Published in final edited form as:
J Autoimmun. 2009 ; 33(3-4): 208–213. doi:10.1016/j.jaut.2009.09.007.

Self heat-shock protein 65-mediated regulation of autoimmune arthritis

Malarvizhi Durai, **Min-Nung Huang**, and **Kamal D. Moudgil**
Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA

Abstract

Heat-shock proteins (Hsps) have been invoked in the pathogenesis of a variety of autoimmune diseases. The mycobacterial heat-shock protein 65 (Bhsp65) has been studied extensively as one of the antigenic triggers of autoimmunity in experimental models of, as well as patients with, rheumatoid arthritis. As Hsps are highly conserved and immunogenic, it is generally anticipated that self Hsps might serve as the endogenous targets of the immune response initiated by the homologous foreign Hsps. Contrary to this expectation, studies in the rat adjuvant arthritis (AA) model have revealed that priming of the self (rat) hsp65 (Rhsp65)-directed T cells in the Lewis rat leads to protection against AA instead of disease induction or aggravation. The arthritis-protective attribute of the self hsp65 is also evident following spontaneous priming of the anti-Rhsp65 T cells during the natural course of AA. Furthermore, immunization of rats with human hsp60, or with Bhsp65 peptides that are crossreactive with the corresponding self hsp65 peptides, leads to protection against AA. Importantly, high levels of T cell reactivity against self hsp60 in patients with juvenile idiopathic arthritis positively correlate with a favorable outcome of the disease. Thus, immune response against self hsp65 in autoimmune arthritis is protective rather than being pathogenic.

Keywords

Adjuvant arthritis; Autoimmunity; Heat-shock protein 65; Immunoregulation; Juvenile Idiopathic Arthritis

The immune system of a healthy individual is endowed with the ability to control aberrant self-directed immune responses. This is accomplished via diverse mechanisms of central and peripheral tolerance that cooperate effectively to suppress the initiation and progression of a potentially harmful anti-self response [1-5]. However, the efficacy of this protective arsenal can be compromised under certain conditions involving the convergence of a highly susceptible genetic background, a defective tolerogenic mechanism, and a potent environmental trigger for autoreactivity [6-9]. One of the frequent culprits in this regard is the microbial agent that provides ample ligands for the activation of the innate and adaptive immune responses [10, 11]. These immune effector responses might inadvertently target self

Correspondence and reprint request to: Kamal D. Moudgil, Associate Professor, Department of Microbiology and Immunology, University of Maryland School of Medicine, Howard Hall Room 323 C, 660 West Redwood St., Baltimore, MD 21201; Fax: 410-706-2129; kmoud001@umaryland.edu.

components that fortuitously mimic the microbial antigens, leading to the initiation of autoimmunity [10, 11]. The eventual outcome of this initial breach of self tolerance is dependent in large part on the responsiveness of the host's immune regulatory mechanisms. The adjuvant-induced arthritis (AA) model of human rheumatoid arthritis (RA) recapitulates several of the features of induction of autoimmunity highlighted above. AA can be induced in the Lewis (RT.1^{l}) rat by immunization with heat-killed *M. tuberculosis* H37Ra (Mtb) [12]. The mycobacterial heat-shock protein 65 (Bhsp65) is one of the major targets of the immune response of arthritis rats [13-16]. The region 180-188 of Bhsp65 harbors an arthritogenic T cell determinant [13]. The T cells primed by Mtb/Bhsp65 are believed to induce autoimmune arthritis via recognition of cartilage-resident self proteins. Unlike the Lewis rat, the Wistar Kyoto (WKY) (RT.1^{l}) rat, the Brown Norway (BN) (RT.1^{n}) rat, and the Fischer F344 (RT.1^{lvl}) rat are relatively resistant to the induction of AA following Mtb challenge [15-17].

