, uncouplers of oxidative phosphorylation, inhibitors of electron transport components, steroid hormones, prostaglandins, etc., induce a very similar response at the cell level[47].

The synthesis of heat shock proteins correlates with the acquisition of thermotolerance to elevated temperatures and stresses that are otherwise lethal. HSP protect organisms not only from lethality but also from heat-induced developmental defects. Furthermore, some, but not all, of the HSP are developmentally induced in many organisms[46,71]. Recently, considerable progress has been made towards understanding the functions of HSP. It has been shown that HSP play a key role in many cellular activities even when temperature or stress is not administered to a cell. For example, it has been demonstrated that members of the hsp70 family maintain mitochondrial proteins in an unfolded, trans-locationally competent state and function inside mitochondria as they are being transported[11,12,62]. The induction of HSP is also involved in preventing the denaturation of proteins in mammalian and bacterial cells[20]. Additionly, HSP have been shown to be present in abnormal levels in several pathologic conditions, such as neurodegenerative disease[9], cancer[88], etc. Analyses of highly conserved structural and functional properties of the hsp70 family also suggest a potential role in cross-linking cellular proteins to actin filaments[82]. Members of the hsp60 and hsp70 families of bacterial and lower eukaryotic pathogens often act as immunodominant antigens, and immunological reactions to these highly conserved proteins have been implicated in the pathology of several autoimmune diseases[15,40,85] and inflammatory processes associated with pathogens[24,72,89].

Hsp70 in parasitic organisms

With respect to structure and function, HSP are among the most highly conserved proteins known. HS genes are induced very early during infection in parasitic organisms and play a critical role during adaptation to the new environment. An understanding of the role of HSP both in adaptation to the host environment and as antigens requires a detailed analysis of their regulation. In most organisms, as well as in parasites, several genes coding for *hsp70* exist, and only some are stress inducible. Therefore, it is crucial to have precise information from both structural and transcriptional analyses in order to assign to each particular protein a specific role in the host/parasite relationship. This is particularly important for hsp70 since this protein is probably the most conserved protein in all organisms thus far analyzed.

Constitutive hsc70 and inducible hsp70

In the last few years, the presence of hsp70 has been intensively investigated in several parasites. In germinating cells of the fungus *Candida albicans*, under non HS conditions, 28, 58, and a 66 kDa HSP have been described (the latter probably a member of the hsp70 family), while in a nongerminating variant only hsp18 and hsp22 were detectable at 23 °C (ref. 12). While

many members of HS genes from pathogens have been cloned and sequenced, most of the studies have been done at the structural and not the transcriptional level. Thus, there is a lack of data on the mechanisms of HS gene regulation in parasites when genes such as *hsp70* genes are actively transcribed at the onset of infection. Particularly, under more physiological conditions such as those that employ animal cells infected with various parasites. Such information is critical since it would provide a more comprehensive understanding of the mechanisms of action of their protein products, their cellular localization, its importance for survival in the host environment, and the role they play as antigens.

Transcription[7,55] and protein data[77] obtained in studies with the dimorphic fungal pathogen *Histoplasma capsulatum* have shown that maximal HS response, which varies between 34 and 37 °C, correlates with the level of virulence of the strain. Expression studies of cloned *hsp70* and *hsp82* genes have demonstrated that 34 °C is the temperature of maximal HS mRNA transcription in nonvirulent strains, while in virulent strains maximal induction occurs at 37 °C. Similar results have been found at the protein level[77]. With the assumption that exposure to stresses such as low pH or hydrogen peroxide are conditions that *H. capsulatum* finds in the intraphagolysosomal environment of macrophages (MΦ) when it infects humans[16], Kamei et al.,[34] analyzed cytosolic proteins by SDS-PAGE and fluorography. Such analyses have shown the induction, among other proteins, of hsp70. However, a more direct analysis of induction of hsp70 during macrophage infection has still not been performed.

