Published in final edited form as:

Trends Immunol. 2008 September ; 29(9): 412–418. doi:10.1016/j.it.2008.06.003.

Regulation of autoimmune arthritis by self heat-shock proteins

Kamal D. Moudgil^{1,3} and Malarvizhi Durai^{1,2}

¹Department of Microbiology and Immunology, University of Maryland School of Medicine, Howard Hall 323C, 660 West Redwood St., Baltimore, MD 21201, USA

²Department of Pathology, Johns Hopkins Medical Institutions, 664G Ross Research Bldg., 720 Rutland Ave, Baltimore, MD, 21205, USA.

Abstract

Heat-shock proteins (hsps) are highly conserved and immunogenic, and they are generally perceived to be attractive initiators or targets of a pathogenic immune response and as such have been implicated in the pathogenesis of autoimmune arthritis. However, studies in animal models and arthritis patients have unraveled the disease-regulating attributes of self hsp65. We propose that the self hsp65 induces a protective and beneficial immune response owing to its ubiquitous distribution, stress-inducibility, and participation in tolerogenic processes. In contrast, the foreign hsp65 that does not influence the above processes, and which resides admixed with microbial ligands for innate receptors, generates an inflammatory pathogenic response. The regulatory properties of self hsps need be fully explored and might be utilized for therapeutic purposes.

Heat-shock proteins and autoimmune arthritis

Immune response to mycobacterial heat-shock protein 65 (Bhsp65) has been invoked in the pathogenesis of autoimmune arthritis in animal models as well as humans. Considering both the highly conserved nature of heat-shock proteins (hsps) and the prevailing notion about the 'pathogenic' self-reactivity in autoimmunity, the mammalian self hsp65 might represent a favored endogenous target antigen in autoimmune arthritis (for uniformity, we have used the term hsp65 to refer to both hsp65 and hsp60). On the contrary, the results of studies by ourselves and others in the rat adjuvant arthritis (AA) model (Table 1) suggest that both the spontaneously developing and the deliberately induced immune responses to self hsp65 afford protection against arthritis. Furthermore, the protective self hsp65-directed T cells can be activated by the cross-reactive epitopes within Bhsp65 leading to the downmodulation of AA. The observations in juvenile idiopathic arthritis (JIA) patients, showing that high immune reactivity to self hsp65 positively correlates with remission from and better prognosis of acute arthritis, further support the conclusions drawn from animal studies (Table 1). With notable progress made in the recent years in certain key areas of tolerance and autoimmunity (e.g., modulation of self antigen expression and thymic selection by autoimmune disease dampening, CD4+CD25+ T regulatory cells (Treg), and toll-like receptors (TLRs) and autoimmunity) coupled with the increasing realization about the limitations of the Th1-Th2 paradigm in explaining the pathogenesis of autoimmunity, it is imperative to address the conceptual and mechanistic aspects of immune regulation by self hsps in contemporary settings.

Opinion regarding the immunoregulatory role of self hsps

In this article, we present our viewpoint on why self hsp65 is favored to induce a protective immune response, while foreign (microbial) hsp65 is more likely to generate a pathogenic

³Corresponding author: Moudgil, K.D. (E-mail: kmoud001@umaryland.edu) Fax +1-410-706-2129.

effector response. We propose that self hsp65, because of its ubiquitous distribution and stress-inducibility, coupled with its participation in thymic selection and tolerogenic processes, induces a protective and beneficial immune response. We suggest that during cellular stress (infection, trauma, etc.), self hsps are upregulated which then generates a regulatory response to prevent or limit potential immunopathology caused by the stressors. In contrast, the foreign hsp65, which does not influence the above processes and that resides alongside microbial ligands for innate immune receptors in the cell wall, generates an inflammatory pathogenic response following microbial challenge. Moreover, differential processing and presentation of the corresponding epitopes within the homologous hsp65 proteins might introduce additional disparities in the timing, the specificity and the type of immune response induced. Thus, despite their conformational and sequence conservation, self and foreign hsp homologs induce differential immune responses largely as a result of their disparate physiological characteristics. Furthermore, the stress-dependent expression patterns of self hsps may make them uniquely suited to induce a regulatory immune response.

