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Heat-shock proteins can promote as well as regulate autoimmunity

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Abstract

Heat-shock proteins (Hsps) are among the most highly conserved and immunogenic proteins shared by microbial agents and mammals. Under physiological conditions, the ubiquitously distributed Hsps maintain the integrity and function of other cellular proteins when cells are exposed to stressful stimuli. However, owing to their conserved nature and stress inducibility, Hsps may become targets of immune response. The T cells and/or antibodies induced by a microbial Hsp may crossreact with the corresponding mammalian Hsp (molecular mimicry) and trigger an autoimmune response, which if unchecked can lead to immune pathology and clinical manifestations. Furthermore, enhanced expression of Hsp under stress can unveil previously hidden antigenic determinants that can initiate and perpetuate autoimmune reactivity. Also, the innate immune mechanisms activated by an Hsp can reinforce and even direct the type of adaptive immune response to that protein. Hsps have been implicated in the induction and propagation of autoimmunity in several diseases, including rheumatoid arthritis, atherosclerosis and type 1 diabetes. However, Hsps possess immunoregulatory attributes as well and therefore, are being exploited for immunomodulation of various immune-mediated disorders.

Keywords

Autoimmunity; Heat-shock proteins; Immunomodulation; Inflammation; Molecular mimicry; Stress proteins

Take-home messages:

- Hsps are highly conserved as well as immunogenic proteins
- Hsps are involved in the pathogenesis of a variety of immune-mediated disorders, including autoimmune diseases and graft rejection.
- Hsps may mediate immune pathology via the induction of a pro-inflammatory immune response, but they also have the potential to induce a protective anti-inflammatory immune response.
- Hsps are being exploited for immunomodulation of autoimmunity and other immune disorders.

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Heat shock proteins (Hsps) are a group of molecular chaperones that are highly conserved from prokaryotes to higher eukaryotes [1]. These proteins protect other cellular proteins in different sub-cellular compartments from stress-induced damage. Hsps are categorized under distinct families, for example, small Hsps (e.g., Hsp10), Hsp40, Hsp60, Hsp70, Hsp90 and Hsp110. Hsp60 is a mitochondrial chaperonin that is involved in protein folding. Here, we highlight the role of Hsp60 in the pathogenesis and immunomodulation of autoimmune diseases. (For simplicity, Hsp60 proteins of diverse origin are referred below as hsp65.)

1. Initiation and propagation of immune pathology by Hsps

Hsp65 has been linked to the induction and perpetuation of several immune-mediated diseases (Table 1). The priming of humoral and/or cellular immune response against microbial or self Hsp is a critical component of the disease-related immune events in these disorders. The precise mechanisms by which immune response to Hsp65 results in immunopathology are not fully defined. However, three of the proposed mechanisms are discussed below.

1.1 Activation of innate immunity

Hsps can activate macrophages and dendritic cells (DC) [2] and these early innate responses in turn can be funneled into and direct the type of adaptive immune response to Hsp65. Furthermore, antibodies to Hsp65 and immune complexes consisting of Hsp65 and anti-Hsp65 antibodies can trigger potentially pathogenic effector mechanisms via activation of the complement system.

1.2 Stress-induced Hsp expression as well as altered antigen processing and presentation

The expression of endogenous Hsps is significantly increased when cells are exposed to an inflammatory environment or other stressors. For example, Hsp65 expression can be enhanced by various atherogenic chemicals and other risk factors [3-6]. These self Hsps may further propagate the ongoing inflammation, and also constitute an attractive target for T cells and antibodies induced by foreign Hsps. In addition, inflammation can alter the antigen processing of Hsps to reveal certain epitopes more efficiently or display neodeterminants, including hidden (cryptic) epitopes [7], which can prime an immune response leading to immune pathology.

1.3 Molecular mimicry

Hsps are highly conserved proteins that are good immunogens as well. Hsp65 family is among the most highly conserved families of proteins with more than 97% homology among prokaryotic Hsp65s, and more than 70% homology between prokaryotic and human Hsp65. Moreover, microbial Hsp65s possess B- and T-cell epitopes that are cross-reactive with self Hsp65s [1,5]. Accordingly, the T cells and antibodies induced by microbial Hsps may target the related self Hsps and lead to tissue damage via ‘molecular mimicry’. For example, anti-microbial Hsp65 antibodies that are cross-reactive with self Hsp65 as well as autoantibodies against self Hsp65 are involved in the pathogenesis of atherosclerosis [4,5].