AA is a self-limiting disease. However, the immunological basis of the spontaneous regression of autoimmune inflammation in AA is not fully defined. The results of our studies based on self (rat) hsp65 (Rhsp65), the self homologue of Bhsp65, provide a conceptual framework for linking the inflammation and regulation of autoimmunity [15, 18, 19]. (For uniformity of nomenclature, we have referred to the rat hsp60 as rat hsp65.) Inflammation is an integral component of the processes involved in the initiation of autoimmune arthritis. However, with the progression of arthritis, inflammation both upregulates the cellular expression of self Hsps, including Rhsp65, and modulates the antigen processing and presentation events, culminating in the triggering of regulatory immune responses [15, 18, 19](Fig. 1). The self hsp65-directed T cell repertoire can also be recruited by Bhsp65, which possesses several disease-regulating epitopes spanning different regions of the protein [14-16, 20, 21]. The regulatory attribute of self Hsp unravels the 'brighter' side of these proteins in contrast to their 'darker' side, exemplified by their role as self antigenic targets in autoimmune pathology [22, 23]. The dual role of self hsps in autoimmunity [22, 23] parallels that of several other biological molecules such as cytokines [24], many of which possess both pro-inflammatory and anti-inflammatory properties that manifest under distinct sets of conditions. We present below the results of studies by others and us that highlight the regulatory aspect of self Hsp65 in AA. Also discussed in brief are studies by other investigators in patients with juvenile idiopathic arthritis (JIA) that support the concept of self hsp65-mediated regulation of autoimmune arthritis.

I. Shaping of the T cell repertoire against self hsp65 and the role of these T cells in the pathogenesis of AA

The development of mature T cell repertoire occurs via positive and negative selection events in the thymus, and efficient induction of self tolerance is critical for the prevention of autoimmunity [1-5]. Like other self antigens, self Hsps are also expected to influence the thymic selection of the T cell repertoire. The experiments conducted in our laboratory in the AA model have revealed that subsets of T cells specific for self hsp65 (Rhsp65) escape tolerance induction in the thymus and make it into the periphery of naive Lewis rats, demonstrating that self tolerance to Rhsp65 is incomplete. In this regard, Rhsp65 is different from certain other self proteins that are efficient tolerogens [25-27]. The T cell response of Lewis rats immunized with Rhsp65 is targeted predominantly to the C-terminal epitopes of that protein. The *in vivo*-primed T cells specific for the C-terminal epitopes of Rhsp65 can be restimulated efficiently by the epitopes generated from endogenous hsp within heat-stressed APC [18]. Furthermore, the pretreatment of Lewis rats with Rhsp65 affords protection against AA and this protection also is attributable primarily to the epitopes in the C-terminal region of the protein (Table 1). The protection against AA can be adoptively transferred to naïve syngeneic recipients via the T cells primed by the C-terminal epitopes of Rhsp65. Thus, we have offered experimental support both for in vivo priming of the T cells by the C-

terminal epitopes of Rhsp65 during AA and for the likely role of these T cells in the natural recovery from the acute disease [15, 18, 19]. On the basis of the above observations, we have proposed a model for the role of self hsp65 in regulation of AA [15, 18, 19, 28]. We suggested that inflammation following the onset of AA upregulates the expression as well as the processing and presentation of Hsps. This in turn leads to activation of the T cells against the C-terminal epitopes of Rhsp65 and subsequent recovery from acute AA (Fig. 1) [15, 18, 19]. Our results not only provide evidence for the self hsp65-mediated regulation of acute AA, but also offer novel insights for developing better antigen-specific immunotherapeutic approaches for human RA.

II. Vaccination with human hsp60 (Hhsp60) induces protection against AA

The protective effect of Rhsp65 in arthritis is further supported by studies showing the inhibition of AA in rats by DNA vaccination with Hhsp60, which is over 97% homologous to Rhsp65 (Table 1). Quintana et al. [14] tested the efficacy of DNA vaccination using plasmids encoding self (Hhsp60) or foreign (Bhsp65) Hsp. Their results showed that Hhsp60 was more effective than Bhsp65 in suppressing AA. Although both Hsps could prime self hsp60-reactive T cells, the immunization of rats with Hhsp60 activated T cells that produced much higher amounts of IL-10 and/or TGF-β compared to that produced by the T cells activated by Bhsp65. This difference in cytokine production constituted an important factor that rendered Hhsp60 superior to Bhsp65 in protection against AA. In another study, vaccination of rats with a plasmid carrying the gene for Hhsp60 followed by mapping of the T cell epitopes using overlapping hsp60 peptides showed that the T cell response was directed predominantly against the regulatory peptide, Hu3 [21]. The Hu3-reactive T cells secreted mostly IL-10. Immunization of rats with the Hu3 peptide or the adoptive transfer of splenocytes derived from Hu3 peptide-vaccinated rats into naïve syngeneic rats prior to Mtb injection inhibited the development of AA [21]. However, the mycobacterial counterpart of Hu3 lacked arthritis-regulating properties. Thus, the protective effect of self hsp60 can be induced by immunization with the whole protein or with the functionally relevant peptide epitope of the protein (Table 1).

