Chapter 12
Hsp60 in Inflammatory Disorders

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Abstract Heat shock proteins (HSP) including HSP60 are immunogenic proteins shared by particular microbial agents and mammals. HSP60 has been implicated in multiple inflammatory disorders and autoimmune diseases mostly through its interactions with the immune system. Such diseases include inflammatory bowel disease, chronic obstructive pulmonary disease, Hashimoto's thyroiditis, myasthenia gravis, multiple sclerosis and even atherosclerosis plaques among others. It is present in the cytosol, cell membrane, cell surface as well as in the extracellular space and in the circulation. As a super antigen, HSP60 has the dual role as an immunomodulator and as a biomarker, a node molecule in balance between health and disease. Deciphering the mechanisms of HSP60 interactions with the immune system could lead to the development of new therapeutic strategies.

Keywords Autoimmunity · Homeostasis · HSP60 · Immune system · Immunoregulation · Inflammatory disorders

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Abbreviations

AChR	Muscle acetylcholine receptor
APC	Antigen-presenting cells
CD	Crohn's disease
COPD	Chronic obstructive pulmonary disease
CR	Chemokine receptor
CREB	cAMP response element-binding protein
CSF	Cerebral spinal fluid
ERK	Extracellular signal–regulated kinases
HSP	Heat shock proteins
HSP60	Heat shock protein 60
HT	Hashimoto's thyroiditis
IBD	Inflammatory bowel disease
IFNɣ	Interferon gamma
IL	Interleukin
LPS	Lipopolysaccharides
MG	Myasthenia gravis
MHC	Major histocompatibility complex
MS	Multiple sclerosis
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PBMC	Peripheral blood mononuclear cells
PKB	Protein kinase B
TG	Thyroglobulin
TLR	Innate toll-like receptor
TPO	Thyroid peroxidase
Tregs	T regulatory cells
UC	Ulcerative colitis

12.1 Introduction

Molecular chaperones constitute a large group of molecules highly conserved during evolution. They play many roles during the cell cycle and organismal growth by being involved primarily with protein homeostasis (Rappa et al. 2012). Evolutionary data suggest that the primitive chaperones evolved from archaea to reach the complex forms we detected today in humans, probably as a consequence of the constant exposure of living creatures to environmental stressors. Throughout evolution, molecular chaperones were physiologically involved as part of the defensive system against aggressors (temperature elevation, tumors, infections, etc.). For instance, the regulation of their levels could play an important role in maintaining cell viability both under normal and stress conditions. In pathologic conditions, these proteins are recognized as potentially useful biomarkers and therapeutic

targets (Rappa et al. 2012; Barone et al. 2016). Likewise, the immune system as the role to protect against aggressors, such as infectious agents. In this respect, it is probably that the chaperoning and the immune system interact to ensure organismal homeostasis both in normal and pathological conditions. A considerable amount of data, over the last decades, have shown that exploring the cytoprotective and immunoregulatory characteristics of chaperones/heat shock proteins (HSP) can open a new avenue for drug discovery and treatment of many important human pathologies (Campanella et al. 2016; Ghosh et al. 2010; Czarnecka et al. 2006).

In this chapter, we review the interaction between the immune system and the chaperoning system with special attention to heat shock proteins 60 (HSP60), focusing on some chronic inflammatory disorders such as IBD, COPD, HT, MG and MS. It is well established that, HSP60 has the capacity to act as a self-antigen, foreign antigen, a carrier of other functional molecules, and as a ligand for innate TLR (Quintana and Cohen 2011). Then, HSP60 has the dual role as an immune modulator and a biomarker, thus giving the possibility to modulate immunity for therapeutic purposes, and to monitor the immune response in health and disease.