2. Immunoregulation of autoimmunity by Hsps

Hsps are endowed with immunoregulatory attributes, which are revealed in part through studies on the natural course of disease in experimental models as well as patients, and from investigations into experimental modulation of the disease using different therapeutic approaches (Table 2). Understandably, different set of conditions would facilitate pathogenic versus regulatory functions of Hsps. However, the precise conditions controlling these differential outcomes of the immune response to Hsps are not fully defined. In this regard, some of the factors proposed and elaborated by others and us [7-10] include the induction of T cell tolerance by self Hsp leading to pre-emptive deletion of potentially autoreactive T cells;

the contribution of microbial toll-like receptors (TLR) and other ligands of innate immune receptors to the pathogenicity of foreign (microbial) Hsps; and the differential activation of DCs by foreign versus self Hsp leading to the generation of a pro-inflammatory versus a regulatory response, respectively. Furthermore, molecular mimicry or enhanced expression of Hsps and their antigenic determinants can contribute not only to pathogenic mechanisms (described in section 1), but also to regulation of autoimmunity. Broadly, the regulatory effector mechanisms recruited to control pathogenic responses include [7,8,10-13] the induction of immune tolerance, deviation of the cytokine response from Th1 to Th2 type, suppression of IL-17 response, apoptosis of pathogenic T cells, and activation and expansion of regulatory T cells (e.g., CD4+CD25+ (Treg)-, IL-10-secreting (Tr1)-, or TGF-β-secreting (Th3)- regulatory T cells).

3. Autoimmune diseases and other conditions displaying the pathogenic/ protective role of immune response to Hsp65

3.1 Induction and regulation of autoimmune arthritis by Hsp65

Adjuvant arthritis (AA) is inducible in the Lewis rat by immunization with heat-killed *M. tuberculosis* (Mtb). In arthritic rats, mycobacterial Hsp65 (Bhsp65) is the major target of the immune response [7,9,10,13-15]. The Bhsp65-primed T cells derived from arthritic rats, for example the A2b T cell clone, can adoptively transfer disease to syngeneic naive recipient rats [14]. The arthritogenic T cells are directed against the determinant region 180-188 of Bhsp65, and the polyclonal T cells primed by Bhsp65 peptide 180-188 as well as the A2b clone can be restimulated by the longer versions of this epitope, 176-190/ 177-191 [7,9,10,14,15]. Unlike the T cells, serum antibodies cannot transfer AA to naïve rats, so the role of antibodies in mediating pathogenic events in this disease is not clear. Immune response to Hsp65 has also been linked with arthritis in streptococcal cell wall-induced arthritis (SCWIA) in rats and Pristane-induced arthritis (PIA) in mice. Moreover, studies conducted in patients with rheumatoid arthritis (RA) have revealed synovial fluid T cells that are reactive against microbial Hsp65 and secrete pro-inflammatory cytokines [16]. Taken together, the above studies highlight the role of Hsp65 in the initiation and progression of autoimmune arthritis. However, another set of studies in the AA model and in patients with juvenile idiopathic arthritis (JIA)/juvenile chronic arthritis (JCA) have unraveled the regulatory role of self Hsp65 in this disease. In AA, in contrast to the pathogenic A2b T cell clone, the A2c T cell clone reactive against the same Bhsp65 epitope, 180-188 is protective against arthritis. In our studies in the AA model, we observed that during the course of arthritis there is diversification of response to Bhsp65, particularly the C-terminal determinants of the protein [7,15]. The peptides corresponding to these C-terminal determinants as well as the T cells primed by them can induce protection against arthritis in recipient rats. Furthermore, arthritic rats spontaneously raise T cell response to self (rat) Hsp65 (Rhsp65) about the peak phase of AA, and the T cells primed by Rhsp65 can induce protection against AA [7]. Interestingly, the corresponding C-terminal determinants of the two homologous Hsp65 proteins (Bhsp65 and Rhsp65) are crossreactive [7,15]. On the basis of these results we suggested that it is the inflammation-induced upregulation of the expression of Rhsp65 in vivo that triggers the diversification of response to Bhsp65 observed in vitro, and induces natural recovery from acute AA [7,15]. The significance of self Hsp reactivity of AA-regulating T cells is further underscored by studies on the 256-270 epitope of Bhsp65, which is crossreactive with the corresponding epitope of Rhsp65 [10] and can induce protection against AA. Moreover, it has been shown that antibodies reactive against Bhsp65/Rhsp65 can induce protection against AA [13,17]. Results of the studies on immunomodulation of AA by different vaccination regimen using Hhsp65, which is highly homologous to Rhsp65, offer additional support to the regulatory role of self Hsp65 in AA [9,18]. The disease-regulating T cell epitope for rats has been identified within the region 31-50 (Hu3 epitope) of Hhsp65 [9]. Furthermore, juvenile arthritis patients raise T cell response