Experimental evidence and the proposed mechanisms

1. Self hsp shapes the antigen-directed T cell repertoire in the thymus and activates regulatory and protective T cells in the periphery

The mature T cell repertoire of an individual is shaped in the thymus by the complex processes of positive and negative selection [1]. As the endogenous self hsp but not the foreign microbial hsp has the opportunity to participate in selection of the developing T cells, the self hsp would imprint epitope-specific tolerance and select those T cell subsets that are not harmful to the host. We have shown in the Lewis rat that negative selection of self (rat) hsp65 (Rhsp65) is incomplete, and that Rhsp65-reactive T cells exist in the mature repertoire of otherwise normal animals [2]. This situation contrasts with the profound tolerance to many other antigens, for example, self lysozyme. We propose that this 'incomplete' tolerance to self hsp65 serves a useful purpose by preserving those T cell subsets that can recognize and respond ('sense') via antigen-induced activation and clonal expansion in response to the altered expression levels of self hsp under stressful conditions. We further suggest that following repeated episodes of stress leading to the induction of self hsp, the host immune system gets 'programmed' to mount a protective response to pre-empt or minimize the harmful effects of repeated stress responses.

The AA-protective characteristic of the peripheral T cell repertoire in the Lewis rat is borne out by studies by others [3,4] and us [2] showing that the priming and expansion of self hsp65-reactive T cells using Rhsp65 [2] or the closely related human hsp65 [3,4] affords protection against AA (Table 2). Furthermore, the findings that the AA-resistant rats both express higher levels of self hsp65 in their synovial tissue and in the periphery [5], and raise an earlier and vigorous T cell response to self hsp65 compared to that of AA-susceptible rats [6], provide additional evidence for the regulatory role of self hsp65. At present, there is no information on the relative frequency or the relative suppressive efficacy of Treg from arthritis-susceptible versus arthritis-resistant rat strains.

There is limited information on the thymic selection of the T cell repertoire against self hsp65 in rats. As for Rhsp65 [2], incomplete negative selection has also been reported for some other self proteins [1,7]. However, the major difference between Rhsp65 and non-hsp self proteins is the factor of stress inducibility, which enforces a regulatory outcome of the frequent priming of the self hsp65-specific T cells under stressful conditions to limit potential immune pathology. In contrast, activation of the residual self-specific T cells by other self proteins may lead to autoreactivity, which when unchecked can result in immune pathology. Thus, the physiological immune effects of upregulation of self hsps under stress are different from the passive release of non-hsp self proteins following cellular injury or death.

2. Arthritic Lewis rats spontaneously raise a T cell response to self hsp65 that drives the regulatory diversification of the response to Bhsp65

Arthritic Lewis rats in the late phase of AA showed a diversification of the T cell response to Bhsp65 C-terminal determinants (BCTD) that correlated with spontaneous recovery from acute arthritis [8]. Moreover, the T cells primed by a mixture of peptides representing the BCTD afforded protection against AA [8]. We tested whether endogenous self hsp65 was involved in this regulatory epitope spreading. Our results showed that arthritic Lewis rats spontaneously raised a T cell response to Rhsp65, and the timing of emergence of Rhsp65-reactivity approximately coincided with the initiation of epitope spreading to Bhsp65 [2]. Furthermore, the peptides comprising the C-terminal determinants of Rhsp65 are crossreactive with the corresponding C-terminal epitopes of Bhsp65 [9]. Because of this crossreactivity, the activated T cells directed against the C-terminal epitopes of either hsp homologue can suppress AA [2, 9]. In addition, the 'dominant' C-terminal epitopes naturally generated from endogenous Rhsp65 within heat-stressed naïve antigen-presenting cells (APC) could efficiently restimulate the T cells against the 'cryptic' BCTD that are targeted during epitope spreading [9], highlighting an example of differential processing of cross-reactive homologous hsp epitopes. The APC process the two hsp proteins differently such that the C-terminal determinants are efficiently processed and presented ('immunodominant') from native Rhsp65, but poorly processed and presented ('cryptic'/'subdominant') from native Bhsp65 [2,9]. We have previously reported a similar observations for defined T cell determinants within another pair of self (mouse lysozyme) versus foreign (hen eggwhite lysozyme) protein [10]. Multiple factors have been implicated in differential antigen processing and presentation [11], including differences in the amino acid residues in the flanking regions of the core determinants, differences in the rate of unfolding of different determinant regions and the relative access of the determinants to the proteolytic enzymes, and the influence of the adjacent epitopes on the binding of a particular T cell determinant to the major histocompatibility complex (MHC) molecule. The above-mentioned results support a model (Figure 1) whereby the observed spreading of the T cell response to Bhsp65 during the course of AA is actually triggered in vivo self hsp65-reactive T cells primed following the enhanced expression of endogenous hsp65 under the inflammatory milieu of arthritis [2]. Importantly, the epitope spreading observed in AA [8] is unique in being regulatory as opposed to the pathogenic epitope spreading reported in some other autoimmune diseases. Moreover, it offers a conceptual framework for understanding the natural regression of autoimmune inflammation in AA. The precise reasons for the epitope spreading to be regulatory in AA but not in EAE or diabetes remain to be fully defined. As discussed above, we propose that the stress inducibility of self hsp65 is a major factor in directing the inflammation-induced epitope spreading in AA to a regulatory phenotype.

