Heat Shock Proteins

Volume 20

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Heat Shock Proteins: key mediators of Health and Disease. Heat shock proteins (HSP) are essential molecules conserved through cellular evolution required for cells to survive the stresses encountered in the environment and in the tissues of the developing and aging organism. These proteins play the essential roles in stress of preventing the initiation of programmed cell death and repairing damage to the proteome permitting resumption of normal metabolism. Loss of the HSP is lethal either in the short-term in cases of acute stress or in the long-term when exposure to stress is chronic. Cells appear to walk a fine line in terms of HSP expression. If expression falls below a certain level, cells become sensitive to oxidative damage that influences aging and protein aggregation disease. If HSP levels rise above the normal range, inflammatory and oncogenic changes occur. It is becoming clear that HSP are emerging as remarkably versatile mediators of health and disease. The aim of this series of volumes is to examine how HSP regulation and expression become altered in pathological states and how this may be remedied by pharmacological and other interventions.

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Heat Shock Proteins in Neuroscience
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Heat shock proteins (HSP) family members are the principal pathways involved in degradation and clearance of these misfolded protein aggregates. This highly-conserved protein family plays a critical role in preventing the misfolding of protein or refolding of partially denatured or misfolded proteins. HSP are also involved in autophagy mechanism, known as chaperone-mediated autophagy (CMA), in which small protein aggregates are targeted to lysosome for their degradation. Therefore, they are considered as intracellular lifeguards or guardians of proteome, as well as protein quality control. Importantly, they are constitutively expressed in the nervous system. Several experimental evidences suggest that HSP play vital role for inhibition of amyloidogenic protein assembly or reducing the risk of formation of toxic oligomeric assemblies of amyloid beta protein (Aβ), tau, mutant huntingtin (mHTT), α-synuclein (α-Syn) and promote their degradation through ubiquitin system.

The book *Heat Shock Proteins in Neuroscience* provides the most comprehensive review on contemporary knowledge on the role of HSP in signaling pathways relevant to a number of diseases. Using an integrative approach, the contributors provide a synopsis of novel mechanisms, signal transduction pathways. To enhance the ease of reading and comprehension, this book has been subdivided into various section including; Part I, reviews current progress on our understanding of Neurological Aspects of HSP; Part II, focuses on Aspects of HSP in Neurodegenerative Diseases and Disorders, Part III, emphasizes the importance of HSP in Multiple Sclerosis and Part IV, gives a comprehensive update of the Development of HSP-Based Therapies for Neurological Disorders.

Key basic and clinical research laboratories from major universities, academic medical hospitals, biotechnology and pharmaceutical laboratories around the world have contributed chapters that review present research activity and importantly project the field into the future. The book is a must read for graduate students, medical students, basic science researchers and postdoctoral scholars in the fields of Neurology and Neurosciences, Translational Medicine, Clinical Research, Human
Physiology, Biotechnology, Cell & Molecular Medicine, Pharmaceutical Scientists and Researchers involved in Drug Discovery.

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Prof. Dr. Alexzander A. A. Asea is a highly innovative and accomplished world renowned clinical and basic research scientist and visionary executive leader who has exceptional experience spearheading clinical and basic science research, training, education, and commercialization initiatives within top ranked academic biomedical institutes. Prof. Dr. Asea’s initial findings studying the effects of Hsp72 on human monocytes lead to the proposal of a novel paradigm that Hsp72, previously known to be an intracellular molecular chaperones, can be found in the extracellular milieu where it has regulatory effects on immuno-competent cells – a term now called chaperokine. Prof. Asea has authored over 255 scientific publications including peer-reviewed articles, reviews, books, book chapters, editorials, and news headliners in a wide range of biomedical-related disciplines. Prof. Asea is the series editor of the widely successful book series Heat Shock Proteins (Springer Nature Publishing) and is an editorial board member of numerous scientific peer-reviewed journals. Currently, Prof. Dr. Asea is at the University of Toledo College of Medicine and Life Sciences in Toledo, USA.

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Part I
Neurological Aspects of HSP
Chapter 1
Hsp60 Friend and Foe of the Nervous System

Antonella Marino Gammazza, Celeste Caruso Bavisotto, Francesca Rappa, Federica Scalia, Everly Conway de Macario, Alberto J. L. Macario, and Francesco Cappello

Abstract
Hsp60 belongs to the subgroup of molecular chaperones named chaperonins and, typically, resides and functions in the mitochondria but it is also present in extramitochondrial sites. It chaperones client peptides as they fold to achieve the native conformation and also displays anti-stress roles by helping stress-damaged proteins regain a functional shape. Thus, Hsp60 is central to the integrity and functionality of mitochondria and energy production. All cells in the nervous system depend on Hsp60 so when the chaperonin malfunctions the consequences on nervous tissues are usually devastating, causing diverse diseases. These are the Hsp60 chaperonopathies, which can be genetic or acquired with the former caused by gene variants and the latter by various post-transcriptional mechanisms. All forms of chaperonopathies, i.e., by defect, by excess, and by mistake, associated with Hsp60 have been described, and some illustrative examples are discussed here. It is clear that this chaperonin is key to neuromuscular physiology but, when qualitatively and/or quantitatively abnormal causes diseases, often very serious.