In contrast, an extensive literature exists concerning survival of prokaryotic pathogens such as *Salmonella*, *Listeria*, *Mycobacterium*[23,29,45] in murine MΦ as well as studies such as the expression of 65 and 67 kD heat-regulated proteins and a 70 kD heat shock cognate (hsc70) protein expressed by *Leishmania donovani*[67]. Immunoblotting of murine marrow-derived MΦ were infected with promastigotes of *L. donovani* and tested with antibodies for the presence of 65 and 67 and hsp70 kDa proteins. Rey-Ladino et al. speculated that a rapid and high level of expression of inducible hsp60-related and *Leishmania* hsp70 in infected MΦ suggests that these proteins are involved in pathogenesis and may be a target of the immune response.

It has been shown that in promastigotes of *L. major*, the parasitic form present in the sandfly, most of the HSP are constitutively expressed at low temperatures[80]. Four *hsp70* genes have been detected and shown to be arranged in tandem with intergenic regions of ca. 380 nucleotides. The coded proteins are highly homologous to *hsp70* genes of *Trypanosoma brucei*, having 89% homology at the amino acid level, and contain miniexons which are *trans*-spliced under HS conditions at 37 °C (ref. 41). These hsp70 are induced both in vitro and in vivo in response to a temperature shift to 37 °C. However, a fifth *hsp70* gene located at a different locus is unaffected by a temperature shift upwards. In *L.donovani*, the cognate gene, *hsc70*, coding for an antigen related to the hsp70 family, is constitutively expressed during all stages of the life cycle[49].

Four genes coding for *hsp70* have been cloned from schistosomes[70] that are constitutively expressed in larvae and adult organisms. At least one *hsp70* sequence in *S. mansoni*, is heat shock inducible[33]. Furthermore, the schistosomulum stage of *S. mansoni* (the form in which cercariae differentiate in humans) predominantly synthesizes *hsp70* at 37 °C and cannot induce a HS response in the first 3 hours of this differentiated stage. However, fully differentiated schistosomula can induce an additional *hsp70* gene, whose sequence and regulatory elements have been determined[61]. Further analyses show stage-specific expression of the *hsp70* gene which is constitutive in miracidia sporocysts and adult worms but not in cercariae and termination of transcription during the sporocyst/cercaria transformation[59]. The *hsp70* gene can be transiently induced by stress and temperature (a shift to 42 °C) during cercariae/schistosomula transformation and in the adult worm. Neumann et al. have proposed that, since removal of the tail from the parasite is necessary for full induction of *hsp70*, an inhibitory factor may be present in the tail of cercariae[60]. Yet another example is provided by the tick-borne tropical parasite *Theileria annulata*, in which high levels of hsp70 are present at 28 °C and are further inducible by HS[53].

In the hemoflagellate *T. cruzi*, which causes Chagas disease and sleeping sickness in humans, epimastigotes, the form found in insect vectors, differentiate into trypomastigotes (bloodstream form) before infection. Experimentally, epimastigotes produce a classical HS response at temperatures from about 37 to 41 °C. Under these conditions, this form induces 10 major peptides with three dominant bands of 60 to 83 kDa which are also present in exponentially growing and stationary cells at normal temperatures[8,14]. Furthermore, in trypomastigotes most of the HSP synthesized at 39 °C are also present at 24 °C. In agreement with the Yost and Lindquist hypothesis[91], it has been suggested that the induction of HSP in trypomastigotes could prepare *Trypanosoma* to infect the mammalian host[1], similar to what has been generally proposed for the presence of high constitutive levels of HSP in the vector form of other parasites. Requena et al.[66] have shown that an *hsp70* mRNA of 2.2 kb is constitutively transcribed from the *hsp70* cluster at 28 °C and after HS its abundance is increased about 4-fold. A similar increase was observed when the parasite reached stationary phase at 28 °C. In *T. brucei*, transcripts of *hsp70* and *hsp83* were 25- to 100-times more abundant in trypomastigotes at 37 °C than in insect stages[86]. In *T. brucei*, a 23 kb heat shock 70 locus coding for several clustered *hsp70* genes has