Using the AA model, Lopez-Guerrero et al [29] have reported the immunomodulatory effects of recombinant vaccinia virus expressing the Hhsp60 gene (Table 1). The challenge of rats with this virus on day 7 after the induction of AA reduced the severity of arthritis but induced both T cell and antibody responses to Hhsp60. In another study, the disease-protective effects of vaccinia virus expressing Bhsp65 or Hhsp60 were compared [30]. Both Hsps were effective in the prevention of AA. However, in the therapeutic regimen, Hhsp60 induced higher protection against AA than that offered by Bhsp65, although both constructs induced optimal T cell responses against the respective protein [30]. The results of another vaccinia virus-based study provided support to the protective effect of Bhsp65 [31]. In that study, soluble Bhsp65 aggravated the disease development [31]. Taken together, the above findings provide a convincing rationale for the possible therapeutic use of Hhsp60 in autoimmune arthritis.

III. Arthritis-protective effects of the crossreactive T cells directed against self and bacterial hsp65

The initial studies on the pathogenesis of AA revealed that arthritis can be induced in Lewis rats by the passive transfer of T cells derived from syngeneic arthritic rats [32, 33], and that the pathogenic T cells include subsets of T cells reactive against Bhsp65. Subsequently, it has been demonstrated that a T cell clone (A2b) specific for the epitope region 180-188 of Bhsp65 could adoptively transfer AA to naïve recipients [13, 34] and that this T cell clone was crossreactive with an epitope of the cartilage proteoglycan [35]. However, Bhsp65 per se

failed to induce the disease (AA) in rats, although the T cells from arthritic rats responded to Bhsp65 upon in vitro restimulation. Surprisingly, immunization of naïve rats with Bhsp65 afforded protection against subsequent induction of AA by Mtb injection. The disease-protective effect of Bhsp65 has also been documented in other models of arthritis besides AA [13, 36-38], namely streptococcal cell wall-induced arthritis in rats [39], Pristane-induced arthritis [40] and dimethyl dioctadecyl ammonium bromide (DDA)-induced arthritis [41].

Reports from different laboratories have further highlighted the protective effects of Bhsp65-derived peptides in AA [15, 19, 20, 42, 43]. There is increasingly realization that the disease-protective effect of Bhsp65 is mediated in part via T cells that are crossreactive with self hsp65 (Table 1). Van Eden et al. showed that a T cell line directed against the Bhsp65 epitope 256-270 was capable of transferring protection against AA and that these T cells are crossreactive with the mammalian hsp60 homologue [20]. Furthermore, the pretreatment of rats with Bhsp65 peptide 256-270 afforded protection against AA and the T cells from these rats responded to heat-stressed syngeneic antigen presenting cells (APC) expressing endogenous (rat) hsp65.

We have previously reported the spreading of T cell response to Bhsp65 C-terminal determinants (BCTD) during the course of AA, as well as the protection against AA following pretreatment of rats with a mixture of peptides comprising BCTD [15, 18, 19]. Moreover, the epitope spreading to Bhsp65 was accompanied by spontaneous induction of T cell response to the corresponding C-terminal epitopes of the homologous self hsp65 (Rhsp65) [15]. We have also shown that the T cells activated by peptides comprising BCTD could adoptively transfer protection against AA [19], and that these T cells can be restimulated by the naturally processed C-terminal epitopes of recombinant self hsp65 as well as the endogenous hsp65 expressed within heat-stressed APC. These findings led us to suggest that the diversification of T cell response to BCTD within Bhsp65 during the course of inflammatory AA was the result of upregulation of the display of BCTD coupled with spontaneous induction of the T cell response to the crossreactive C-terminal epitopes of self hsp65 [15, 18, 19]. Thus, the activation of T cells reactive against the C-terminal determinants of Bhsp65/Rhsp65 constitutes a critical mechanism of protection/recovery in AA.