Keywords
Acquired chaperonopathies · Alzheimer’s disease · Central nervous system · Chaperonins · Chaperonopathies · Genetic chaperonopathies · Hsp60 ·
1.1 Introduction

Molecular chaperones have been found in ancestral life forms (including Bacteria and Archaea) and are structurally much conserved (Macario and Conway de Macario 1997). One of the oldest groups of molecular chaperones is the Hsp60 family (Gupta 1995); they have been called “chaperonins“ to differentiate them from other chaperones (Hemmingsen et al. 1988) because they have unique molecular characteristics:
for instance, they can form very large, ~1 MDa, macromolecular complexes able of accommodating inside them the client proteins, i.e., proteins that must be folded to achieve the correct and functional final conformation (Skjærven et al. 2015; Koldewey et al. 2017). Although a third group has been recently identified and is under characterization (Rowland and Robb 2017), classically, two groups of chaperonins are described. Group I chaperonins are found in bacteria as well as organelles of endosymbiotic origin (chloroplasts and mitochondria). In humans, Group I chaperonins are represented by Hsp60 (or HSP60, or Cpn60, or HSPD1) that works along with its co-chaperone (or co-chaperonin) Hsp10 (or HSP10, or Cpn10, or HSPE1). Group II chaperonins are found in the eukaryotic cytosol and in archaea. In human cells, this group is represented by TRiC (TCP-1 Ring Complex), also called CCT (Chaperonin Containing TCP-1) (Horwich et al. 2007; Macario et al. 2013).

It is well established that molecular chaperones constitute a physiological system distributed throughout the body and they are present in all cellular compartments as well as extracellularly (Marino Gammazza et al. 2016). Moreover, many conditions have been identified in which one or more components of the chaperoning system are abnormal and are at the basis of pathogenic mechanisms that lead to manifest cell and tissue pathology and disease (Macario and Conway de Macario 2005). In various neurological diseases, Hsp-chaperones are present in the affected brain tissue, but their possible pathogenic or protective roles are still under investigation. Clarification of this issue will have an impact on how different neurodegenerative diseases are approached both from a clinical and a therapeutic point of view. These proteins may be used as indicator of disease status and/or as biomarkers to be measured, for example, periodically in the patient’s follow up. Furthermore, the chaperones may represent a target for therapies if actively contribute to disease initiation and/or progression (Macario and Conway de Macario 2005). Here, we focused our attention on Hsp60, a chaperonins with multiple roles in health and disease, with special attention to the Nervous System.

### 1.2 HSP60 and Chaperonopathies

Molecular chaperones, including the chaperonins, have canonical and non-canonical functions, the former pertain to protein homeostasis whereas the non-canonical functions are relevant to various other cellular activities unrelated to protein homeostasis (Horwich et al. 2007; Cappello et al. 2008; Macario et al. 2013; Henderson et al. 2013). In principle, all these canonical and non-canonical functions are cytoprotective and maintain health. However, chaperones can also be etiopathogenic when abnormal in structure, properties, and/or location. Diseases caused by abnormal chaperones are the chaperonopathies, and these can be genetic or acquired (Macario and Conway de Macario 2005). A series of diseases have been identified in which Hsp60 plays an etiopathogenic role, and these are the Hsp60 chaperonopathies that are considered in this chapter. We will refer only to Hsp60, classically considered a mitochondrial chaperonin but nowadays it is known that also occurs
and functions in extramitochondrial locations (Cappello et al. 2008; Henderson et al. 2013). Hsp60 is a very important molecule for life. Experiments were done to generate knock-outs in animal models, e.g., in mice (Christensen et al. 2010; Berger et al. 2016), and in zebrafish (personal data, unpublished), and in cells (Tang et al. 2016) but they were unsuccessful because absence of Hsp60 is incompatible with life. Although all the organs of the human body may suffer from a lack of functional Hsp60 in their cells, the neuraxis is probably the first to be damaged as a result of Hsp60 deficiency. In fact, there are a number of indirect proofs that Hsp60 is crucial for nervous cell homeostasis. Mutations of the hsp60 gene have been found associated with severe nervous system disease, such as the mitochondrial Hsp60 chaperonopathy (MitCHAP-60 disease), spastic paraplegia 13 (SPG13), and hypomyelinating leukodystrophy 4 (HLD4) (Bross and Fernandez-Guerra 2016). Indeed, this mitochondrial chaperonin assists the folding of a number of neuronal mitochondrial matrix proteins (Magnoni et al. 2013), and its failure determines a malfunction of this fundamental organelle with a deleterious impact on nervous cells, both neurons and glia. Among the latter, particularly affected are the oligodendrocytes and the Schwann cells, which have to produce and transport myelin over long distances. Because the axonal transport system requires energy, a mitochondrial deficit with its consequent energy deficit results in a severe impairment of the nervous tissue homeostasis (Magnoni et al. 2013). Also, Hsp60 participates in Aβ peptide homeostasis, by preventing aggregation of its early oligomeric species and, in turn, amyloid fibrillogenesis (Mangione et al. 2016). Data showing the role of Hsp60 in nervous cell homeostasis will be further discussed below.