been identified[43]. At least six genes have been distinguished that are transcribed in a long polycistronic molecule. However these genes are, in contrast to the heat shock genes of other eukaryotes, encoded by nucleotide sequence blocks that are identical in all six *hsp70* genes. It was shown that, from 5′ to 3′, a diverged cognate *hsc70* gene (gene 1) is separated by about 6 kb of DNA from a cluster of five identical *hsp70* genes (genes 2–6)[43]. Gene 1 is unaffected by HS. These conserved domains are located in the 5′ coding region and are separated by highly diverged nucleotide sequences. Lee et al. have proposed that the nucleotide sequence conservation between *hsp70* gene 1 and *hsp70* genes 2–6 indicates that selective sequence homogenization by gene conversion maintained the amino acid sequence conservation.

In *L.major* four *hsp70* genes have been found arranged in tandem with intragenic regions of about 380 nucleotides[41]. They also demonstrated that in an undifferentiating mutant strain all the *hsp70* genes were normally expressed, implying that members of this gene family are not directly involved in the differentiation process. Promastigotes of cutaneous species of *Leishmania* of South American origin change to become amastigote-like when incubated at 34–37 °C and are antigenically similar to lesion-derived amastigotes. It is interesting to note that both axenic amastigotes and heat shocked promastigotes are more infective to laboratory animals than untreated promastigotes[78]. Furthermore, it has been shown that *hsp70* and *hsp83* genes are induced prior to the morphological changes and remain constitutively expressed in *Leishmania* amastigotes. Similar conditions may exist in the vector just before taking a new blood meal when the promastigotes experience nutritional deprivation[83]. Thus, the presence of HSP prior to transmittance may pre-adapt the parasite for additional stress in the mammalian host[76]. Three additional members of the *hsp70* family, which are located on different chromosomes, were isolated[73]. Their sequences were related to those of previously characterized *hsp70* genes of trypanosomatids. However, two of these genes were constitutively expressed in promastigotes and not inducible, while a third was differentially expressed between non-infective and infective promastigote stages in the absence of a HS. Recently, a *hsp70* gene containing an N-terminal sequence characteristic of a mitochondrial targeting signal has been isolated[74]. This *hsp70* gene is localized and expressed in mitochondria in all stages of *L. major*.

Several members of the *hsp70* gene family have been cloned and sequenced from *P. falciparum*[2,4,38,39,64]. The coded proteins share 50 to 70% aminoacid identity. The cloned *hsp70* genes are constitutively expressed and are detected in all blood stages of *Plasmodium*. In culture-derived cells of *P. falciparum* a HS to 37 °C plus glucose induced the synthesis of *P. falciparum* -hsp and glucose regulated protein (grp) of *P. falciparum*, which are similar in sequence to hsp70 and grp78[36]. By employing immuno-gold electron microscopy the authors showed that Pfhsp was localized in the nucleus whereas Pfgrp was found primarily in the cytoplasm. Kumar et al.[35] also studied the expression of the hsp70-like protein induced by the exoerythrocytic stages of *P. berghei* cultured in HepG2-A16 hepatoma cells and those of *P. falciparum* in human hepatocytes transplanted under the kidney capsule of CB-17/Icr scid/scid mice[37]. These proteins were not detected in the sporozoite form of malaria, but markedly induced in the hepatic stages of the parasite.

