Current Immunology Reviews, 2017, *13*, 44-55

REVIEW ARTICLE

HSP60 is a Ubiquitous Player in the Physiological and Pathogenic Interactions between the Chaperoning and the Immune Systems

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Abstract: HSP60 participates in many interactions between the system integrated by all chaperones and closely associated molecules (chaperoning system or CS) and the immune system (IS). These interactions occur constantly to maintain normal cell physiology but, occasionally, they are perturbed and become mediators of pathologic events that may lead to disease. This switch to pathology may be initiated by various factors, genetic or acquired, which cause qualitative and/or quantitative modifications of HSP60, or immune crossreactivity between the human and microbial chaperonin orthologs, or a break in the balance between the pro- and anti-inflammatory actions of the chaperonin. Thus, autoimmune and chronic inflammatory pathologies may occur. Likewise, a perturbation of the CS-IS interactions, *e.g.*, those that take place during ageing, may favor carcinogenesis. HSP60 may be commandeered by tumor cells to assist its high-rate protein synthesis and, also, to be an emissary among the devices tumor cells utilize to avoid anti-tumor immune reactions. Here, we briefly discuss the canonical and non-canonical functions of HSP/chaperones; and HSP60 as a multifunctional molecule, its migration itinerary, and its possible roles during carcinogenesis and in certain chronic inflammatory and autoimmune diseases. We examine the potential of HSP60 as a biomarker useful for diagnosing and monitoring the progression of the various conditions in which it actively participates. Lastly, we discuss the use HSP60 as target for controlling its activity when it is an etiopathogenic factor, or as a therapeutic agent to correct its deficiency.

ARTICLE HISTORY

Received: November 18, 2016
Revised: January 26, 2017
Accepted: March 20, 2017

DOI:
10.2174/1573395513666170412170540

Keywords: Chaperoning system, HSP60, immune system, inflammation, autoimmunity, cancer, exosomes.

1. INTRODUCTION

One may envision the entire set of chaperones, co-chaperones, chaperone co-factors, and chaperone receptors and close interactors of an organism as a physiological system, the chaperoning system (CS) [1]. Evolutionary data suggest that the primitive CS evolved from a very simple form in ancient unicellular organisms (*e.g.*, primitive archaea) to reach the complexity we see today in humans, for instance, most likely as a consequence of the constant exposure of living creatures to environmental stressors. The data indicate that throughout evolution the CS played important roles in cell physiology and was, and to some extent still is in today's living beings whether simple or complex, part of a defensive system against aggressors. Likewise, the immune system (IS) evolved, starting later with respect to the CS, also to defend against aggressors, such as infectious agents. The CS and the IS probably complemented each other over the millennia and interacted to ensure cell and organismal homeostasis and survival. It is, therefore, no surprise that today in complex, multicellular organisms such as humans, both systems interact extensively in health and in disease.

In this article, we review only a few representative examples extracted from the very many available of CS-IS interaction in disease, focusing on the HSP/chaperone HSP60 (also designated HSPD1 in humans, and Hsp60 or Cpn60 in prokaryotes). We also discuss participation of the CS, specifically HSP60, in carcinogenesis. In this respect, there are also many contact points between the CS and the IS, since cancer cells voraciously use components of the CS to meet the demands of fast growth and to block or misdirect the IS and, thereby, avoid anti-tumor immune defenses.

2. HEAT SHOCK PROTEINS/CHAPERONES: CANONICAL AND NON-CANONICAL FUNCTIONS

Molecular chaperones are active during the cell cycle and organismal growth and are involved primarily with protein

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1875-631X/17 $58.00+.00

homeostasis [2]. Many chaperones are heat shock proteins (HSPs), whose genes are expressed during various stress conditions caused by environmental changes, temperature elevation, infections, tumors, etc. This is likely due to the fact that protein folding is severely affected by heat and other stressors and, therefore, some chaperones act to prevent or correct the damage caused by misfolding. Although many genes encoding chaperones are not induced by typical stressors, it is common to use the terms HSP and chaperone as synonyms, and we will do the same here.

Regulated overexpression and/or inhibition of HSPs play an important role in maintaining cell viability under normal and stress conditions. Because of the characteristic changes in their levels in various pathologic conditions, HSPs are potentially useful biomarkers and therapeutic targets [2, 3]. For example, HSP90 has shown promise for diagnosing, assessing prognosis, and treatment of various types of cancer [4, 5]. Similarly, HSP70, HSP60, and small HSPs have potential for the treatment of neurodegenerative diseases, ischemia, autoimmunity, and graft rejection, just to mention a few illustrative examples [6]. A considerable amount of data, accumulated over the last few decades, have shown that exploring the cytoprotective and immunoregulatory roles of HSPs can open new avenues for drug discovery and treatment of many important diseases [7-9].