1.3 HSP60 Genetic Chaperonopathies Affecting Primarily the Neuromuscular System

Hsp60 is endogenously expressed in astrocytes, neurons, microglia, oligodendrocytes, and ependymal cells (D’Souza and Brown 1998), and it has been found increased in the injured PNS neurons (Ousman et al. 2017). Identifications of genetic mutations on Hsp60 showed that many neurodegenerative disorders are chaperonopathies, diseases caused by mutations in molecular chaperone genes of Group I and II (Macario and Conway de Macario 2002, 2005). Hereditary spastic paraplegias (HSP or SPG-) are a group of neurodegenerative disorders characterized by a remarkable genotype-phenotype heterogeneity (Hansen et al. 2002; Fink 2006). Predominant symptoms are weakness of the lower limbs and spasticity. Many studies have been carried out to understand this disease. HSP heterogeneity was immediately evident when individuals from a French family showed an incomplete and age-dependent penetrance for an autosomal dominant mutation on the SPG13 locus (Fontaine et al. 2000), namely the same locus that in 2002 had been characterized as the “house” of the hsp60 and its co-chaperonin hsp10 genes on chromosome 2 (2q33.1) (Hansen et al. 2002).
The first mutation detected was G → A at position 292 of the cDNA of Hsp60 (Hansen et al. 2002). In accordance with the updated sequence deposited (build 30) (Hansen et al. 2003) it is a replacement of the valine residue at position 98 with an isoleucine (V98I) (Bross et al. 2008). To characterize the mutant protein, various experiments were performed in vitro and in vivo. The ATPase activity of the Hsp60 was affected by the mutation V98I, which in turn affected the chaperonin function of assisting the folding of a client-model protein. The greater the number of ATPase subunits mutated within the complexes, the lower was the activity of the chaperone. Since the patients were heterozygous, their cells produced hetero-complexes of both mutated and wild type proteins with a reduced chaperoning activity and a consequent reduction of folded proteins. In view of the presence of others chaperones and chaperonins in the cells, a compensatory mechanism could be in operation by these other chaperones and chaperonins –not mutated, at least in some tissues. However, this compensatory mechanism may be insufficient due to the unique and extended morphology of the neurons (Bross et al. 2008). 23 Danish HSP patients have been identified to bear the p.Gln461Glu (Q461E) mutation in the HSPD1 gene (Hansen et al. 2007). An *Escherichia coli* model was used to demonstrate that, upon deletion of groES/groEL genes (these are the bacterial equivalents of the eukaryotic Hsp60 and Hsp10 chaperonins) and subsequent complementation with eukaryotic Hsp10 and Hsp60-Q461E reduced the growth of *E. coli*. The protein function was mildly compromised and the mutation showed a low penetrance (Hansen et al. 2007).

Other in vivo experiments have been carried out with Cos-7 Hsp60-V98I and Hsp60-wild type transfected cells: the length of mitochondria was shorter (30%) but their number was higher in cells harboring the mutation (Miyamoto et al. 2016). Until now we have been discussing Hsp60 mutations, but we have to bear in mind that the HSP diseases have a multi-factorial etiology, in which both the genetic background and the environment affect the onset and the progression of the disease. Single-nucleotide polymorphisms (SNPs) and copy number variants (CNVs) are the two genetic features that are distinctive of individuals in what pertains to disease predisposition; for instance, some SNPs can act as “modifier genes” contributing to the phenotype occurrence (Bross et al. 2007). An illustrative case consisted of a *spastin* exonic deletion that caused autosomal dominant HSP (SPG4) in a large pedigree, in which some affected individuals with significantly earlier onset also harbored the p.[Gly563Ala] substitution in Hsp60. This is a case that exemplifies a pathogenic interaction of two HSP causative genes: the *hsp60* gene polymorphism seems to modify the SPG4 phenotype, although it may be that polymorphisms in the *spastin* gene can have a similar phenotype-modifying effect (Hewamadduma et al. 2008).

