Immune Properties of HSP70

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Abstract In addition to their conventional chaperon activity, numerous studies have reported that heat shock protein 70 (HSP0) exhibit immune properties and especially the capacity (i) to induce the presentation and cross-presentation of associated or client proteins and, (ii) to control myeloid cell activation. Several studies were focused on the identification of HSP70-binding elements that contribute to their immune properties. A general consensus was reached on the nature of the endocytic receptors involved in the internalization of extracellular HSP70 with belong, for most of them, to the innate immunity receptor family. However, the nature of signaling receptors recruited by HSP70 remains unclear, because the stimulatory versus regulatory properties of HSP70 remains a subject of debate. Nevertheless, these unique immune properties allowed developing innovative prophylactic and therapeutic vaccines, especially in the treatment of cancers and chronic viral infections. Although HSP70 constitute potent vaccine vehicles in different preclinical models, clinical studies remain disappointing. The fact that the immune properties of HSP70 have not been totally clarified may explain their relative efficacy in human. In this review are presented the main immune properties of HSP70 related to the HSP70-binding elements identified to date, and discuss our current knowledge on their intrinsic immune properties.

Keywords Adaptive immunity · Hsp70 · Immune regulation · Innate immune receptors · Innate immunity · Myeloid cell · Vaccine

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Abbreviations

A2M	alpha2 macroglobulin
Ac-LDL	acetylated low-density lipoprotein
ADP	adenosine dipohosphate
AGE	advanced glycation end product
AIF	apoptosis inducing factor
APAf-1	apoptotic peptidase activating factor 1
APC	antigen-presenting cell
APOER	apolipoprotein E receptor
ATP	adenosine triphosphate
Bax	Bcl-2-associated X protein
BCR	B cell receptor
BiP	binding immunoglobulin protein
CCL	C-C motif ligand
CCR	C-C chemokine receptor
CD	cluster of differentiation
CLEC8A	C-type lectin domain family 8 member A
CLEVER-1	common lymphatic endothelial and vascular endothelial receptor-1
CTL	cytotoxic T cell
DAMP	danger-associated molecular pattern
DC-SIGN	dendritic cell-specific ICAM-grabbing non-integrin
EBV	Epstein-Barr virus
EGF-like	and link domain-containing scavenger receptor-1
ER	endoplasmic reticulum
ERK	Extracellular signal-regulated kinases
FAT	fatty acid translocase
FEEL-1	fasciclin EGF-like laminin-type
HBV	hepatitis B virus
HCV	hepatitis C virus
Her2/Neu	human epidermal growth factor receptor 2/proto-oncogene Neu
HLA	human leukocyte antigen
HMGB1	high–mobility group box 1
HPV	human papilloma virus
HSP	heat shock protein
IFN	interferon
IL	interleukin
IRAK	IL-1 receptor-associated kinase
IRE1α	inositol requiring enzyme 1α
JAK	Janus kinase
LBP	LPS-binding protein
LDL	low-density lipoprotein
LRP1	low density lipoprotein receptor-related protein 1
MAGE-1	melanoma-associated antigen 1

Mart-1	melanoma antigen recognized by T-cells 1
MD2	myeloid differentiation factor 2
MDSC	myeloid-derived suppressive cells
MHC	major histocompatibility complex
MyD88	Myeloid differentiation primary response 88
NBD	nucleotide-binding domain
NF-κB	nuclear factor-kappa B
NK	natural killer
Ox-LDL	oxidized low-density lipoprotein
PAMP	pathogen-associated molecular pattern
PDZK	PDZ domain-containing protein 1
PRM	pattern recognition molecule
PRR	pattern recognition receptor
PSA	prostate-specific antigen
PTX3	pentraxin 3
RAP	receptor-associated protein
SBD	substrate-binding domain
SIGLEC	sialic-acid-binding immunoglobulin-like lectins
SP-D	surfactant protein D
SREC	scavenger receptor expressed by endothelial cells
STAT	signal transducers and activators of transcription
TAB1	TAK1-binding protein 1
TAK1	TGFβ-activated kinase
TAM	tumor-associated macrophages
TCR	T cell receptor
Th	helper T cell
TLR	toll-like receptor
TNF	tumor necrosis factor
TNFSF	TNF superfamily
TRAF	TNF receptor-associated factor
Trp2	tyrosinase-related protein 2
TSP-1	thrombospondin 1

Introduction

Heat shock proteins (HSP) are involved in 3D-folding of newly synthesized proteins and protect them against endogenous and exogenous assaults. Besides their classical biochemical properties, several studies have demonstrated that HSP70, one of the largest and highly conserved family of HSP, exhibit intrinsic immune properties. The potential immune properties of HSP70 were first hypothesized in the 1970s by showing that proteins with an apparent molecular weight of 70 kDa and isolated from tumor cells may induce a protective immune response in vivo, in the absence

of adjuvant. Thereafter, a huge quantity of studies aimed at deciphering, in vivo and in vitro, the biological mechanisms involved in the immune properties of HSP. Most of the immune properties of HSP were first elucidated for gp96 (HSP90B1), a member of the HSP90 family, and thereafter for HSP70. One of the most remarkable immune properties of HSP70 is their ability to mediate the cross-presentation of exogenous antigens and to initiate protective antitumor immune responses. In agreement with the cross-presentation process, HSP70 have been shown to interact with different immune receptors, especially innate receptors. However, the exact nature of the endocytic and signaling receptors engaged by HSP70, as well as whether HSP70 exhibit stimulatory or regulatory immune properties, remain a subject of debate. Nevertheless, and whatever the mechanism involved, HSP70 constitute interesting vehicles to induce, in vivo, antigen-specific cytotoxic responses. This review thus addresses the immune properties of HSP70, with a focus on the innate immune receptors engaged, and the consequences on vaccine strategies.

HSP70 Family of Chaperones

Common Features of HSP70

The 70 kDa heat shock proteins (HSP70s) constitute a family of highly conserved proteins that are ubiquitously expressed in prokaryotes and eukaryotes. Prokaryotes express three HSP70 proteins (DnaK, HscA (Hsc66), and HscC (Hsc62)) whereas eukaryotes express several HSP70s. As an example, the human family comprises 13 proteins, that differ from each other by their amino acid sequence, their levels of expression and their localization (Radons 2016). The mostly expressed inducible human HSP70s are Hsp70–1 (encoded by the *HSPA1A* gene) and Hsp70–2 (*HSPA1B*); these two genes are closely located in the genome (Brocchieri et al. 2008). In line with the topic of this review, it is important to mention human HSP70–14 (also referred to as Hsp70L1), a stress-induced HSP identified in dendritic cells, exhibits potent immunostimulatory properties by favoring Th1 responses (Wan et al. 2004). Interestingly, the levels of HSP0L1 are elevated in some tumors (Yang et al. 2015).