12.2 Hsp60 a Multifaceted Molecule that Speaks with the Immune System in Many Voice

HSP60 is one of the most studied HSP, especially in its interaction with the immune system. It was first identified as a protein capable to stimulate human monocyte proinflammatory cytokine synthesis without inducing monocyte activation (Tsan and Gao 2009). This led to assign to the chaperonin proinflammatory properties acting via the same receptors as lipopolysaccharides (LPS) (Tsan and Gao 2009). HSP60 is classically described as an intracellular chaperone, typically a mitochondrial protein, assisting the folding of polypeptides into proteins and their transport inside the cell (Campanella et al. 2014). HSP60 functions as a highly connected chaperone with links to most cellular proteins (Borges and Ramos 2005) since it has been found in the cytosol, cell membrane and surface as well as in the extracellular space and in circulation (Marino Gammazza et al. 2016). The chaperonin was described as a dominant antigen recognized during infections (Van Eden et al. 2005) and has been studied in various immunologic mechanisms involved in tumors, transplantation, tissue regeneration, autoimmune and inflammatory diseases (Rappa et al. 2012; Coelho and Faria 2012; Pei et al. 2016). The immunological relevance of HSP60 was recognized also in physiological conditions by having the capacity to induce self-reactive B and T cell clones even in health status (Coelho and Faria 2012). Although HSP60 represents a fundamental molecule in the intracellular chaperone network, evidence is lacking for the immune-specific function of the chaperonin inside the cell. Besides, the participation of other molecular chaperones, such as HSP70 and HSP90, in the cell biology of antigen processing and presentation (Bendz et al. 2007; Kunisawa and Shastri 2006), and

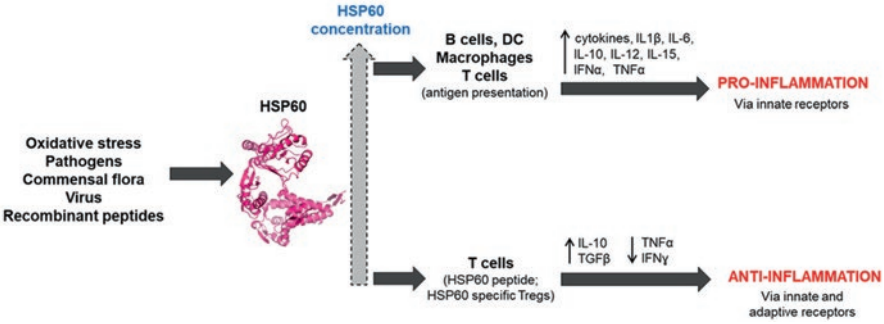

Fig. 12.1 HSP60 can interact with the innate ad the adaptive immune system. HSP60 can act as immunogen-antigen or as auto-antigen. HSP60 can also act as a sort of hormonal factor on cells very near its origin (paracrine effect) or far away (endocrine-like effect). Immune effects of HSP60 are mediated 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 stress factors, 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 (Source: modified from Quintana and Cohen 2011). In hot pink a tridimensional model of an HSP60 monomer created using SWISS-MODEL (https://swissmodel.expasy.org/)

in T cell polarization, through their interactions with transcription factors have been published (Bohen et al. 1995; Pratt 1997). HSP90, for example, controls the activity of the ligand-activated transcription factor aryl hydrocarbon receptor (Tsuji et al. 2014), which has been proven recently to play an important role in the differentiation of FoxP3+ T regulatory cells (Treg), Th17 and Tr1 cells (Quintana et al. 2008; Gandhi et al. 2010). At the cell surface, HSP60 as the capacity to interact with TLR2, TLR4 and major histocompatibility complex (MHC) molecules triggering innate and adaptive immune responses (Coelho and Faria 2012) (Fig. 12.1). Increased amounts of HSP60 on the cell's surface was considered to serve as a danger signal for the immune system leading to the activation and maturation of dendritic cells and the generation of a antitumor T-cell response (Pockley et al. 2008; Quintana and Cohen 2011) (Fig. 12.1). Moreover, Hsp60 was found in the extracellular space (Davies et al. 2006) and may be exported outside cells through vesicles like exosomes (Campanella et al. 2014; Thery et al. 2009) or might be released intact or fragmented from damaged or dead cells (Quintana and Cohen 2011). In particular, the immunological activities of exosomes affect immunoregulation mechanisms including modulating antigen presentation, immune activation, immune suppression, immune surveillance, and intercellular communication (Greening et al. 2015; Cappello et al. 2006). The presence of HSP60 in circulation has been linked with various inflammatory conditions (Quintana and Cohen 2011; Henderson and Pockley 2010) even if the exact mechanisms by which HSP60 is secreted into the extracellular space are not well understood. However, it is clear that extracellular HSP60 is a link between body tissue and the immune system acting in paracrine and endocrine fashion (Henderson and Pockley 2010;