A genetic interaction has been reported also in two siblings (Yamamoto et al. 2018). Patient 1 showed a progressive paraplegia due to an unknown leukodystrophy and a novel HSPD1 mutation (p.Leu47Val) was detected. Patient 2 did not have any abnormalities in the brain, in line with the fact that he did not have an Hsp60 mutation. However, he showed neuropsychiatric symptoms and a de novo HIP1 (Huntingtin-interacting protein 1) variant (p.Glu465Lys). Both patients and their
mother carried a MECP2 variant (p. Arg167Trp), indicating an X-linked recessive inheritance, which could have a phenotype-modifier effect (Yamamoto et al. 2018). Another neurodegenerative disorder associated with Hsp60 mutation is the MitCHAP-60 disease (Magen et al. 2008). It is a hypomyelinating leukodystrophy allelic to the pure autosomal-dominant SPG13 but, unlike SPG13, it is associated to a recessive missense mutation on the Hspd1 gene. The first missense mutation identified in a large inbred Israeli Bedouin kindred was a g.1512A/G in exon 2 at position 86 of the Hsp60 cDNA sequence, resulting in an aspartic acid/glycine exchange at the 29 amino acid position (Asp29Gly or Asp3Gly in the mature form). The mutation segregated with the disease. In this case as well, in vivo complementation assay with E. coli showed a reduced ability of the Hsp60-Asp29Gly mutant protein to support E. coli survival, in particular at higher temperatures (Magen et al. 2008). On a molecular basis, the mutated protein showed a propensity to form oligomers (heptamers and tetradecamers, especially) that were unstable and tended to rapidly dissociate upon dilution with impairment of ATPase ability (Parnas et al. 2009). Some alterations regarding mitochondrial morphological changes were tested on Cos-7 cells (Miyamoto et al. 2015). Disturbed interactions between HSPD1 gene with the Asp29Gly missense mutation and ACADS homozygous variation have been evaluated in an additional case, demonstrating that also for the onset and progression of MitCHAP-60 the contribution of a particular genetic background cannot be excluded (Kusk et al. 2016).

1.4 HSP60 Acquired Chaperonopathies

In acquired chaperonopathies, Hsp60 carries abnormalities that modify the chaperone’s structure and/or makes the chaperone unavailable for functioning when needed (Macario and Conway de Macario 2007). Acquired chaperonopathies include diverse conditions characterized by chronic inflammation and autoimmunity, and various types of cancer (Cappello et al. 2014). In these pathologies, Hsp60 plays a pathogenic role as autoantigen, or as inducer of inflammatory cytokines, or as facilitator of cancer development and growth (Cappello et al. 2014). Moreover, a situation due to acquired protein defects such as those occurring in ageing, including progressive protein damage due to oxidation and other aberrant post-translational modifications, expands the pool of proteins requiring assistance from chaperones. A quantitative chaperonopathy by defect may develop because of the excessive demand on an impoverished chaperoning system. This chaperone deficiency, in turn, increases protein damage leading to protein aggregation and precipitation with formation of irreversible protein deposits and, ultimately, cell pathology or even cell death (Macario and Conway de Macario 2007). These conditions may occur in Alzheimer disease (Marino Gammazza et al. 2016). Moreover, a defect in Hsp60 function caused, for example, by oxidative stress may also contribute to neuronal cell death, epileptogenesis, and chronic epilepsy (Marino Gammazza et al. 2015). In the following sections, we report the results obtained in our laboratories, and also in
those of others, pertaining to Hsp60 involvement in autoimmune diseases of the nervous system such as myasthenia gravis and multiple sclerosis, in brain tumours, and in some neural disorder such as temporal lobe epilepsy and Alzheimer’s disease.

1.4.1 Hsp60 Involvement in Autoimmune Diseases of the Nervous System: Myasthenia Gravis and Multiple Sclerosis

Hsp60 is a multifunctional molecule highly conserved during evolution (Lindquist 1986, and other reports mentioned earlier). It has a key role in cell homeostasis and survival, but because it is a ubiquitous protein and very similar in structure across a wide range of species, including bacteria, archaea, and eukaryotes simple or complex, it can become immunogenic in humans (Rajaiah and Moudgil 2009; Marino Gammazza et al. 2012, 2014). The chaperoning system (CS) and the immune system (IS) share common functions in organism defense, respectively against various types of stress and against external pathogens (Pockley et al. 2008; Marino Gammazza et al. 2012, 2017; Cappello et al. 2014). Most probably, the two systems evolved in parallel to coordinately protect the organism but under certain circumstances, non-physiological events can cause a failure of the mechanism for self-/non-self-discrimination and autoimmunity develops (Marino Gammazza et al. 2014). Hsp60 has also been described as an antigen that can suppress or induce remission of disease in experimental models of inflammatory disorders (van Eden et al. 2005). In a mouse model of skin allograft, Hsp60 modulated the host rejection of the allograft, which demonstrated the possible role of the chaperonin in autoimmunity, as an autoantigen (Birk et al. 1999). Other studies have reported miscellaneous of roles of Hsp60 via interaction with cell-surface receptors, such as CD14, CD40, thus causing either pro- or anti-inflammatory effects (Henderson and Pockley 2010; Quintana and Cohen 2011).