HSP70s display a common functional domain structure: (i) a 44 kDa N-terminal nucleotide binding domain (NBD) that binds and hydrolyzes ATP, (ii) a middle protease-sensitive domain and, (iii) a 28 kDa C-terminal substrate-binding domain (SBD) that can interact with 6–9 amino acid-long peptides; SBD has preferential affinity for neutral and hydrophobic amino acids. HSP70s are monomeric chaperons that participate, in physiological situations, to the folding of neo-synthesized proteins, to their transport and to the assembly of multi-protein complexes. The binding/release of polypeptides is dependent on the ATPase activity: the ADP- and

ATP-bound states favor peptide binding and release, respectively. HSP70s also regulate the activity of proteins and prevent their aggregation. Indeed, HSP70s exhibit an « unfoldase » activity, meaning that they recognize unfolded or aggregated proteins and unfold them before native refolding (Radons 2016). The expression of HSP70s is strongly upregulated by cellular stress, such as heat, toxic chemicals (such as heavy metals), ischemia, irradiations, infection, inflammation and nutrient deprivation. In stressed cells (induced by endogenous or exogenous challenges), HSP70s promote cell survival and allow cells to restore cellular homeostasis.

HSP70s act as anti-apoptoti proteins via their capacity to prevent mitochondrial translocation and activation of Bax (Yang et al. 2012) and to inhibit assembly of the death-inducing signaling complexes (Guo et al. 2005). HSP70s also inhibit the activity of different pro-apoptotic molecules (such as Apaf-1, AIF and caspase 3) (Beere et al. 2000; Ravagnan et al. 2001). HSP70s can protect cells against apoptosis induced by endoplasmic reticulum stress via their interaction with the ER stress sensor protein IRE1α (Wei et al. 2013).

Intracellular, Membrane and Extracellular HSP70

As the other HSP, members of the HSP70 family are mainly expressed in intracellular compartments, including cytosol, endoplasmic reticulum (such as binding immunoglobulin protein (BiP) or Grp78) and mitochondria (mtHsp70 or Grp75), to exert their chaperon activities. Nevertheless, HSP70s can be expressed at the membrane surface or present, in a soluble form, in different biological fluids. Membrane HSP70s (mHSP70) accumulate at the surface of infected and tumor cells but not living cells (Multhoff et al. 1995; Multhoff and Hightower 1996; Poccia et al. 1996). Different mechanisms have been proposed to explain the membrane relocalization of proteins devoid of secretion signals, such as the release of secretory granules (Evdonin et al. 2004; Mambula and Calderwood 2006a, b) and of exosomes (Gastpar et al. 2005; Lancaster and Febbraio 2005). The apparently « passive » accumulation of HSP70 at the cell membrane appears similar to the one reported for other intracellular molecules, such as PTX3 (Jaillon et al. 2009), or danger molecules, such as histones and nucleic acids (Cunin et al. 2016) that have also been reported to accumulate at the surface of stressed cells. Accordingly, mHSP70 may have an important role in the recognition of stressed and tumor cells by innate immune cells (Multhoff 2007; Radons and Multhoff 2005). Based on their biochemical properties, one can suspect that mHSP70 are associated to membrane proteins; however, to date, the nature of these putative mHSP70-associated molecules remains unknown.

Another explanation should be that extracellular HSP70s may bind to altered molecules, such as oxidized proteins, that are present at the surface of stressed cells, in a way similar to the one of bridging molecules (Henson 2017). HSP70s can be

released by numerous cell types in response to various stimuli (Campisi and Fleshner 2003). Different ways of secretion of HSP70 have been described:

– A passive release by necrotic cells (Fleshner and Johnson 2005),
– The disruption of HSP70-containing secretory vesicles/exosomes released via a non-classical secretion pathway (MacKenzie et al. 2001) and,
– A release via the secretion of lysosomal endosomes (Baraldi et al. 2004; Mambula and Calderwood 2006b).

HSP70s can be used as biomarkers. As an example, mHSP70s are considered as selective markers of aggressive tumors. Circulating HSP70 have been proposed as biomarkers of inflammation in healthy subjects (Gehrmann et al. 2014a; Marotta et al. 2007). HSP70s have been detected in the serum of patients suffering from cancer or chronic infection (Pockley et al. 2014) and are proposed as biomarkers of tumor outcome after treatment (Gehrmann et al. 2014a, b). These data suggest that the immune properties of HSP70s may depend not only on the nature of the chaperoned peptides but also on their localization. Indeed, membrane and extracellular HSP70 may influence the nature and amplitude of signals delivered to immune cells present in the close vicinity or at distance.

HSP70 Induce Protective Immune Responses Against Tumors and Microbial Pathogens

Most of the studies on the use of HSP70s as vaccine vehicles have been performed in preclinical models of tumor growth. Nevertheless, and based on their vaccine potential, HSP70 have been also used for the induction of protective immune responses against microbes. In this paragraph, we focus on the antitumor immune responses induced by HSP70s.

HSP70s Mediate Antigen Presentation and Cross Presentation

The pioneering studies by the group of PK Srivastava demonstrated that tumor-derived gp96 initiate protective tumor-specific CTL responses, suggesting that HSP may act as major tumor-rejection antigens (Suto and Srivastava 1995; Tamura et al. 1997). This capacity to induce protective antitumor immune responses has been extended to other HSP families, especially HSP60 and HSP70 families (Castellino et al. 2000). The capacity of HSP70s to induce protective antitumor immune responses was mainly reported in both prophylactic and therapeutic murine models of tumor development (reviewed in (Srivastava 2002). Moreover, HSP70s also induce cross-presentation of human tumor antigens (Castelli et al. 2001; Milani et al. 2002; Noessner 2006; Noessner et al. 2002), the rationale for their use in

tumor vaccines in humans. In fact, numerous studies have clearly demonstrated that the specificity of the anti-tumor immune response was determined by the chaperoned peptides (Binder and Srivastava 2005; Ishii et al. 1999; Suto and Srivastava 1995). Biochemical analysis have shown that peptides associated to HSP70 are very diverse, deriving from self-proteins, tumor, microbial or minor histocompatibility antigens (Srivastava 2002). As an example, HSP70s isolated from melanoma cell lines can chaperon peptides derived from the tumor antigens Mart-1, gp100, Trp2 and gp100 (Castelli et al. 2001; Noessner et al. 2002).