The arthritis-regulating activity of hsp65-primed T cells has been attributed to the production of predominantly anti-inflammatory/immunoregulatory Th2 (IL-10)/Th3 (TGF-β) cytokines [14, 16, 20, 21, 42]. In our study on the AA-protective effect of the Rhsp65 peptide 465-479 (R465), we observed that the regulatory effect of this peptide was mediated primarily via the Th1 cytokine IFN-γ [44]. These results are supported by similar findings of protection mediated via IL-12/IFN-γ in various other models of autoimmunity [24, 45, 46]. The control of Th1-mediated autoimmunity by IFN-γ highlights the complexities as well as limitations of the simplified Th1-Th2 concept of cytokine-based regulation of autoimmunity [47, 48]. In addition to cytokines, CD4+CD25+ T regulatory cells (Treg) [3-5] also mediate disease regulation in autoimmunity. In this regard, Hsps have been shown to activate Treg [49, 50]. However, the precise role of Treg in Hsp65 peptide-induced downmodulation of AA or in natural recovery from AA is not yet defined.

IV. Antibodies against hsp65 are protective against AA

There is increasing evidence supporting the role of antibodies in the pathogenesis of AA, which is generally believed to be a predominantly T cell-mediated disease. Ulmansky et al. [16, 17] showed that the resistance to induction of AA in naïve BN rats, or in Lewis rats recovered from arthritis, was due to the presence of antibodies against hsp65. Moreover, the resistance to AA could be transferred to naïve susceptible rats by infusion of these antibodies. These antibodies are produced naturally in BN rats, but acquired during AA in

Lewis rats. The kinetics of antibodies against Bhsp65 epitopes was different in resistant versus susceptible rat strains: arthritis-susceptible rats had antibodies to a few epitopes within Bhsp65 and acquired more epitope reactivities during the disease progression, whereas resistant rats had those antibodies naturally throughout. These results suggest that similar to epitope spreading involving the T cell epitopes, spreading also targets the antibody epitopes during the course of AA. The vaccination of Lewis rats with peptide 6 (a.a. 31-46) or peptide 7 (a.a. 37-52) of Bhsp65, or peptide 5 of self hsp65 (a.a. 61-80) (rat homologue of bacterial peptide 6) (Table 1), led to the production of antibodies against both bacterial peptide 6 and Bhsp65 as well as protection against AA. These three peptides share a common amino acid motif (VEWGP), which might contribute to the AA-protective effect of these peptides [16]. Antibodies to peptide 6 were shown to induce production of IL-10 by the mononuclear cells [16]. In an independent study, we reported the kinetics, epitope specificity and the functional attributes of antibodies directed against Bhsp65, self hsp65 and peptides representing different regions of these two proteins in Lewis versus WKY rats. We observed that the AA-resistant WKY rats mounted a vigorous antibody response to both Bhsp65 and self hsp65 after immunization with Mtb, whereas the AA-susceptible Lewis rats developed antibodies against relatively fewer epitopes of Bhsp65. In Lewis rats, antibodies to self hsp65 did not appear until the recovery phase of AA [51]. The functional significance of anti-hsp65 antibodies was evident following serum adoptive transfer experiments. Serum from arthritic Lewis rats in the late phase of AA afforded protection against AA to the recipient rats. Studies by other investigators [29, 30], showing that protection induced by vaccinia virus expressing Hhsp60 is associated with the generation of cell-mediated and humoral immune response to self hsp, have provided indirect support for the protective effect of antibodies to self hsp65 in AA.