The immunogenic roles of Hsp60 are developed at extracellular level. Hsp60, is often classified as a mitochondrial protein, but it can be present outside cells since it is secreted into the extracellular space and thereby gain the blood circulation (Novo et al. 2011; Marino Gammazza et al. 2014; Campanella et al. 2015, 2016), with secretion occurring in two ways, as a soluble, free form or through extracellular vesicles, such as exosomes (Caruso Bavisotto et al. 2013, 2017a). Extracellular Hsp60 has been postulated to have effects on neutrophils and macrophages (Cappello et al. 2011; Tomasello et al. 2011). Thus, it would constitute a link between immune cells and would coordinate the activity of the immune system (Quintana and Cohen 2011). Several experimental and clinical observations have confirmed that Hsp60 is a key molecule in the regulation of some autoimmune and inflammatory diseases, including type 1 diabetes (Quintana et al. 2003, 2004; Verrijn Stuart et al. 2012), atherosclerosis (Rahman et al. 2017), inflammatory bowel disease (Tomasello et al. 2011, 2012).
Given the important role of Hsp60 in cellular and extracellular mechanisms and its constitutive expression in the human central nervous system (CNS), it is not surprising that any malfunctioning of this chaperonin leads to pathogenic conditions, as seen in different neurodegenerative diseases (D’Souza and Brown 1998; Graziano et al. 2018; Vilasi et al. 2018; Caruso Bavisotto et al. 2018).

There is evidence of crosstalk between the immune system and the CNS (Ransohoff and Engelhardt 2012). These findings focused on pathological conditions, such as neuroinflammation and autoimmunity, which are believed to contribute to the pathogenesis of neurodegenerative conditions, including age-related dementia and multiple sclerosis (Saikali et al. 2010; Li et al. 2017; Janelidze et al. 2018). In this regard, little is known about the involvement of the chaperonin Hsp60 as immunogenic protein in altering the homeostasis of nervous tissue. As stated before, Hsp60 is constitutively and highly expressed by activated microglia, and these cells release in the extracellular environment the chaperonin, determining the production of pro-inflammatory factors, through the binding to toll-like receptor 4 (TLR-4) of target cells and, consequently stimulating neuronal cell death (Zhang et al. 2012, 2017; Cheng et al. 2014).

So it is clear that some Hsp60 key functions pertain to immune system regulation and are exercised in part extracellularly. Therefore, in order to understand these functions and their alterations in chaperonopathies, one must examine the pertinent molecular events that take place in the microenvironment around the nervous tissue cells. This is not an easy task because the nervous tissue is extremely complex and poorly understood. For example, knowledge of interactions and crosstalk between neural cells is still fragmentary. The nervous tissue is believed to be a site of immune privilege but immune phenomena are common in it (Fabriek et al. 2005). This tenet, and the concept that the blood-brain barrier (BBB) permeability is strictly regulated (Andreone et al. 2017) have led to the idea that a regulated connection must exist between the brain and the systemic circulation and, thus, with other organs. Therefore, it would be appropriate to study the immunological role of Hsp60 in the CNS in a way strictly connected to the environment of its cells, and to the two sides of the BBB. Furthermore, if one takes into account the roles of Hsp60 inside mitochondria and in oxidative stress in future research, it may be possible to make progress in the understanding of the chaperonin’s participation in the pathogenesis of two major CNS diseases: myasthenia gravis and multiple sclerosis.

Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction mediated by specific antibodies to the nicotinic acetylcholine receptors (AChR) (Newsom-Davis 1990). The involvement of Hsp60 in the pathogenesis of MG is suggested by the existence of a cross-reactivity among the antigenic epitopes of Hsp60 and AChR, as indicated by a study that demonstrated the presence of high anti-Hsp60 antibody levels in myasthenic patients compared to controls. This is likely explained by the high similarity of the amino acid sequences of human Hsp60 with the orthologs from two common bacterial pathogens, Chlamydia trachomatis and Chlamydia pneumoniae: these sequences share several segments that are identical or of very high similarity. Consequently, the antibodies directed against AChR
considered to be part of the pathogenic factors in MG, may very well be induced by the bacterial Hsp60 as they enter circulation from the infected sites in the body (Marino Gammazza et al. 2012). These anti-bacterial antibodies can be expected to crossreact with the human Hsp60 and AChR because several potentially immunogenic epitopes present in the extracellular region of the AChR (AChRα1) molecule, around and inside its main immunogenic region (MIR), and in the ACh-binding site, are also present in human and bacterial Hsp60 (Cappello et al. 2009; Marino Gammazza et al. 2012). In summary, Hsp60 from humans and C. trachomatis and C. pneumoniae share highly immunogenic/antigenic epitopes with AChR, and antibodies elicited by the bacterial chaperonin as it enters the body from the infected sites will be crossreactive.

