Review Article

Heat Shock Proteins and Regulatory T Cells

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Heat shock proteins (HSPs) are important molecules required for ideal protein function. Extensive research on the functional properties of HSPs indicates that HSPs may be implicated in a wide range of physiological functions including immune function. In the immune system, HSPs are involved in cell proliferation, differentiation, cytokine release, and apoptosis. Therefore, the ability of the immune system, in particular immune cells, to function optimally and in unison with other physiological systems is in part dependent on signaling transduction processes, including bidirectional communication with HSPs. Regulatory T cells (Tregs) are important T cells with suppressive functions and impairments in their function have been associated with a number of autoimmune disorders. The purpose of this paper is to examine the relationship between HSPs and Tregs. The interrelationship between cells and proteins may be important in cellular functions necessary for cell survival and expansion during diseased state.

1. Introduction

Optimal cellular function is regulated by several molecules including heat shock proteins (HSPs). These proteins have chaperone properties and are important in both stressed and unstressed cells. HSPs can be categorized into six diverse highly or less-conserved families. These include HSP10, HSP40, HSP60, HSP70, HSP90, and HSP100 [1–4]. HSP60 is found in the mitochondria [5]. HSP70 is implicated in protein transport assembly and synthesis. It has anti-apoptotic properties that are implicated in intrinsic and extrinsic apoptotic pathways. HSP70 interacts with the mitochondria through death receptor signaling where it binds to death receptors DR4 and DR5 impeding TNF-related apoptosis inducing ligand (TRAIL) [6]. Importantly, HSP70 can bind to either the unphosphorylated C terminus of protein kinase C or Akt prompting rephosphorylation and kinase stabilization [7]. ATP-dependent HSP90 regulates cell survival by stabilizing kinases such as Akt and suppressing apoptosis by inhibiting caspases [8, 9]. The exact structural domains for all human HSPs remains to be determined; however, HSP70 and HSP90 have been well characterized. HSP70 is comprised of an N-terminal nucleotide-binding domain with ATPase activity and a C terminal containing a substrate-binding domain [10–12]. HSP90 on the other hand has three characterized structural domains including an N-terminal nucleotide-binding domain, a middle segment and a C terminus [13–16]. Inter-domain interactions occur by a conserved linker [17].

HSPs are found in intracellular and extracellular spaces as well as in the circulation. Intracellular HSPs including HSP27, HSP70, and HSP90 have direct roles in preventing protein aggregation, induction of cell death pathways, cellular rescue and maintaining receptor interactions [18]. These HSPs may be overexpressed in most cancers promoting the growth and survival of tumor cells. The downregulation of their expression results in a substantial decrease in tumor cells [3, 19–21]. Extracellular HSPs, HSP70, HSP90, and gp96 are peptide carriers, inducers of cytokines, and stimulants for immune cells during stress [18]. These HSPs may be either membrane bound to the plasma membrane or released

into the circulation [9]. In general, there are two sources of extracellular HSPs, pathogen- and human-derived HSPs. Pathogen derived HSPs are found in the extracellular space as a consequence of infection while human-derived HSPs are released into the extracellular space in the event of intracellular traumas such as apoptosis or necrosis [8]. Extracellular HSPs may be attached to the plasma membrane. Additionally, extracellular HSPs induce the maturation of dendritic cells and present peptide molecules to antigen-presenting cells (APCs) thus, linking the innate immune and adaptive immune systems. Cell-free or circulating HSPs, for instance, HSP70, are released into the circulation by glial cells, B cells, PBMCs, or following necrosis [22–25]. These HSPs can be found in the serum and plasma. Cytokines, interferon (IFN)-γ and IL-10, can cause the release of HSP70 from exosomes [26]. Thus, HSP70 may serve as molecular markers of diseases such as acute myocardial infarction where these HSPs are unduly expressed in the circulation [27].

In unstressed cells, HSPs are chaperone proteins that maintain protein configuration and transport. The presence of HSPs is advantageous especially during cell stress owing to the versatility in their functional attributes, encompassing the inhibition of protein processing, regulation, and production [28, 29]. In the incident of stress, the heat shock functional domain and heat shock transcription factors (HSFs), including HSF-1, HSF-2, HSF-3, and HSF-4, are activated ensuing in the stimulation of the heat shock response (HSR) [30]. HSF-1 translocates into the nucleus oligomerizes, phosphorylates, and binds to heat shock elements causing the release of RNA polymerase [31, 32]. The heat shock functional domain is comprised of a nucleotide-binding domain and a peptide-binding domain. Hydrolysis of ATP to ADP occurs when it binds to the adenine nucleotide-binding domain causing a structural change and detachment of substrates. The peptide domain interacts with the hydrophobic substrates [33].

A set of HSPs known as small HSPs have also been identified to have an involvement in the immune system; nonetheless, this paper is limited to HSPs and not small HSPs.

2. Heat Shock Proteins and the Immune System

The immune system is an intricate network of cells and proteins, and bidirectional communication between different components of the immune system is necessary for optimal homeostasis. HSPs are implicated in both the adaptive and innate immune systems. In the innate immune system, HSPs stimulate dendritic cells and macrophages, as these are APCs, they consecutively stimulate adaptive immune cells [34, 35]. HSPs are important in NK cell function as they are known to increase cytotoxic function and cell proliferation [36]. In particular, membrane-bound HSP70 on various cancer cells is recognized by cluster of differentiation (CD)94 on the NK cell, initiating effective cytolysis of the tumor cells [37, 38]. HSPs may induce the secretion of either anti- or proinflammatory cytokines thereby monitoring the immune response [39, 40].