3. Foreign hsp epitopes can recruit cross-reactive T cells against self hsp and induce a protective immune response

Not all immune responses induced by foreign hsps are pathogenic. Immunization of Lewis rats with certain peptides of Bhsp65 using an immunogenic [8,9,12] rather than a tolerogenic [13–15] regimen can induce protection against AA. These Bhsp65 epitopes were found to be cross-reactive with the corresponding T cell epitopes within self hsp65 [8,9,12]. The outcome of this cross-reactivity is puzzling in context of the general view that self-reactivity is potentially harmful. We propose that the observed cross-reactivity represents a case of molecular mimicry that fortuitously translates into functional mimicry largely due to the broader regulatory characteristic of the self hsp-directed T cell repertoire. This cross-reactivity generally involves the corresponding pairs of T cell epitopes (e.g., Bhsp65-Rhsp65 256–270 [12], and C-terminal determinants [8,9]), but it might also extend to non-overlapping regions (e.g., Rhsp 465–479 and Bhsp65 177–191) [16] of homologous hsps. Furthermore, either both [8,9,16] or only one [12] of the two cross-reactive epitopes may show a functional outcome.

A similar foreign-self cross-reactivity has been observed for AA-regulating epitopes within Bhsp70 [17], but not all conserved hsps display regulatory activity. It is conceivable that the T cell response induced by different epitopes of self vs. foreign hsps may yield different outcomes depending on the extent of sequence homology, the relative avidity for the MHC/T cell receptor (TCR), and the cytokine milieu and inflammatory environment elicited by the microbial agent (in the case of AA, Mtb). Such disparate outcome of the priming of T cells by the same epitope but under different local milieu is evident from the pathogenic (e.g., the A2b clone) versus the regulatory response induced by the epitope region 180–188/177–191 of Bhsp65 in the Lewis rat; the former response is induced by Mtb immunization [8,12,18], whereas the latter response follows priming by peptide 177–191 in an adjuvant [16].

4. Enhanced levels of pro-inflammatory cytokines produced by self hsp65-reactive T cells afford protection against arthritis

The epitope region 465–479 of Rhsp65 (R465) induces T cells that secrete predominantly IFN- γ and attenuate AA by decreasing IL-17 expression by the pathogenic T cells [19]. The production of higher levels of IFN- γ by the AA-regulating T cell clone A2c compared to the arthritogenic clone A2b further supports the regulatory potential of this Th1 cytokine [20]. Similarly, exogenous TNF- α administration can suppress arthritis in rats [21]. AA is a typical Th1-mediated disease, and therefore, its control by the counter-regulatory Th2 and Th3 cytokines [4,12,13,22] offers a logical explanation. However, the regulatory effects of the pro-inflammatory cytokines are paradoxical and somewhat difficult to reconcile with [23,24]. We propose that a critical threshold of these Th1 cytokines is essential for disease induction, whereas a relatively higher concentration of the same cytokines (e.g. as occurs during exogenous addition of TNF- α) leads to downmodulation of the disease. We further suggest that as it is difficult to deviate the cytokine profile of an already differentiated T cell, this mechanism is harnessed in situations of restimulation of Bhsp65-reactive Th1 cells in vivo by self hsp65, leading to enhanced IFN- γ and TNF- α secretion and subsequent suppression of the disease process.

5. Upregulation of self hsp expression might favor the selection of naturally occurring Treg (nTreg) and the generation of adaptive Treg (aTreg)

Treg play an important role in the maintenance of peripheral tolerance to self antigens [25–27], and these cells might contribute to the disease-protective effects of self hsps as well [25, 26]. The nTreg are apparently positively selected in the thymus on relatively higher concentrations of self antigen than that mediating the selection of other non-Treg effector T cells [27,28]. Furthermore, conventional non-regulatory CD4+CD25- T cells in the periphery can be induced to develop into aTreg [25]. We propose that the enhanced expression of self hsp under stress can facilitate the generation of antigen-specific subsets of Treg in the thymus as well as in the periphery. Interestingly, hsps can influence Treg activation via TLRs [29]–[30], for example, self hsp65 can activate Treg via TLR2 and enhance their suppressive activity [31]. However, the precise role of Treg in vivo in the AA model is not yet known, largely due to the lack of availability of a rat Treg-depleting antibody.

