Chapter 7
Heat Shock Proteins and Alarmins in Autoimmunity

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Abstract Autoimmunity represents a diverse group of diseases, which demonstrate complex immuno-pathological responses. Heat shock proteins (Hsp) and danger signaling proteins such as HMGB1 and RAGE, grouped as alarmins play a crucial role in autoimmunity. These proteins are present at elevated levels in the patient's plasma. Hsp bind and stabilize large protein complexes such as immune complexes (ICs), which are formed with autoantibodies generated against modified proteins and nucleic acids that are released from apoptotic and dead cells. Alarmins protect nucleic acids from degrading and enhance ICs capability to produce proinflammatory cytokines. Our current understanding of the role of Hsp in disease is largely based on the studies performed in innate immune cells. In autoimmunity, $CD4^+$ T cells are a major contributor of pathology in inflamed tissue. Activation of $CD4^+$ T cells by ICs triggers upregulation of a large set of genes that encode Hsp and also the HMGB1. HMGB1 associates with the low affinity Fc receptor, which trigger the release of proinflammatory cytokines from ICs ligation. This chapter will address our current understanding of the role and interplay of Hsp with other alarmins in autoimmune pathology. Additionally, it will also address the possible role of low affinity Fc receptors in triggering Hsp and alarmins mediated responses in autoimmune pathology.

Keywords Alarmins · Autoimmunity · $CD4^+$ T cells · Heat shock proteins · HMGB1 · Immune cells · Immune complexes

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Abbreviations

17-AAG	allyamino-17-dimethoxygeldamycin
APC	antigen presenting cells
BCR	B cell receptor
CpG	cytosine-phosphate- guanine
DAMPs	damage-associated molecular patterns
DC	dendrocyte
ER	endoplasmic reticulum
Grp-78	glucose-regulated protein 78
HMGB1	high mobility group box 1
Hsp	heat shock proteins
HSPB1	heat shock protein family B member 1
ICs	immune complexes
IFNs	interferons
JIA	juvenile idiopathic arthritis
LPS	lipopolysaccharides
MAP	mitogen activated protein kinase
MMP	matrix metalloproteinase
PAMPs	pathogen-associated molecular patterns
pDCs	plasmacytoid dendritic cells
PRRs	pattern recognition receptors
PS	phosphotidyl serine
RA	rheumatoid arthritis
RAGE	receptor for advanced glycation end-products
SLE	systemic lupus erythematosus
TCR	T cell receptor
T_E	effector T cells
TLRs	toll-like receptors
TRAF-6	TNF receptor associated

7.1 Introduction

In mild stress innate immune cells produce heat shock proteins (Hsp), which provide protection from subsequent severe stress. Thus, a major role of these proteins is to act as “molecular chaperons” that guide protein folding and prevent protein aggregation. In autoimmunity, both innate and adaptive immune responses are heightened, which is accompanied by formation of large protein complexes with a tendency to precipitate and enhance cell signaling. This necessitates upregulation of the synthesis of chaperon proteins that regulate protein folding. Mis-folded proteins expose the internal buried peptide domains that are prone to chemical modifications and mis-folded proteins also tend to get precipitated. Protein modifications, such as