According to the classical view of antigen presentation, antigen-derived peptides are presented into the MHC class I (MHC-I) and MHC class II (MHC-II) molecules of antigen-presenting cells (APC) to epitope-specific T cell receptors (TCR) expressed by CD8+ and CD4+ T cells, respectively. This interaction is the basis of the antigen-specificity of the adaptive immunity. Although different myeloid cells are able to present antigens to T cells (such as macrophages and neutrophils), dendritic cells are the only APC able to prime naive T cells and to initiate immune responses (Banchereau et al. 2000; Cella et al. 1997).

The process by which some exogenous antigens are endocytosed by APCs, gain access to the MHC class I pathway, and stimulate CD8+ T cells is called cross-presentation (Heath and Carbone 2001; Yewdell et al. 1999). Indeed, the classical view of antigen presentation claimed that exogenous antigens endocytosed by APC are mainly loaded into the MHC-II molecules for recognition by CD4+ T cells, while, in contrast, endogenous antigens (self and viral proteins) are loaded in the MHC-I molecules for recognition by CD8+ T cells. Antigen cross-presentation has revolutionized our view of the induction of antigen-specific immune responses and allowed to propose « conventional » vaccine approaches for the treatment of cancers. The HSP-mediated antigen cross-presentation is dependent on three essential mechanisms:

– A receptor-mediated internalization of the antigens by professional APCs, especially dendritic cells,
– The functional maturation and activation of professional APC, rendering them able to prime naive T cells and,
– An intracellular trafficking allowing exogenous antigens/peptides to get access to the MHC-I presentation pathway.

One of the most important, and also most debated immune property of HSP, is their capacity to activate APCs. Indeed, the priming of CD8+ T cells by exogenous antigens requires that APC are fully activated (Banchereau et al. 2000), a status that may require CD4+ T cell help (Heath and Carbone 1999). The cross-presentation of an antigen by non-activated dendritic cells maintains or induces antigen-specific tolerance; this process is referred to as cross-tolerance. Ideally, the induction of functional CD8+ T cell responses against exogenous antigens may require both antigen cross-presentation in MHC-I molecules to CD8+ T cells and presentation in MHC-II molecules to CD4+ T cells. In agreement with the classical view of antigen presentation, HSP70 also induce antigen-specific CD4+ T cell activation, as evidenced by the

induction of humoral immune responses and the interaction of microbial HSP70 with HLA-DR and their peptide fragments (Haug et al. 2007). Moreover, HSP70 chaperon both MHC-I and MHC-II epitopes (Stocki et al. 2010; 2011).

Potential Immunomodulatory Roles of Extracellular HSP70

The fact that (i) HSP70s mediate the cross-presentation of chaperoned peptides and (ii) that the cross-presentation requires receptor-mediated internalization of the peptide/chaperone complex, suggest that HSP70s have to be released in the extracellular milieu (Stocki and Dickinson 2012). According to their capacity to induce effective immune response, several studies reported that HSP70s are immunostimulatory molecules and, more interestingly, can induce the maturation and activation of dendritic cells, rendering them fully functional (reviewed in (Kuppner et al. 2001; Milani et al. 2002; Srivastava 2002). The term chaperokine was attributed to HSP to define this unique capacity of chaperons to activate immune cells (Asea et al. 2000). However, the intrinsic potential of HSP70s to activate APC remains a subject of debate (Borges et al. 2012) (see paragraph 5).

HSP70-Binding Elements

Antigen cross-presentation requires that exogenous antigens are internalized via endocytic receptors [3, 4]. Accordingly, HSP70s bind to dendritic cells and macrophages (Arnold-Schild et al. 1999; Todryk et al. 1999; Wassenberg et al. 1999) before being internalized in a receptor-dependent manner (Arnold-Schild et al. 1999; Basu et al. 2001; Binder et al. 2000; Castellino et al. 2000; Singh-Jasuja et al. 2000; Sondermann et al. 2000; Wassenberg et al. 1999). Most if not all HSP70-binding elements identified are innate immune receptors. These results are in agreement with the fact that, in an immunological point of view, extracellular HSP70 can be viewed (or detected) as a danger signal (modified self) released by altered/dying cells. These motifs are detected by the innate immune system.

By opposition to the adaptive immunity, innate immunity is defined as a non-antigen specific system. It is involved in numerous processes, such as antimicrobial activity, induction and resolution of inflammation, maintenance of tissue homeostasis and wound healing. The innate immune system includes a large variety of molecular and cellular actors, such as epithelial barriers, numerous soluble molecules (including the complement system) and innate lymphoid and myeloid cells. The most remarkable characteristic of innate immune cells is their capacity to discriminate self from non self (microbes) and altered or modified self (such as the detection of biochemical modification of cell surface molecules). The recognition of non self

and modified self is mediated by a restricted number of molecules (compared to the TCR and BCR repertoires) called pattern recognition molecules (PRM); this term is now preferred to the ancient nomenclature pattern-recognition receptor (PRR). PRM recognize microbial moieties called pathogen-associated molecular patterns (PAMPs) and motifs expressed by altered self and called danger-associated molecular patterns (DAMPs). Remarkably, a same PRM can detect different PAMPs and DAMPs and exhibiting diverse biochemical characteristics (such as nucleic acids, lipids, proteins or glucids). Innate immune cells also orchestrate the adaptive immune response via the production of soluble immune mediators (cytokines and chemokines) and the priming/activation of antigen-specific lymphocytes, thanks to the antigen-presenting functions of myeloid cells.

PRM can be classified into three families, based on their functions: (i) endocytic receptors, involved in ligand recognition and internalization, (ii) signaling receptors, involved in ligand-induced cell activation and (iii) bridging molecules (also called opsonins), that bind to and favor the recognition of extracellular ligands by innate cells. To date, and except some individual cases, most of the HSP70-binding elements are endocytic and/or signaling PRM.