6. The humoral response to self hsp65 is geared to be protective in AA

Both the AA-susceptible (Lewis) and the AA-resistant (BN and WKY) rats given a potentially arthritogenic challenge with *Mycobacterium tuberculosis* H37Ra (Mtb) raised an antibody response not only to the foreign Bhsp65, but also to self Rhsp65 [32–34]. The response gradually diversified in Lewis rats, but contracted in WKY or BN rats [32,34], eventually becoming focused on specific non-overlapping epitopes of Bhsp65 and Rhsp65. Following serum adoptive transfer, these antibodies induced protection against AA [32–34]. We suggest that the humoral response to self hsp65 also is regulatory, and thereby, cooperative with the

protective anti-hsp65 T cell response. These antibodies might act by producing immunosuppressive cytokine (IL-10) [32,33] or by lowering the antigen concentration via neutralization and so deviating the response to a protective Th2 type [35]. In addition, antibodies can modulate antigen processing and the epitope-directed T cell response [36].

7. The differential response to microbial versus self hsps is attributable in part to other microbial ligands of innate receptors

Infections have long been associated with the pathogenesis of autoimmune diseases [37,38]. Hsps represent one of the major immunogenic antigens of pathogens. Invariably, these hsps reside in the microbe along with various pathogen-associated molecular patterns (PAMPs), which in turn are recognized by the pattern recognition receptors on the cells of the mammalian host e.g. TLRs and NOD (Nucleotide Oligomerization Domain) receptors [39]. This physical constellation leads to the induction of an inflammatory immune response against hsps and other antigens of the pathogen. A robust anti-microbial immune response serves to contain the infection, but it can sometimes also be pathogenic for the host [40]. As applicable to AA, the arthritogenicity of Mtb but not of purified Bhsp65 might be attributable to one or more of the innate receptor ligands provided by Mtb. In contrast to the microbial hsps, the endogenous self hsps that are induced under stressful stimuli in the absence of foreign PAMPs are much less likely to induce a pathogenic effector response. The enhanced expression and/or release of self hsps serves to alert the host system, and any immune response thus initiated is apparently downregulated effectively via mechanisms of peripheral tolerance. We suggest that the physical milieu of foreign versus self hsp65 is a major factor in determining the type of immune response generated by these homologous proteins.

Clinical relevance of the regulatory role of self hsp65 in autoimmune arthritis

As hsp65 has also been invoked in the pathogenesis of human autoimmune arthritis [41], the observations in the AA model regarding the regulatory role of self hsp65 are of direct relevance to arthritis patients. JIA patients showed increased levels of hsp65 expression in the synovium, and increased levels of the T cell response to self hsp65 [42,43]. Furthermore, enhanced T cell response to self hsp65 at the onset of JIA positively correlated with a favorable prognosis, suggesting its regulatory role [42,44]. Another cohort of JIA patients raised a pro-inflammatory response to the *Escherichia coli* hsp dnaJ, but an immunosuppressive response with IL-10 secretion to self dnaJ [45]. In addition, self dnaJ epitopes enhanced the frequency as well as the activity of Treg [45]. Functionally disparate responses to microbial versus self hsp65 have been reported in rheumatoid arthritis (RA) patients as well [46]. In addition, it has been shown that Treg are functionally compromised in RA patients [47], and that the number and/or function of Treg can be restored by conventional anti-TNF- α treatment [47] as well as by hsp dnaJ peptide-based immunotherapy [48]. Furthermore, the TLRs expressed on synovial tissue of RA patients [49] provide an immunomodulatory link with both hsps and Treg.

Beneficial aspects of the anti-self immunity in other immune disorders

Considering the ubiquitous and conserved nature of hsps, it is intuitive and rather expected, that the principle of regulation of autoimmunity by self reactivity would be harnessed to control other autoimmune diseases besides arthritis. In fact, immune response to self hsp65 has been shown to downmodulate diabetes in the non-obese diabetic mouse [50]. Moreover, pilot clinical trials have revealed the beneficial effects of treatment with self hsp65 peptide, p277 [51]. Similarly, it has been demonstrated that a level of autoreactivity is beneficial by maintaining the homeostasis and functional integrity of the central nervous system under physiological and pathological conditions [52]. Additional evidence for immune regulation by self hsp65 has been provided by studies in organ transplantation [22,53]. A therapeutic benefit of overexpressed hsp70 in hemorrhage-induced dysfunction has also been reported [54].