HSP/chaperones are a large group of molecules for the most part highly conserved during evolution that play many roles: protect cells against the damaging effects of a variety of stressors on the one hand and, as molecular chaperones, maintain protein homeostasis. Molecular chaperones are central components of the molecular machinery that monitors and maintains the health of the proteome [10]. Chaperones defend cells and tissues from the dangerous consequences of protein aggregates, promoting their degradation by, for instance, the ubiquitin-proteasome pathway [10, 11]. In addition, HSP/chaperones perform a variety of functions unrelated to protein homeostasis but, nonetheless, key to cell physiology and survival. Thus, it may be said that HSP/chaperones have canonical and non-canonical functions, with the former pertaining to protein homeostasis and the latter encompassing a range of roles in cell physiology other than protein-quality control, all important in one way or another in health and disease; therefore, HSP/chaperones have potential in diagnosis, monitoring, and therapy [7]. Typically, HSP/chaperones assist other proteins to fold correctly as they emerge from the ribosome, to re-fold when partially denatured by stressors, to go in the direction of degradation when irreversibly damaged, and to translocate from the place of their synthesis to their final destination in the cell. Distinctive functional domains in chaperone molecules recognize a polypeptide in need of assistance, build a chaperoning complex, form a network with other chaperoning complexes, or interact with a protein-degrading machine like the ubiquitin-proteasome system. HSP/chaperones also play 'extrachaperoning' roles, namely non-canonical functions, such as participation in IS regulation, cell differentiation, gene expression, DNA replication, signal transduction, programmed cell death, cellular senescence, and carcinogenesis [12-18].

3. HSP60: A MULTIFUNCTIONAL MOLECULE THAT INTERACTS WITH THE IMMUNE SYSTEM

HSP60 is typically a mitochondrial protein, which together with its co-chaperone HSP10 (also designated HSPE1 in humans, and Hsp10 or Cpn10 in prokaryotes), assists protein folding inside the organelle [12-15]. The presence and integrity of HSP60 in mitochondria are crucial for cell survival as demonstrated by inducing HSP60 knockdown with *siRNA* oligonucleotides, which leads to apoptosis [12-18], and by the occurrence of at least two serious human genetic chaperonopathies associated with mutations of the *HSP60* gene [19, 20].

HSP60 has been found also in extramitochondrial sites, such as the cytosol [21], intracellular vesicles [22], plasma membrane [23], and the surface of normal and tumor cells [24, 25]. In the cytosol, HSP60 can have either pro-survival or pro-apoptotic action depending on tissue distribution, and interactors such as Bax, Bcl-2, or caspase 3 [21, 26-28]. The events of cell stress and cell death are linked and HSP60 induced in response to stress appears to function at key regulatory points in the control of apoptosis [12, 26-28]. At the cell's surface, HSP60 can interact with TLR2, TLR4, and MHC molecules triggering innate and adaptive immune responses, (Table **1**) [7, 29, 30]. HSP60 was found in the extracellular space, soluble in the peripheral blood, and may be exported outside cells through microvesicles, *e.g*. exosomes, which derive from endosomes and multivesicular bodies [24, 25, 31, 32]. Exosomes participate in immunoregulatory mechanisms by modulating antigen presentation, immune activation, immune suppression, immune surveillance, and intercellular communication [33]. Intercellular communication by chaperones via exosomes contributes to maintenance of protein homeostasis at the organismal level [34], and alterations of the exosome's properties and cargo are a component of the cellular response to environmental stress [35]. Hence, exosomal HSP60 (as other HSPs) may be considered key players in intercellular cross-talk, acting in paracrine and endocrine modes [24, 25, 36]. HSP60 was found to be a target self-antigen in pathological autoimmunity (see later), and there are consistent experimental data showing that HSP60 also displays dominant immunoregulatory properties [29, 37].

It has been shown that extracellular HSP60 has effects on neutrophils and macrophages [38]. In macrophages, HSP60 can interact with cell-surface receptors, such as CD14, CD40, and Toll-like-receptors (TLRs), causing in turn either pro- or anti-inflammatory effects. For example, HSP60 can induce secretion of cytokines from professional antigen-presenting cells, with consequent activation of T cells [22, 39-42]. Examples of the physiological interactions thus far delineated between Hsp60 and the IS are illustrated, in Table **1** and Fig. (**1**). These interactions probably reflect functional complementation of two physiological systems that have evolved to cooperate in supporting and preserving cell physiology, maintaining proteomic homeostasis, and defending the cell and the organism against stressors, as mentioned earlier in this review and other publications.

Table 1. HSP60-immune system interactions: target cells, effects, and receptors[a].

Cell Type(s)	Effect	Receptor	References	Note
Macrophages and dendritic cells	Production of IFN-α, which in turn is responsible for antigen-dependent T-cell release of IFN-γ	Unknown (independent of TLR4)	[44, 45]	It has been reported that HSP60 has no effect on expression of 96 common cytokine genes, including IFN-γ in macrophages (see footnote)[b]
Macrophages	TNF-α production	TLR4	[46]	Autologous HSP60 may serve as danger signal (antigen) to innate immune system
Neutrophilic granulocytes	Chemoattractant; enhances production of reactive oxygen and release of primary granule enzymes	Unknown	[47]	HSP60, which is released by damaged tissue, may promote early innate defense mechanisms against invading pathogens
Peripheral blood-derived mononuclear cells	Enhanced secretion of TNF-α	Unknown	[48]	HSP60 released upon tissue damage might play a role in regulation of bacteria-induced inflammation
T cells	Enhances population of Tregs	TLR2 (7) and/or TCR	[49-51]	HSP60 can downregulate adaptive immune responses by upregulating Tregs
T cells	Proliferation	CD45 RA^+ RO^-	[52]	May be relevant for pathogenesis of atherosclerosis
T cells	Downregulation of chemokine receptor expression (CXCR4 and CCR7)	TLR2	[53]	HSP60 can regulate T-cell behavior in inflammation
$CD4^+$ T cells	Determines IL-10 secretion	CD30	[54-56]	May have clinical relevance in treatment of type II diabetes and juvenile
Immune system cells	Immunomodulation	Unknown	[24, 57]	Extracellular HSP60 may have a role in the cross talk between tumor cells and the immune system
Natural Killer cells	Stimulation of migration and cytolytic activity of natural killer cells	Unknown	[58]	Exosome-carried HSP60 may have a role in a antitumor response of NK cells