Endocytic Receptors

CD91

CD91 was the first HSP-binding element identified, initially as a gp96 receptor (Binder et al. 2000). CD91 was then reported as a receptor for human HSP70s on macrophages (Basu et al. 2001). CD91, also known as the α2 macroglobulin (A2M) receptor, low density lipoprotein receptor-related protein 1 (LRP1) or apolipoprotein E receptor (APOER), is an endocytic and signaling receptor belonging to the lipoprotein receptor family. CD91 is expressed by numerous cell types, including hepatocytes, fibroblasts, keratinocytes, smooth muscle cells and myeloid cells (Herz and Strickland 2001). CD91 is a multimeric receptor consisting of a 420 kDa α subunit, a 85 kDa β subunit and a 39-kDa associated molecule. CD91 binds to the activated form of α2M, a soluble molecule that binds to and inhibits a wide variety of proteinases and growth factors. CD91 is also suspected involved in lipid metabolism and can bind, in addition to activated A2M, tissue-specific plasminogen-activator−inhibitor complex and urokinase-PAI1 complex.

The identification of CD91 as an HSP-binding structure was mainly based on competitive binding assays with the CD91 ligand A2M and with a neutralizing anti-CD91 mAb. The role of CD91 in HSP70-mediated antigen presentation to CD4+ and CD8+ T cells was confirmed by several independent studies (Fischer et al. 2010; Salimu et al. 2015; Tobian et al. 2004a).

Scavenger Receptors

Scavenger receptors represent a family of non-related cell-surface glycoproteins that recognize a large repertoire of ligands, ranging from bacteria and yeast to self (native proteins) and modified-self such as oxidized LDL (Ox-LDL) and apoptotic cells (reviewed in (Yamada et al. 1998; Yu et al. 2015)). Some scavenger receptors can also bind chemically modified LDL (acetylated LDL) that constitutes reliable tools in identifying HSP-binding elements, especially as binding competitors. Scavenger receptors thus represent an important family of endocytic PRM through their ability to bind endogenous and exogenous danger molecules (Jeannin et al. 2008). Different studies reported that scavenger receptor-binding molecules, such as modified LDL (OxLDL or AcLDL), apoliprotein B, fucoidan and poly[IC], inhibited the binding of HSP70 to human APCs, suggesting that these PRM are the main cell surface HSP70 binding elements on human APCs (Delneste et al. 2002; Facciponte et al. 2007; Theriault et al. 2006; Theriault et al. 2005).

The first identified HSP70-binding scavenger receptors was SR-E1. Initially identified as an Ox-LDL receptor expressed by endothelial cells; this molecule, also known as LOX-1 or CLEC8A (C-type lectin domain family 8 member A), binds multiple ligands, including advanced glycation end products (AGE), activated platelets, and apoptotic cells. SR-E1 also binds exogenous ligands, such as virus and bacteria. In addition to endothelial cells, SR-E1 is constitutively expressed by macrophages and dendritic cells (Delneste et al. 2002). By using a collection of scavenger receptor-expressing CHO cells, we have identified SR-E1 as one of the main HSP70-binding element on human macrophages and dendritic cells. Interestingly, the in vitro and in vivo cross-presentation of an antigen coupled to HSP70 is dependent on SR-E1 internalization (Delneste et al. 2002). Finally, the in vivo targeting of an exogenous antigen to SR-E1, by coupling to an anti-SR-E1 antibody, induced an antigen-specific CD8+ T cell response (Delneste et al. 2002).

SR-E1 also exhibits features of C-type lectins (Sawamura et al. 1997) which represent another important family of highly conserved PRM. Several studies have underlined the important role played by C-type lectins in mediating antigen cross-presentation, both in vitro and in vivo. Among these PRM, one can mention CD205 (DEC205) (Bozzacco et al. 2007) and CD209 (DC-SIGN) (Garcia-Vallejo et al. 2013). However, no binding of HSP70s to some other C-type lectins, such as CD209 (Theriault et al. 2005) and CLEC7A (dectin-1), was reported so far. A binding of HSP70 family members was also reported to other scavenger receptors:

- SR-A1 (Facciponte et al. 2007); SR-A1 (CD204) is a 220–250 kDa trimeric molecule that shares a collagen-like domain, essential for ligand binding. SR-A1 binds numerous endogenous and exogenous ligands, such as AGE products, as well as microbes and microbial moieties.
- SR-B1 (Fischer et al. 2010). Also known as CD36, platelet glycoprotein 4, fatty acid translocase (FAT) or glycoproteins 88 (GP88), IIIb (GPIIIB), or IV (GPIV), SR-B1 preferentially accumulates in caveolae. SR-B1 bind modified LDL (OxLDL and AcLDL), unmodified low density lipoproteins (LDL), very low

density lipoproteins (VLDL) and apoptotic cells. SR-B1 is a heavily N-glycosylated protein with the C-terminal cytoplasmic tail that interacts with the multisubunit adaptor protein PDZK.
– SR-F1 (Facciponte et al. 2007; Gong et al. 2010; Theriault et al. 2006). Also known as SREC-I, SR-F1 mediates the internalization of OxLDL (as well as AcLDL). SR-F1 can also interact with SREC-2, a homologous of SR-F1, via its extracellular domain; although the role played by this heterophilic interaction remains unclear, it is suppressed by SR-F1 ligands.
– SR-H1 (Theriault et al. 2006). Also known as FEEL-1, CLEVER-1 or stabilin-1 (STAB1), SR-H1 binds AcLDL, AGEs as well as Gram-negative and Gram-positive bacteria (Adachi and Tsujimoto 2002; Tamura et al. 2003).

These endocytic receptors are involved in the internalization and presentation of associated antigens to CD4+ and CD8+ T cells (Facciponte et al. 2007; Gong et al. 2010).

CD40

In 2001, the group of T Lehner reported a very elegant study showing that CD40 is a binding and uptake receptor for *Mycobacterium tuberculosis* HSP70 (MtbHSP70), but not human HSP70 (Binder 2009; Wang et al. 2001). CD40, a membrane molecule belonging to the TNF receptor superfamily (TNFRSF5), is constitutively expressed by APCs, including macrophages, dendritic cells and B lymphocytes. Triggering CD40 on myeloid cells induces the production of inflammatory cytokines. The engagement of CD40 on B cells induces their maturation, antibody isotype switching, and their differentiation into plasma cells. The ligand of CD40 is a member of the TNF superfamily, TNFSF5, also called CD40 ligand (CD40L) or CD154. CD154 is mainly expressed by activated CD4+ T cells and acts as a costimulatory molecule for B cells and myeloid cells; the engagement of CD154 participates to the T cell priming process.