Overexpression of hsp70 has been shown to downregulate certain key components of the TCR/CD3-mediated signal transduction pathway, but to enhance the Fas-mediated apoptotic cell death [55,56]. However, in parallel there is evidence for the pathogenic role of foreign and self hsps in atherosclerosis [57], type I diabetes [51], and organ transplantation [22,53], presenting the complex dual functional characteristics of the immune response to hsps. Space constraints, however preclude a detailed discussion of these and related issues influencing the induction and propagation of autoimmunity.

Limitations of the current experimental model systems for mechanistic studies involving hsp65

Studies in the rat AA model have provided invaluable insights into the role of hsp65 in the pathogenesis of arthritis as well as regulation of autoimmunity. However, in-depth examination of mechanisms involved in these processes are limited largely by the lack of key immunological reagents for the rat immune system and the non-availability of knock-out rats. For reasons not yet defined, AA cannot be induced readily in the mouse without resorting to drastic measures (e.g., continued IL-4 neutralization in vivo) [58]. Many other models of arthritis in mice are based on antigens whose expression patterns are not influenced by stress as significantly as that of hsps. Moreover, hsp65-gene knockout in mice is embryonically lethal, pre-empting the development of hsp65-deficient mice. We hope that the recent triumphs consisting of sequencing of the rat genome [59] and the generation of the first knock-out rat [60] would pave the way for new avenues of research into the pathogenesis and immunotherapy of autoimmune arthritis using the rat models.

Concluding remarks

The microbial and self hsps possess many similarities in their sequence, structural conformation and function, but these homologous proteins exist in different milieus, both locally and more widely. Consequently, self hsps influence the selection and subsequent activation of T cells in vivo such that the default immune response against them is regulatory or protective in nature. In contrast, microbial hsps, which are excluded from shaping or programming the specific T cell repertoire as well as co-residing with ligands for innate receptors (i.e. PAMPs), are more likely to induce a pathogenic response. However, considering the reported role of self hsps as targets of autoimmune attack in some disorders, the self-foreign hsp model presented here cannot explain all seemingly conflicting evidences. We hope that future developments will further clarify the proclivity of self hsps to induce immunoprotective responses by filling the gaps regarding the identification of Treg-inducing epitopes of self hsps, the subtle differences in the intracellular signaling following recognition of self versus foreign hsps, and the influence of conditionally expressed self hsps within specific target organs on immune responsiveness to these antigens. The predominantly regulatory attribute of the immune response to self hsps is already being exploited and should be explored further for therapeutic purposes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank our laboratory members, Md. Younus Mia, Eugene Y. Kim, and Shailesh R. Satpute for their helpful discussions and suggestions. This work was supported by grants from the National Institutes of Health (NIH), Bethesda, MD, the Arthritis Foundation National Office (Atlanta, GA), the Maryland Chapter of the Arthritis Foundation and the Maryland Arthritis Research Center (MARRC), Baltimore, MD.