Campanella et al. 2012). Tsan and collaborators (Tsan and Gao 2009) suggested that the immunomodulatory role of the chaperonin on the innate immune system results from the presence of bacterial contaminants in preparations of recombinant mammalian HSP60 (Tsan and Gao 2009). However, it has been demonstrated that HSP60 on its own can trigger the activation of innate immune receptors (Henderson et al. 2010). In a review of Quintana and Cohen, they reported that TLR4 signaling, in macrophages and dendritic cells, is activated in response to four sources of HSP60: (1) bacterial HSP60, (2) bacterial or self-HSP60 molecules that bear LPS or other bacterial ligands (3) self-HSP60 molecules produced by infected, transformed, damaged or stressed cells, and (4) peptides of HSP60 (Quintana and Cohen 2011). Moreover, in macrophages the chaperonin when released extracellularly, can interact also with other cell-surface receptors, such as CD14, CD40, causing in turn either pro- or anti-inflammatory effects (reviewed in Quintana and Cohen 2011 and Henderson and Pockley 2010) (Fig. 12.1).

A number of studies reported that HSP60 can induce secretion of cytokines from professional antigen-presenting cells (APC), with consequent activation of T cells (Quintana et al. 2008; Osterloh et al. 2008) (Fig. 12.1). For example, it has been demonstrated that HSP60 can regulate T-cell behavior in inflammation via TLR2 and the down-regulation of chemokine receptor expression (CXCR4 and CCR7) (Zanin-Zhorov et al. 2005). Self-HSP60 can be recognized from T cells as specific antigens both in health and in autoimmune disease and the effect of the chaperonin on these cells is varied and unexpected (Nussbaum et al. 2006). The chaperonin was recognized as a co-stimulator of CD4+CD25+ Tregs via innate TLR2 signaling, with specific changes in Protein kinase B (PKB), Pyk2, p38, extracellular signal–regulated kinases (ERK) and T-bet signaling related to Tregs and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) signaling related to T effector cells (Zanin-Zhorov et al. 2006).

Finally, HSP60 can activate, via TLR4 and MyD88 signaling, naive mouse B cells to proliferate, to secrete interleukin (IL)-10 and IL-6, and to upregulate the expression of MHCII and other activation molecules (Cohen-Sfady et al. 2005). Activated B cells present antigens to allogeneic T cells to enhance T cell secretion of both IL-10 and IFNɣ (Cohen-Sfady et al. 2005) (Fig. 12.1). Furthermore, HSP60 can trigger the production of polyclonal IgG3 antibodies (Cohen-Sfady et al. 2005), and have the capacity to play as a second signal to activate specific IgG3 antibodies to LPS (Cohen-Sfady et al. 2005; Quintana and Cohen 2011). Thus, extracellular HSP60 impacts B cell function in terms of cytokine expression, antigen presentation and antibody secretion. These interactions probably reflect the complementarity of two biological mechanisms, the chaperoning and the immune system, that have evolved to defend the cell and the organism, as mentioned earlier in this chapter and other publications. Probably, there is an evolutionary advantage to having an immune system that recognizes and responds to HSP60 in different ways from birth, since upregulation of HSP60 represents a sign of cell stress, the chaperonin might allow the body to respond and to restore tissue homeostasis (Cohen 2007).

12.3 HSP60 in Chronic Inflammatory and Autoimmune Diseases

As mentioned earlier in this chapter, HSP60 is a linking molecule in intercellular immune networks. It has the capacity to interact with both the innate and the adaptive immune systems in mammals (Quintana and Cohen 2011; Marino Gammazza et al. 2017). Many publications reported that some immunological properties of HSP60 arise from its high degree of structural similarity with the prokaryotic counterpart of the chaperonin, triggering the failure of the mechanism of self-non-self-discrimination leads to induction of autoimmunity (Marino Gammazza et al. 2014) and inflammation (Tomasello et al. 2011) and, consequently, to chronic inflammatory disorders (Cappello et al. 2011). Because of these physiological characteristics, HSP60 can be involved in the pathogenesis of a variety of human diseases. Here, we report our work together with the findings of other laboratories, to better illustrate the protein involvement in some chronic inflammatory and autoimmune disorders.