HSPs may serve as immunogens released in response to an inflammatory episodes which associates with particular surface receptors to induce adaptive immune reactions [39, 41–44]. Antigenic binding of HSPs occurs via interactions between hydrophobic residues such as the V436 in DnaK and bound peptides hence, mutations in these residues may obscure adaptive immunity as a consequence of loss in binding abilities of the peptide [45]. Similarly, HSPs increase the effectiveness of cross-presentation between antigens and APCs in the extracellular milieu, perpetrating in the presentation of peptides to major histocompatibility complex class one (MHCI) or MHCII molecules on T cells. CD91, an HSP receptor, is a requisite for this process and increases T-cell-mediated responses related to T helper (Th)1, Th2, and Tregs [9]. Thus, in the presence of tumors, concomitant relations between the extracellular HSPs and the APCs following internalization of the tumor peptides via CD91 pathway generate both anti-and proinflammatory immune response mediated by T cells [46].

Incidentally, extracellular HSPs may have potent cytokine-related properties necessary for immune response. They act via the association with pattern recognition receptors (PRR) including toll-like receptors (TLRs) and CD14. CD14 is a lipopolysaccharide membrane protein receptor lacking a transmembrane or an intracellular domain [47]. CD14 is a necessary stimulant for HSP60 and HSP70. Following stimulation, these extracellular HSPs are endocytosed causing calcium influx and phosphorylation [48]. Myeloid differentiation primary response gene (MYD) 88 associates with the cytoplasmic domain of the TLR while interleukin (IL)-1 receptor-associated kinase (IRAK) is recruited, phosphorylated, and released. IRAK interacts with the tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6). This is sequentially followed by the stimulation of transforming growth factor-β-activated protein kinase 1 (TAK1) [49]. TAK1 stimulates IκB Kinase (IKK) and this phosphorylates IκB prompting the movement of nuclear factor kappa-light-chain enhancer of activated B cells (NFκB) to the nucleus where it binds to target genes. NFκB modulates the transcription of cytokine genes including TNF-α, IL-1β, IL-6, and IL-12 [50]. The generation of NFκB elicits sequences of events altering the expression of cytokines, chemokines, cell adhesion molecules, growth factors, anti-apoptotic proteins, and immune receptors [51]. HSP may repress NFκB successively decreasing TNF-α [52]. This occurs via mitogen-activated protein kinases (MAPK) pathway originating in the phosphorylation of c-Jun, which sequentially stimulates activator protein (AP)-1 and upregulates proinflammatory IL-18. Similarly, excess secretion of IL-18 is regulated by HSR which suppresses IL-18, by inhibiting JNK/MAPK signaling [53].

The multifaceted nature of HSPs incorporates the regulation of reactive oxygen species (ROS) and some chemokines from stimulated monocytes, macrophages, and dendritic cells. Equally, heightened HSP antigen presentation in APCs is correlated with an increase in CD86, CD40, and MHC molecules [9, 54]. HSP60 expressively inhibits chemotaxis and in combination with TLRs upregulates anti-inflammatory reactions while altering B-cell activity [55,

56]. Involvement of HSPs in the mechanism of danger-activated molecular pattern (DAMPS) is controversial as they have been described as DAMPs while elsewhere they have been implicated in the dampening of DAMPS owing to their interactions with TLRs thus, inducing proinflammatory responses [57, 58]. DAMPS are intracellular endogenous molecules secreted following necrosis with the ability to induce nonspecific adaptive immune responses following dendritic cell activation. DAMPS may also interact with pattern recognition receptors (PRRs) resulting in the presence of inflammatory cytokines [59]. In mice, macrophages stimulated with LPS release high mobility group box 1 (HMGB1) sequestering a proinflammatory response which effectively prompts apoptosis [60]. In the presence of HSP, HMGB is not translocated to the nucleus averting induction of the apoptotic pathway; thus, cell death is aborted [61]. Similarly, HSF-1 binds to the promoter regions of cytokine genes such as TNF-α and IL-1β in mice, inhibiting TNF-α expression in macrophages [62]. TNF-α is involved in the TRAIL death receptor pathway and perhaps obscures their production preventing nonspecific cell death that may be harmful to the immune response or arouse an overreactive immune response that activates autoimmunity.

In autoimmune diseases, HSPs may be important in regulating T-cell-related cytokine dominance from a primarily proinflammatory to an anti-inflammatory state [40, 63–65]. High incidence of HSP70 decreases endotoxin-induced protein and mRNA levels of TNF-α in heat-induced peritoneal macrophages [66] suggesting an influence of HSPs on the transcription of these genes. However, overexpression of HSP70 by peripheral blood macrophages decreases LPS-induced TNF-α, IL-1β, IL-10, and IL-12 [67]. Additionally, HSP70 alters proinflammatory cytokine production increasing endotoxin tolerance and survival [68].

3. The Role of Heat Shock Proteins in Regulatory T Cell Function

Regulatory T cells (Tregs) are a subset of CD4$^+$ T cells with suppressive functions. Two main groups of Tregs have been characterized based on their site of development, that is, in the thymus (natural Tregs (nTregs)) and in the periphery (inducible Tregs (iTregs)) [69]. NTregs are derived from bone marrow progenitor cells transported to the thymus where they differentiate into nTregs following negative and positive selection. Following maturation, these cells migrate to the periphery [70]. NTregs can be differentiated from other T cells owing to the exclusive expression of forkhead box P3 (FOXP3) which is necessary for nTreg-suppressive function [71, 72]. In mice nTregs can be differentiated from iTregs owing to the presence of high levels of neuropilin-1 on mice nTregs [73]. Other essential effector and costimulatory molecules that are expressed by these cells include CD39, CD73, cytotoxic T-lymphocyte antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), CD28, CD80/86, CD40, OX40 (CD134), and 4-IBB (CD137) [74–76]. ITregs are generated from naive CD4$^+$ T cells subsequent to induction by IL-10 and TGF-β resulting in two populations of iTregs,

type 1 Tregs (Tr1), and T helper 3 (Th3) cells, respectively [77–79]. Suppressive function of these cells occurs via IL-10 and TGF-β. Peripheral Tregs can also be generated through interactions between IL-4 or IL-13 and the IL-4Rα [80]. Although, FOXP3 is a characteristic marker of nTregs, Th3 cells can also be induced to generate FOXP3 [81–83]. Upon activation of the T cell receptor, Tregs suppress dendritic cells, B cells, macrophages, osteoblasts, mast cells, NK cells, NKT cells, CD4$^+$ T, and CD8$^+$ T cells [84].