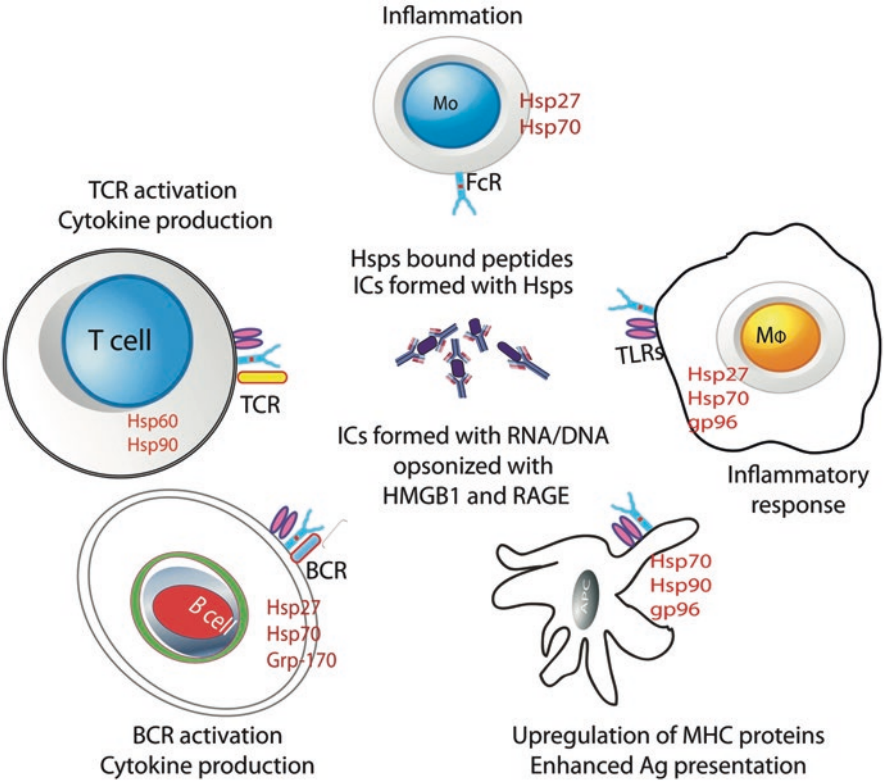

Fig. 7.1 Hsp expressed on immune cells that contribute to autoimmunity. Biochemical modification to Hsp leads to the autoantibody production and ICs formation. Hsp bind to ICs formed with autoantigens generated in autoimmune pathology. ICs opsonize with alarmins such as HMGB1 and RAGE engage immune receptors on the cell surface, enhance signaling and production of proinflammatory cytokines

altered glycation, citrullination, ubiquitination and undesired phosphorylation leads to the development of neo-epitopes that are recognized as foreign and which trigger autoantibody development (Fig. 7.1). Hsp typically reside in the cellular organelles, but they also exist in the extracellular fluid. Enhanced apoptosis is associated with several autoimmune disorders, however it is the necrotic cell death that releases Hsp in the extracellular fluid (Srivastava 2002b). Hsp27 and Hsp70 provide resistance to apoptosis mediated stress (Samali and Orrenius 1998; Xanthoudakis and Nicholson 2000). Many Hsp bind to cell surface glycoproteins that act as scavenger receptors on monocyte derived DCs, myeloid DCs, macrophages, monocytes and B cells (Arnold-Schild et al. 1999; Binder et al. 2000). Various Hsp expression is observed in different immune cell types (Fig. 7.1). These cells contribute to the various immunological responses that are altered and enhanced in autoimmunity. Hsp recognize lipopolysaccharides (LPS) and lipoteichoic acids by pattern recognition. They also bind to membrane phospholipids i.e. phosphatidylserine (PS). Target proteins that

are recognized by Hsp such as pattern recognition receptors (PRRs) play a critical role in the pathogenesis of autoimmune disorders. Toll-like receptors (TLRs) particularly those recognizing nucleic acids, such as nucleic acid binding TLRs (NA-TLRs) recognize ligands in the endosomal compartment and trigger nucleic acid sensing pathways, both in the plasmacytoid dendritic cells (pDCs) and B cells (Blasius and Beutler 2010). TLR2 and TLR4 act as receptors for Hsp. Hsp70 transduce signals via TLR2 and TLR4, which then activate mitogen-activated protein (MAP) kinase cascade and NF-κB signaling pathway (Asea et al. 2002; Vabulas et al. 2002). Toll/IL-1 receptor signaling pathway is activated by Hsp70, which utilizes TNF receptor associated factor (TRAF)-6, a key intracellular signaling pathway protein that drives proinflammatory cytokine production and is a known key contributor of auto-inflammation. Folding of TLR proteins is dependent on dimerization of Hsp, gp96 (also known as glucose-regulated protein-94, Grp-94). All TLR proteins except TLR3 are client proteins for gp96 (Liu et al. 2010). For the host immune system, extracellular Hsp act as pathogen-associated molecular patterns (PAMPs), and are self-adjuvants that promote innate immune responses. In autoimmunity, cells succumb to immunogenic variant of apoptosis, and expose calreticulin on the cell surface. These cells also expose or release Hsp70 and Hsp90, which facilitate the uptake of the dying cells by phagocytes. Further, dying cells release high mobility group binding 1 (HMGB1) protein, which is recognized by DCs through receptor for advanced glycation end-products (RAGE), TLR2 or TLR4. HMGB1 is essential for the cell death to be immunogenic. Surface-expression of calreticulin, Hsp70 and Hsp90 affect the function of DCs that is to present antigen and these proteins also bind to CD91 and TLRs.

Small peptides of 10–30 aa in length with low affinity and specificity associate to Hsp. Antigenic peptides of both viral and bacterial origin associate with cytosolic Hsp70, Hsp90, ER calreticulin and gp96 (Navaratnam et al. 2001; Nieland et al. 1996; Rapp and Kaufmann 2004; Zugel et al. 2001). These interactions lead to productive peptide presentation by MHC class I (MHC I). Peptide loaded MHC I heavy (H) chains associate with β2-macroglobulin and this complex is transported via Golgi to the cell surface, where it is recognized by T cells leading to enhanced development of proinflammatory T_E cells. Hsp70, Hsp90, gp96, and calreticulin transport peptides from proteasomes to MHC I (Srivastava et al. 1994). Interferons (IFNs) are key cytokines in SLE pathology and these cytokines induce Hsp70 and Hsp90, which provide an extraordinary efficiency for antigen cross-presentation, a mechanism that contribute to the autoimmunity. IFN-γ is a cytokine produced by T_H1 cells, and it induces expression of Hsp proteins in addition to MHC I (Anderson et al. 1994). Hsp in particular gp96 contribute to the folding of nascent peptides in ER. Hsp-peptide complexes are capable of priming T cell responses and synergize response with other molecules such as HMGB1 and dsDNA (Gallo and Gallucci 2013). These studies propose diverse roles and mechanisms by which Hsp contribute to the autoimmune pathology.