References

1. Anderson MS, et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002;298(5597):1395–1401. [PubMed: 12376594]
2. Durai M, et al. The T cells specific for the carboxyl-terminal determinants of self (rat) heat-shock protein 65 escape tolerance induction and are involved in regulation of autoimmune arthritis. *J Immunol* 2004;172(5):2795–2802. [PubMed: 14978079]
3. Lopez-Guerrero JA, et al. Modulation of adjuvant arthritis in Lewis rats by recombinant vaccinia virus expressing the human 60-kilodalton heat shock protein. *Infect Immun* 1993;61(10):4225–4231. [PubMed: 8406810]
4. Quintana FJ, et al. DNA fragments of the human 60-kDa heat shock protein (HSP60) vaccinate against adjuvant arthritis: identification of a regulatory HSP60 peptide. *J Immunol* 2003;171(7):3533–3541. [PubMed: 14500649]
5. Miyata M, et al. Significance of endogenous heat shock protein in adjuvant arthritis. *J Rheumatol* 1999;26(10):2210–2214. [PubMed: 10529142]
6. Mia MY, et al. The dynamics of articular leukocyte trafficking and the immune response to self heat-shock protein 65 influence arthritis susceptibility. *J. Clin. Immunol.* 2008(In Press)
7. Kyewski B, Klein L. A central role for central tolerance. *Annu Rev Immunol* 2006;24:571–606. [PubMed: 16551260]
8. Moudgil KD, et al. Diversification of T cell responses to carboxy-terminal determinants within the 65-kD heat-shock protein is involved in regulation of autoimmune arthritis. *J Exp Med* 1997;185(7):1307–1316. [PubMed: 9104817]
9. Durai M, et al. The regulatory C-terminal determinants within mycobacterial heat shock protein 65 are cryptic and cross-reactive with the dominant self homologs: implications for the pathogenesis of autoimmune arthritis. *J Immunol* 2004;173(1):181–188. [PubMed: 15210773]
10. Moudgil KD, Sercarz EE. Dominant determinants in hen eggwhite lysozyme correspond to the cryptic determinants within its self-homologue, mouse lysozyme: implications in shaping of the T cell repertoire and autoimmunity. *J Exp Med* 1993;178(6):2131–2138. [PubMed: 8245785]
11. Sercarz EE, et al. Dominance and crypticity of T cell antigenic determinants. *Annu Rev Immunol* 1993;11:729–766. [PubMed: 7682817]
12. Anderton SM, et al. Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. *J Exp Med* 1995;181(3):943–952. [PubMed: 7869052]
13. Prakken BJ, et al. Peptide-induced nasal tolerance for a mycobacterial heat shock protein 60 T cell epitope in rats suppresses both adjuvant arthritis and nonmicrobially induced experimental arthritis. *Proc Natl Acad Sci U S A* 1997;94(7):3284–3289. [PubMed: 9096385]
14. Yang XD, et al. Treatment of adjuvant arthritis in rats: vaccination potential of a synthetic nonapeptide from the 65 kDa heat shock protein of mycobacteria. *J Autoimmun* 1990;3(1):11–23. [PubMed: 1691914]
15. Satpute SR, et al. Mycobacterial Hsp65-IgG-expressing tolerogenic B cells confer protection against adjuvant-induced arthritis in Lewis rats. *Arthritis Rheum* 2007;56(5):1490–1496. [PubMed: 17469108]
16. Durai M, et al. T cells against the pathogenic and protective epitopes of heat-shock protein 65 are crossreactive and display functional similarity: novel aspect of regulation of autoimmune arthritis. *J Rheumatol* 2007;34(11):2134–2143. [PubMed: 17937454]
17. Wendling U, et al. A conserved mycobacterial heat shock protein (hsp) 70 sequence prevents adjuvant arthritis upon nasal administration and induces IL-10-producing T cells that cross-react with the mammalian self-hsp70 homologue. *J Immunol* 2000;164(5):2711–2717. [PubMed: 10679112]
18. Quintana FJ, et al. Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J Immunol* 2002;169(6):3422–3428. [PubMed: 12218165]
19. Kim EY, et al. Regulation of autoimmune arthritis by the pro-inflammatory cytokine interferon-gamma. *Clin Immunol* 2008;127(1):98–106. [PubMed: 18276192]
20. Jacob CO, et al. Heterogeneous effects of IFN-gamma in adjuvant arthritis. *J Immunol* 1989;142(5):1500–1505. [PubMed: 2493048]

21. Kim EY, et al. Exogenous tumor necrosis factor- α induces suppression of autoimmune arthritis. *Arthritis Res Ther* 2008;10(2):R38. [PubMed: 18380898]
22. Granja C, et al. T-cell autoreactivity to Hsp in human transplantation may involve both proinflammatory and regulatory functions. *Hum Immunol* 2004;65(2):124–134. [PubMed: 14969767]
23. Gor DO, et al. TH1-TH2: a procrustean paradigm. *Nat Immunol* 2003;4(6):503–505. [PubMed: 12774069]
24. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007;13(2):139–145. [PubMed: 17290272]
25. Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. *Nat Rev Immunol* 2003;3(3):253–257. [PubMed: 12658273]
26. Mukherjee R, et al. CD4+CD25+ regulatory T cells generated in response to insulin B:9–23 peptide prevent adoptive transfer of diabetes by diabetogenic T cells. *J Autoimmun* 2003;21(3):221–237. [PubMed: 14599847]
27. Andersson J, et al. CD4+CD25+ regulatory T cells are activated in vivo by recognition of self. *Int Immunol* 2007;19(4):557–566. [PubMed: 17369190]
28. Jordan MS, et al. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2001;2(4):301–306. [PubMed: 11276200]
29. Vabulas RM, et al. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 2001;276(33):31332–31339. [PubMed: 11402040]
30. Caramalho I, et al. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med* 2003;197(4):403–411. [PubMed: 12591899]
31. Zanin-Zhorov A, et al. Heat shock protein 60 enhances CD4+ CD25+ regulatory T cell function via innate TLR2 signaling. *J Clin Invest* 2006;116(7):2022–2032. [PubMed: 16767222]
32. Ulmansky R, et al. Resistance to adjuvant arthritis is due to protective antibodies against heat shock protein surface epitopes and the induction of IL-10 secretion. *J Immunol* 2002;168(12):6463–6469. [PubMed: 12055266]
33. Herskho AY, et al. Susceptibility and resistance to experimental adjuvant arthritis. *Front Biosci* 2004;9:3268–3275. [PubMed: 15353356]
34. Kim HR, et al. Antibody responses to mycobacterial and self heat shock protein 65 in autoimmune arthritis: epitope specificity and implication in pathogenesis. *J Immunol* 2006;177(10):6634–6641. [PubMed: 17082575]
35. Constant S, et al. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J Exp Med* 1995;182(5):1591–1596. [PubMed: 7595230]
36. Simitsek PD, et al. Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. *J Exp Med* 1995;181(6):1957–1963. [PubMed: 7539034]
37. Pordeus V, et al. Infections and Autoimmunity: A Panorama. *Clin Rev Allergy Immunol*. 2008
38. Lo WF, et al. Molecular mimicry mediated by MHC class Ib molecules after infection with gram-negative pathogens. *Nat Med* 2000;6(2):215–218. [PubMed: 10655113]
39. Osterloh A, Breloer M. Heat shock proteins: linking danger and pathogen recognition. *Med Microbiol Immunol* 2008;197(1):1–8. [PubMed: 17638015]
40. Krieg AM, Vollmer J. Toll-like receptors 7, 8, and 9: linking innate immunity to autoimmunity. *Immunol Rev* 2007;220:251–269. [PubMed: 17979852]
41. Celis L, et al. Clonal expansion of mycobacterial heat-shock protein-reactive T lymphocytes in the synovial fluid and blood of rheumatoid arthritis patients. *Arthritis Rheum* 1997;40(3):510–519. [PubMed: 9082939]
42. De Graeff-Meeder ER, et al. Recognition of human 60 kD heat shock protein by mononuclear cells from patients with juvenile chronic arthritis. *Lancet* 1991;337(8754):1368–1372. [PubMed: 1674762]