[a]Source: References [1, 7].

[b]Reports of effects of HSP60 on immune system components (*e.g.*, T and B cells, macrophages, monocytes, dendritic cells, neutrophils, microglia) must be examined with caution, because often it is unclear whether the effects observed are due exclusively to the HSP60 molecule or to contaminants such as lipopolysaccharide, lipoproteins, and flagellin, or to both. We have focused here on reports of work in which either highly purified human HSP60 or human HSP60 peptides that were synthesized *de novo* were used. Abbreviations: IFN, interferon; TNF, tumor necrosis factor; TLR4, Toll-like receptor 4; IL-10, interleukin-10.

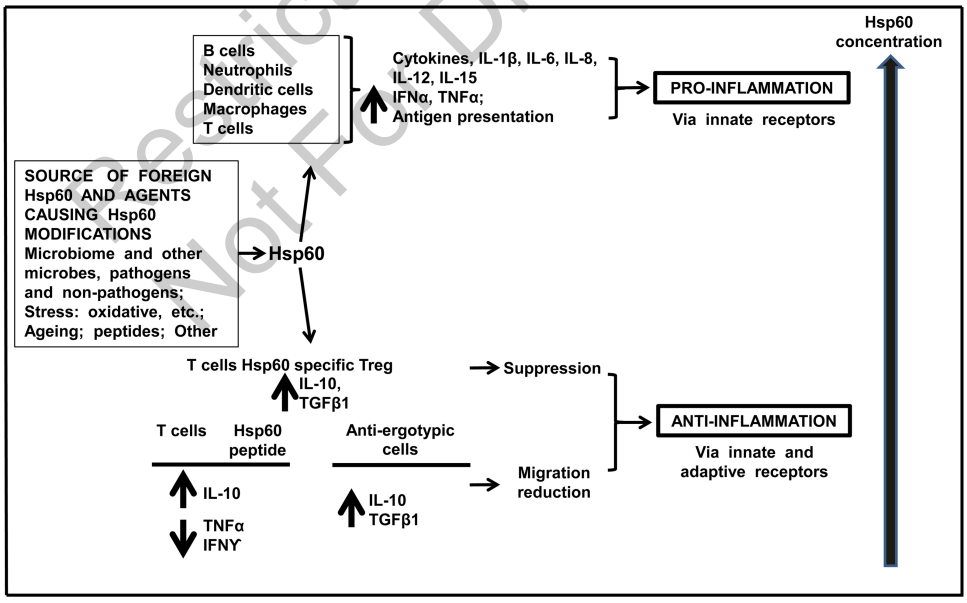

Fig. (1). HSP60 (Hsp60) can interact with the two arms of the IS, innate and adaptive. Hsp60 (here Hsp60 stands for the 60 kDa chaperonin from any species) can act as immunogen-antigen when foreign (*e.g.*, from an intestinal bacterium), or as auto-antigen when autologous but increased in concentration and/or modified (*e.g.*, abnormal post-translational modification) to the point that it appears foreign to the IS. Hsp60 can also act as a sort of hormonal factor on cells very near its origin, *i.e.*, paracrine effect, or far and very far away, *i.e.*, endocrine-like effect. Immune effects of Hsp60 are mediated by innate TLR signaling and adaptive receptors. The local concentration of Hsp60 is an important factor that determines the choice of pro-inflammatory or anti-inflammatory pathways. An increase in the Hsp60 levels caused by oxidative stress, for instance, can lead to a pro-inflammatory effect via cytokine cascade or via B-cell activation, but it can also lead to an anti-inflammatory effect by increasing IL-10 and TGFβ and decreasing of TNFα by T-cells. **Symbols:** Short thick vertical arrows pointing upward indicate increase of pro-inflammatory factors (top) or anti-inflammatory factors (bottom); likewise, the short thick vertical arrow pointing downward indicates decrease of cytokine levels. The long thick vertical arrow to the far right indicates increasing concentrations of Hsp60 from bottom to top. Source: References [36, 59, 60].

4. HSP60 IN CARCINOGENESIS AND ANTITUMOR IMMUNE RESPONSES

The role of HSP60 in apoptosis is a subject of controversy [21]. Since HSP60 levels are abnormal, usually elevated, in a variety of cancers, this chaperone has potential in diagnosis and in assessing prognosis [7]. Consequently, over the last several years, the possible role of HSP60 in various human cancers has been under scrutiny, including its value as a biomarker in risk prediction, and in screening-diagnosis of human cancer [2].