to Hsp65, and the remission from acute phase of arthritis correlates positively with increased T cell response to self Hsp65 [19]. Similarly, RA patients possess in their synovial fluid T cells that are reactive against self Hsp65 and secrete anti-inflammatory cytokines, pointing to a regulatory T cell subset [16].

3.2 Hsp65-induced immune response in atherosclerosis

The etiology of atherosclerosis is not fully defined, but increasing evidence points to the role of autoimmune reactivity against microbial/self Hsp65 in the induction and propagation of the diseases process. Immunization of normocholesterolemic New Zealand White rabbits with Mtb or Bhsp65 can induce atherosclerosis [20]. A similar immunogenic regimen (Mtb or Hsp65) can lead to the development of atherosclerotic lesions (e.g., fatty-streak formation) in wild type C57BL/6 mice that in addition are fed a diet rich in cholesterol, but not in those fed regular chow diet [21]. The disease can also be induced in low density lipoprotein receptor-deficient recipient mice via the adoptive transfer of Hsp65-reactive T cells derived from the diseased mice as well as via serum antibodies obtained from donor mice challenged with Hsp65, demonstrating the significance of both the cellular and humoral immunity in mediating the pathological damage in atherosclerosis [22]. Antibodies against Hsp65 may induce endothelial cell damage upon binding to cell surface-expressed Hsp65, and the outcome of Hsp65-anti-Hsp65 antibody interaction is significantly influenced by the Hsp65 epitope involved [4]. The involvement of Hsp65 in this disease is further validated by findings in patients with atherosclerosis, who show high titers of serum antibodies against chlamydial Hsp65 (Chsp65) and human Hsp65 (Hhsp65) [5]. In addition, the T cells isolated from human atherosclerotic lesions show significant reactivity against Chsp65 and Hhsp65 [6,23]. In contrast to the pathogenic role of Hsp65, tolerogenic pre-treatment of mice with Hsp65 can downmodulate the course of subsequent atherosclerosis, demonstrating the immunoregulatory attribute of anti-Hsp65 immunity as well [24].

3.3 Immunity to Hsp65 in autoimmune diabetes

The role of Hsp65 in the disease process has been extensively studied in the non-obese diabetic (NOD) mice and in patients with type 1 diabetes (T1D) [25-27]. Hhsp65 and its peptide determinant region 277 (p277) is the focus of T cell response of diabetic mice and patients with diabetes [25,27]. Moreover, treatment of naïve NOD mice with p277 affords protection against diabetes [12]. Besides Hhsp65, Bhsp65 has been identified as one of the target antigens responded to by the diabetic NOD mice [26], and there is an orderly appearance of T cell reactivity to glutamic acid decarboxylase (GAD), Bhsp65, carboxypeptidase, and insulin during the course of diabetes [26]. As a practical application of the above information from experimental models, early phase clinical trials in T1D patients have shown a beneficial effect of p277 treatment in preserving endogenous insulin production and maintaining C-peptide levels [28]. The p277 treatment was associated with Th1 to Th2 deviation.