Several lines of evidence indicate that Hsp60 is also involved in inflammatory lesions of the CNS inflammatory process, such as those occurring in multiple sclerosis (MS). MS is a disorder of the CNS with autoimmune pathogenic components, characterized by demyelination and axon-damaging (Traugott et al. 1983). Local production of antibodies and the presence of oligoclonal bands in the cerebrospinal fluid (CSF) are observed (Giovannoni 2006). Although the aetiology of MS is still under investigation, the pathology is likely to be determined by an inflammatory reaction, caused by recruitment of myelin-reactive T cells, which are autoreactive against Hsp proteins, such as Hsp60. Overexpression of Hsp60 may lead to the inflammatory process which ultimately induces demyelination (Wucherpfennig et al. 1992). In addition, it has been demonstrated that Hsp60 is expressed by glia cells at different stages of MS development, i.e., in early and late active lesions and inactive lesions (Bajramović et al. 1997). The immunogenic activity of Hsp60 is confirmed by the presence of antibodies directed against the protein in CSF (Prabhakar et al. 1994) and in serum (Quintana et al. 2012; Efthymiou et al. 2016). Furthermore, peripheral blood mononuclear cells (PBMC) from MS patients stimulated by peptides derived from Hsp60 were reactive and released pro-inflammatory cytokines consistent with a T helper1-like pattern with typical features of autoimmune responses (Ruiz-Vázquez and de Castro 2003). The results summarized above clearly indicate a meaningful association between autoantibodies against Hsp60 and the development of various autoimmune and inflammatory diseases of the CNS. This is an exciting area of research, particularly if one thinks that it may provide the basic molecular knowledge necessary to develop therapeutic means targeting the antibodies and the immune phenomena that characterize these diseases.

1.4.2 Hsp60 Involvement in Brain Tumors

The World Health Organization (WHO) classification of brain tumors recognize different types based on histological and molecular genetic aspects (Louis et al. 2016). Even if there are few reports on this topic, it is already clear that Hsp60 is involved in the carcinogenic process in CNS tumors, most likely due to its anti-apoptotic properties. Tumors in which one or more chaperones play a pathogenic
role by favoring tumor development, growth, and metastasization, are grouped under the heading chaperonopathies by mistake or collaborationism (Macario et al. 2013). These terms reflect the fact that a molecular chaperone, whose functions are supposedly all in favor of the organism, has turned against the latter. The advantage of looking at these tumors as chaperonopathies by mistake or collaborationism is that the implicated chaperone becomes prominent in patient management as a biomarker useful in diagnosis, assessing prognosis and response to treatment, and as a target for treatment. This concept provides a novel platform to look at the tumor and the patient with promising alternatives for patient management.

Hsp60 is elevated in various human cancers, including glioblastoma, and likely acts as a regulator of surviving stability and as a moderator of p53 (Ghosh et al. 2008). Overexpression of Hsp60 was demonstrated in immune-morphological experiments carried out to investigate the tissue levels of Hsp60 in a various brain tumors (Rappa et al. 2013). High tissue levels of Hsp60 were found in a group of neuroepithelial malignancies, including two types of brain tumors that occur in children and young adults, pylvocistic astrocytoma and medulloblastoma, and grades II and IV astrocytomas. Each evaluation was carried out in comparison with normal tissue. Similar studies have been conducted on glioblastoma multiforme (GMB), the most common and lethal human adult neoplasm of the nervous system. The aggressiveness of this kind of tumor is associated with poor sensitivity to radiotherapy and chemotherapy. Increased levels of Hsp60 in GMB were seen as compared with normal brain tissue (Rappa et al. 2013). The localization of Hsp60 in the tumor mass, unlike in the normal cells, was diffuse in the cytoplasm and this observation is in agreement with other studies, suggesting that a localization change of Hsp60 is typical in cancerous cells and is correlated with a tumor progression (Ciocca and Calderwood 2005; Rappa et al. 2012; Caruso Bavisotto et al. 2018). Hsp60 is implicated in the cellular proliferation and tumor maintenance in glioblastomas because it is a regulator of a mitochondrial immunophilin, cyclophilin D (CypD), a pro-apoptotic component of the mitochondrial permeability transition pore. The interaction between Hsp60 and CypD leads to a blockage of CypD-dependent cell death in glioblastomas and thereby favors the survival of the tumor cells (Ghosh et al. 2010). Other authors have shown that Hsp60 silencing is followed by an increase of reactive oxygen species (ROS) and interruption of cell growth in a human glioblastoma cell line (U87), which suggests that blocking Hsp60 could be a potential therapeutic way for glioblastoma treatment (Tang et al. 2016; Graziano et al. 2018; Caruso Bavisotto et al. 2018).