V. Modulation of AA by other Hsp family members

We have elaborated above the role of self/foreign hsp65 in protection against AA. The protective effects of other hsp family members such as mycobacterial hsp70 (Bhsp70) [52-54], mycobacterial hsp90 (Bhsp90) [55] and mycobacterial hsp10 (Bhsp10) [56] have also been documented. Wendling et al. [54] reported that the conserved Bhsp70 sequence 111-125 was found to be immunogenic and that it induced T cells that are crossreactive with the homologous rat sequence. The T cells reactive against peptide 111-125 produced IL-10, and the nasal administration of this peptide induced protection from subsequent induction of AA. Similar results were obtained by Tanaka et al. [53], who reported that pretreatment with peptide 234-252 of Bhsp70 produced high amounts of IL-10 and suppressed the development of AA. The transfer of a T cell line specific for peptide 234-252 also inhibited AA, suggesting that the T cells recognizing the conserved hsp70 peptide play a critical role in protection against arthritis.

VI. The disease-regulating attributes of human heat-shock protein 60 (Hhsp60) in juvenile idiopathic arthritis (JIA)

JIA is comprised of a heterogeneous group of clinical disorders characterized by chronic arthritis with disease onset before the age of 16 years [57]. Based on the International League of Associations for Rheumatology (ILAR) criteria, JIA is currently classified into 7 different disease categories, including systemic arthritis (SA), oligoarthritis (OA), rheumatoid factor (RF)-positive polyarthritis (PA), RF-negative PA, enthesitis-related arthritis, psoriatic arthritis and undifferentiated arthritis [57]. Most of the studies examining the role of Hhsp60 in JIA [58-65] are focused mainly on OA, PA and SA (Table 2). The overall results support the notion that Hhsp60 is regulatory in the OA category of JIA (OA-JIA). The regulatory function of Hhsp60 is associated with cell-mediated immune response characterized by cytokine deviation to a Th2-type. However, in a couple of studies, no correlation was

observed between the T cell response to Hhsp60 and disease activity in JIA patients [66, 67]. Furthermore, the Hhsp60-specific humoral response may not be specific for JIA and the significance of the role of humoral response in the pathogenesis of JIA is not yet clear. In regard to adult rheumatoid arthritis (RA), the role of Hhsp60 in regulation of the disease activity is rather inconclusive because both positive [68, 69] and negative [70, 71] associations have been reported for the specificity of Hhsp60-induced T cell activation, with or without Th2 cytokine deviation. It is a pleasure to contribute this paper to this special issue of the Journal of Autoimmunity devoted to the lifetime achievements of Dr. Noel Rose to autoimmunology [72-77], to his longstanding dedication to patient care, including the development of American Autoimmune Related Disease Association (AARDA) [78]. This volume is part of the Journal's commitment to recognize outstanding contributions in the field of autoimmunity [79-81].

Acknowledgments

My lab members and I are thankful to Noel Rose for his advice, encouragement and support over the years. Noel's creative contributions to the field of autoimmunity and his generous sharing of ideas have had a deep imprint on our thinking and experimental work. We also thank Rajesh Rajaiah, Yinghua Yang, Steva Nkrumah-Komeh and Hua Yu for their helpful critique and suggestions. This work was supported by grants from the Arthritis Foundation (Atlanta, GA) and the National Institutes of Health (Bethesda, MD).