7.1.1 Hsp in Arthritis

Hsp role in arthritis is controversial, as both protective and inflammatory responses from these proteins in this disease has been observed (Pockley et al. 2008). Hsp influence both innate and adaptive immune responses in rheumatoid arthritis (RA). It has been argued that the context such as cell type and the environment govern Hsp responses where they happen. Serological studies have implicated Hsp40, Hsp60, Hsp70, Grp-78, and Hsp90 in modulating immune responses in RA. In arthritis, inflammatory responses in the joints trigger up-regulated expression of Hsp in the cells that form synovial tissue in the joints. Several studies have shown increased membrane expression of Hsp60 and Hsp70 in the fibroblasts like synovial cells (synovial fibroblasts) isolated from arthritis-affected joints of RA and juvenile idiopathic arthritis (JIA) subjects (Boog et al. 1992; Nguyen et al. 2006; Sedlackova et al. 2009). Levels of soluble Hsp60 are elevated in oligo and polyarticular JIA. ICs isolated from JIA patients contain Hsp40 and mitochondrial peptides along with IgG and IgM rheumatoid factor (RF) (Moore et al. 1995). In oligoarticular JIA, disease remission is associated with proliferative T lymphocyte responses to human Hsp60 (Massa et al. 2007; Prakken et al. 1996). Studies have further shown that Hsp60 successfully suppressed adjuvant arthritis (Van Eden et al. 2007). Elevated levels of Hsp70 are present in both the inflamed synovium and synovial fluid of RA patients. Increase in the extracellular Hsp70 in arthritic joints is profoundly correlative to the disease (Sedlackova et al. 2009). Expression of Hsp70 was also observed on the surface of DCs and in the extracellular compartment in rheumatoid joints (Martin et al. 2003). Both Hsp60 and Hsp70 of mycobacterial origin trigger proliferation of T cells isolated from either SF or peripheral blood of RA and JIA patients (Sedlackova et al. 2006). Citrullination of filaggrin and myelin occurs in RA patient's sera and this modification is a marker for disease activity in RA. In RA patient's, antibodies to hyper-citrullinated Hsp90 are observed (Travers et al. 2016). Another Hsp gp96, a member of Hsp90 is highly expressed in RA synovial tissue and protein levels correlate with the joint lining thickness and inflammation. Furthermore, gp96 is significantly increased in RA SF, demonstrating that this endoplasmic reticulum-localized chaperone is released during chronic inflammation. In addition to signaling through TLR2, gp96 also induces the expression of TLR2. The concentration of gp96 in RA SF correlates with the level of TLR2 expressed on synovial macrophages. These observations suggest that in RA gp96 is released and functions as an endogenous TLR2 ligand, capable of promoting the self-perpetuating activation of synovial macrophages (Huang et al. 2009). These studies establish that several Hsp influence the disease in RA pathology and utilize multiple mechanisms, including the signaling via surface TLR proteins.

A limited number of studies have also suggested the participation of TLR signaling proteins in $CD4^+$ T cell responses (Gelman et al. 2004; Mills 2011). Extracellular Hsp70 binds to the cell surface receptors via TLR2 and TLR4, which than induces IL-10 production (Asea et al. 2002; Borges et al. 2012). Hsp60 has also been shown to enhance regulatory T cells (Tregs) function via TLR2 signaling (Zanin-Zhorov

et al. 2006). IL-10 is an anti-inflammatory cytokines produced by Tregs and it counter balances the inflammatory response triggered by cytokines produced by Th1 and/or Th17 cells (Niu et al. 2012). Both of these cell types participate and contribute to RA disease pathology. Thus these data suggest that by regulating the Treg production both Hsp60 and Hsp70 regulate immune responses in RA.