43. Boog CJ, et al. Two monoclonal antibodies generated against human hsp60 show reactivity with synovial membranes of patients with juvenile chronic arthritis. *J Exp Med* 1992;175(6):1805–1810. [PubMed: 1316935]
44. de Kleer IM, et al. The spontaneous remission of juvenile idiopathic arthritis is characterized by CD30 + T cells directed to human heat-shock protein 60 capable of producing the regulatory cytokine interleukin-10. *Arthritis Rheum* 2003;48(7):2001–2010. [PubMed: 12847694]
45. Massa M, et al. Differential recognition of heat-shock protein dnaJ-derived epitopes by effector and Treg cells leads to modulation of inflammation in juvenile idiopathic arthritis. *Arthritis Rheum* 2007;56(5):1648–1657. [PubMed: 17469159]
46. van Roon JA, et al. Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. *J Clin Invest* 1997;100(2):459–463. [PubMed: 9218524]
47. Ehrenstein MR, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J Exp Med* 2004;200(3):277–285. [PubMed: 15280421]
48. Prakken BJ, et al. Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. *Proc Natl Acad Sci U S A* 2004;101(12):4228–4233. [PubMed: 15024101]
49. Roelofs MF, et al. Identification of small heat shock protein B8 (HSP22) as a novel TLR4 ligand and potential involvement in the pathogenesis of rheumatoid arthritis. *J Immunol* 2006;176(11):7021–7027. [PubMed: 16709864]
50. Quintana FJ, et al. DNA vaccination with heat shock protein 60 inhibits cyclophosphamide-accelerated diabetes. *J Immunol* 2002;169(10):6030–6035. [PubMed: 12421990]
51. Raz I, et al. Treatment of new-onset type 1 diabetes with peptide DiaPep277 is safe and associated with preserved beta-cell function: extension of a randomized, double-blind, phase II trial. *Diabetes Metab Res Rev* 2007;23(4):292–298. [PubMed: 17124720]
52. Schwartz M, Ziv Y. Immunity to self and self-maintenance: a unified theory of brain pathologies. *Trends Immunol.* 2008
53. Pockley AG, Muthana M. Heat shock proteins and allograft rejection. *Contrib Nephrol* 2005;148:122–134. [PubMed: 15912031]
54. Kiang JG, et al. Geldanamycin prevents hemorrhage-induced ATP loss by overexpressing inducible HSP70 and activating pyruvate dehydrogenase. *Am J Physiol Gastrointest Liver Physiol* 2006;291(1):G117–G127. [PubMed: 16565416]
55. Liossis SN, et al. Overexpression of the heat shock protein 70 enhances the TCR/CD3- and Fas/Apo-1/CD95-mediated apoptotic cell death in Jurkat T cells. *J Immunol* 1997;158(12):5668–5675. [PubMed: 9190915]
56. Liossis SN, Tsokos GC. Heat-shock of normal t-cells and T-cell lines downregulates the TCR/CD3-mediated cytoplasmic Ca²⁺ responses and the production of inositol trisphosphate. *Immunopharmacol Immunotoxicol* 1997;19(4):511–521. [PubMed: 9436050]
57. Afek A, et al. Immunization of low-density lipoprotein receptor deficient (LDL-RD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis. *J Autoimmun* 2000;14(2):115–121. [PubMed: 10677242]
58. Yoshino S, et al. Successful induction of adjuvant arthritis in mice by treatment with a monoclonal antibody against IL-4. *J Immunol* 1998;161(12):6904–6908. [PubMed: 9862723]
59. Gibbs RA, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* 2004;428(6982):493–521. [PubMed: 15057822]
60. Zan Y, et al. Production of knockout rats using ENU mutagenesis and a yeast-based screening assay. *Nat Biotechnol* 2003;21(6):645–651. [PubMed: 12754522]