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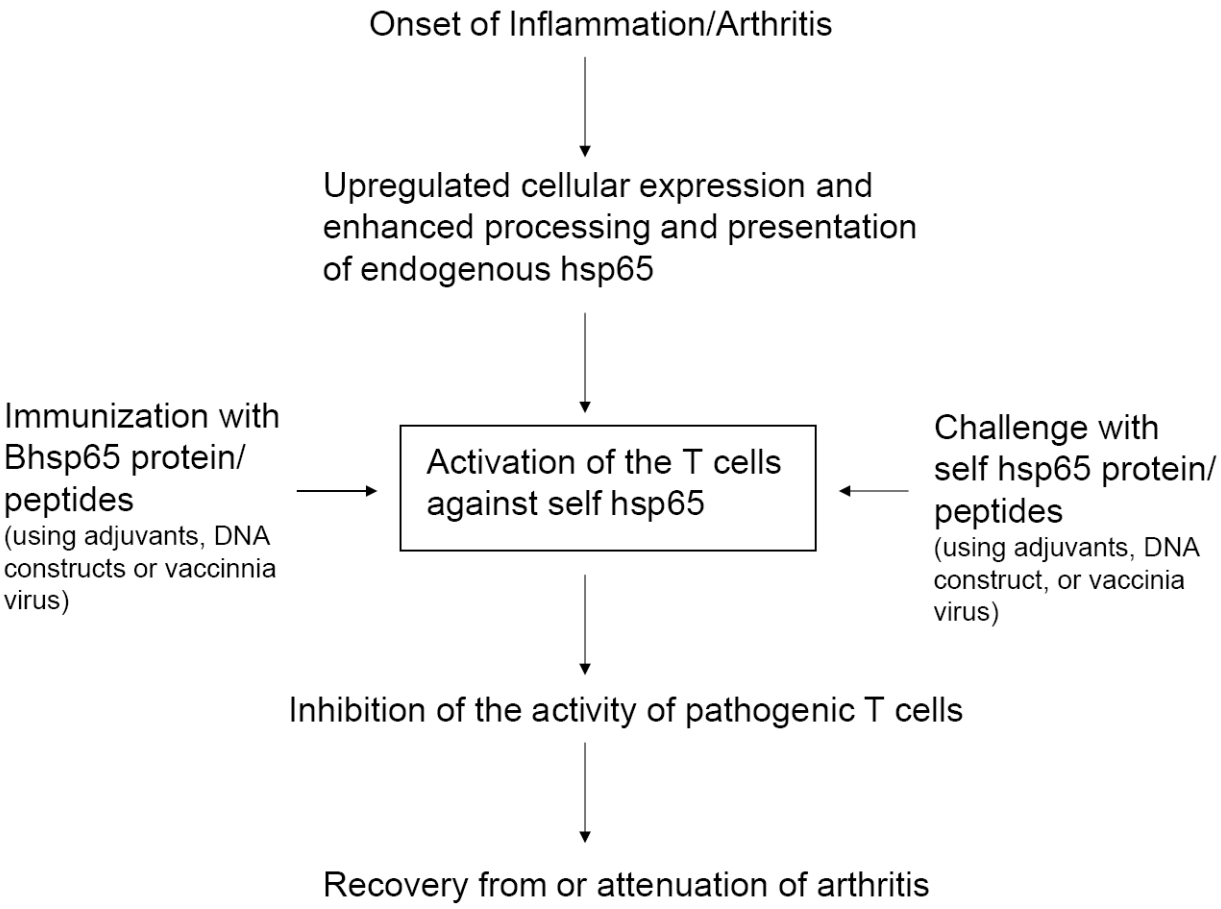

Figure 1. Regulation of adjuvant arthritis (AA) by self hsp65-reactive T cells

The onset of AA (inflammation) leads to upregulation of the cellular expression of self hsp65 as well as enhancement of the processing and presentation of this self hsp. This in turn leads to priming of the self hsp65-reactive T cells available in the mature repertoire ('incomplete' tolerance). This subset of T cells can also be activated following immunization with self hsp65 or by the crossreactive homologous Bhsp65. The T cell reactivity against self hsp65 is focused on the C-terminal epitopes of the protein. The *in vivo* priming of the T cells against self hsp65 or its C-terminal epitopes leads to downregulation of AA. Furthermore, challenge with Bhsp65 or its peptides (e.g., certain C-terminal epitopes and peptide 256-265) results in activation of the T cells that are crossreactive with self hsp65 as well as capable of inhibiting the progression of AA. Thus, despite the appreciable sequence homology (approximately 50%) between bacterial and self homologues of hsp65, the self hsp65 is involved in immune modulation rather than induction of AA.