HSP and infectious diseases

It has been proposed that the HS response of parasites plays an essential role during host invasion[50,65,87]. In fact, HSP have been detected during the course of many parasitic (e.g., *Leishmania*, *Trypanosoma*, *Plasmodium*, *Giardia*, *Schistosoma*, nematodes, etc.) and pathogenic fungal (*Histoplasma*, *Candida*, etc.) infections. While the overall HS response in these organisms is very similar to that seen in *Saccharomyces cerevisiae* and mammalian cells, specific differences exist. For example, in higher eucaryotic cells, experimental conditions are such that the increase in temperature is transient and the temperature must be lowered to ensure survival. On the other hand, in lower eucaryotes such as dimorphic fungal pathogens and parasites there is a transient cessation of growth after HS followed by resumption of growth, presumably as a result of adaptation to the new environmental condition. In fact dimorphic fungi, which exist in soil as saprobic organisms, and parasitic organisms, which reside in poikilothermic animals, must shift from environments where the temperature is 22–28 °C and rapidly adjust to the temperature of a homeothermic mammalian host at 37 °C, an elevated temperature that remains constant after the shift upwards. Furthermore, it is reasonable to assume that during host invasion, when the parasite makes contact with host cells, the stress response is elicited independently of the temperature shock. Consistent with this hypothesis Fields et al.[22] have shown that with *Salmonella* cultured at 37°C, HSP are induced during MΦ infection without changing the temperature of incubation.

The ability of organisms that have been pre-treated with moderate heat to withstand a lethal HS is amply documented and correlates with conditions that induce optimal synthesis of HSP, a condition called thermotolerance. However, experiments on eucaryotic cells are often performed under non-physiological experimental conditions, such as exposure to a sudden change to very high temperatures followed by a rapid shift to lower temperatures in order to ensure survival of the organism; in reality, under natural conditions, temperature

changes are gradual. Thus, with most eucaryotic organisms, thermotolerance plays a central physiological role in the maintenance of several cell properties, such as protein aggregation (dimerization), mRNA processing, etc. For example, Patriarca and Maresca[63] have shown that induction of thermotolerance in *S. cerevisiae* protects ATPase activity at high temperatures, whereas mitochondrial ATPase becomes nonfunctional within minutes if cells are shifted directly from 30 °C to an elevated temperature of 44 °C. On the other hand, thermotolerance probably plays a minor role in parasites because these organisms face a sudden and drastic environmental temperature change upon infection. In organisms such as these the HS response is not an artificial phenomenon. On the contrary, at the onset of host invasion, the changes in temperature and in environment are abrupt, and parasites have no time to induce a thermotolerant state. Thus, the entire cellular apparatus such as mRNA maturation machinery, membrane structures, protein folding and transport, etc., must remain functional to allow the organism to survive and undergo proper morphogenic adaptation. This could be achieved either by the evolution of temperature resistant structures or by the existence of an innate constitutively thermotolerant state before host invasion. Thus, in these organisms the presence of temperature-sensitive enzymes or structures (e.g., spliceosome, see below) whose activities are dependent on high levels of HSP during adaptation and the phase transition at 37 °C may severely hamper the survival of these organisms during the process that leads to virulence.

In *H. capsulatum*, a dimorphic fungus that exists in a multicellular filamentous state in nature and as unicellular budding yeasts in human tissue, the transition between the multicellular and unicellular phases can be reversibly induced in the laboratory by shifting the temperature from 25 °C (mycelia) to 37 °C (yeast). There are major differences in the manner in which pathogens such as *H. capsulatum* retain functional RNA processing at elevated temperature ranges. In *Drosophila*[90], HeLa cells[5], *Caenorhabditis elegans*[25] and *S. cerevisiae*[91], mRNA splicing is blocked at high temperatures. However, Yost and Lindquist[90] demonstrated that spliceosome activity can remain functional if thermotolerance is induced and hypothesized that the spliceosome is a temperature-sensitive structure whose activity is protected by the presence of HSP. Thus, in all these organisms physiological fluctuations of temperatures that gradually induce HSP ensure that their spliceosomes remain functional at high temperatures. However, it has been shown that in *H. capsulatum*, even when phase transition is induced under non-physiological temperatures up to 42 °C, hsp82 and β-tubulin[55], *cdc2*[19] and *Ole1*[25] mRNA are properly spliced, suggesting that temperature-resistant splicing is a characteristic feature of this organism[55].