The involvement of HSP60 in tumorigenesis has been investigated in various ways, among which the monitoring of its quantitative levels and distribution in cells has provided good correlative data. For example, the progression of breast carcinoma, prostate cancer, and colorectal carcinoma are typically accompanied by an increase in the levels of HSP60 and/or in the expression of the *HSP60* gene. Also, it has been shown in gastric cancer, that elevated levels of HSP60 are significantly correlated with MMP-9 and, in parallel, with depth invasion, lymph node metastases, and disease progression [43].

These quantitative variations may be ascribed to the high concentrations of mutant and misfolded oncoproteins in the cancer cells and/or altered signaling pathways occurring in tumor cells that induce the transcription of the *HSP60* gene [61, 62]. In summary, tumor cells need more HSP60 than normal counterparts. On the other hand, for some type of cancers elevated levels of HSP60 are cytoprotective for normal cells whereas decreased levels are associated with carcinogenesis as occur in renal-cell carcinoma [63] and bladder tumors [64]. In these two types of tumors, the loss or decrease of HSP60 expression is correlated with poor prognosis. Recent data indicate that HSP60 is fiber-type specific in the posterior muscles of trained mice and high HSP60 levels are correlated with mitochondrial biogenesis [65]. These data open interesting avenues to investigating the relationship of HSP60 and exercise and its implication in cancer cachexia.

Because of the numerous functions of HSP60, it is not surprising that its levels and intracellular distribution are altered during carcinogenesis. Under normal cellular conditions, HSP60 participates in a broad cell-survival program and this process is selectively exploited by cancer [66]. HSP60 levels must increase to meet the demand, as observed in ovarian [67], pancreatic [68], and large bowel [69] cancers. In these tumors, HSP60 promotes cell transformation via regulation of proto-oncogenes such as *c-myc* [70], or by orchestrating a cytoprotective pathway centered on interaction with other proteins, such as p53 and survivin and, thereby, abrogating the apoptotic response and favoring the survival of tumor cells [12, 15, 66]. An example of the importance of HSP60 overexpression during carcinogenesis is given by the evidence that genetic ablation of HSP60 by siRNA triggers cyclophilin D-dependent mitochondrial permeability transition, caspase-dependent apoptosis, and suppression of intracranial glioblastoma growth *in vivo* [12, 71]. In what regards caspase-dependent apoptosis, it has been reported that HSP60 affects the caspase-3 (C3) activation cascade with pro-apoptotic or pro-survival functions in the cytosol by either establishing interactions with C3 [21] or forming, as in the mucoepidermoid carcinoma cells, a stable complex with pro-C3 and acting as an anti-apoptotic agent, with a subsequent cytoprotective effect in these cells [26]. The HSP60 effect on apoptosis in cancer cells may favor cell viability and alter the response to the host's protective mechanisms and to therapy. As emerged from the data discussed, the role or roles of HSP60 in cell survival are still controversial and depend on tissue type, stress signals, and apoptosis inducers. In this regard, some studies, using *in vitro* models, indicate that HSP60 can have a pro-apoptotic effect, by acting in combination with the co-chaperone HSP10 to promote caspase activation [72, 73]. Table **2** summarizes reports on the variations of HSP60 levels in various types of cancer.

As mentioned earlier, HSP60 was classically considered an intracellular chaperone; however, under normal physiological conditions, it can also occur in non-canonical locales, such as the cell's surface and the extracellular milieu and bloodstream [22, 57, 74, 75]. In these locations, HSP60 is involved in the regulation of the IS and this becomes particularly important in cancer. Many studies have demonstrated that HSP60 is on the surface of not only normal but also tumor cells [22, 24] and HSP60 released from cancer cells (see following Section) could be a mediator of tumor immunity or the opposite, HSP60 could block or misdirect the IS [24].

As mentioned above, HSP60 interacts with both, adaptive and innate immunity components, (Fig. **1**). In what pertains to the innate immune response, HSP60 can bind cell-surface receptors in macrophages and dendritic cells, for instance. Furthermore, HSP60 can participate in antigen cross-presentation and for this reason this chaperonin could be considered a chaperokine [76-78]. On the cell's surface or in circulation, HSP60 can form complexes with other cellular proteins, which might be relevant to the establishing of immune tolerance and thus play a role in cancer progression and metastasis [79]. For example, interaction with major histocompatibility-complex molecules leads to induction of maturation of monocyte-derived dendritic cells, to T-cell polarization, and to inducing monocytes and macrophages to synthetize pro-inflammatory cytokines, such as TNF-α, IL-12 and IL-15 [80-82]. In relation to this ability of HSP60 to interact with the IS, it can be said that this chaperone plays opposing roles: it may induce pro-inflammatory effects, determining the secretion of IL-6 from macrophages [83] and favor the maturation of the dendritic cells [82] or, on the other hand, HSP60 may display anti-inflammatory activity [84, 85].

