

## Review

# Heat shock proteins and their immunomodulatory role in inflammatory arthritis

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## Abstract

Autoimmune diseases, including inflammatory arthritis, are characterized by a loss of self-tolerance, leading to an excessive immune responses and subsequent ongoing inflammation. Current therapies are focused on dampening this inflammation, but a permanent state of tolerance is seldom achieved. Therefore, novel therapies that restore and maintain tolerance are needed. Tregs could be a potential target to achieve permanent immunotolerance. Activation of Tregs can be accomplished when they recognize and bind their specific antigens. HSPs are proteins present in all cells and are upregulated during inflammation. These proteins are immunogenic and can be recognized by Tregs. Several studies in animal models and in human clinical trials have shown the immunoregulatory effects of HSPs and their protective effects in inflammatory arthritis. In this review, an overview is presented of the immunomodulatory effects of several members of the HSP family in general and in inflammatory arthritis. These effects can be attributed to the activation of Tregs through cellular interactions within the immune system. The effect of HSP-specific therapies in patients with inflammatory arthritis should be explored further, especially with regard to long-term efficacy and safety and their use in combination with current therapeutic approaches.

**Key words:** heat shock proteins, inflammatory arthritis, regulatory T cells, rheumatoid arthritis, juvenile idiopathic arthritis

### Rheumatology key messages

- HSPs are upregulated at sites of inflammation, i.e. in synovial tissue, in patients with RA and JIA.
- Targeting Tregs to achieve tolerance could be a potential strategy in patients with inflammatory arthritis.
- Immunization with HSPs leads to control of inflammatory arthritis by activating Tregs.

## Introduction

RA and JIA are characterized by persistent inflammatory synovitis, resulting in destruction of joint cartilage and bone, functional disability, decreased quality of life and cardiovascular disease [1, 2]. The fundamental problem in autoimmune diseases, such as RA and JIA, is faulty regulation of the inflammatory process. The immune system of healthy people is populated with T cells and B cells expressing receptors that can bind self-antigens. Chronic activation of these self-reactive lymphocytes results in chronic inflammation. Failure of the immune system to downregulate these potentially dangerous cells leads to autoimmune diseases. Therefore, a rational

therapeutic goal in diseases of unregulated inflammation is to re-establish physiological regulation. Competing targeted therapeutics, such as anti-CD3, CTLA4-Ig, anti-TNF- $\alpha$  or B cell-depleting antibodies, are oriented towards suppression of the inflammatory response. Therefore they are less safe, more costly in side effects and do not address the fundamental problem, a disturbed immune tolerance.

HSPs have been increasingly studied in inflammatory arthritis models and could be a potential new therapeutic target, as they appear to be involved in immune tolerance in a physiological manner. It seems as if the immune system is poised towards HSP recognition, with a natural inclination towards control of inflammatory responses. In this review we will summarize the immunoregulatory function of HSPs and their role in diseases like RA and JIA. We will also discuss how these properties can be translated into new therapies and summarize the trials that have already been performed in this field.

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## HSPs and immune regulation

In systemic autoimmune diseases, immunological self-tolerance is disturbed. This results in chronic inflammatory responses directed towards self-tissues. Several cells and parts of the immune system are thought to play a role in the maintenance of self-tolerance and a balanced immune response.

Roughly 20 years ago it was discovered that so-called Tregs are critical for the development of immunological tolerance. Tregs are part of our adaptive immune system, as they are a subset of specialized CD4<sup>+</sup> Th cells defined by the expression of the IL-2 receptor  $\alpha$ -chain (CD25) and the transcription factor FoxP3 [3]. It is thought that in healthy organisms these cells control self-reactive or exaggerated immune responses. Impaired Treg function has been identified in diseases like RA and diabetes mellitus type 1 [4].

Treg activation is antigen dependent. Activation is established when an antigen bound in the antigen binding cleft of an MHC class II molecule, presented by an antigen-presenting cell (APC), binds to the T cell receptor, in conjunction with appropriate co-stimulatory molecules. When activated by their specific antigen, Tregs produce anti-inflammatory cytokines or start other mechanisms that lead to inactivation of the effector cells, leading to inhibition of the inflammatory reaction [5–7].

Tregs are important in the control of autoimmunity [8]. Targeting these cells is an important new focus of immunotherapeutic approaches in autoimmune diseases. Since Tregs are antigen specific, there is good reason for the use of their specific antigens for triggering their actions. Previous research has indicated that HSPs can serve as an antigen. Several family members of the HSPs form self-antigens that can activate disease-suppressive Tregs. We will further introduce this family of proteins and summarize their role in immune responses, including their interaction with Tregs.