7.1.2 Hsp in Systemic Lupus Erythematosus

SLE is an autoimmune pathology that is linked to aberrant nucleic acid recognition. Disturbances in several immunological pathways contribute to SLE pathology. ICs formed with nucleic acids and autoantibodies trigger innate immune activation, leading to the plasma B cell development producing autoantibodies and activating complement pathways that lead to apoptosis. Elevated levels of ICs formed with nucleic acids are present in the plasma of SLE patients. ICs are central player in tissue damage, and are often present together with hyper responsive $CD4^+$ T cells, which contribute to the breakdown of B cell tolerance. The defective clearance of ICs is a confounding factor for SLE pathology (Davies et al. 1990). Apoptotic blebs on the cell membrane, in particular on lymphocytes accumulate and release modified proteins and nucleic acids, which trigger humoral responses. This process also contributes to the epitope spreading. In SLE, autoantibodies and T cell clones against Hsp are observed. Both abnormalities in the expression and/or localization of endogenous Hsp contribute to autoimmune response in SLE. High levels of both Hsp72 (inducible form of Hsp70) and Hsp90 are present in SLE patients, which correlate with disease activity (Dhillon et al. 1993, 1994). Hsp90 is largely present in the cytoplasm, but under stress it is released into the extracellular compartment. One of the Hsp90 variant with a unique hydrophobic terminal (HSP90N) localizes to the cell membrane, both in the lymphocytes and monocytes. The membrane bound HSP90N will assist in retaining high density of solubilized signaling proteins on the cell surface, thus enhancing the signaling responses such as those observed in B cell receptor (BCR) and T cell receptor (TCR) complexes during disease activity. As such the formation of immunological synapse is mandatory for TCR signaling, which results in the accumulation of signaling proteins at a single site where the CD3 complex is located (Dustin 2002). This is usually the site where the antigen-presenting cell (APC) makes a contact with T cell during antigen presentation. A role for Hsp in upregulating the costimulatory molecules on APCs are proposed. Hsp70, Hsp90, calreticulin or gp96 enhance the expression of costimulatory proteins, CD80, CD86, CD40 and MHC II (Basu et al. 2000; Srivastava 2002a). Hsp90 co-precipitates with MHC I molecules suggesting a role for Hsp in class I antigen presentation. High-density of protein complexes and membrane rafts accumulate at a single site in $CD4^+$ T cells forming "innate immune synapse" (Chauhan and Moore 2011). These are also the site where ICs bind to FcγRIIIa on activated $CD4^+$ T cells (Chauhan et al. 2015). Also Hsp90 containing ICs are observed in SLE and this protein is present within the immune deposits formed in the kidney of SLE patients

associated with glomerulonephritis (Manderson et al. 2004). In SLE mouse model MRL/lpr elevated expression of Hsp90 is observed. These mice also show age dependent expression of Hsp72 in several tissues. Chronic expression of gp96 on the surface of DCs induces cell activation and SLE-like phenotypes in mice (Han et al. 2010). IgM isotype antibodies against Hsp90 are found in 35% of SLE patients and 26–50% show IgG subclasses (Conroy et al. 1994; Fink 1999; Hightower and Hendershot 1997). High levels of Hsp90 autoantibodies observed in SLE patients correlate with C3 levels suggesting complement consumption (Kenderov et al. 2002). Hsp90 deposition is also observed in the kidney biopsies of patients with lupus nephritis (Kenderov et al. 2002). Extracellular Hsp90 present in the serum of SLE patients correlate and associate with levels of extracellular self-DNA and DNA-ICs. Hsp90 bound to self-DNA triggers the production of proinflammatory cytokines (Okuya et al. 2010). Hsp90 opsonized ICs are more potent in producing IFN-α (Saito et al. 2015). Hsp90 inhibitor 17-AAG, suppresses IFN-α, TNF-α, and IL-6 production post TLR7 and TLR9 activation (Saito et al. 2015). Both TLR7 and TLR9 associate with Hsp90 as observed by co-precipitating experiments (Saito et al. 2015). NA-TLRs drive expression of IFNs that drives SLE pathology (Christensen et al. 2006). Hsp90 empowers the chaperoned ligands to activate pro-inflammatory immune responses. Unmethylated single-stranded DNA containing a cytosine–phosphate–guanine (CpG) motif binds to endosomal TLR9 receptors in pDCs and B cells, which results in IFN-α responses that drive disease pathology (Blasius and Beutler 2010; Shrivastav and Niewold 2013). In human pDCs, Hsp90-CpG-A ODN complexes are more potent to enhance IFN-α production compared to the monomeric CpG-A ODN (Okuya et al. 2010). The cellular localization of TLR9 discriminates between self-DNA and viral-DNA (Barton et al. 2006). The recognition of modified self-nucleic acids by NA-TLRs is critical for BCR activation and autoantibody production (Pelka et al. 2016). The extracellular Hsp90 is able to chaperone CpGs in early endosomes leading to robust IFN responses. Therefore, Hsp are crucial player in the pathogenesis of SLE. Hsp90 associates and deliver TLR7 and TLR9 from ER to early endosome, where they recognize their ligands. In addition, gp96 an ER resident Hsp is shown to be a master immune chaperon for TLR1, 2, 4, 5, 7, and 9 in macrophages (Yang et al. 2007). Macrophages lacking do not respond to the TLR ligands (Yang et al. 2007). Grp-170 is a large Hsp that modulate NF-κB signaling pathways and IFN-β cytokine produced upon TLR3 signaling. Grp-170 binds to immunoglobulins in B cells and compensate for gp96 in the assembly of immunoglobulins (Liu and Li 2008) Forced expression of gp96 on the cell surface in DCs induces lupus like disease in mice and upregulate the myeloid differentiation primary response 88 (MyD88) signaling (Liu et al. 2003). It will be of significant interest to determine whether the Hsp play a role in recruiting endosomal NA-TLRs to the cell surface, where they can bind to altered self-nucleic acid (Chauhan 2017). Thus it is safe to conclude that in SLE, extracellular Hsp contribute to the disease pathology by enhancing the nucleic acid sensing via TLRs, one of the several key mechanisms that contribute to the SLE pathology. In SLE, Hsp are critical in inducing both T and B cell responsiveness, which will result in tolerance breakdown.