HSP60 released by tumor cells caused a persistent activation of TLR2 and was critical in the constitutive activation of transcription factor Stat3, leading to the release of immunosuppressive cytokines and chemokines perpetuating tumor progression and metastatization [79]. Other studies have shown that extracellular HSP60 triggers B cells through a pathway involving TLR signaling, and can activate naive cells upregulating the expression of MHC class II molecules and, thereby, allow the cell to dodge apoptosis [86]. Therefore, this could be considered a mechanism used by tumors to escape immune-surveillance. It has been reported that due to its specific functions, tumor-derived HSP60 and a DNA vaccine encoding this chaperonin

Table 2. Examples of works reporting on the mechanisms of carcinogenesis involving HSP60[a].

Cell Line(s) / Tissue(s)	Cancer Type	Regulation	Interactors / Factors Related	Prognosis	Reference
Ovarian tumor tissue	Epithelial ovarian carcinoma	Over-expression	N.D.[b]	Good	[67]
Primary human pancreatic adenocarcinoma cells	Human pancreatic carcinoma	Surface expression	N.D.	N.A.	[68]
Colorectal cancer; lymph node metastasis	Large bowel carcinoma	Over-expression	N.D.	Poor	[69]
Early prostatic adenocarcinoma tissue; advanced cancer tissue; human prostatic epithelial cell lines	Prostatic cancer	Over-expression	N.D.	Poor	[88]
Lymphoblastoid cell lines	N.A.	Over-expression	c-Myc	N.A.	[70]
Breast adenocarcinoma cells	Breast cancer	Over-expression	Survivin	N.A	[66]
Human primary glioblastoma cell	Glio-blastoma	Over-expression	Cyclophilin D	Poor	[12]
Mucoepidermoid carcinoma cell line (NCI-H292)	Muco-epidermoid carcinoma	Over-expression	Procaspase-3	N.A.	[26]
Gastric carcinoma tissue	Gastric carcinoma	Over-expression	Matrix metallo-peptidase 9 (MMP-9)	Poor	[43]
Clear cell renal cell carcinoma (ccRCC)	Kidney cancer	Down-regulation	N.D.	N.A.	[63]
Bladder carcinoma tissue	Bladder carcinoma	Down-regulation	N.D.	Risk of recurrence	[64]

[a]Source: References [1, 7].
[b]N.D., not determined; N.A., not applicable.

can be used as anti-tumor vaccines under appropriate conditions [87]. Considering the various ways in which HSP60 interacts and modulates the IS, future studies should focus on characterizing the correlation between immune response and HSP60 in patients with cancer as the foundation to design a personalized therapy.

5. HSP60 TRAFFICKING

The type of mechanism of HSP60 secretion into the extracellular space probably determines the type of effect of the chaperonin on the IS. HSP60 secretion by tumor cells occurs via an active mechanism involving a lipid raft-exosome pathway [24, 57]. A multistage process, Fig. (**2**), has been proposed for the translocation of HSP60 from inside the cell into the extracellular space, including, initially, accumulation of the chaperonin in the cytoplasm from where it reaches the plasma-cell membrane and the Golgi apparatus. At the plasma-cell membrane, lipid rafts internalize HSP60 into early endosomes and then multivesicular bodies (MVB) from where the chaperonin is secreted via extracellular vesicles (EVs), such as exosomes. The exosomes are vesicles that are produced by virtually all cell types and are present in body fluids and secretions such as blood; urine; cerebrospinal fluid; breast milk; saliva; bronchoalveolar lavage; and ascitic, and amniotic liquids [89]. Cancer cells release a large amount of EVs, which can carry proteins, lipids, and nucleic acids associated with cancer progression [90], including angiogenesis [91] and metastatization [92]. Consequently, EVs constitute a biomarker amenable to potentially sensitive and specific laboratory assays.

HSP60 is carried by exosomes in their membrane and probably also inside them [24]. This exosomal chaperonin possibly mediates interactions between the tumor and immune cells and other tissues. The exosomal HSP60 can reach other cells near and far through the circulation and its presence in the blood, urine, and other biological fluids could be a marker of cancer presence and growth activity. For example, the levels of HSP60-containing exosomes were considerably elevated in the blood of colon cancer patients before operation but decreased to normal levels a week after surgical removal of the tumor mass [93].

Post-translational modifications of HSP60 may regulate its functions. For instance, acetylation alters the activity of the chaperonin, disturbing protein folding [94] and, possibly, promoting cell death [95]. Other post translational modifications, such as nitration, may affect HSP60 trafficking in the cell, favoring its translocation into exosomes and subsequent secretion into the extracellular space, which is followed by passage into the circulation to finally interact with the IS [8]. The impact on the IS of