Tregs suppressive mechanisms transpire through cytokine secretion, cytolysis, metabolic destruction, and altering of APCs function. The secretion of inhibitory cytokines such as IL-10, TGF-β, and IL-35 suppresses immune function. The inhibitory effects of IL-10 occur via its association with APCs to suppress inflammation hence, in the absence IL-10-secreting Tregs there is an increase in inflammation [85]. IL-35 on the other hand suppresses the expansion of T cells into other T helper subsets, as well as B cells and macrophages [86, 87]. TGF-β is necessary for the survival of Treg subsets [88]. Tregs may suppress the function of other cells via granzyme-mediated killing following the release of granzymes into the target cells [89, 90]. Similarly, metabolic disruption involving induction of adenosine and the production of cyclic adenosine monophosphate (cAMP) may be a vital mechanism for suppressing overreactive cells [91]. The versatility in Treg effector function allows them to modulate innate immune cells in particular APCs. This entails the engagement of surface molecules such as CTLA-4 and LAG-3 with MHCII molecules on the APCs conferring inhibitory responses that avert the stimulation of other conventional T cells [92].

As HSPs regulate an extensive component of the immune system, it is likely that they have a role in the optimal function of most immune cells. Importantly, the chaperoning effects of HSPs are necessary for the induction of certain T-cell phenotypes, importantly, Th1, Th2, and Tregs. This presupposes that HSPs are important in Treg function. To date, the following HSPs have been investigated in relation to Tregs, HSP60, HSP70, and HSP90. HSPs are important in the induction, proliferation, suppressive function, and cytokine production of Tregs.

HSP60 employs TLR2-signaling pathway in regulating Treg function. TLR2 is expressed on the surfaces of Tregs [93] hence, association between the TLR2 on the Tregs and the HSP stimulates a sequence of events that affect the functional properties of Tregs. Incidentally, increasing levels of HSP60 are correlated with proportional elevations in the intensity of CD4$^+$ CD25$^+$ Treg-directed suppression on the production of TNF-α and IFN-γ [94]. An increase in HSP60 increases ligand binding of the HSP and the TLR2, thus, increasing suppression. This may represent an autoreactive inflammatory response causing autoimmunity [95]. HSP60 also causes an increase in Treg secretion of TGF-β and IL-10 [94]. HSP60 enhances the differentiation of cord blood cells into CD4$^+$ CD25$^+$ Foxp3$^+$ Tregs [96]. Similarly, costimulatory signals from p277 also increase the activity of CD4$^+$ CD25$^+$ Tregs [94]. Therapeutic administration of HSP60 increases the presence of nTregs, and this is often correlated with a decrease in atherosclerotic plaques, the

generation of Tregs, and an increase in the production of TGF-β [97, 98]. The concentration of HSP60 affects Treg suppression and proliferation. Hence, with respect to TLR2 on Tregs, strong ligand binding results in Treg proliferation while relatively low levels or interactions of ligands and TLR2 on the Treg result in an increase in Treg suppression [99].

Equally, HSP70 in Tregs promotes heightened suppressive function in Tregs [100]. HSP70 confers its activity via TLR4 pathway inducing a surge in the regulatory activities of Tregs. The TLR4-signaling pathway is important in Treg function, and this may be important for FOXP3 induction and suppression of inflammatory reactions [101]. TLR4 interactions with HSP70 may also augment effector T cell suppression by Tregs as this has been confirmed in coculture experiments with other ligands [102]. Additionally, the type of Tregs present following HSP administration may be dependent on the type of inflammatory response occurring at the time. For example in the mice model of arthrosclerosis, immunization with HSP70 produces a significant amount of CD4$^+$ CD25$^+$ Foxp3$^+$ Tregs [97]. Similarly, adoptive transfer of HSP70 peptide epitopes such as B29 induces antigen-specific FoxP3$^+$ or LAG-3$^+$ CD4$^+$ CD25$^+$ Tregs that are effective in either aborting or suppressing arthritis in mice [103]. B29 is highly immunogenic peptides with conserved sequences that are presented to T cells by MHC II molecules. Immunization with HSP70 increases IL-10 producing Tregs [104]. HSP70 derived from mycobacterium tuberculosis stimulates the proliferation of Tregs by acting through dendritic cells causing a surge in IL-10 while dampening TNF-α release [105]. Additionally, HSP70 has anti-inflammatory properties including down-regulating inflammatory cytokine production, increasing cell and tissue tolerance of cytokine-related cytotoxicity, and influencing the permeability of the epithelial barrier [106].

HSP90 is important for conserving proteins involved in signal transduction, via a multichaperone complex [107]. HSP90 can be regulated by histone deacetylases (HDACs) such as HDAC6, and hypoacetylation of HSP90 occurs in the presence of excessive HDAC6 [108]. HDAC6 belongs to the Class II family of HDACs that are necessary for the removal of acetyl from histones and are found in the nucleus and cytoplasm [109]. In Tregs, the removal of HDAC6 results in the overexpression of HSP90 acetylation resulting in an increase in HSF1-related genes instigating an increase in the suppressive function of Tregs [100]. This may be important in treating patients with colitis. Mice deficient in HDADC6 are more likely to have increased levels of Treg suppression due to the presence of HSP90 and excess Foxp3 [100]. Similarly, mice deficient in HDAC9 have an increased expression of Foxp3 [110]. The presence of HDAC9 has been observed to decrease Foxp3 via deacetylation and incidentally Treg function. HSP70 not only acts via the TLR4 to regulate Tregs, but also may inhibit HDAC9 ultimately enhancing the release of Tregs and effective Treg repression [111]. Acetylation is a necessary posttranslational modification process for protein production. Hence, increased acetylation of Foxp3 may avert ubiquitination, increase its regulatory effects, stability, and promote DNA binding [112, 113]. Therapeutic strategies involving the use of HSPs to enhance the availability of Foxp3$^+$ Tregs may be important in autoimmune diseases while in diseases like cancer it may be necessary to inhibit Foxp3 acetylation [112].