7.1.3 Other Alarmins in Autoimmunity

Alarmins mediated signal enhancement in lymphocytes is crucial for the pathogenesis of autoimmune disorders. In addition to Hsp, several additional alarmins have been recognized, which include HMGB1, S100 proteins (notably S100A8/A9 and S100A12), serum amyloid A, purine metabolites (uric acid and ATP), altered matrix proteins and IL-33. In this chapter I will focus on the role of HMGB1 and RAGE, since these proteins opsonize and enhance the IC mediated immune responses. HMGB1 is critical in driving the host inflammatory response to invading pathogens and sterile inflammation in autoimmunity. Elevated levels of HMGB1 are present in the sera of RA and SLE patients, and in the SF of RA patients (Hamada et al. 2008; Lu et al. 2015). Invading pathogens trigger release of HMGB1 from macrophages, DCs and NK cells, which are at the frontline of host defense. However during autoimmunity, HMGB1 is secreted in the extracellular space by molecular mechanisms triggered by downstream cellular signaling events. We have shown that the FcγRIIIa ligation by ICs is one of such singling event that promotes HMGB1 expression and its subcellular redistribution Fig. 7.1. HMGB1 acts as a DNA chaperon that stabilizes nucleosome formation. It is released in the extracellular milieu by necrosis or actively secreted by inflammatory macrophages and monocytes. It has also been observed that the monocytes exposed to apoptotic blebs release HMGB1 (Qin et al. 2006). Extracellular HMGB1 upon binding to RAGE, decrease mTOR activity, which enhances the autophagy (Kang et al. 2010). Nuclear HMGB1 regulates the expression of heat shock protein family B (small) member 1 (HSPB1) and promote mitophagy (Tang et al. 2011). Reactive oxygen species production is triggered upon Fc-receptor signaling, and this could trigger translocation of HMGB1 to the cytosol resulting in the release of beclin by inhibiting Bcl2 (Tang et al. 2010). Beclin plays a critical role both in apoptosis and autophagy by forming the phosphotydylionositol 3-phosphate complex, which mediates vesicle trafficking. The increase in cytosolic HMGB1 also promotes autophagy. Extracellular levels of HMGB1 protein is important in SLE pathology, since HMGB1 binds to ICs and protects nucleic acids present in these complexes from degradation by nuclease such as three prime repair exonuclease 1 (Lee-Kirsch et al. 2007). This enhances the TLR mediated immune response triggered by RNA/DNA-ICs in pDCs and B cells. HMGB1 coordinate cellular responses through RAGE, TLRs, TIM-3, CXCR4 and CD24-Siglecs G/10. HMGB1 binds to TLR4 on synovial fibroblasts of RA patients, present in inflammatory cytokine milieu and signal via TLR4 to mediate tissue damage (Yang et al. 2010). HMGB1 protein functions as a modulator for the immunogenic potential of nucleic acids and other PAMPs and DAMPs. A cross-talk between FcRs expressed on the cell surface and surface TLRs trigger production of proinflammatory cytokines i.e. TNFα, IL-1β, IL6, and IL-23 in human DCs (den Dunnen et al. 2012). We have shown that the human naïve $CD4^+$ T cells upon activation via FcγRIIIa cosignaling upregulate the expression of HMGB1 and TLRs (Chauhan 2017; Chauhan et al. 2016). In addition, this signal also promotes the movement of HMGB1 protein towards the cell surface, where it associates and colocalizes with FcγRIIIa protein Fig. 7.1. MyD88 the downstream signaling protein of TLR signaling pathway is