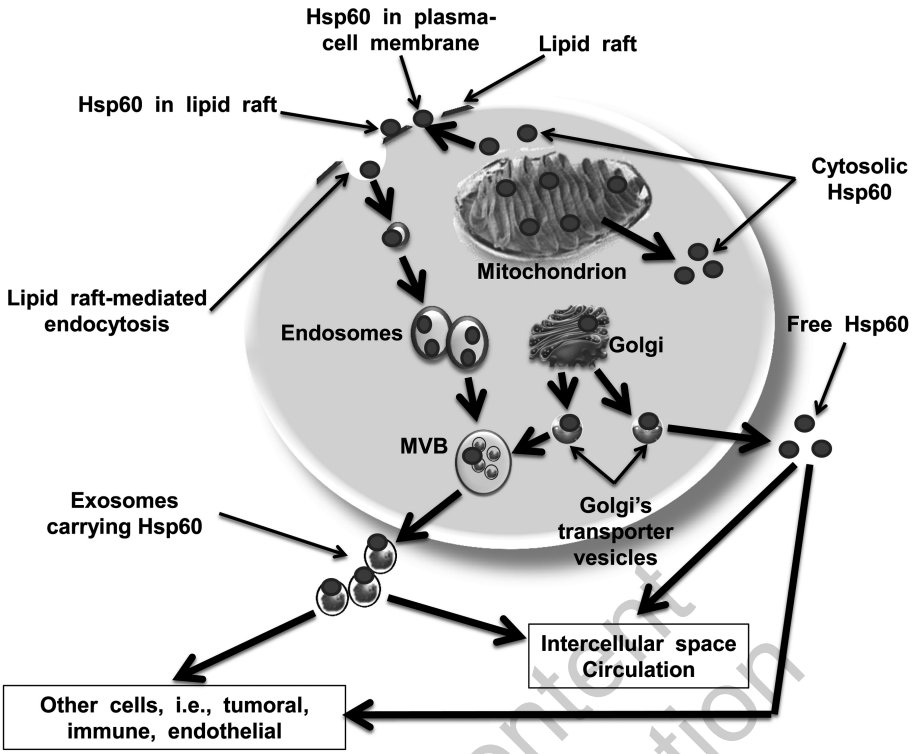

Fig. (2). Proposed multistage process for the translocation of HSP60 from the inside to the outside of the cell and for its migration near and far from its origin. HSP60 (Hsp60, solid ovals) localizes in normal cells mainly in the mitochondria, but in tumor cells the chaperonin also accumulates in the cytosol and reaches the plasma-cell membrane and the Golgi apparatus. At the plasma-cell membrane, lipid rafts internalize HSP60 toward multivesicular bodies (MVB) from where it is secreted via exosomes. In these, it is located in the membrane and probably also inside. HSP60-loaded exosomes can reach other cells near and far through the circulation. The Golgi may also participate in HSP60 secretion via transport vesicles moving to both MVB and the extracellular space. HSP60 released in the extracellular space via Golgi (free HSP60) can thus reach other cells in the vicinity and distant via circulation. The mechanisms behind the migration of cytosolic HSP60 to the plasma-cell membrane have not yet been determined, but post-translational modifications might be involved. Cytosolic HSP60 would be of two types: molecules released by the mitochondria and molecules that never entered the organelle and remained in the cytosol after synthesis in the ribosome. Thus, it may be hypothesized that the latter have the mitochondrial import signal, whereas those released by the mitochondria do not. This point needs further research. Thick arrows indicate proposed HSP60 displacements. It has not been established at what stages in this long route is HSP60 present as monomer or as oligomer, *e.g.*, heptamer or tetradecamer, although it is probable that in mitochondria and the cytosol the latter would tend to predominate, considering the monomers' tendency to oligomerize. Source: Reference [24].

HSP60-carrying exosomes is not yet fully elucidated, but data already available indicate that, under certain conditions, the chaperonin helps to induce a strong anti-tumor cellular immune response [58]. It cannot be excluded that under other conditions HSP60 in exosomes may have an opposite effect, namely, block the anti-tumor immune response.

6. HSP60 IN CHRONIC INFLAMMATORY AND AUTOIMMUNE DISEASES

HSP60 serves as a linking molecule in intercellular immune networks for its capacity to interact with both the innate and the adaptive immune system as proved by several data reported in the literature, some of which are reviewed here, Fig. (**1**). Extracellular HSP60 serves also as a link between the IS and infectious agents and provides communication between immune cells and other cells in the body. This chaperonin has the capacity to play roles as self-antigen, foreign antigen, carrier of other functional molecules, and ligand for toll-like receptor (TLR) signaling [60]. It has been proposed, that some immunological properties of HSP60 stem from its high degree of structural similarity with prokaryotic Hsp60, and this similarity is the basis of the failure of the mechanism of self-non-self-discrimination, which leads to induction of autoimmunity [96] and inflammation [38] and, thereby, to chronic inflammatory disorders. Human HSP60 can interact with, and activate immune receptors and cells. Therefore, the effects of HSP60 on immune cells that have been reported can, at least in some well controlled experiments, safely be ascribed to this chaperone instead of LPS or other bacterial contaminants [49, 53, 86]. Because of these physiological characteristics, HSP60 when abnormal in structure/function and/or quantity can be involved in the pathogenesis of a variety of human diseases. This is illustrated by some chronic inflammatory and autoimmune disorders, such as inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), Hashimoto's thyroiditis (HT), myasthenia gravis (MG), and multiple sclerosis (MS), (Table **3**).