HSPs are housekeeping proteins found in all living organisms. They are highly conserved proteins with high homology between mammalian and microbial HSPs. The family members are classified according to their molecular weight (e.g. HSP60 is 60 kDa). Major HSP families are HSP40, HSP60, HSP70, HSP90, HSP100 and the small HSP family (20–25 kDa). They function as intracellular chaperones involved in several intracellular processes. Their main role is protein folding. Furthermore, they are associated with peptide binding to MHC molecules, protein synthesis and transport. HSP10 and HSP40 function as co-chaperones for HSP60 and HSP70, respectively [9, 10]. The HSP70 family is large and contains at least eight chaperone proteins. Most eukaryotes have several different HSP70 proteins, found in a variety of cellular compartments. For instance, binding immunoglobulin protein (BiP) can be found in the endoplasmic reticulum, while HSC70 is found in the cytoplasm [11, 12]. An overview of the mentioned HSP members is provided in Table 1. HSPs are ubiquitously expressed and are upregulated during inflammation, necrosis and exposure to heat stress or toxic treatments [13, 14]. Several studies have shown

that these proteins are immunogenic and can be recognized by cells of both the innate and adaptive immune systems [15].

As HSPs are self-antigens present in our own cells, it is remarkable that they are recognized by our immune system and generate immunological reactions. It seems as if the immune system has a vested interest in responding to these proteins. A possible explanation is that proteins like HSPs have a signal function, alarming the immune system when tissues are stressed due to infection or inflammation [17]. There is evidence that HSPs can activate the innate immune system by binding as pathogen-associated molecular patterns to certain Toll-like receptors (TLRs), which are present on dendritic cells and macrophages. HSP60 activates Tregs via TLR2 [18]. Extracellular HSP70 can regulate innate immune cell function, binding to cell surface receptors through TLR2 and TLR4 to induce IL-10 production [19, 20].

Given the fact that these cells are APCs, the adaptive immune system can be activated in this way as well. However, HSP60 and HSP70, the most well-studied HSPs, have also been found in the circulation of healthy individuals [21]. The seemingly contradictory nature of some of the effects of HSPs on the immune system is still open for further studies. Accumulating findings, however, indicate that HSPs have potential for the attenuation or regulation of inflammatory responses and can be seen as key players involved in the homeostasis that controls autoimmunity [22–26]. In support of that possibility are the more recent findings on anti-inflammatory responses, by activating Tregs and increasing IL-10 release [10, 18, 27–29].

The mechanisms of Treg activation by several HSP members have been investigated in *in vitro* studies and animal models. Many experiments with regard to HSPs have been performed with microbial HSPs. It is known that because HSPs are highly conserved molecules, the molecular structure of mammalian (human) and microbial HSPs is very similar. At the level of the adaptive immune system, this can lead to cross-reactivity. Thus this cross-reactivity induced by exposure to microbial HSPs during an infection or in an artificial way (e.g. immunization) can lead to activation of self-HSP-reactive T cells and subsequently to immunoregulatory activities, as already mentioned [30–32].

In conclusion, several members of the HSP family are able to regulate immune responses and activate Tregs via both the innate and adaptive immune systems.

## HSPs in inflammatory arthritis

Until now, the exact role of HSPs in autoimmune diseases like inflammatory arthritis has not been clearly defined. In earlier studies, both pro-inflammatory and anti-inflammatory effects induced by several HSP families were described [33]. A likely explanation for this is that studies performed with HSPs that produced pro-inflammatory effects were performed with recombinant HSPs produced in bacteria. Bacterial contaminants are thought to be responsible for the described pro-inflammatory effects. In the

TABLE 1 The HSP families

HSP family	Relevant family members	Alternative names	Common microbial homologues	Cellular location	Functions	References
HSP10	HSP10	CPN10, HSPE1	GroES ( <i>Escherichia coli</i> )	Mitochondrion	Co-chaperone HSP60 activities	[10]
HSP40	dnaJA dnaJB dnaJC	HDJ2, HSDJ HSC40 CSP	dnaJ ( <i>E. coli</i> )	Cytosol	Co-chaperone HSP70 activities	[10, 16]
HSP60 HSP65	HSP60 CPN60		GroEL ( <i>E. coli</i> )	Mitochondrion	Refolding and assembly of protein structures	[10]
HSP70	HSP70-1 HSC70 BiP mHSP70	HSPA1A HSPA8, HSP73 HSPA5, GRP78 HSPA9, GRP75	dnaK ( <i>E. coli</i> )	Cytosol/nucleus Cytosol/nucleus ER Mitochondrion	Refolding and transport through sub-cellular organelle membranes	[10, 16]
HSP90	HSPC4	GRP94	htpG	Cytosol	Maturation and stabilization of cellular proteins involved in signal transduction and transcriptional regulation	[16]

BiP, binding immunoglobulin protein; CPN, chaperonin; CSP, cysteine string protein; ER, endoplasmic reticulum; GRP, glucose-regulated protein; HDJ, DNA homologue; HCS, heat shock cognate protein; htpG, high-temperature protein G; mHSP70, mitochondrial HSP70.

absence of foreign pathogen-associated molecular patterns, using purified bacterial HSPs, there is often no pro-inflammatory effector response [34–37]. Previously we discussed some of the controversies related to the possible function of HSPs as damage-associated molecular patterns [38, 39].