In the *S. cerevisiae ssa1 ssa2* double mutant that carries mutations in two members of the hsp70 family, unspliced mRNAs do not accumulate in cells shifted directly from 25 to 41 °C, indicating that hsp70 may be one of the proteins involved in the protection of the splicing events[91]. In this particular mutant, which is about 100-fold more thermotolerant than wild-type cells, the presence of high constitutive expression of hsp70 at a temperature of 25 °C has been correlated with its ability to survive lethal temperatures and to protect mRNA processing. Mutations in two classes of yeast *HSP* affect the pattern of RNA splicing during the HS response. With specific *hsp70* mutants that overproduce other HSP at normal temperatures, splicing at high temperatures is constitutively protected; as a result, induction of thermotolerance is not required. These results indicate that members of the hsp70 family help to maintain normal cellular processes at higher temperatures. It is intriguing that mycelial cells of *H. capsulatum* constitutively have high levels of hsp70 (ref. 79). Whatever the mechanism, it is reasonable to speculate that with parasites it is crucial that the spliceosome remains functional at high temperatures, since only accurate mRNA processing will allow adaptation to the host environment, subsequent morphogenesis and virulence.

In trypanosomes, in which many genes are part of polycistronic transcription units and mRNA maturation involves a *trans*-splicing event in which a mini-exon is joined to the 5′ end of a protein-coding exon, the machinery responsible for β-tubulin mRNA matur-ation is disrupted by HS (ref. 57). However, Muhich and Boothroyd[58] have shown that in *T. brucei trans*-spliced *hsp70* and *hsp83* mRNAs are not sensitive to disruption by severe HS and has proposed that *T. brucei* may have evolved a mechanism by which *trans*-splicing of HS genes is specifically protected by the disruption effect[58]. However, a more detailed analysis of mRNA maturation and the role of hsp70 in other parasites is necessary to elucidate differences with other eukaryotic cells.

In several parasitic organisms a shift to high temperatures (37 °C) results in both a HS response and a developmentally regulated morphologic transition. Thus far, it is not clear whether expression of the HS gene family is part of the process of differentiation itself, or whether it is an epiphenomenon involved in adaptation to the new environmental temperature and extant conditions. In fact, parasites must adapt not only to higher temperatures, but also to different conditions such as redox potential, presence or absence of growth nutrients, hormones, factors present in serum, etc., and must change their capacity to protect themselves from oxidative products and digestive enzymes produced by human cells such as MΦ, etc.

We have shown that HS gene transcription can be profoundly modified in *H. capsulatum* by altering the ratio of membrane saturated/unsaturated fatty acids[6,51].

When levels of unsaturation of membrane fatty acids were increased by addition of oleic acid, HS gene transcription was abolished. Mycelia treated in this manner still retained the ability to transform to the yeast phase although at a much slower rate, 20 rather than 4 days, and they were not virulent. Thus, at least in this pathogenic fungus, HSP do not appear to be directly involved in morphogenesis.

Hsp70 as an antigen

Stress proteins play an important role in parasite-host interactions and are of immunological importance since some of them (hsp60, hsp70, and hsp90) are among the most dominant antigens synthesized at the onset of infection in humans by a large spectrum of pathogens[18,54,92] and are recognized by the immune system[79,93]. Furthermore, in the last few years there has been a better understanding of antigen processing and antigen presentation, suggesting that particularly hsp60 and hsp70 are involved in the molecular processes that lead to the assembly mechanisms.