IBD is a complex pathology that results from the interaction of environmental and genetic factors leading to immunological responses and inflammation. Crohn's disease (CD) and ulcerative colitis (UC) are common types of IBD of the colon and small intestine. CD can also affect the mouth, esophagus, stomach and the anus, whereas UC

Table 3. Examples of diseases in which molecular mimicry between microbial and human HSP60 may be involved in generation of autoimmunity[a].

Organ / System	Pathologic Condition	Pathogen	Putative Epitope	Main Mediators of Interaction with HSP60	Ref. [b]
Central nervous	Multiple sclerosis	*Helicobacter pylori*	N.D.	T cell (postulated)	[114]
Heart	Ischemic disease	*Chlamydia pneumoniae*	N.D.	IgG/IgA; IgG	[115, 116]
Lung	Asthma	*Chlamydia pneumoniae*	N.D.	CD14/toll-like receptor 4 complex	[117, 118]
Muscle	Myasthenia gravis	*Chlamydia trachomatis*	LIVEKIMQSSSEV (489-501) FEKISKGANPVEIRRG (128-143) VIAELKKQSKPVTTPEE (155-171) AIATGGAGEE (317–326)	N.D.	[119, 120]
Colon and small intestine	Ulcerative colitis; Crohn's disease	*Mycobacterium tuberculosis; Yersinia enterocolitica; Escherichia coli*	N.D.	IgA; IgA; T regulatory cells (Tregs)	[103-109, 121]
Vessels	Athero-sclerosis	*Escherichia coli; Chlamydia pneumoniae*	N.D.	DCs, CD4+T cells; T helper 1 cells	[122-125]
		Helicobacter pylori	HuHSP60 Glu^{141}-Leu^{160}	T helper 1 cells	[126, 127]
		Porphyromonas gingivalis	*P. gingivalis*, peptide19	N.D.	[128, 129]

[a]Source: References [1, 7]
[b]Abbreviations: Ref., reference; N.D.: Not determined

primarily affects the colon and the rectum [97]. IBD is considered a high-risk condition for cancer development and HSP60 could be implicated in the pathogenesis of UC and CD by triggering and/or maintaining inflammation [38]. However, the role of the chaperonin is still controversial. It has been demonstrated by performing comparative proteomics that, in UC, HSP60 levels are low in colonic biopsy samples, suggesting the implication of colonocyte mitochondrial dysfunction in this pathology [98, 99]. On the contrary, an increase in HSP60 and HSP10 levels was observed in biopsy specimens from both CD and UC [100, 101]. Furthermore, an old study found that control and CD tissues showed similar quantitative patterns of HSP60 [102].

Many data suggest a cause-effect connection between bacterial infections and IBD; a connection that can be attributed to the high conservation of the HSP60 sequence between species (molecular mimicry), including humans and bacteria. In 1992, it was shown that increased IgA anti-HSP60 antibody levels in serum from CD and UC patients, after stimulation with *Mycobacterium tuberculosis* Hsp65, can occur as the result of chaperonin release from injured gut epithelium, or as a result of increased intestinal permeability that facilitates mucosal access of luminal antigens; both mechanisms can lead to the production of anti-bacterial Hsp65 antibodies crossreactive with human HSP60 [103]. Administration of *Yersinia enterocolitica* Hsp60 induced UC-like lesions and autoimmune responses in mice [104, 105], and IBD-specific T cell epitopes were found in various regions of the human HSP60 and in even more regions of the bacterial Hsp65 sequences [106]. Furthermore, it has been demonstrated that a humanized monoclonal antibody against HSP60 (prozumab), developed from an antibody against Hsp65, suppressed murine colitis by inducing an anti-inflammatory response through IL-10 secretion from human peripheral blood mononuclear cells (PBMC) [107]. Other studies showed that low levels of antibodies against *Escherichia coli* and mycobacterial Hsp65 were detected in patients with CD and in both active and inactive UC, whereas no difference was found in the levels of anti-HSP60 antibodies [108, 109]. Moreover, pediatric CD was also associated with autoimmune response to HSP60-derived T-cell epitopes after stimulation of biopsy samples with an HSP60/Hsp65-derived peptide [98]. It is then possible that an abnormal immune response to bacterial Hsp65 can contribute to a dysregulation of host defenses against certain intestinal bacteria [109], and it has been hypothesized that the use of specifically-designed probiotics can counteract gut microbiota imbalance and chaperoning system malfunction in IBD [110].

COPD is a chronic inflammatory disease of central and peripheral airways and lung parenchyma characterized by an increased number of inflammatory cells such as tissue lymphocytes, macrophages, and neutrophils [111]. Tobacco smoking is the most common cause of COPD, with a number of other factors such as air pollution and genetics playing also etiopathogenic roles [112]. Long-term exposure to those irritants causes an inflammatory response in the lungs with narrowing of the small airways and destruction of lung tissue [113]. However, HSP60 can be involved in maintaining the inflammatory status since in severe and very severe COPD there was a positive correlation between the number of

neutrophils and elevated HSP60 expression. All of these cause acute exacerbations of COPD producing a number of virulence factors, among which Hsp60 is believed to be critical for the inflammatory pathology [130]. Like mycobacterial Hsp65, chlamydial Hsp60 (cHsp60) has about 50% amino acid identity with human HSP60 and can be abundantly synthesized from invaded host cells during persistent chlamydial infection and released into the site of infection and the circulation [131]. cHsp60 induces an inflammatory response through activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) [132], involving p38 mitogen-activated protein kinase (MAPK) and granulocyte macrophage colony-stimulating factor (GM-CSF) in the lung epithelial cells; all of them contributing significantly to COPD pathology [133].