4. Conclusion

In summary, despite the limited amount of research on Tregs and HSPs, the available literature suggests an involvement of HSPs in the suppressive function and cytokine production of Tregs. HSPs may indirectly or directly stimulate Tregs, via acetylation, TLR, ligation or act as costimulatory molecules via the induction of other cells or molecules to stimulate the Tregs. These may involve cytokines, antigens, and APCs. Hence, HSPs acting are therefore essential in inducing Foxp3 expression, cytokine secretion, and mediating Treg suppressive effects. Additionally, peptides such as B29 may have therapeutic potential as they are able to suppress inflammation and maintain tolerance. Thus, the therapeutic advantage of HSPs relates to their potential use in diseases where the function of Tregs is impaired, importantly, in the management of autoimmune diseases.

References

[1] B. Henderson, "Integrating the cell stress response: a new view of molecular chaperones as immunological and physiological homeostatic regulators," *Cell Biochemistry and Function*, vol. 28, no. 1, pp. 1–14, 2010.

[2] H. H. Kampinga, J. Hageman, M. J. Vos et al., "Guidelines for the nomenclature of the human heat shock proteins," *Cell Stress and Chaperones*, vol. 14, no. 1, pp. 105–111, 2009.

[3] C. Sarto, C. Valsecchi, F. Magni et al., "Expression of heat shock protein 27 in human renal cell carcinoma," *Proteomics*, vol. 4, no. 8, pp. 2252–2260, 2004.

[4] G. Tomasello, C. Sciume, F. Rappa et al., "Hsp10, Hsp70, and Hsp90 immunohistochemical levels change in ulcerative colitis after therapy," *European Journal of Histochemistry*, vol. 55, no. 4, article e38, 2011.

[5] R. Rajaiah and K. D. Moudgil, "Heat-shock proteins can promote as well as regulate autoimmunity," *Autoimmunity Reviews*, vol. 8, no. 5, pp. 388–393, 2009.

[6] Q. Pang, T. A. Christianson, W. Keeble, T. Koretsky, and G. C. Bagby, "The anti-apoptotic function of Hsp70 in the interferon-inducible double-stranded RNA-dependent protein kinase-mediated death signaling pathway requires the Fanconi anemia protein," *FANCC*, vol. 277, pp. 49638–49643, 2002.

[7] T. Gao and A. C. Newton, "The turn motif is a phosphorylation switch that regulates the binding of Hsp70 to protein kinase C," *Journal of Biological Chemistry*, vol. 277, no. 35, pp. 31585–31592, 2002.

[8] A. L. Joly, G. Wettstein, G. Mignot, F. Ghiringhelli, and C. Garrido, "Dual role of heat shock proteins as regulators of apoptosis and innate immunity," *Journal of Innate Immunity*, vol. 2, no. 3, pp. 238–247, 2010.

[9] S. K. Calderwood, S. S. Mambula, and P. J. Gray Jr., "Extracellular heat shock proteins in cell signaling and immunity," *Annals of the New York Academy of Sciences*, vol. 1113, pp. 28–39, 2007.

[10] R. Schlecht, A. H. Erbse, B. Bukau, and M. P. Mayer, "Mechanics of Hsp70 chaperones enables differential interaction with client

proteins," *Nature Structural and Molecular Biology*, vol. 18, no. 3, pp. 345–351, 2011.

[11] S. Rüdiger, A. Buchberger, and B. Bukau, "Interaction of Hsp70 chaperones with substrates," *Nature Structural Biology*, vol. 4, no. 5, pp. 342–349, 1997.

[12] A. Cegielska and C. Georgopoulos, "Functional domains of the Escherichia coli dnaK heat shock protein as revealed by mutational analysis," *Journal of Biological Chemistry*, vol. 264, no. 35, pp. 21122–21130, 1989.

[13] C. Prodromou, S. M. Roe, R. O'Brien, J. E. Ladbury, P. W. Piper, and L. H. Pearl, "Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone," *Cell*, vol. 90, no. 1, pp. 65–75, 1997.

[14] T. Gidalevitz, C. Biswas, H. Ding et al., "Identification of the N-terminal peptide binding site of glucose-regulated protein 94," *The Journal of Biological Chemistry*, vol. 279, pp. 16543–16552, 2004.

[15] S. Vogen, T. Gidalevitz, C. Biswas et al., "Radicicol-sensitive peptide binding to the N-terminal portion of GRP94," *Journal of Biological Chemistry*, vol. 277, no. 43, pp. 40742–40750, 2002.

[16] S. F. Harris, A. K. Shiau, and D. A. Agard, "The crystal structure of the carboxy-terminal dimerization domain of htpG, the Escherichia coli Hsp90, reveals a potential substrate binding site," *Structure*, vol. 12, no. 6, pp. 1087–1097, 2004.

[17] J. Jiang, K. Prasad, E. M. Lafer, and R. Sousa, "Structural basis of interdomain communication in the Hsc70 chaperone," *Molecular Cell*, vol. 20, no. 4, pp. 513–524, 2005.

[18] E. Schmitt, M. Gehrmann, M. Brunet, G. Multhoff, and C. Garrido, "Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy," *Journal of Leukocyte Biology*, vol. 81, no. 1, pp. 15–27, 2007.