In a series of elegant experiments, Gomez et al.[28] showed that in *H. capsulatum*, two antigens, HIS-62 (hsp60) and HIS-80 (hsp70), induce cellular immune responses in mice that are able to mediate protective immunity against challenge with *H. capsulatum*[17]. Gomez et al. probed cell wall and membrane extracts for the presence of immunoreactive proteins using monoclonal antibody (MAb) directed against hsp70; hsp70 has been demonstrated to elicit a cellular immune response both in vivo and in vitro. Although the 80 kDa antigen has sequence similarity with hsp70, it differs antigenically, since murine T cells responded to 80 kDa antigen, but not to a purified hsp70. Presumably the 80 kDa protein contains an epitope recognized by T cells that is not present in hsp70. It has also been shown that mice immunized with viable yeast cells mounted a delayed-type hypersensitivity response to the 80 kDa antigen, indicating that it is indeed a target of the cellular immune response to *H. capsulatum*[26,27]. In a recent report, Jevons et al.[32] described a murine monoclonal IgG antibody, MAb 69F, that also recognized an 80 kDa antigen that was identical to the protein isolated by Gomez et al.[27] based on molecular mass and N-terminal amino acid sequence. Enzymatic and chemical studies showed the molecule to be *O*-glycosylated, heat-inducible, and of cytoplasmic origin or membrane-bound but not in the cell wall. Of interest is that MAb 69F did not appreciably stain mycelia of *H. capsulatum* grown at 25°C, but when mycelial cells were incubated at 37 °C for 24 hours they exhibited the same general staining pattern as yeast cells. The MAb used by Gomez et al.[81,82] was directed primarily against hsp70 and recognized a band on Western blot at 80 kDa whereas MAb 69F predominantly recognized the 80 kDa band and was reactive with cytoplasmic antigens prepared from *Aspergillus fumigatus*, *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis* and the duboisii variant of *H. capsulatum*, suggesting that the two MAbs are directed against different epitopes[32].

A similar situation occurs in candidiasis. While the pathogenesis of infections caused by *C. albicans* is unclear, an immunodominant 47 kDa antigen has been described and thought to play a role in candidiasis[54]. This antigen is a breakdown product of hsp90, and antibody directed against it is protective in an animal model of infection. Related hsp90s have also been identified in infections caused by *C. parapsilosis*[54] and *Aspergillus fumigatus*[54].

A 70 kDa surface protein of *T. cruzi* was found to react strongly with sera from chronically infected mice[21]. A gene coding for 71 kDa was isolated by screening a cDNA library obtained with rabbit antiserum raised against the purified serum surface antigen. However, the cloned *hsp70* -like gene did not react with antibodies elicited by the cloned protein, leaving uncertain the antigenic properties of this hsp70 homologue. In a subsequent study designed to investigate the role of hsp70 as specific target of the human humoral immune response to *T. cruzi* infection, Engman et al. found that hsp70 is in fact a major antigen in Chaga's disease[22]. However, antibodies to *T. cruzi* hsp70 did not react with human hsp70, despite the 73% amino acid homology.

Sera of patients chronically infected with *T. cruzi* and *L. brasiliensis* were shown to react on western blot with a 70 kDa polypeptide[44]. The target of the immune reaction was a cross-reactive antigen, a member of the hsp70 family. Screening of an expression library demonstrated the presence of a common pattern characteristic of *T. cruzi* - *L. brasiliensis* mixed infection. However, approximately 70% of cDNA clones were found to be members of hsp70 and hsp90 by immunoscreening expression libraries with serum of a patient infected with visceral leishmaniasis[13]. While patients with visceral leishmaniasis recognized the recombinant *Leishmania* hsp70 and hsp90, sera of patients infected with *T. cruzi* did not, although hsp70 and hsp90 of *T. cruzi* and *Leishmania* spp. have more than 80% amino acid identity.