12.3.1 Inflammatory Bowel Disease (IBD)

IBD is a complex of continuum pathologies that result from the interaction of environmental and genetic factors leading to immunological responses and inflammation. Crohn's disease (CD) and ulcerative colitis (UC) are the most common types of IBD of the colon and small intestine. CD can also affect the mouth, oesophagus, stomach and the anus, whereas UC primarily affects the colon and the rectum (Baumgart and Carding 2007). IBD is considered a high-risk condition predisposing to cancer development and the chaperonin could be implicated in the pathogenesis of UC and CD by triggering and/or maintaining inflammation (Tomasello et al. 2011). However, the role of HSP60 is still controversial. In fact, comparative proteomics analysis showed colonocyte mitochondrial dysfunction due to low levels of HSP60 observed in biopsy specimens from both CD and UC (Peetermans et al. 1995; Rodolico et al. 2010). On the contrary, it has been reported that control and CD tissues showed similar quantitative patterns of the protein (Baca-Estrada et al. 1994). Interestingly, published data indicate a cause-effect relationship between bacterial infections and IBD due to the high conservation of the HSP60 sequence between humans and bacteria. For example, IBD-specific T cell epitopes were found in many regions of HSP60 and of Hsp65 sequences (Bene et al. 2002). An old work in 1992, reported increased levels of anti HSP60 IgA antibody in serum from CD and UC patients, after stimulation with the *Mycobacterium tuberculosis* homolog Hsp65, probably as a result of HSP60 release from damaged gut epithelium, or as a result of increased intestinal permeability that facilitates mucosal access of luminal antigens leading to the production of cross-reactive anti-bacterial HSP60 antibodies (Stevens et al. 1992). Moreover, administration of Yersinia enterocolitica HSP60 induced UC-like lesions and autoimmune responses in mice (Sukegawa

et al. 2000; Yagita et al. 1999). Recently, it has been demonstrated that prozumab, a monoclonal antibody against the human chaperonin, developed from an antibody against HPS65, suppressed murine colitis by inducing IL-10 secretion from human peripheral blood mononuclear cells (PBMC) (Ulmansky et al. 2015). Moreover, 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 (Huszti et al. 2004; Bene et al. 2002). Other studies showed that pediatric CD was associated with an autoimmune response to HSP60-derived T-cell epitopes after stimulation of biopsy samples with an HSP60/65-derived peptide (Puga et al. 2009). These data suggested that an abnormal immune response to bacterial HSP65 can contribute to a dysregulation of host defences against certain component of the intestinal flora (Bene et al. 2002), and it is reasonable to hypothesized that the use of specific probiotics can counteract gut microbiota imbalance and HSP malfunction and deregulation in IBD (Bellavia et al. 2013).

12.3.2 Chronic Obstructive Pulmonary Disease (COPD)

COPD is a chronic inflammatory disease of the central and peripheral airways as well as the lung parenchyma. It is characterized by an increased number of inflammatory cells such as tissue lymphocytes, macrophages, and neutrophils (Di Stefano et al. 2009). Tobacco smoking is the most common cause of COPD, together with a number of other factors such as air pollution and genetics (Decamer et al. 2012). Long-term exposure to these irritants causes an inflammatory response in the lungs resulting in the narrowing of the small airways and breakdown of lung tissue (Gamble et al. 2007). However, the chaperonin can be involved in maintaining the inflammatory status, since in severe COPD there was a positive correlation between the number of neutrophils and elevated HSP60 levels (Cappello et al. 2011). Recently, it has been proved that in human bronchial epithelial cells, stimulation with HSP60 showed pro-inflammatory properties by the up-regulation of IL-8, IL-10, and CREB (Sangiorgi et al. 2017). Regarding the sequence homologies between bacterial and human HSP60, Chlamydia pneumoniae has been established as a common cause of acute exacerbations of COPD producing HSP60 as a critical proinflammatory factor (Rupp et al. 2004).