A subsequent study by the same group showed that the binding of MtbHSP70 to the extracellular domain of CD40 was localized in the N-terminal nucleotide-binding domain in its ADP (peptide-binding) state (Becker et al. 2002). To date, CD40 has been mainly reported as a signaling receptor (reviewed in (Banchereau et al. 1994); its role in myeloid cell activation by HSP70 is discussed in the paragraph “Signaling receptors”.

C-type Lectins

As mentioned above, HSP70 can bind to the C-type lectin/scavenger receptor SR-E1 which is constitutively expressed on myeloid cells. Studies have also reported that HSP70 can bind to the C-type lectin CD94 (Gross et al. 2003a; Moser et al. 2002). CD94/NKG2 is a family of receptors mainly expressed on natural killer (NK) cells

and a subset of CD8+ T cells. The consequences of HSP70 binding to CD94 on the biology of NK cells is detailed in the paragraph 4.

Signaling Receptors

HSP70 can efficiently cross-prime naive T cells, a process that required an optimal activation of APCs. However, some of the endocytic receptors identified are not signaling molecules, such as SR-E1, suggesting that signaling receptors are recruited by HSP70s. Numerous studies were thus focused on identifying HSP70 signaling receptors.

TLRs

Through their pivotal role in the activation of myeloid APCs, members of the Toll-like receptor (TLR) family are crucial in the initiation of innate and adaptive immune responses. The induction of in vivo immune responses by HSP70 suggested that they can activate myeloid cells and members of the TLR family rapidly emerged as candidate molecules. TLRs are type I integral membrane glycoproteins belonging to the IL-1 receptor (IL-1R) superfamily. TLR and IL-1R have a conserved region of ≈ 200 amino acids in their cytoplasmic domain, known as Toll/IL-1R (TIR) domain. The TIR domain is required for the intracellular signaling induced by TLR ligands. TLR can sense a large variety of microbes and microbial moieties, as well as host motifs. They are localized either at the cell surface or in endosomes where there are specialized in detecting microbes or nucleic acids, respectively. After ligand binding, TLRs dimerize and undergo a conformational change required for the recruitment of downstream signaling molecules that include the adaptor molecule myeloid differentiation primary-response protein 88 (MyD88), IL-1R-associated kinases (IRAKs), TGFβ-activated kinase (TAK1), TAK1-binding protein 1 (TAB1), TAB2 and TNF-receptor-associated factor 6 (TRAF6). The engagement of TLRs generates potent activation signals for myeloid cells, as evidenced by the production of numerous pro-inflammatory cytokines, chemokines and interferons (IFNs). TLR agonists also induce the maturation of dendritic cells, a process required for naïve T cell priming. TLR agonists may also participate to the activation of innate and conventional lymphoid cells.

Asea et al. reported that TLR2/TLR4 are involved in the activation of myeloid cells by HSP70 (Asea et al. 2000, 2002), as evidenced by the production of the inflammatory cytokines IL-1β, TNFα and IL-12, and an elevated expression of the costimulatory molecule CD86. The HSP70-induced signaling was mediated via the MyD88/IRAK/NF-kB signaling pathway. The role played by TLR4 in the activation of dendritic cells and in the induction of a Th1 response by HSP70s was confirmed by others (Fang et al. 2011).

TLR4 was initially described as an element of the multimeric LPS receptor which includes, in addition to TLR4, the binding elements CD14 and LBP and the accessory molecule MD2. Accordingly, the activation of APCs induced by HSP70 requires CD14, in addition to TLR4; however, no data supported a direct binding of HSP70 to CD14 (Asea et al. 2000; Moroi et al. 2000). In line with the capacity of HSP70 to signal via TLR2/TLR4, several endogenous ligands have reported to bind to these molecules, including, in addition to other HSP (HSP60, HSP96), HMGB1, surfactant protein D (SP-D), fibrinogen, fibronectin, and hyaluronic acid (Bryant et al. 2015).

CD91

CD91 is phosphorylated in response to HSP, triggering signaling cascades that ultimately lead to the activation of NF-κB (Pawaria and Binder 2011). The stimulatory function of CD91 was confirmed in another study showing that the proliferation and cytokine production by CD4+ T cells in response to APC pulsed with complexes between HSP70 and antigenic peptides was inhibited by CD91 siRNA (Fischer et al. 2010). Accordingly, the CD91 ligand A2M acts as an adjuvant to prime CD8+ T cells in vivo (Kropp et al. 2010).

CD40

MtbHSP70 induced a CD40-mediated production of numerous cytokines and chemokines, such as CCL3 (MIP1α) involved in the recruitment and activation of polymorphonuclear cells, CCL4 (MIP1β) chemotactic for numerous immune cells and, CCL5 (RANTES), chemotactic for T cells. This property can be explaind by the adjuvanticity of MtbHSP70 (Wang et al. 2001). The binding of MtbHSP70 to CD40 induces an intracellular signaling via p38, associated with the internalization of the complex HSP70-CD40. P38 is a transduction molecule involved in the signaling cascade downstream CD40 that is involved in the production of pro-inflammatory cytokines, such as TNFα and IFNγ (Pullen et al. 1999).

CCR5

C-C chemokine receptor type 5 (CCR5), also known as CD195, acts as a receptor for the C-C chemokines CCL3, CCL4, and CCL5, three chemokines induced by MtbHSP70. CCR5 is mainly involved in the attraction of T cells in specific tissues and organs. The signaling via CCR5 induces dendritic cell activation and aggregation and participates to the formation of the immune synapse between dendritic cells and T lymphocytes (Floto et al. 2006). The binding of MtbHSP70 to CCR5 induces a Ca2+ signaling and the engagement of CCR5 participates in the

generation effector immune responses (MacAry et al. 2004). In this study, the authors showed that the activation by MtbHSP70 was not dependent on TLR signaling.