Table 1

The involvement of self hsp60-directed T cell repertoire in regulation of adjuvant arthritis (AA) in rats

Origin of hsp60/Epitope region	Reference	Remarks
(I) Rat hsp65 (Rhsp65)	18	Immunization of Lewis rats (= rats) with native Rhsp65 in IFA s.c. induces protection against AA
(II) Rhsp65 determinants:		
5 C-terminal determinants	18	Pre-treatment of rats with a mixture of peptides (in IFA) representing these 5 determinants affords protection against AA
465-479	19, 44	This peptide primes a predominantly Th1-type (IFN-γ) of response, which suppresses subsequent AA
256-270	20	Treatment of rats with Bhsp65 256-270 downmodulates AA; this peptide is crossreactive with its self hsp65 homologue, (Rhsp65 256-270), which does not have AA-regulatory activity
61-80	16	Injection of rats with this peptide reduces the severity of AA; sera of young Brown-Norway rats, old Lewis rats and post-arthritic Lewis rats possess antibodies reactive against this peptide.
(III) Human hsp60 (Hhsp60)	14, 29, 30	DNA vaccination of rats with the construct encoding Hhsp60, or injection with vaccinia virus expressing Hhsp60 induces protection against AA
(IV) Hhsp60 determinant:		
Hu3	21	DNA vaccine containing a DNA fragment encoding the epitope 31-50 suppresses the development of AA; it involves a Th2/Th3 cytokine shift in response to the arthritogenic epitope Bhsp65 176-190; this epitope has the same sequence in Rhsp65

IFA= incomplete Freund's adjuvant; s.c.= subcutaneously; Th1= T helper 1

Table 2

The immune response to human heat-shock protein 60 in juvenile idiopathic arthritis

JIA classification*	Experimental parameter of interest	Experimental evidence	Association with the disease activity	Notable observations		Ref
Oligoarthritis JIA	T cell response	Positive	Predicts disease remission with favorable prognosis	**1**	Significant T cell proliferative response of PBMCs and/or SFMCs in OA-JIA, but not in non-JIA patients	58-61
				2	The T cell proliferative response was observed at active phase of the disease, not at remission phase	58, 59 61, 62
				3	High IL-10/IFN-γ ratio at active phase, but not at remission phase	62
				4	Increased expression of CD30 on $CD4^+$ T cells in PBMCs and SFMCs from patients with active disease, paralleled by high IL-10 production	
			Correlates with the duration of current arthritis	**1**	T cell proliferative response correlated with the duration of ongoing arthritis, but not with the overall duration of the disease	60
		Negative	-	**1**	No increased proliferative response of PBMCs compared with healthy controls	67
	Antibody response	Negative	-	**1**	No increased antibody titers compared with the healthy controls	64, 65
	Synovial expression of Hhsp60	Positive	Unknown	**1**	Increased Hhsp60 expression in the synovium compared with non-JIA patients	59, 60, 63
Polyarthritis, mainly RF-negative JIA	T cell response	Positive	Unknown	**1**	Increased T-cell proliferative response of PBMCs	61, 67
				2	Low IL-10/INF-γ ratio of the cytokine production from the T cells at active and remission phases of the disease	61, 62 62
				3	No expression of CD30 on $CD4^+$ T cells in PBMCs and SFMCs	
	Antibody response	Positive	Possibly no correlation with the disease activity	**1**	Not an exclusively disease-specific response	65
				2	The antibody titer in the SF is higher than that in the serum at active phase of the disease	65
		Negative	-	**1**	No increased antibody titer compared with the healthy controls	64
	Synovial expression of Hhsp60	Positive	Unknown	**1**	Increased Hhsp60 expression in the synovium compared with non-JIA patients	60
Systemic arthritis	T cell response	Negative	-	**1**	No increased proliferative response of PBMCs compared with healthy controls	67

JIA classification *	Experimental parameter of interest	Experimental evidence	Association with the disease activity	Notable observations		Ref
JIA	Antibody response	Positive	Unknown	**1**	High positivity of the specific antibody could be detected in the sera by ELISA and WB	64
		Negative	-	**1**	No increased antibody titer compared with the healthy controls	64, 65

* The International League of Associations for Rheumatology (ILAR) classification for JIA

Abbreviations: ELISA: enzyme-linked immunosorbent assay; Hhsp60: heat-shock protein 60; JIA: juvenile idiopathic arthritis; PBMCs: peripheral blood mononuclear cells; RF: rheumatoid factor; SF: synovial fluid; SFMCs: synovial fluid mononuclear cells; WB: Western blotting.