also up-regulated upon FcγRIIIa cosignaling and associate with FcγRIIIa protein (Chauhan 2017). Also TLR4 binds to MyD88, which then localizes to cell surface (Barton and Kagan 2009; van Egmond et al. 2015). FcγRIIIa cosignaling contributes to the formation of Myddosomes like signaling complex on the surface in human $CD4^+$ T cells Fig. 7.1 (Hamerman et al. 2016). Co-precipitation of these proteins confirmed their association in activated $CD4^+$ T cell lines (Chauhan 2017). A synergistic co-signaling among FcγRIIIa and TLR9 in the human naïve $CD4^+$ T cells upregulates the secretion of proinflammatory cytokines IFN-γ and IL-17A, which contribute to autoimmunity (Chauhan 2017). In addition, Hsp72 during oxidative stress translocate to the nucleus, where it interacts with HMGB1, which prevent oxidative stress (H_2O_2)-induced HMGB1 cytoplasmic translocation and its release. The nuclear Hsp72-HMGB1 interaction may be a universal nuclear stress response to various adverse stimuli (Tang et al. 2007). These studies strongly suggest a role for HMGB1 protein in regulating autoimmune pathology and this protein is a known therapeutic target for various immune pathologies.

Stressed cells markedly enhance RAGE expression, which is implicated in the pathogenesis of a variety of inflammatory diseases. RAGE binds to HMGB1 and regulate the inflammatory responses in autoimmune pathology (Sims et al. 2010). RAGE is a multi-ligand receptor that recognizes non-AGE ligands such as HMGB1, S100, Mac1 and several surface TLRs (Chen and Nunez 2010). RAGE mediates the cytokine activity of HMGB1. Activated platelets trigger NETosis from HMGB1 activation, which result in the engagement of RAGE on neutrophils. NETosis is one of the established mechanism that contributes to the autoimmune pathology (Papayannopoulos 2018). RAGE is expressed by T lymphocytes and it plays a role in the activation and differentiation of these cells. The evidence that soluble RAGE prevented joint inflammation and bone loss in arthritic mice model suggest its involvement in this disease (Hofmann et al. 2002). RAGE expressing cells show matrix metalloproteinase-mediated inflammation and higher binding to S100 protein. The FcγRIIIa cosignaling in human naïve $CD4^+$ T cells upregulates RNA transcripts that encode S100, Fig. 7.2. RAGE also binds to PS in the outer plasma membrane. Lupus mice B6-MRL Fas lpr/j with *Ager* mutation (deletion of RAGE) show delayed apoptosis of T lymphocytes. *Ager* exacerbates autoimmunity, organ injury and lymphoproliferative disorder. RAGE/RAGE ligand interactions are associated with the cell survival and inflammation mediated by the phosphorylation of the extracellular signal-regulated kinase (ERK) and an increase in NF-κB p65 subunit expression (Bierhaus et al. 2001). Cardiovascular pathology observed in SLE patients is linked to RAGE (Nienhuis et al. 2009). In 2005, Means et al. showed that TLR7 and TLR9 recognize DNA/RNA-ICs and trigger upregulation of IFN signature genes and contribute to SLE pathology (Means et al. 2005). Another study that followed soon showed that HMGB1 was an essential component of DNA-ICs that produce IFN-α in both pDCs and B cells. This study also showed that indeed the CpG-A ODN-HMGB1 complexes utilize RAGE to produce IFN-α in pDCs, and other RAGE ligands such as S100 does not show similar affect (Tian et al. 2007). A role for RAGE in the subcellular localization and/or retention of DNA-TLR9 complexes in the endosome has also been shown (Tian et al. 2007). It is now

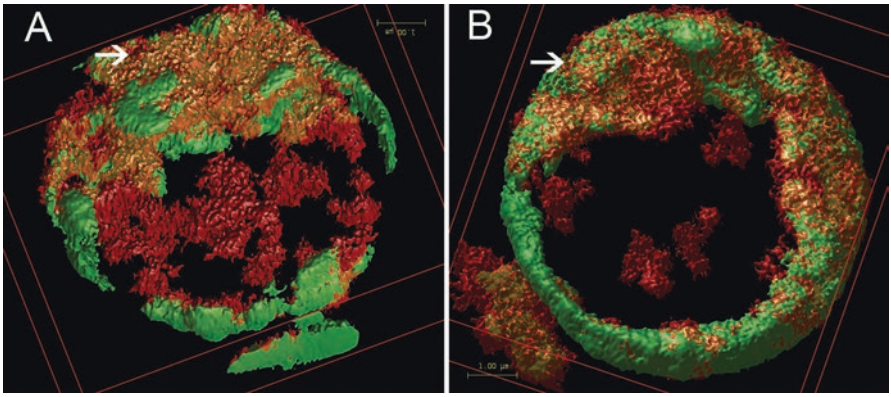

Fig. 7.2 HMGB1 and MyD88 in CD4⁺ T cells upon FcγRIIIa cosignaling moves to cell surface and colocalize with IC staining. (**a**) 3D confocal image shown with double staining of IC binding (Alexa Fluor 488, green) and HMGB1 (Alexa Fluor 594, red). (**b**) 3D confocal image shown with double staining of IC binding (Alexa Fluor 488, green) and MyD88 (Alexa Fluor 594, red)