Cooperation Between Endocytic and Signaling Receptors

Except some HSP70-binding elements, such as CD91 and CD40 which can act as both endocytic and signaling receptors, several studies suggest that the activation of innate immune cells by HSP70 requires cooperation between an endocytic receptor and a signaling receptor. This mechanism of interaction is observed in several examples of innate immune cell activation by non self and altered self. Nevertheless, in most cases, this model is supported by indirect in vivo experiments using HSP70 as a vaccine carrier molecule. As an example, Gong et al have demonstrated that the induction of antitumor immunity by HSP70 isolated from tumor-dendritic cell fusions is dependent on functional SR-F1 and TLR2/TLR4 expression by dendritic cells (Gong et al. 2009). In a similar manner, we have reported that targeting in vivo a vaccine antigen to SR-E1 is not sufficient to induce a protective antitumor response and that protection was only observed when antigen targeting was associated with the use of a TLR-activating adjuvant (Delneste et al. 2002). Interestingly, Mizukami et al showed that the cross-priming capacity of HSP70s was mediated by a TLR-independent mechanism, while the MyD88/IRAK signaling was required to induce tumor rejection (Mizukami et al. 2012).

Hsp70 in the Regulation of Innate Immune Cell Activation

HSP70s, as other members of the HSP superfamily, induce antigen-specific immune responses, in vitro and in vivo, a process that requires activation of APCs. As a consequence, several studies have reported that HSP70 induce the activation of innate immune cells, and especially myeloid cells.

Activation of Myeloid Cells

In addition to their role as sentinels of the innate immune system, macrophages and dendritic cells also act as professional antigen-presenting cells. Although macrophages are only able to present antigens to memory T cells, dendritic cells have the unique capacity to prime naive T cells. HSP70s can induce not only the cross-presentation but also the presentation of chaperoned peptides and coupled antigens to CD4+ and CD8+ T cells. The fact that HSP70s induce protective immune CD4+ and CD8+ T cell responses suggests that they are able to activate APCs. It is

important to mention that antigen presentation and cross-presentation induced by HSP70s involve the same endocytic and signaling receptors. As an example, CD91 and scavenger receptors are involved in the activation of antigen-specific memory CD4+ T cells (Fischer et al. 2010). The role of HSP70 in the induction of CD4+ T cells has been also reported by other studies (Mycko et al. 2004; Tobian et al. 2004a, b; Wang et al. 2006).

As mentioned above, extracellular HSP70s induce the production of pro-inflammatory cytokines (TNFα, IL-1β, IL-6) and C-C chemokines by myeloid cells (Asea et al. 2000, 2002; Redzovic et al. 2015; Wang et al. 2001). HSP70s also induce the production of IL-12 (Vabulas et al. 2002), a pro-Th1 cytokine, and of IL-15 (Redzovic et al. 2015), a cytokine involved in the activation of innate and adaptive cytotoxic lymphoid cells, as well as type I IFNs by plasmacytoid cells (Jacquemin et al. 2017). The activation of myeloid cells is also associated with an increase of expression of MHC-II and of the costimulatory molecules CD40, CD80 and CD86 (Asea et al. 2002; Fang et al. 2011; Wang et al. 2002) which provide stimulatory signals required for optimal T cell activation.

Interestingly, HSP70s were reported to induce dendritic cell maturation (Kuppner et al. 2001; Vabulas et al. 2002; Wang et al. 2002), a process required to prime naive T cells against neo-antigens. Importantly, the capacity of HSP70 to activate and induce the maturation of dendritic cells supports their ability to generate immune responses in vivo, in the absence of adjuvant (Srivastava et al. 1998). As a consequence of their dendritic cell-stimulatory activity, HSP70s have been reported able to convert T cell tolerance to autoimmunity in vivo, in a murine model of model of autoimmune diabetes (Millar et al. 2003). Studies also reported that an intratumoral infusion of HSP70 increase the infiltration of cytotoxic lymphoid and the production of IFNγ (Shevtsov et al. 2014).

Activation of Innate Lymphoid Cells

NK cells are innate lymphoid cells that play a pivotal role in the destruction of virus-infected cells and tumoral cells. Their activation is dictated by a delicate balance between stimulatory and regulatory signals (Narni-Mancinelli et al. 2013). The overexpression of HSP70 by tumor cells has been reported as a marker of tumor immunogenicity (Clark and Menoret 2001) and in vivo studies showed that tumors secreting HSP70s display increased immunogenicity, with induction of strong and specific CTL responses (Massa et al. 2004). Interestingly, CD94+ CD3- NK cells can recognize and kill membrane HSP70-positive tumor cells in a granzyme-dependent manner (Moser et al. 2002; Multhoff et al. 1995). The interaction of HSP70 with CD94 was demonstrated by competitive binding experiments (Gross et al. 2003a, b).

Moreover, in the absence of HLA-E expression, the activation of NK cells with the HSP70 peptide TKD render them cytotoxic against target cells (Bottger et al. 2012; Gross et al. 2008). More intriguingly, Massa et al reported that the interaction

of HSP70 with NK cells may also participate in the initiation of antigen-specific T cell responses. More precisely, the authors demonstrated that the adjuvant activity of HSP70 requires NK cells at the site of dendritic cell-HSP70 interaction whereas its ability to induce antigen cross-presentation of chaperoned peptides is independent of NK cells (Massa et al. 2005). A recent study reported that HSP70-positive exosomes derived from genotoxic drug-treated tumor cells induce the production of cytokines by $CD56^{high}$ NK cells in a TLR2-dependent manner (Vulpis et al. 2017).

The Immune Properties of HSP70: An Unsolved Mystery

Innate immunity receptors act as immune sensors able to discriminate self from non-self and modified self. In most cases, the elimination of modified self does not activate immune cells, maintaining tissue homeostasis and preventing the initiation of a potentially harmful autoimmune response. Internalization without recruitment of a signaling receptor may lead to tolerance, as reported for the internalization of apoptotic cells. As an example, the interaction of TSP-1 with CD91 and CD36, two HSP70-binding elements, may participate to the tolerogenic status of dendritic cells after apoptotic cell phagocytosis (Poon et al. 2014). In agreement with these studies, the presentation of apoptotic-cell-derived antigens requires the presence of a TLR agonist in the same cargo as that of apoptotic cells (Blander and Medzhitov 2006).