In *P. falciparum*, a protein defined as Ag63 was shown to contain a region of tandemly repeated amino acid sequence that is similar to that found in hsp70 of *P. chabaudi*[78], and *Brugia pahangi*[75] but not in Ag361 which is another member of hsp70 of *P. falciparum*[64]. However, in contrast to other malarial antigens, the repeated regions of Ag63 are not immunogenic in humans. Recently, Pf72/hsp70–1, a major immunogen corresponding to hsp70 that is present in a fraction of the blood stage of *P. falciparum*, has been characterized and shown to experimentally protect Saimiri monkeys[3]. The antigen was found to be present in all blood stages and

one of its epitopes was also detected on the surface of infected hepatocytes. Fifty-two percent of individuals living in West Africa tested positive by ELISA for humoral levels of antibody against Pf72/hsp70–1. All were found to have statistically significant levels of anti Pf72/hsp70–1 antibodies (70% in adults and 26% of children). The total anti-Pf72/hsp70–1 antibody level was related with age and exposure to malaria. However, T cell responses did not correlate with either antibody level or age. Ten peptides whose sequences corresponded to the C terminal part of Pf72/hsp70–1 were tested as potential T cell epitopes. Three of these peptides more frequently triggered T cell proliferation in individuals continuously exposed to the parasite. None of these peptides reacted with T cells of Europeans who had no exposure to plasmodia. Since some of these peptides are similar to human hsc70 and hsp70 sequences, it is possible that cross-reactivity exists between the HSP of *P. falciparum*, human, and other microorganisms. Until these problems are solved, these peptides cannot be used in a polyvalent vaccine against malaria.

It has been shown that human sera of individuals infected with *S. mansoni* show reactivity with a hsp70 antigen[30] mainly towards nonconservative sequences[31]. In fact, antibodies present in sera of patients infected with *S. japonicum* do not react with the antigen of *S. mansoni*. In mice, antibodies against this hsp70 antigen appear early as a result of infection with *S. mansoni*[30]. Hsp70s of other parasites have been reported to react with sera of infected patients. In screening of an expression λ cDNA library of *Entamoeba histolytica*, 49 positive clones were isolated that reacted with sera of patients infected with invasive amoebiasis[62]. Sixteen of these clones were highly homologous to eukaryotic hsp70s, and one had 70% sequence identity to the human hsp70. Another example is given by the nematode *Brugia malayi*, in which a member of a multigene *hsp70* family reacts with antibodies active against hsp7075. By using a λ clone (OvG15) encoding for a hsp70 protein of *Onchocerca volvulus* that is recognised by sera of individuals living in areas endemic for lymphatic filariasis, Rothstein and Rajan[69] have isolated a corresponding gene from *B. malayi* which is developmentally regulated and heat-inducible.

Conclusion

In parasites, unlike most organisms, HS is a physiological phenomenon that is part of their life cycle. As a result of an infection, all parasites induce high levels of HSP whose sequences are astonishingly conserved in evolution. In recent years, a great deal of attention has focused on these proteins because their sequences are highly conserved and because of their potential role as immunogens. During the process of infection, HSP of parasites play a central role in the process of differentiation, adaptation and protection from the host's killing mechanisms such as reactive oxygen meabolites, low pH, etc. On the other hand, mammalian T cells proliferate in response to parasitic HSP. It is now accepted that HSP are the target of humoral and cell-mediated immune responses. However, the reasons why these proteins are immunodominant are still not clear. One possibility that has been proposed is that the abundance with which they are induced is the reason for their immunodominance[92]. Other possibilities include the presence of conserved epitopes, preferential processing, an intrinsic role as virulence factors, etc. Thus far there is no experimental evidence to support any of these theories.

While the host's immune system may be able to discriminate between its own hsp70 and those of the parasite, a deranged immune system may ultimately cause autoimmune diseases in humans. It is feasible that induction of HSP by the pathogen and the concomitant recognition by the immune system of hsp70 is the result of an equilibrium established between mammals and pathogens during evolution. In fact, while the immune system recognizes a highly conserved protein such as hsp70 that could be confused with the mammalian 'self' hsp70 proteins during the process of antigen-presentation, these proteins may not be eliminated or modified by the pathogen as a self-defense mechanism against the host unless its own HS response and its capacity to adapt are greatly impaired. In yeast, for example, it has been shown that certain *hsp70* genes are essential and this is probably the case in most eucaryotes and pathogens.

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* To whom reprint requests should be addressed.

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