12.3.3 Hashimoto's Thyroiditis (HT)

HT is characterized by a prolonged autoimmune response against thyroid tissue that alters significantly the morphology of the gland (Ahmed et al. 2000) and causes primary hypothyroidism in humans (Vanderpump and Tunbridge 2002). The development and progression of the disease include increased levels of antibodies to

thyroglobulin (TG) and thyroid peroxidase (TPO), two proteins localized within the thyroid gland cells (Lorini et al. 2003). Because of the interaction of the antibodies with TG and TPO inflammation develops, the gland is destroyed, and the patient develops hypothyroidism (Ahmed et al. 2000). A bioinformatics analysis, conducted in our laboratories, showed that there are regions in the HSP60 sequences with a high degree of similarity with portions of the TG and TPO molecules, supporting the idea that autoantibodies against TG and TPO are likely to recognize HSP60 exposed on the plasma membrane of oncocytes (Marino Gammazza et al. 2014). Moreover, peripheral blood mononuclear cells PBMC from HT patients after stimulation with recombinant HSP60 produce IL-2 and IFN-γ, suggesting that circulating HSP60 levels might be considered as good candidates forrr biomarkers in HT (Tonello et al. 2015).

12.3.4 Myasthenia Gravis (MG)

MG is a T cell-dependent, B cell-mediated autoimmune disease in which autoantibodies against the muscle acetylcholine receptor (AChR) attack the receptor at the neuromuscular junction (Astarloa and Martinez Castrillo 1996). The humoral immune response to HSP60 in MG is still in need of more scrutiny. Seroreactivity to HSP60 was detected in MG patients thus suggesting the involvement of the chaperonin in the development of the disease (Astarloa and Martinez Castrillo 1996). Moreover, a bioinformatics analysis conducted in our laboratory showed that HSP60 proteins from humans, Chlamydia trachomatis and Chlamydia pneumoniae, share sequence segments of high similarity with AChR subunit α1 (Marino Gammazza et al. 2012; Cappello et al. 2010) indicating that AChRa1 autoantibodies production could be elicited and/or maintained by self- and/or bacterial HSP60 (Marino Gammazza et al. 2012; Cappello et al. 2010).

12.3.5 Multiple Sclerosis (MS)

MS is a chronic inflammatory demyelinating disease of the central nervous system with unknown etiology and pathogenesis (Ruiz-Vazquez and de Castro 2003). A common structural motif (“2-6-11” motif) of the chaperonin is able to elicit the immune response of PBMC from MS patients by the release of pro-inflammatory cytokines consistent with a Th1-like pattern (Ruiz-Vazquez and de Castro 2003). Antigen arrays conducted on cerebral spinal fluid (CSF) and serum samples of patients with untreated relapsing-remitting MS showed the presence of different antibody signatures targeting epitopes of different proteins including HSP60 (PMID: Quintana et al. 2012). Moreover, there are experimental evidences suggesting that Helicobacter pylori is a trigger of MS and that the anti-HSP60 seropositivity correlated with age of disease onset (Efthymiou et al. 2016).

12.4 Conclusions

In physiological conditions, HSP60 has the capacity to function as a homeostatic molecule participating in the fine-tuning of inflammation allowing the body to respond and to restore tissue homeostasis. In pathological conditions, as in chronic inflammatory or autoimmune disease, HSP60 can function eliciting autoantibodies production or stimulating the immune cells to produce pro-inflammatory factors and thereby perpetuating inflammation. On this basis, the regulation of HSP60 levels or effects, for example through appropriate peptides or in combination with other modulatory agents, can be considered as new strategies for autoimmune and chronic inflammatory disease treatment. Since it is still unclear what determines the immune regulatory role or the pro-inflammatory activities of the chaperonin, further studies and scientific efforts are necessary to better elucidate the molecular pathways involved and their physiological significance. However, there are no doubts that HSP60 represent a node molecule in the balance between health and disease.

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