[19] U. Banerji, M. Walton, F. Raynaud et al., "Pharmacokinetic-pharmacodynamic relationships for the heat shock protein 90 molecular chaperone inhibitor 17-allylamino, 17-demethoxygeldanamycin in human ovarian cancer xenograft models," *Clinical Cancer Research*, vol. 11, no. 19, pp. 7023–7032, 2005.

[20] K. Nanbu, I. Konishi, M. Mandai et al., "Prognostic significance of heat shock proteins HSP70 and HSP90 in endometrial carcinomas," *Cancer Detection and Prevention*, vol. 22, no. 6, pp. 549–555, 1998.

[21] P. Workman, "Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone," *Cancer Letters*, vol. 206, no. 2, pp. 149–157, 2004.

[22] A. Clayton, A. Turkes, H. Navabi, M. D. Mason, and Z. Tabi, "Induction of heat shock proteins in B-cell exosomes," *Journal of Cell Science*, vol. 118, no. 16, pp. 3631–3638, 2005.

[23] I. Guzhova, K. Kislyakova, O. Moskaliova et al., "In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance," *Brain Research*, vol. 914, no. 1-2, pp. 66–73, 2001.

[24] C. Hunter-Lavin, E. L. Davies, M. M. F. V. G. Bacelar, M. J. Marshall, S. M. Andrew, and J. H. H. Williams, "Hsp70 release from peripheral blood mononuclear cells," *Biochemical and Biophysical Research Communications*, vol. 324, no. 2, pp. 511–517, 2004.

[25] G. I. Lancaster and M. A. Febbraio, "Mechanisms of stress-induced cellular HSP72 release: implications for exercise-induced increases in extracellular HSP72," *Exercise immunology review.*, vol. 11, pp. 46–52, 2005.

[26] M. A. Bausero, R. Gastpar, G. Multhoff, and A. Asea, "Alternative mechanism by which IFN-γ enhances tumor recognition: active release of heat shock protein 72," *Journal of Immunology*, vol. 175, no. 5, pp. 2900–2912, 2005.

[27] B. Dybdahl, S. A. Slørdahl, A. Waage, P. Kierulf, T. Espevik, and A. Sundan, "Myocardial ischaemia and the inflammatory response: release of heat shock protein 70 after myocardial infarction," *Heart*, vol. 91, no. 3, pp. 299–304, 2005.

[28] F. U. Hartl and M. Hayer-Hartl, "Protein folding. Molecular chaperones in the cytosol: from nascent chain to folded protein," *Science*, vol. 295, no. 5561, pp. 1852–1858, 2002.

[29] E. A. Craig, B. D. Gambill, and R. J. Nelson, "Heat shock proteins: molecular chaperones of protein biogenesis," *Microbiological Reviews*, vol. 57, no. 2, pp. 402–414, 1993.

[30] S. Lindquist, "The heat-shock response," *Annual Review of Biochemistry*, vol. 55, pp. 1151–1191, 1986.

[31] C. Sõti, E. Nagy, Z. Giricz, L. Vígh, P. Csermely, and P. Ferdinandy, "Heat shock proteins as emerging therapeutic targets," *British Journal of Pharmacology*, vol. 146, no. 6, pp. 769–780, 2005.

[32] K. D. Sarge, S. P. Murphy, and R. I. Morimoto, "Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress," *Molecular and Cellular Biology*, vol. 13, no. 3, pp. 1392–1407, 1993.

[33] A. Gragerov and M. E. Gottesman, "Different peptide binding specificities of hsp70 family members," *Journal of Molecular Biology*, vol. 241, no. 2, pp. 133–135, 1994.

[34] J. G. Facciponte, I. J. MacDonald, X. Y. Wang, H. Kim, M. H. Manjili, and J. R. Subjeck, "Heat shock proteins and scavenger receptors: role in adaptive immune responses," *Immunological Investigations*, vol. 34, no. 3, pp. 325–342, 2005.

[35] U. Kumaraguru, C. D. Pack, and B. T. Rouse, "Toll-like receptor ligand links innate and adaptive immune responses by the production of heat-shock proteins," *Journal of Leukocyte Biology*, vol. 73, no. 5, pp. 574–583, 2003.

[36] G. Multhoff, "Activation of natural killer cells by heat shock protein 70," *International Journal of Hyperthermia*, vol. 25, no. 3, pp. 169–175, 2009.

[37] C. Gross, I. G. Schmidt-Wolf, S. Nagaraj et al., "Heat shock protein 70-reactivity is associated with increased cell surface density of CD94/CD56 on primary natural killer cells," *Cell Stress & Chaperones*, vol. 8, pp. 348–360, 2003.

[38] S. Stangl, C. Gross, A. G. Pockley, A. A. Asea, and G. Multhoff, "Influence of Hsp70 and HLA-E on the killing of leukemic blasts by cytokine/Hsp70 peptide-activated human natural killer (NK) cells," *Cell Stress and Chaperones*, vol. 13, no. 2, pp. 221–230, 2008.

[39] M. F. Tsan and B. Gao, "Heat shock proteins and immune system," *Journal of Leukocyte Biology*, vol. 85, no. 6, pp. 905–910, 2009.

[40] W. Van Eden, R. Van Der Zee, and B. Prakken, "Heat-shock proteins induce T-cell regulation of chronic inflammation," *Nature Reviews Immunology*, vol. 5, no. 4, pp. 318–330, 2005.

[41] W. Van Eden, G. Wick, S. Albani, and I. Cohen, "Stress, heat shock proteins, and autoimmunity: how immune responses to heat shock proteins are to be used for the control of chronic inflammatory diseases," *Annals of the New York Academy of Sciences*, vol. 1113, pp. 217–237, 2007.

[42] J. M. van Noort, M. Bsibsi, P. Nacken, W. H. Gerritsen, and S. Amor, "The link between small heat shock proteins and the

immune system," *The International Journal of Biochemistry & Cell Biology*, vol. 44, no. 10, pp. 1670–1679, 2012.