accepted that the surface expression of TLR9 protein is essential for the recognition of altered self-DNA (Barton et al. 2006; Chauhan 2017). It is thus important to explore the role for Hsp and RAGE in subcellular localization of NA-TLRS and joint signaling events to produce proinflammatory cytokines in various immune cells. Collectively, evidence suggests that alarmins interact with Hsp and nucleic acid containing ICs and contribute to inflammatory responses in autoimmunity. Both of these protein groups are critical contributor for the generation of sterile inflammatory responses.

7.1.4 Hsp in Activated CD4⁺ T Cells

Naïve CD4⁺ T cells express TCR, which upon engaging pep-MHC on APCs trigger cellular proliferation. In addition to this primary signal, a secondary co-stimulatory signal is delivered by CD28. In the absence of CD28 cosignal these cells become anergic and die via apoptosis (Esensten et al. 2016). However, in autoimmunity naïve CD4⁺ T cells differentiate into T_E subsets in the absence of CD28 cosignal. The proinflammatory effector T cell (T_E) subsets generated upon TCR activation produce several proinflammatory cytokines such as IFN-γ, IL-17, IL-21, and TNF-α. Many of these cytokines in particular IFN-γ and IL-21 are efficient B cell help provider. Of particular interest of these T_E subsets are follicular helper T_E cells (Tfh) that form cytoconjugates with B cells in the germinal centers (GCs) and drive the development of plasma B cells that produce autoantibodies. A role for Hsp in adaptive immune response has not yet been fully addressed. We have shown that the FcγRIIIa cosignaling successfully substitute the CD28 requirement for the

generation of T_E cells (Chauhan et al. 2015, 2016; Chauhan and Moore 2011). We have also shown that this cosignal drives the generation of Tfh subset in vitro and $CD4^+$ T cells in vivo bind to ICs, suggesting the expression of FcγRIIIa on these cells (Chauhan et al. 2012). These cells also express B cell CLL/lymphoma 6 (Bcl6), a transcription regulator of Tfh and produce IFN-γ and IL-21. Our RNA-seq data of human naïve $CD4^+$ T cells that were activated via FcγRIIIa cosignaling showed upregulation of a large subset of Hsp RNA transcripts, Fig. 7.2. This increase was observed over the transcript levels present in paired $CD4^+$ T cells from canonical CD28 cosignaling (Chauhan 2017). These data suggest that the FcγRIIIa cosignaling by upregulating the expression of Hsp contribute to stress response. A statistically significant increase in gene transcripts of DNAJP5, DNAJB6, DNAJC2, DNAJC5, DNAJC5B, HSF2, HSFY2, HSP90AB3P, HSPB7, HSPE1, S100A7A, S100A7L2, and S100G was noted (Chauhan 2017). S100A2, S100A5, and S100Z were overexpressed upon CD28 cosignaling (Fig. 7.3). Transcripts that encode proteins, which regulate protein folding, refolding and negative regulation of inclusion body assembly were also upregulated (Chauhan 2017). FcγRIIIa cosignaling upregulated genes that contributes to proteasome assembly and ubiquitination. An alternate mechanism by which Hsp can contribute to T cell activation is by forming ICs with autoantibodies against chemically modified Hsp (Fig. 7.3). Combined these studies points to the role for Hsp in the regulation of $CD4^+$ T cells responses during autoimmunity (Srivastava et al. 1998). Lck is a Src kinase that is a major abundant signaling protein in the TCR complex. CD28 protein primarily utilizes the Lck for downstream signaling (Esensten et al. 2016). CD28 bound Lck phosphorylates phosphoionositide-dependent kinase 1, which activates protein kinase C Φ and mediates signaling events that lead to activation of NF-κB, AP-1, and NF-At transcription factors (Dodson et al. 2009; Esensten et al. 2016). A single study have shown that Hsp90 protects degradation of Lck in activated TCR and regulate ubiquitination of Lck (Giannini and Bijlmakers 2004). FcγRIIIa mediated signaling phosphorylates Lck both in human naïve $CD4^+$ T cells and Jurkat cells (Chauhan and Moore 2011). Thus Hsp90 upregulation likely stabilizes the primary substrate for cosignaling protein CD28 in $CD4^+$ T cells. Also an indirect role for Hsp70 in enhancing the expression of CD28 on T cells has been shown (Kumar et al. 2016). These studies advocate for a role of Hsp proteins in influencing the outcome of $CD4^+$ T cell by altering the threshold of TCR signal during immune responses in autoimmunity.