Figure 1. The endogenous self hsp65 drives the regulatory epitope spreading in autoimmune arthritis

The inflammation accompanying the onset of AA, which occurs at about 8–10 d following an arthritogenic challenge with Mtb, upregulates the cellular expression of endogenous self (rat) hsp65. It then takes another 8–10 d for the optimal priming of self hsp65-reactive T cells to occur, which corresponds to the time point just preceding the peak phase of AA. The T cell response to self hsp65 is directed mostly to its C-terminal determinants (RCTD). These T cells in turn are re-stimulated by the corresponding and cross-reactive C-terminal epitopes of Bhsp65 (BCTD) when tested in the late phase of AA, manifesting as epitope spreading. Thus, the endogenous hsp65 is the actual stimulus *in vivo* for the observed diversification of T cell response to Bhsp65. The appearance of the hsp65 C-terminal directed T cell response correlates with the beginning of spontaneous recovery from acute arthritis. (AA= adjuvant arthritis; Bhsp65= mycobacterial hsp65; BCTD= Bhsp65 C-terminal determinants; Mtb= *Mycobacterium tuberculosis* H37Ra (heat-killed); Rhsp65= rat hsp65; RCTD= Rhsp65 C-terminal determinants.)

Table 1

Experimental evidence supporting the regulatory role of self heat-shock protein 65 (hsp65) in autoimmune arthritis

The adjuvant arthritis (AA) model	
1.	The self hsp65-reactive T cells exist in the mature repertoire of naïve rats, and the priming and expansion of these T cells by immunization with Rhsp65 induces protection against AA
2.	In Mtb-induced AA, Bhsp65 can have protective effect against arthritis possibly via induction and expansion of self hsp65-reactive regulatory T cells.
3.	Arthritic rats have T cell response to self hsp65, which correlates with the regulatory epitope spreading to mycobacterial hsp65 (Bhsp65)
4.	The AA-protective epitopes within Bhsp65 prime T cell subsets that are cross-reactive with self hsp65
5.	Pre-treatment with the pathogenic epitope of Bhsp65 induces protection against AA in part by activating self hsp65-reactive T cells
6.	Arthritis-resistant rats both express higher levels of self hsp65 in the synovial tissue and the periphery and raise higher level of T cell response to self hsp65 compared to the arthritis-susceptible rats
7.	Antibodies produced during the course of AA are protective against arthritis and display anti-self hsp65 reactivity
Arthritis patients	
1.	In juvenile idiopathic arthritis (JIA) patients, increased immune reactivity of peripheral and synovial T cells to self hsp65 positively correlates with better prognosis of the disease
2.	Exposure to self DnaJ heat shock protein epitopes increases the frequency and suppressive activity of CD4+CD25+ T regulatory cells (Treg) of JIA patients

Table 2

Proposed explanations for the immunoregulatory attributes of self heat-shock proteins (hsps)

1. Self hsp65 is involved in shaping of the T cell repertoire, enabling it to preserve and later expand the protective T cell subsets
2. Mycobacterial hsp65 epitopes regulate autoimmune responses by activating self hsp65-cross-reactive T cells through regulatory epitope spreading
3. Self hsp65-primed or restimulated T cells produce high levels of pro-inflammatory cytokines that induce suppression of adjuvant arthritis (AA)
4. Upregulation of self hsp65 under stress favors the selection of natural CD4+CD25+ T regulatory cells (nTregs) and the generation of adaptive Tregs (aTreg)
5. As for the T cell response, the humoral response to self hsp65 also is geared to be protective so as to develop a functionally cooperative response that is beneficial to the host
6. Unlike self hsp, the foreign hsp in association with microbial ligands for innate receptors is more likely to induce a pathogenic inflammatory response