[43] Y. Bulut, K. S. Michelsen, L. Hayrapetian et al., "Mycobacterium tuberculosis heat shock proteins use diverse toll-like receptor pathways to activate pro-inflammatory signals," *Journal of Biological Chemistry*, vol. 280, no. 22, pp. 20961–20967, 2005.

[44] J. S. H. Gaston, "Heat shock proteins as potential targets in the therapy of inflammatory arthritis," *Biotherapy*, vol. 10, no. 3, pp. 197–203, 1998.

[45] P. A. MacAry, B. Javid, R. A. Floto et al., "HSP70 peptide binding mutants separate antigen delivery from dendritic cell stimulation," *Immunity*, vol. 20, no. 1, pp. 95–106, 2004.

[46] S. Pawaria, M. N. Messmer, Y. J. Zhou, and R. J. Binder, "A role for the heat shock protein-CD91 axis in the initiation of immune responses to tumors," *Immunologic Research*, vol. 50, pp. 255–260, 2011.

[47] F. Stelter, "Structure/function relationships of CD14," *Chemical Immunology*, vol. 74, pp. 25–41, 2000.

[48] A. Asea, S. K. Kraeft, E. A. Kurt-Jones et al., "HSP70 stimulates cytokine production through a CD 14-dependant pathway, demonstrating its dual role as a chaperone and cytokine," *Nature Medicine*, vol. 6, no. 4, pp. 435–442, 2000.

[49] T. Kawai and S. Akira, "TLR signaling," *Cell Death & Differentiation*, vol. 13, pp. 816–825, 2006.

[50] T. S. Blackwell and J. W. Christman, "The role of nuclear factor-kappa B in cytokine gene regulation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 17, pp. 3–9, 1997.

[51] A. R. Brasier, "The NF-kappaB regulatory network," *Cardiovascular Toxicology*, vol. 6, pp. 111–130, 2006.

[52] D. Sun, D. Chen, B. Du, and J. Pan, "Heat shock response inhibits NF-kappaB activation and cytokine production in murine Kupffer cells," *The Journal of Surgical Research*, vol. 129, pp. 114–121, 2005.

[53] Y. Wang, C. Li, X. Wang, J. Zhang, and Z. Chang, "Heat shock response inhibits IL-18 expression through the JNK pathway in murine peritoneal macrophages," *Biochemical and Biophysical Research Communications*, vol. 296, no. 3, pp. 742–748, 2002.

[54] S. K. Calderwood, S. S. Mambula, P. J. Gray Jr., and J. R. Theriault, "Extracellular heat shock proteins in cell signaling," *FEBS Letters*, vol. 581, no. 19, pp. 3689–3694, 2007.

[55] M. Cohen-Sfady, G. Nussbaum, M. Pevsner-Fischer et al., "Heat shock protein 60 activates B cells via the TLR4-MyD88 pathway," *Journal of Immunology*, vol. 175, pp. 3594–3602, 2005.

[56] A. Zanin-Zhorov, G. Nussbaum, S. Franitza, I. R. Cohen, and O. Lider, "T cells respond to heat shock protein 60 via TLR2: activation of adhesion and inhibition of chemokine receptors," *The FASEB Journal*, vol. 17, no. 11, pp. 1567–1569, 2003.

[57] P. Matzinger, "The danger model: a renewed sense of self," *Science*, vol. 296, no. 5566, pp. 301–305, 2002.

[58] W. van Eden, R. Spiering, F. Broere, and R. van der Zee, "A case of mistaken identity: HSPs are no DAMPs but DAMPERs," *Cell Stress & Chaperones*, vol. 17, pp. 281–292, 2012.

[59] H. Kono and K. L. Rock, "How dying cells alert the immune system to danger," *Nature Reviews Immunology*, vol. 8, no. 4, pp. 279–289, 2008.

[60] D. Tang, Y. Shi, L. Jang, K. Wang, W. Xiao, and X. Xiao, "Heat shock response inhibits release of high mobility group box 1 protein induced by endotoxin in murine macrophages," *Shock*, vol. 23, no. 5, pp. 434–440, 2005.

[61] A. Hasegawa, H. Iwasaka, S. Hagiwara, and T. Noguchi, "Relationship between HMGB1 and tissue protective effects of HSP72

in a LPS-induced systemic inflammation model," *Journal of Surgical Research*, vol. 169, no. 1, pp. 85–91, 2011.

[62] I. S. Singh, J. R. He, S. Calderwood, and J. D. Hasday, "A high affinity HSF-1 binding site in the $5'$-untranslated region of the murine tumor necrosis factor-alpha gene is a transcriptional repressor," *The Journal of Biological Chemistry*, vol. 277, pp. 4981–4988, 2002.

[63] W. van Eden, "Immunoregulation of autoimmune diseases," *Human Immunology*, vol. 67, no. 6, pp. 446–453, 2006.

[64] W. Van Eden, F. Hauet-Broere, S. Berlo et al., "Stress proteins as inducers and targets of regulatory T cells in arthritis," *International Reviews of Immunology*, vol. 24, no. 3-4, pp. 181–197, 2005.

[65] W. van Eden, U. Wendling, L. Paul, B. Prakken, P. van Kooten, and R. van der Zee, "Arthritis protective regulatory potential of self-heat shock protein cross-reactive T cells," *Cell Stress & Chaperones*, vol. 5, pp. 452–457, 2000.

[66] Y. M. Snyder, L. Guthrie, G. F. Evans, and S. H. Zuckerman, "Transcriptional inhibition of endotoxin-induced monokine synthesis following heat shock in murine peritoneal macrophages," *Journal of Leukocyte Biology*, vol. 51, no. 2, pp. 181–187, 1992.

[67] X. Z. Ding, C. M. Fernandez-Prada, A. K. Bhattacharjee, and D. L. Hoover, "Over-expression of hsp-70 inhibits bacterial lipopolysaccharide-induced production of cytokines in human monocyte-derived macrophages," *Cytokine*, vol. 16, pp. 210–219, 2001.