3.4 Involvement of Hsp65 in other diseases with an autoimmune component

Immune response to Hsp65 has been invoked in the pathogenesis of psoriasis [29], autoimmune intestinal inflammation [30], autoimmune demyelination (e.g., experimental autoimmune encephalomyelitis ; EAE) [31], Kawasaki disease [32], Behcet's disease [33], juvenile dermatomyositis [34] and Sjögren's syndrome (SS) [35]. The precise etiology of many of these diseases is not fully clear, but there is evidence for an autoimmune reaction stimulated by an infectious agent or an environmental toxin. Adoptively transferred CD8+ T cells originally primed by microbial Hsp65 but crossreactive with self Hsp65 can induce autoimmune intestinal inflammation in mice, and the immune pathology involves recognition of self Hsp65 expressed in the intestine [30]. The inflammation is restricted primarily to the small intestine, and the disease can be induced in germ-free mice, excluding the involvement of microbial Hsp65 in

the restimulation of the pathogenic CD8+ T cells. In EAE, the T cells reactive against myelin basic protein as well as Bhsp65 are enriched in the spinal cord of the diseased rats [31]. Furthermore, EAE can be modulated by immunization with Bhsp65, supporting the role of Hsp65 in autoimmune demyelination. The results of a study in Kawasaki disease suggest that self Hsp65 plays a regulatory rather than a pathogenic role in the disease process [32]. Similarly, pretreatment of NOD mice with mammalian Hsp65 affords protection against experimental Sjögren's syndrome [35]. Thus, there is evidence for both a pathogenic and a protective role of Hsp65 in several diseases involving autoimmune reactivity.

3.5 Immune reactivity to Hsp65 in organ transplantation

Studies conducted in patients with renal, cardiac and other allograft transplants have revealed a dual (pathogenic/protective) role of immune responses to Hsp65 [8,36,37]. For example, in one study, patients with renal transplant were followed serially, and at defined time points, their peripheral blood mononuclear cells (PBMC) were collected and tested for T cell proliferative and cytokine response [36]. There was increased T cell proliferative response to Hsp65 in the early phase compared to the late phase post-transplant. Furthermore, in the late phase, there was a shift in the cytokine response to a Th2 type (IL-4). IL-4 had a differential effect on Hsp65-specific T cell proliferation, suppressing proliferative response in the early phase but enhancing it in the late phase post-transplant.

3.6 Concluding remarks

In addition to its role in the above-mentioned disorders, immune response to Hsp65 is involved in the pathogenesis of various infectious diseases (e.g., infection by *C. pneumoniae*, gastritis induced by *H. pylori*), tumors (e.g., breast cancer), and traumainduced inflammation (e.g., central nervous system trauma). However, a discussion of these other disorders is beyond the scope of this article. Nevertheless, these examples, along with those of autoimmune diseases discussed above in more detail, illustrate the broad impact of Hsp65 immunity not only in mediating tissue inflammation, organ pathology, and clinical disease, but also in inducing remission from and offering protection against inflammation associated with autoimmunity and other diseases. In addition, other Hsps (e.g., Hsp10 and Hsp70) besides Hsp65 have been shown to possess immunomodulatory properties in autoimmune arthritis and some other disorders. A comprehensive understanding of the factors determining the pathogenic versus protective immune effects of Hsps would advance our understanding of disease pathogenesis as well as facilitate the development of new immunomodulatory regimen for the treatment of autoimmune diseases.

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Table 1

Involvement of Hsp65 in the pathogenesis of autoimmune and other immune-mediated disorders