However, most of innate endocytic receptors also recognize non self (microbes and microbial moieties) that induce a huge activation of immune cells. The concept of cooperation between endocytic and signaling receptors has thus emerged to explain this dichotomy. Innate cell activation induces the recruitment of signaling PRM after the binding of ligands to the endocytic receptor. In a similar manner, HSP70 endocytosis precedes signaling in myeloid cells (Kuppner et al. 2001; Moroi et al. 2000). In contrast to early apoptotic cells, necrotic cells induce a pro-inflammatory response, thanks to the release of stimulatory endogenous molecules called alarmins. These alarmins, such as HMGB1, IL-33 and ATP, activate immune cells and participate in the initiation of antigen-specific immune responses. Based on the studies showing that HSP70s activate APCs, it has been proposed that HSP70 may be considered as a danger signal. However, other authors refute to classify HSP70 as a DAMP (van Eden et al. 2012). Indeed, circulating HSP70 are detected in the serum of healthy subjects, without signs of inflammation. Moreover, different studies argue that the stimulatory activities of HSP70s are associated to the presence of contaminating molecules in HSP70 preparation.

Do HSP70 Interact with a Limited Number of Receptors?

To date, HSP70s have been reported to interact with a large variety of immune receptors. However, and even though most studies concur to recognize that HSP70 are able to induce antigen (cross) presentation, different studies refuted the HSP70-binding capacity to some receptors. Although this initial study was confirmed by subsequent studies (Binder and Srivastava 2004; Salimu et al. 2015), authors contested the fact that CD91 may be considered as an HSP-binding structure, either by using $CD91^{null}$ cells or by demonstrating that the binding of HSP to CD91 was not altered by the conventional CD91 ligand activated α2 macroglobulin ($A2M^{*}$) or the CD91 antagonist molecule, receptor-associated protein (RAP) (Berwin et al. 2002). Moreover, although highly expressed on macrophages, the expression of CD91 is very low on dendritic cells, suggesting that its role in in vivo T cell priming should be marginal. Moreover, Theriault et al. reported that scavenger receptors, but not CD40 and CD91, are the main HSP70-binding elements (Theriault et al. 2005). In a similar manner, Bendz et al have shown that the cross-presentation capacity of different dendritic cell subsets was equivalent, irrespective of the level of CCR5 expression (Bendz et al. 2008).

HSP70 are endogenous molecules that, theoretically, cannot activate APCs. Nevertheless, several studies reported that HSP70s can directly activate myeloid cells (and, at a lower extent, lymphoid cells) via innate receptors, such as TLR4. All the studies reporting a direct stimulatory activity of HSP70 claimed that this process was independent of contaminating molecules, especially endotoxins (Wang et al. 2010). However, independent studies reported that contaminating endotoxins were responsible for the activation of myeloid cells (Bausinger et al. 2002; Gao and Tsan 2003, 2004). Another study reported that calcium signaling induced by human HSP70 and MtbHSP70 may be caused by contaminating nucleotides (Bendz et al. 2008).

The Immunoregulatory Properties of HSP70

HSP70s are highly conserved molecules which can be released in the extracellular milieu. Consequently, the initiation of HSP70-specific immune responses remains exceptional. Contrary to the vast majority of studies, some authors have reported that HSP70 exhibit regulatory properties (reviewed in (Borges et al. 2012; van Eden et al. 2005). The initial study on a potential regulatory role for HSP70s was the demonstration that MtbHSP70 has an anti-inflammatory role in an in vivo model of

autoimmune arthritis model (van Eden et al. 1998). The anti-inflammatory and protective roles of HSP70s and of selected HSP70 peptides have been thereafter reported in different models of severe or chronic inflammation (Vinokurov et al. 2012; Yurinskaya et al. 2009), infection (Kimura et al. 1998) and skin allografts (Borges et al. 2010). Extracellular HSP70s also induce endotoxin tolerance in macrophages (Aneja et al. 2006).

At the cellular level, HSP70 have been also reported to inhibit the maturation of dendritic cells, to induce the differentiation of monocyte-derived dendritic cells into tolerogenic cells (Motta et al. 2007; Stocki and Dickinson 2012) and to potentiate the suppressive activity of myeloid derived suppressive cells (MDSC) (Diao et al. 2015). By inducing suppressive myeloid cells, HSP70s increase the immunosuppressive activity of CD4+ CD25+ FoxP3+ regulatory T cells (Wachstein et al. 2012); regulatory T cells (Treg) have the unique capacity to dampen inflammation and to maintain an immunoregulatory environment (Josefowicz et al. 2012). HSP70s also favor the production of immunosuppressive Th2 cytokines by CD4+ T cells (Tsan and Gao 2004).

Macrophages are involved in numerous processes, such as immune surveillance and wound healing. A binary classification has thus been proposed to define their functional polarization with M1 and M2 cells representing the extremes of a continuum of polarization profiles. M1 cells exhibit antimicrobial and antitumor properties while M2 cells, mainly involved in tissue homeostasis and repair, exhibit immunoregulatory and protumoral properties. In established solid tumors, tumor-associated macrophages (TAM) exhibit a M2 phenotype (Mantovani et al. 2017). As examples, SR-A1 promotes tumor progression in murine models of ovarian and pancreatic cancer (Neyen et al. 2013a, b). HSP70s favor the polarization of macrophages into regulatory M2 cells (Lopes et al. 2014). An intriguing study recently reported that HSP70 regulates the M2-like polarization of tumor-associated macrophages in a SR-A1-dependent manner, favoring in vivo glioma regression (Zhang et al. 2016). However, whether this mechanism can be extended to other HSP70-binding elements, and especially scavenger receptors and C-type lectins, remains unknown.

The immunoregulatory activity of HSP70 was demonstrated to be dependent on the induction of the suppressive cytokine IL-10 by myeloid cells ((Detanico et al. 2004; Kimura et al. 1998; van Eden et al. 2005; Wendling et al. 2000). HSP70-induced IL-10 may then favor the generation of regulatory cells (Treg) as Treg cell depletion completely abolished this effect (Hauet-Broere et al. 2006). The capacity of HSP70 to favor a M2 polarization was also suspected dependent on the induction of IL-10 (Lopes et al. 2016). More recent studies have shown that the capacity of HSP70 to inhibit the production of IL-10 is driven by a down-regulation of the transcription factors C/EBPβ and C/EBPδ (Borges et al. 2013); this inhibition was correlated with a decreased production of pro-inflammatory cytokines and abrogated upon pretreatment of cells with ERK and JAK2/STAT3 inhibitors. These results are in agreement with studies reporting the pivotal role played by STAT3 in the establishment of an immunoregulatory environment (Yu et al. 2007). More intriguingly, Chandarwakar et al reported that the regulatory versus stimulatory activity of HSP

was dependent on their concentrations with low doses being efficient to initiate antitumor immune responses and high doses being inefficient or even immunosuppressive (Chandawarkar et al. 1999).