[68] B. Gao and M. F. Tsan, "Induction of cytokines by heat shock proteins and endotoxin in murine macrophages," *Biochemical and Biophysical Research Communications*, vol. 317, no. 4, pp. 1149–1154, 2004.

[69] C. J. Workman, A. L. Szymczak-Workman, L. W. Collison, M. R. Pillai, and D. A. Vignali, "The development and function of regulatory T cells," *Cellular and Molecular Life Sciences*, vol. 66, pp. 2603–2622, 2009.

[70] E. M. Shevach, "CD4$^+$CD25$^+$ suppressor T cells: more questions than answers," *Nature Reviews Immunology*, vol. 2, no. 6, pp. 389–400, 2002.

[71] S. Sakaguchi, "Naturally arising Foxp3-expressing CD25$^+$CD4$^+$ regulatory T cells in immunological tolerance to self and non-self," *Nature Immunology*, vol. 6, pp. 345–352, 2005.

[72] S. Sakaguchi, "Naturally arising CD4$^+$ regulatory t cells for immunologic self-tolerance and negative control of immune responses," *Annual Review of Immunology*, vol. 22, pp. 531–562, 2004.

[73] M. Yadav, C. Louvet, D. Davini et al., "Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo," *The Journal of Experimental Medicine*, vol. 209, no. 10, pp. 1713–1722, 2012.

[74] S. Huang, L. Li, S. Liang, and W. Wang, "Conversion of peripheral CD4$^+$CD25- T cells to CD4$^+$CD25$^+$ regulatory T cells by IFN-γ in patients with Guillain-Barré syndrome," *Journal of Neuroimmunology*, vol. 217, no. 1-2, pp. 80–84, 2009.

[75] W. Chen, W. Jin, N. Hardegen et al., "Conversion of peripheral CD4$^+$CD25- naive T cells to CD4$^+$CD25$^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3," *Journal of Experimental Medicine*, vol. 198, no. 12, pp. 1875–1886, 2003.

[76] K. Kretschmer, I. Apostolou, D. Hawiger, K. Khazaie, M. C. Nussenzweig, and H. von Boehmer, "Inducing and expanding regulatory T cell populations by foreign antigen," *Nature Immunology*, vol. 6, no. 12, pp. 1219–1227, 2005.

[77] M. H. Kaplan, Y. L. Sun, T. Hoey, and M. J. Grusby, "Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice," *Nature*, vol. 382, pp. 174–177, 1996.

[78] R. A. Seder, R. Gazzinelli, A. Sher, and W. E. Paul, "Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon γ production and diminishes interleukin 4 inhibition of such priming," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 21, pp. 10188–10192, 1993.

[79] F. Cottrez and H. Groux, "Specialization in tolerance: innate CD4+CD25+ versus acquired Tr1 and Th3 regulatory T cells," *Transplantation*, vol. 77, no. 1, Supplement, pp. S12–S15, 2004.

[80] A. Skapenko, J. R. Kalden, P. E. Lipsky, and H. Schulze-Koops, "The IL-4 receptor alpha-chain-binding cytokines, IL-4 and IL-13, induce forkhead box P3-expressing CD25+CD4+ regulatory T cells from CD25-CD4+ precursors," *Journal of Immunology*, vol. 175, pp. 6107–6116, 2005.

[81] Y. Carrier, J. Yuan, V. K. Kuchroo, and H. L. Weiner, "Th3 cells in peripheral tolerance. I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF-beta T cell-transgenic mice," *Journal of Immunology*, vol. 178, pp. 179–185, 2007.

[82] E. Maggi, L. Cosmi, F. Liotta, P. Romagnani, S. Romagnani, and F. Annunziato, "Thymic regulatory T cells," *Autoimmunity Reviews*, vol. 4, no. 8, pp. 579–586, 2005.

[83] L. S. Taams and A. N. Akbar, "Peripheral generation and function of CD4+CD25+ regulatory T cells," *Current Topics in Microbiology and Immunology*, vol. 293, pp. 115–131, 2005.

[84] E. M. Shevach, "Mechanisms of foxp3+ T regulatory cell-mediated suppression," *Immunity*, vol. 30, pp. 636–645, 2009.

[85] D. K. Sojka, Y. H. Huang, and D. J. Fowell, "Mechanisms of regulatory T-cell suppression—a diverse arsenal for a moving target," *Immunology*, vol. 124, no. 1, pp. 13–22, 2008.

[86] G. S. Whitehead, R. H. Wilson, K. Nakano, L. H. Burch, H. Nakano, and D. N. Cook, "IL-35 production by inducible costimulator (ICOS)-positive regulatory T cells reverses established IL-17-dependent allergic airways disease," *Journal of Allergy and Clinical Immunology*, vol. 129, pp. 207–215, 2012.

[87] L. W. Collison, C. J. Workman, T. T. Kuo et al., "The inhibitory cytokine IL-35 contributes to regulatory T-cell function," *Nature*, vol. 450, pp. 566–569, 2007.

[88] S. Yamagiwa, J. D. Gray, S. Hashimoto, and D. A. Horwitz, "A role for TGF-β in the generation and expansion of CD4+CD25+ regulatory t cells from human peripheral blood," *Journal of Immunology*, vol. 166, no. 12, pp. 7282–7289, 2001.

[89] M. Czystowska, L. Strauss, C. Bergmann, M. Szajnik, H. Rabinowich, and T. L. Whiteside, "Reciprocal granzyme/perforin-mediated death of human regulatory and responder T cells is regulated by interleukin-2 (IL-2)," *Journal of Molecular Medicine*, vol. 88, no. 6, pp. 577–588, 2010.

[90] X. Cao, S. F. Cai, T. A. Fehniger et al., "Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance," *Immunity*, vol. 27, no. 4, pp. 635–646, 2007.