1.4.3 Hsp60 Involvement in Temporal Lobe Epilepsy and Alzheimer’s Disease

Epilepsy is one of the most common chronic neurologic disorders affecting approximately 1% of the world population (Pitkänen and Sutula 2002). This disease has deleterious effects on the quality of life affecting independent living, education,
employment, mobility, and personal relationships. Epilepsy is characterized by spontaneous recurrent seizures caused by abnormal, synchronized, high frequency neuronal discharges as the result of excessive neuronal activity in the cortex of the brain (Van Liefferinge et al. 2013). Some cases of epilepsy occur after brain injury or stroke, and in some cases of brain tumors or infections or birth defects but the cause of most cases of epilepsy is still unknown (Goldberg and Coulter 2013). Seizures are controllable with medication in about 70% of cases (Eadie 2012) and in those patients whose seizures do not respond to medication, surgery, neurostimulation, or dietary changes may help (Bergey 2013; Martin et al. 2016). The period between the initial injury and the occurrence of the first epileptic seizure is named epileptogenesis, a clinically silent period of 5–10 years in which a cascade of neurobiological events and histological and biochemical changes occur (Van Liefferinge et al. 2013). Inflammation develops at the site of injury, involving glial and endothelial cells (Ravizza et al. 2008). At the later stage of epileptogenesis, sprouting of new axons and synapses, and angiogenesis occur changing the nervous tissue microarchitecture (Arellano et al. 2004). Neuronal excitability can be affected by mitochondrial alterations such as depletion of ATP, generation of ROS, elevated oxidative stress, disruption of Ca²⁺ homeostasis, dysregulation of excitotoxicity, and alterations in biosynthesis and metabolism of neurotransmitters (Wu et al. 2010).

The most common type of epilepsy in adult humans is temporal lobe epilepsy (TLE), characterized by a progressive development of spontaneous recurrent seizures from temporal lobe foci and unique morphological alteration in the hippocampus (Liu et al. 2008; Sendrowski and Sobaniec 2013). Existing data suggest the involvement of Hsp in neuronal damage caused by status epilepticus, although their role in neurodegeneration during epilepsy still remains unclear (Stringer et al. 1997; Bidmon et al. 2004; Kim et al. 2013). For example, in animal models of epilepsy, increased Hsp70 expression during acute (Yang et al. 2008) and chronic phases (Kharlamov et al. 2011) has been documented. In TLE patients, complete remission of mesial TLE seizures post-surgery was associated with decreased Hsp70 expression in CA4 and subiculum and decreased Hsp90 expression in the granular layer (Kandratavicius et al. 2014). Higher Hsp70 serum levels in patients with TLE as compared to controls were observed, and were predictive of higher frequencies of seizures in the TLE group (Chang et al. 2012). In contrast to other Hsp, Hsp60 levels and expression have only been sporadically studied in animal models of CNS diseases or neurological patients. The association between Hsp60 in the CNS and TLE is poorly understood. Hsp60 induction could be considered as a protective mechanism against epileptic seizures as supported by the observation that a loss of function of Hsp60 leads to an increased vulnerability to oxidative stress (Liu et al. 2008), which in turn can affect neuronal excitability and seizure susceptibility (Waldbaum and Patel 2010). Overexpression of Hsp60 was associated with increased activity of mitochondrial complex I after 3,4-dihydroxy-L-phenylalanine (L-DOPA) administration to rats (Calabrese et al. 2007). Hsp60 can be induced by mitochondrial DNA depletion (Czarnecka et al. 2006) and this chaperonin can interact directly with other mitochondrial proteins such as aldehyde dehydrogenase 2, ATP synthase, dihydrofolate reductase, and human carbonic anhydrase II (Cappello
et al. 2014). Likewise, Hsp60 associates with pro-caspase 3 favoring cell survival (Campanella et al. 2008; Caruso Bavisotto et al. 2017b) and some of these Hsp60 interactors are affected by oxidative stress leading to the metabolic alterations that characterize TLE (Rowley and Patel 2013). Other data indicate that Hsp60 levels decrease in the rat hippocampus in a pilocarpine model of TLE (Liu et al. 2008). In our laboratories, it has been demonstrated in an animal model of partial complex (limbic) seizures induced electrically, Hsp60 increased in the hippocampus of the stimulated rats and the circulating levels of the protein were negatively correlated with the onset of epileptic seizures (Marino Gammazza et al. 2015). Moreover, circulating Hsp60 levels increased in patient affected by TLE after epileptic seizures (Marino Gammazza et al. 2015). These results demonstrate that Hsp60 is increased in both animals and patients with TLE in affected tissues, and in plasma in response to epileptic seizures, and point to it as biomarker of hippocampal stress potentially useful for diagnosis and patient management.