Can SIGLEC4/15 Reconciliate Stimulatory and Regulatory Immune Properties of HSP70?

In 2015, Fong et al reported that the immune properties of extracellular HSP70s are mediated via the receptors Siglec-5 and Siglec-14 (Fong et al. 2015). Siglecs are transmembrane sialic acid-binding immunoglobulin-like lectins mainly expressed on leukocytes (Macauley and Paulson 2014; Schwarz et al. 2015). Siglec-5 and Siglec-14 belong to the rapidly evolving CD33-related Siglecs (CD33rSiglecs) family (Angata 2006). Siglec-5 and Siglec-14 are immune-suppressive and immune-activating paired receptors. This study reported that HSP70s bind Siglec-5 and Siglec-14 in a sialic-acid independent manner and that Hsp70 suppresses inflammation through Siglec-5 while, in contrast, its augments inflammation through Siglec-14. Interestingly, Siglec-5 and Siglec-14 can interact with other HSP70-binding elements, such as TLR and scavenger receptors/C-type lectins which have been defined as HSP70-binding elements. In addition to shed new light on the complexity of the biology of extracellular HSP70, this study provides an elegant molecular demonstration of the dichotomous immune properties of HSP70s and may help clarifying contradictory studies on the immune properties of HSP70s.

HSP70-Based Strategies to Induce Protective Immune Responses

Even though the intrinsic immune properties of HSP70 remain unclear, most authors agree on the fact that HSP70s represent unique vehicles to induce protective antitumoral and antimicrobial CD8+ and CD4+ T cell responses in vivo (Blachere et al. 1997; SenGupta et al. 2004; Udono et al. 1994; Udono and Srivastava 1993), thanks to their capacity to carry exogenous antigens into MHC-I and MHC-II antigen presentation pathways in professional APCs (Castellino et al. 2000; Srivastava 2002). Accordingly, different vaccine strategies have been proposed based on these unique immune properties. A recent review summarized some ongoing antitumoral vaccine clinical trials using HSP (Shevtsov and Multhoff 2016). In this paragraph are only mentioned the different HSP70-based vaccine strategies to induce protective antitumor and antiviral immune responses.

The initial HSP70-based vaccine strategies relied on the peptide-biding capacity of HSP. Vaccines contained HSP70 isolated from tumor (or virus infected) cells (Noessner et al. 2002). In this approach, the antigen specificity is determined by the

chaperone-assisted peptides, allowing proposing individual vaccines irrespective of the nature of the vaccine antigens and of the MHC restriction (Suto and Srivastava 1995). HSP70s can be also isolated from the supernatants of apoptotic or stressed tumor cells (Chen et al. 2009; Masse et al. 2004) or from HSP70-transfected tumor cells (Massa et al. 2004). An original approach was the use of HSP70 isolated from dendritic/tumor cell fusion which induce potent antigen-specific antitumor immune responses, superior to the one of HSP70 isolated from tumor cells. (Enomoto et al. 2006). Another peptide-based approach is to reconstitute HSP-peptides complexes with immunodominant tumor antigen peptides (Blachere et al. 1997). Vaccinations with dendritic cells pulsed with tumor-derived HSP70 can also induce protective immune responses (Toomey et al. 2008).

Other strategies are based on the capacity of HSP70 to target dendritic cells in vivo, allowing the antigen to get access to the antigen presentation pathways. In this case, HSP70, coupled to the vaccine antigen, is used as a vaccine vehicle. Vaccine antigens can be chemically coupled to HSP70 (Delneste et al. 2002) or produced as a recombinant fusion molecule (Zhang and Huang 2006). Fusion proteins, consisting in HSP70 coupled to a vaccine antigen, have been validated using several different tumor antigens, such mesothelin, MAGE-1, PSA, carcinoembyonic antigen and Her2/neu (Ge et al. 2009; Yuan et al. 2014; Dong et al. 2013; Jiang et al. 2013; Pakravan et al. 2010; Wu et al. 2005) as well as viral antigens, such as the HPV16 E7 antigen and a dominant epitope of the EBV latent protein 2A (Zong et al. 2009, 2013).

Based on its immunostimulatory properties, other strategies use HSP70s as an adjuvant molecule for vaccines using tumor cell lysates (Wang et al. 2010; Li et al. 2010) or DNA vaccines as a source of antigens (Li et al. 2007; Zhang et al. 2007; Farzanehpour et al. 2013; Garrod et al. 2014) or in classical subunit vaccines (Lewis et al. 2014; Li et al. 2010; Shevtsov et al. 2014) to initiate effective immune responses. Nevertheless, in order to ameliorate the efficacy of the vaccine or to overcome the tumor immunosuppressive environment, several studies associated HSP70 vaccines with an adjuvant. In most cases, combining HSP70 vaccine with adjuvant (Delneste et al. 2002) or CD40L (Gao et al. 2012) induced more potent protective immune responses.

Conclusions

The identification of tumor-associated antigens and the presence of circulating tumor specific cytotoxic T lymphocytes (CTLs) in tumor-bearing patients suggested that initiating a protective antitumor immune response is feasible. A lot of work was thus done to propose antitumor vaccine strategies, such as the use of recombinant virus encoding human tumor antigens, tumor cell-derived exosomes or nucleic acid-based vaccines. Following the identification of the antigen cross-presentation process, numerous studies aimed at identifying carrier proteins that may selectively target and activate immature dendritic cells in vivo. HSP70s have thus emerged in

the 1990s as powerful vaccine vehicles to initiate potent and protective antitumoral (and antiviral) CD8+ T cell immune responses. This remarkable property is related to the ability of extracellular HSP70s to interact with innate immune receptors involved in antigen cross-presentation. The initiation of an antigen-specific CD8+ T cells response against a neo-antigen requires that dendritic cells are fully activated. In agreement with their vaccine potentials, the initial studies suggested that HSP70s are intrinsically able to activate APCs. However, the exact nature of PRM involved in the immune properties of HSP70s remains, to date, controversed. Members of the scavenger receptor family appear as the less discussed endocytic receptors. Contrastingly, whether HSP70s are suppressive or stimulatory molecules remain debated. Based on the current view of the capacity of the innate immune system to maintain immune homeostasis in response to modified self, one could hypothesize that HSP70s are not stimulating molecules. Nevertheless, to precisely determine the immune status of HSP70s remains a big challenge that would allow reevaluating their therapeutic use.

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