[91] M. Vaeth, T. Gogishvili, T. Bopp et al., "Regulatory T cells facilitate the nuclear accumulation of inducible cAMP early repressor (ICER) and suppress nuclear factor of activated T cell c1 (NFATc1)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 6, pp. 2480–2485, 2011.

[92] X. Zhu, P. Yang, H. Zhou et al., "CD4+CD25+Tregs express an increased LAG-3 and CTLA-4 in anterior chamber-associated immune deviation," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 245, pp. 1549–1557, 2007.

[93] H. Liu, M. Komai-Koma, D. Xu, and F. Y. Liew, "Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, pp. 7048–7053, 2006.

[94] A. Zanin-Zhorov, L. Cahalon, G. Tal, R. Margalit, O. Lider, and I. R. Cohen, "Heat shock protein 60 enhances CD4+CD25+ regulatory T cell function via innate TLR2 signaling," *Journal of Clinical Investigation*, vol. 116, no. 7, pp. 2022–2032, 2006.

[95] M. Sakaguchi, H. Kitahara, T. Seto et al., "Surgery for acute type A aortic dissection in pregnant patients with Marfan syndrome," *European Journal of Cardio-thoracic Surgery*, vol. 28, no. 2, pp. 280–283, 2005.

[96] J. A. Aalberse, B. Kapitein, S. de Roock et al., "Cord blood CD4+ T cells respond to self heat shock protein 60 (HSP60)," *PloS One*, vol. 6, article e24119, 2011.

[97] G. H. M. Van Puijvelde, T. Van Es, E. J. A. Van Wanrooij et al., "Induction of oral tolerance to HSP60 or an HSP60-peptide activates t cell regulation and reduces atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 12, pp. 2677–2683, 2007.

[98] H. Li, Y. Ding, G. Yi, Q. Zeng, and W. Yang, "Establishment of nasal tolerance to heat shock protein-60 alleviates atherosclerosis by inducing TGF-beta-dependent regulatory T cells," *Journal of Huazhong University of Science and Technology*, vol. 32, pp. 24–30, 2012.

[99] R. Sutmuller, A. Garritsen, and G. J. Adema, "Regulatory T cells and toll–like receptors: regulating the regulators," *Annals of the Rheumatic Diseases*, vol. 66, Supplement 3, pp. iii91–iii95, 2007.

[100] E. F. de Zoeten, L. Wang, K. Butler et al., "Histone deacetylase 6 and heat shock protein 90 control the functions of Foxp3(+) T-regulatory cells," *Molecular and Cellular Biology*, vol. 31, pp. 2066–2078, 2011.

[101] J. Dai, B. Liu, S. M. Ngoi, S. Sun, A. T. Vella, and Z. Li, "TLR4 hyperresponsiveness via cell surface expression of heat shock protein gp96 potentiates suppressive function of regulatory T cells," *Journal of Immunology*, vol. 178, no. 5, pp. 3219–3225, 2007.

[102] I. Caramalho, T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury, and J. Demengeot, "Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide," *Journal of Experimental Medicine*, vol. 197, no. 4, pp. 403–411, 2003.

[103] M. J. van Herwijnen, L. Wieten, R. van der Zee et al., "Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, pp. 14134–14139, 2012.

[104] L. Wieten, S. E. Berlo, C. B. T. Brink et al., "IL-10 is critically involved in mycobacterial HSP70 induced suppression of proteoglycan-induced arthritis," *PLoS ONE*, vol. 4, no. 1, article e4186, Article ID e4186, 2009.

[105] T. J. Borges, B. N. Porto, C. A. Teixeira et al., "Prolonged survival of allografts induced by mycobacterial Hsp70 is dependent on CD4+CD25+ regulatory T cells," *PLoS ONE*, vol. 5, no. 12, article e14264, Article ID e14264, 2010.

[106] M. Jaattela, D. Wissing, K. Kokholm, T. Kallunki, and M. Egeblad, "Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases," *The EMBO Journal*, vol. 17, pp. 6124–6134, 1998.

[107] J. C. Young, I. Moarefi, and F. U. Hartl, "Hsp90: a specialized but essential protein-folding tool," *Journal of Cell Biology*, vol. 154, no. 2, pp. 267–273, 2001.

[108] S. Aoyagi and T. K. Archer, "Modulating molecular chaperone Hsp90 functions through reversible acetylation," *Trends in Cell Biology*, vol. 15, no. 11, pp. 565–567, 2005.

[109] H. Zhang, Y. Xiao, Z. Zhu, B. Li, and M. I. Greene, "Immune regulation by histone deacetylases: a focus on the alteration of FOXP3 activity," *Immunology & Cell Biology*, vol. 90, pp. 95–100, 2012.

[110] R. Tao, E. F. De Zoeten, E. Ozkaynak et al., "Deacetylase inhibition promotes the generation and function of regulatory T cells," *Nature Medicine*, vol. 13, no. 11, pp. 1299–1307, 2007.

[111] E. F. de Zoeten, L. Wang, H. Sai, W. H. Dillmann, and W. W. Hancock, "Inhibition of HDAC9 Increases T Regulatory Cell Function and Prevents Colitis in Mice," *Gastroenterology*, vol. 138, no. 2, pp. 583–594, 2010.

[112] Y. Xiao, B. Li, Z. Zhou, W. W. Hancock, H. Zhang, and M. I. Greene, "Histone acetyltransferase mediated regulation of FOXP3 acetylation and Treg function," *Current Opinion in Immunology*, vol. 22, no. 5, pp. 583–591, 2010.

[113] U. H. Beier, T. Akimova, Y. Liu, L. Wang, and W. W. Hancock, "Histone/protein deacetylases control Foxp3 expression and the heat shock response of T-regulatory cells," *Current Opinion in Immunology*, vol. 23, pp. 670–678, 2011.