To the best of our knowledge, there is little information regarding HSP60 involvement in HT and MG. HT causes primary hypothyroidism in humans [134] and is a disease characterized by a prolonged autoimmune response against thyroid tissue that alters significantly the morphology of the gland [135]. Classical clinical laboratory features include increased levels of antibodies to thyroglobulin (TG) and thyroid peroxidase (TPO), two proteins localized within the thyroid gland cells [136]. Because of the interaction of the antibodies with TG and TPO inflammation develops, the gland is destroyed, and the patient develops hypothyroidism [135]. Regions in the HSP60 molecule have been identified with remarkable structural similarity to portions of the TG and TPO molecules, supporting the notion that autoantibodies against TG and TPO are likely to recognize HSP60 on the plasma membrane of oncocytes [76]. Moreover, PBMC (peripheral blood mononucleated cells) from HT patients after stimulation with recombinant HSP60 produce IL-2 and IFN-γ, an observation that supports the notion that the levels of these two cytokines in response to stimulation with the chaperonin can be considered good candidates as biomarkers for HT [137].

MG is a T cell-dependent, B cell-mediated autoimmune disease in which autoantibodies against the muscle acetylcholine receptor (AChR) attack the AChR at the neuromuscular junction [138]. The humoral immune response to HSP60 in MG is still incompletely understood. Seroreactivity to HSP60 was detected in MG patients suggesting the involvement of the chaperonin in the development of the disease [138]. Moreover, HSP60 proteins from humans and two common pathogens, *Chlamydia trachomatis* and *Chlamydia pneumoniae*, share various potentially highly immunogenic epitopes with AChR subunit α1. Bioinformatics analysis indicated that AChRa1 antibodies could very well be elicited and/or maintained by self- and/or bacterial HSP60 [119, 120].

MS is a chronic, inflammatory, demyelinating disease of the central nervous system with unknown etiology and pathogenesis [139]. A common structural motif ("2-6-11" motif) of HSP60 can elicit an immune response by PBMN from MS patients with release of pro-inflammatory cytokines consistent with a Th1-like pattern [139]. Moreover, there is evidence suggesting that *Helicobacter pylori* is a trigger of MS and that anti-HSP60 seropositivity correlates with age of disease onset [114].

It may be inferred that occurrence of anti-HSP60 antibodies in those conditions discussed above, and probably in other diseases as well, has promise as diagnostic marker and as the target for investigating pathogenic mechanisms and new therapeutic strategies.

CONCLUSION & PERSPECTIVES FOR THE FUTURE

HSP60 plays key roles in physiological, normal cellular processes but it is also involved in pathogenesis through interactions with the IS. Involvement of the IS along with HSP60 in pathogenesis reflects the close relationship that exists between the CS and the IS. HSP60 can function as an autoantigen and elicit autoantibodies, which cross react with other molecules and, thus, be at the root of autoimmune disorders. It can also stimulate cells of the IS to produce pro-inflammatory factors and thereby initiate and/or perpetuate chronic inflammatory diseases.

Another effect on the IS mediated by HSP60 is relevant to cancer. This chaperonin can participate in processes that help tumor cells to grow rapidly and avoid the immune anti-tumor mechanisms.

It is clear from the data discussed that HSP60 is a crucial molecule, involved in a variety of cellular and extracellular events that determine health or disease. This dichotomy seems to be balanced but the balance can be broken by many factors acting upon the CS and/or the IS.

Future research in the laboratory and in clinical settings ought to investigate further the fate of extracellular HSP60, where it goes after being released by tumor cells, for example, and what it does when it reaches its destination. When at least some of the mechanisms involved in HSP60 migration, docking on the target cells of the IS, interaction with receptors, and triggering of intracellular reactions are elucidated, it will be possible to develop anti-cancer therapeutic strategies targeting the chaperonin and/or its interactors. Similar considerations apply to the problem of HSP60 being an etiopathogenic factor in some serious and widespread autoimmune and chronic inflammatory disorders. Future research ought to investigate when and how during the growth of an individual, HSP60 is recognized as an antigen to elicit autoantibodies, and when and how these auto-antibodies reach levels that are pathogenic and cause lesions in certain target tissues. In this regard, the role of foreign Hsp60 from bacteria in the digestive and genitourinary tracts or the skin, in triggering an anti-chaperonin immune response is established but, again, future research should investigate why and how Hsp60 reaches the IS. Elucidation of these aspects of HSP60 autoimmunity will help design means to stop the process at one or more critical points between the entrance of a bacterium in the body and its Hsp60 reaching the IS and triggering a response.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

A.J.L.M. and E.C.de M. were partially supported by IMET; A.J.L.M. and F.C. were partially supported by IEMEST. This work was done under the umbrella of the agreement between IEMEST and IMET signed in March 2012 (this is IMET contribution number IMET 17-198).

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