7.2 Conclusions

A role for Hsp and alarmins in autoimmune pathology is now established. Contribution of the Hsp and alarmins to the inflammatory responses has attracted interest for them to be evaluated as the potential therapeutic target for both autoimmune pathology and cancers. Our current knowledge of Hsp contribution to autoimmune disease pathology is largely based from studying the role of these proteins in

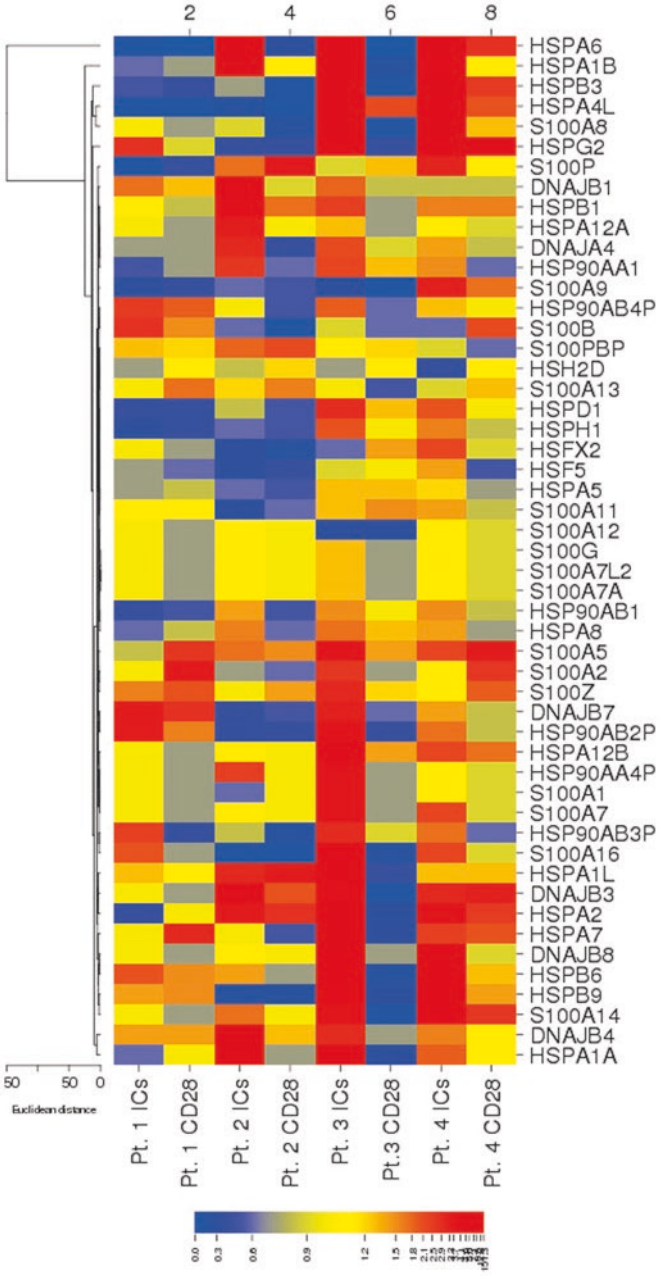

Fig. 7.3 FcγRIIIa cosignaling upregulate the Hsp expression. Relative expression of Hsp RNA transcripts in $CD4^+$ T cells upon FcγRIIIa cosignaling (plate-bound ICs) and CD28 cosignal (plate-bound anti-CD28) in four different subjects. Several transcripts encoding the S100 proteins are overexpressed upon FcγRIIIa cosignaling

innate responses. It remains to be determined how Hsp influence adaptive immune responses and what is the role these adaptive responses in autoimmune pathology. It will also be important to examine if the Hsp mediated responses differ from the traditional responses observed in lymphocytes. Studies from B cells have now conclusively shown that the immune receptors such as BCR and TLRs synergistically upregulate the B cell responses and enhance the production of inflammatory cytokines. It will be of significant interest to examine the role of Hsp in the synergistic responses observed for FcRs and TLR signaling in $CD4^+$ T cell responses. It also remains to be determined whether induced expression of Hsp contributes to the enhanced cell surface-signaling, which is observed during autoimmunity, in particular T and B-lymphocytes. Such studies will enhance our understanding of the role of Hsp mediated events in immune tolerance breakdown. Over past two decades significant advancement in our understanding of the role of Hsp in autoimmune diseases has occurred. However with current advances in T cell based therapies, the role of these proteins in adaptive immunity should be further explored. Hsp may successfully provide effective adjuvant therapy for vaccine development in autoimmunity and cancers.

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