Disease	Host	Origin of Hsp	Effector mechanism	Reference
Autoimmune arthritis (e.g., A A)	Rat	Mtb[a]	Mtb immunization leads to activation of Bhsp65-reactive T cells, which are then restimulated in the joint by a cartilage protein (molecular mimicry)	[7,9,10, 13,15]
Atherosclerosis	Human Rabbit Mouse	*C. pneumoniae*, Human, Mtb	Microbial Hsp65 triggers antibody and T cell responses that are crossreactive (molecular mimicry) with self Hsp65 over-expressed in vascular endothelial cells in atherosclerotic lesions	[4,5,20-23]
Autoimmune diabetes	Mouse, Human	Human, Mouse, Mtb	T cell response against self Hsp65/Bhsp65 is induced spontaneously in NOD mice and in T1D patients. These T cells home to the pancreatic beta islets, where self Hsp65 serves as the target antigen (along with GAD65 and insulin).	[25-27]
Autoimmune intestinal inflammation	Mouse	Mtb, Mouse	CD8+ T cells specific for Hsp65, upon adoptive transfer, migrate to the intestinal mucosa where recognition of self Hsp65 triggers autoimmune inflammation	[30]
Autoimmune demyelination (e.g., EAE)	Rat	Mtb	T cells reactive against myelin-basic protein and its epitope 71-90 as well as Bhsp65 are enriched in the spinal cord of the diseased rats	[31]
Juvenile dermatomyositis	Human	Human, Mtb	The production of TNF-α, IL-1β and IL-10 in response to Hsp65 is significantly increased in active disease compared to inactive disease; both effector and regulatory T cells are present at the inflammatory site	[34]
Renal transplant: graft rejection	Human	Human	A change in the cytokine response to Hsp65 in favor of anti-inflammatory IL-4 with time post-transplant; IL-4 is associated with absence of graft rejection.	[36]

[a] Abbreviations: AA: Adjuvant arthritis; *C. pneumoniae: Chlamydia pneumoniae;* EAE: experimental autoimmune encephalomyelitis; GAD 65: glutamic acid decarboxylase 65; Hsp: heat shock protein; IL : interleukin; Mtb: *Mycobacterium tuberculosis*; NOD: non obese diabetic; TNF-α tumor necrosis factor-alpha; T1D : type 1 diabetes.

Table 2

Hsp65-mediated regulation of autoimmune diseases

Disease	Antigenic challenge	Host	Origin of Hsp	Effector mechanism
AA	Bhsp65-IgG-expressing B cells	Rat	Mtb	Inhibition of proliferation/expansion of Bhsp65-reactive pathogenic T cells and enhanced production of AA-protective antibodies
	Rhsp65 peptide 465–479	Rat	Rat	Upregulation of IFN-γ and suppression of IL-17 by the T cells against the arthritogenic epitope 180-188 of Bhsp65
	Altered peptide ligand of Bhsp65 peptide 180-188	Rat	Mtb	Generation of regulatory T cells secreting TGF-β, IL-4 and IL-10
	Bhsp65 peptide 31-46	Rat	Mtb	Antibody-induced increased production of immunoregulatory IL-10 by mononuclear cells
	Vaccinia virus-human Hsp65	Rat	Human	Induction of specific immune response to human Hsp65, including increased antibody production
	DNA vaccine-human Hsp65	Rat	Human	Downregulation of IFN-γ secretion in response to the epitope 180-188 of Bhsp65, and increased production of TGF-β and IL-10 by Hsp65-reactive T cells
PIA	DNA-Hsp65 vaccine	Mouse	Mlp	Reduction in the level of pro-inflammatory cytokines IL-6 and IL-12 and enhancement of the level of anti-inflammatory cytokine IL-10
Atherosclerosis	Bhsp65	Mouse	Mtb	Suppression of Bhsp65-reactive effector T cells and increased IL-4 production
Diabetes	Hsp65 peptide, p277	Mouse, Human	Human	A shift in the cytokine profile from a pro-inflammatory Th1-type to an anti-inflammatory Th2-type, which also induces the corresponding antibody isotypes
Sjögren's syndrome	Hsp65,peptide 437-470	Mouse	Human	Effects mediated via downmodulation of inflammatory chemotaxis coupled with enhanced anti-inflammatory and regulatory processes

[a] Abbreviations: AA: Adjuvant arthritis; Bhsp65: mycobacterial heat-shock protein 65; Hsp: heat shock protein; IFN-γ, interferon gamma; IgG: immunoglobulin G; IL: interleukin; Mlp: *Mycobacterium leprae;* Mtb: *Mycobacterium tuberculosis;* NOD: non obese diabetic; p277: peptide 437-460 of human Hsp65; PIA= Pristane-induced arthritis; Rhsp65: rat heat shock protein 65; TGF-β transforming growth factor beta.