Alzheimer’s disease (AD) is a common form of dementia and is characterized by a rapid progression from episodic memory deficits to a decline in overall cognitive function, impairing patient ability to carry out activities of daily living with death occurring usually within 10 years after diagnosis (Ballard et al. 2011). AD is characterized by the accumulation in the brain regions involved in memory and learning of extracellular deposits of amyloid-β (Aβ), called senile plaques, and intraneuronal inclusions of abnormal filaments of tau, called neurofibrillary tangles (Goedert et al. 1989; Iwatsubo et al. 1994). The disease has been associated with protein misfolding and subsequent aggregation, which has led to consider the cytoprotective effects of the heat shock response as an attractive target in AD management (Koren et al. 2009). Given that mitochondrial Hsp60 plays a crucial role in assisting the correct folding of other mitochondrial proteins, a deficiency in its concentration and/or function, for example after post-translational modification, together with the increased vulnerability to oxidative stress, may lead to mitochondrial dysfunction (Meriin and Sherman 2005; Campanella et al. 2014, 2015; Caruso Bavisotto et al. 2017a, b). AD has been described as a disorder aggravated by oxidative stress and/or mitochondrial defect characterized by protein conformation abnormalities (Yoo et al. 2001; Swerdlow et al. 2010; Beck et al. 2016). Therefore, Hsp60 may be considered an active player in AD pathogenesis although its role remains still controversial (Marino Gammazza et al. 2016). It has been demonstrated that Hsp60 prevents aggregation by trapping misfolded forms of prion protein scrapie attenuating the progression of the disease (Telling et al. 1995). AD subjects showed a significant decrease of the chaperonin level in the parietal cortex and the same was observed in the cerebellum of a rat model of the disease, suggesting a defect in the protective role of Hsp60 in the AD brain (Yoo et al. 2001; Jiang et al. 2013). In support of the neuroprotective properties of Hsp60, studies in a human neuroblastoma cell line demonstrated that induced expression of the chaperonin prevented intracellular-amyloid-induced inhibition of complex IV and consequently reduced apoptosis (Veereshwarayya et al. 2006). AD can be considered a chaperonopathy by defect because it has been demonstrated that Hsp60 oxidation via Aβ25–35 and Aβ1–42 in fibroblasts derived from AD patients caused an increase in protein mis-
folding and aggregation (Choi et al. 2003; Boyd-Kimball et al. 2005). However, Hsp60 levels were found elevated in lymphocytes from AD patients when compared to controls (Calabrese et al. 2006) and cases of sporadic AD showed a significant increase in expression levels of genes activated by the mitochondrial unfolded protein response (mtUPR), including the Hsp60 gene (Beck et al. 2016). Interestingly, administration of an Aβ amyloid-Hsp60 peptide-conjugate vaccine led to the induction of anti-Aβ-specific antibodies, associated with a significant reduction of cerebral amyloid burden and of the accompanying inflammatory response in the brain of a mouse model of AD (Nemirovsky et al. 2011). It has also been shown that Hsp60 mediates in vitro the translocation of the amyloid precursor protein (APP) to the mitochondria leading to dysfunction of this organelle (Walls et al. 2012). All the findings discussed here show that Hsp60 is implicated in AD pathogenesis and deserves more research to fully elucidate its role and, using the molecular and mechanistic data, develop therapeutic means centered on the chaperonin.

1.5 Conclusions

The data discussed in this Chapter indicate that Hsp60 actively participates in the cellular and tissue phenomena typical of some neurodegenerative diseases, but the detailed molecular mechanisms involved are still not fully understood. Hsp60 is constitutively expressed in many tissues of the body, including the nervous system, under basal conditions, but also responds to stress and actively participates in pathological conditions. The canonical function of Hsp60 consists of assisting in the folding of mitochondrial proteins, or in the re-folding of these proteins when they are partially denatured by stress. The chaperonin is constitutively expressed in astrocytes, neurons, microglia, oligodendrocytes, and ependymal cells and is upregulated when these cells suffer the action of stressors, playing a cytoprotective role. However, Hsp60 genetic variants determine severe diseases, mostly affecting the neuromuscular system while Hsp60 dysregulation and structural modifications (increase, decrease, post-translational modifications) may contribute to the development of diseases such as brain tumors, epilepsy, AD, and various autoimmune and inflammatory disorders of the CNS. Hsp60 functions and interactors vary depending on its cell and tissue localization, so this protein appears to be functionally multifaceted and play a critical role in the maintenance of the balance between health and disease. For this reason, the detection and quantitative determination of Hsp60 levels in circulation and in tissues constitute promising strategies in clinical laboratory pathology and patient management. The possibilities of using Hsp60 as biomarker or as target for therapy are currently under intense scrutiny in our laboratories and in many others. The purpose is to fully elucidate the role of Hsp60 in the physiology and pathophysiology of the nervous system as the first step toward developing novel means for treatment.
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