In arthritis, inflammation in the joint leads to cellular stress and upregulation of self-HSPs in the synovial tissue. Several studies have shown a significantly higher percentage of cells featuring HSP60 and HSP70 membrane expression among fibroblast-like synovial cells derived from arthritis-affected joints in RA and JIA patients compared with those obtained from healthy controls [40–42]. Two studies investigating HSP70 in RA patients show that self-HSP70 was released from fibroblast-like synovial cells in response to TNF- $\alpha$ , which functions as an inflammatory stressor. HSP70 induced IL-10 production in these fibroblasts via TLR4 and downregulated IL-6 and IL-8 via several signal transduction pathways [43, 44]. Furthermore, it seems that T cells from the SF and peripheral blood leucocytes of patients with JIA or RA proliferate in response to mycobacterial HSP60 and HSP70 [45]. In patients with JIA, cells had substantial proliferative responses to self-HSP60, mycobacterial HSP60 and other HSPs, including HSP dnaJ (HSP40) [46–48].

Another study showed that serum concentrations of soluble HSP60 were increased in both active and inactive oligo- and polyarticular disease in JIA patients. Interestingly, the serum concentrations correlated positively with the time required for remission from flare-ups [49]. This might imply that the presence of high levels of soluble HSP60 reflects a high degree of inflammation or

tissue damage and could have prognostic value. Although this might suggest a possible role of soluble protein in the pathogenic mechanism of the disease, studies have shown that patients with JIA developing immune responses to human or mycobacterial HSP60 or human HSP40 had a favourable prognosis. In oligoarticular JIA, remission was associated with a significant T lymphocyte proliferative response to human HSP60 [50, 51]. In a subsequent study, it was shown that T cells recognizing self-HSP60, isolated from JIA patients, express CD25 and CD30 and produce regulatory cytokines like IL-4 and IL-10 [52]. These results suggest that T lymphocyte reactivity to HSP60 may be part of the T cell regulatory mechanisms that control the development of arthritis.

In studies with RA patients, similar observations were made. With regard to HSP60, we found that SF T cells reactive to human HSP60 produced more IL-4 and less IFN- $\gamma$  when stimulated with bacterial HSP60 [53]. In studies with human and bacterial HSP40, it was found that these proteins inhibited CD4<sup>+</sup> and CD8<sup>+</sup> T cells and stimulated the secretion of IL-10. Also, HSP40 reduced TNF- $\alpha$  secretion [54, 55]. Concerning HSP70, potent disease-suppressive Tregs recognizing self-HSP70 have been found. In a recent study by van Herwijnen *et al.* [56] it was revealed that antigen-specific Tregs bind the conserved HSP70 epitope 141–155 of mycobacterial HSP70 (B29) in mice and that the endogenous HSP70 homologs activate these cells *in vivo*. It was shown that this conserved HSP70 epitope (B29) could naturally be bound by murine MHC class II. In a subsequent study, this B29 epitope showed a high binding affinity of all homologous peptides for the RA-associated HLA-DR4 and

HLA-DQ8 molecules [57]. Upon transfer, B29-induced CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T cells suppressed established proteoglycan-induced arthritis in mice. When using lymphocyte activation gene-3 as a selection marker, as few as 4000 cells sufficed to fully suppress arthritis. Furthermore, antibody-mediated *in vivo* depletion of transferred Tregs abrogated disease suppression. Transferred cells exhibited a stable phenotype and were found in joints and draining lymph nodes up to 2 months after transfer [56]. Another member of the HSP70 family, which deserves special attention, is BiP. This stress protein has been investigated for its immunomodulatory properties in patients with RA. In earlier studies, autoantibodies to BiP were found in the majority of RA patients [58]. Similar to other HSPs, BiP is overexpressed in synovial tissue of patients with RA. In addition, some findings in relation to BiP have been contradictory, as there were studies that found BiP stimulated T effector cells and therefore has a role in RA pathogenesis [59, 60]. Not much is known about the change in serum HSP levels during the course of disease. Only one small study reported a difference in serum levels of HSP70 in patients with RA in high and low disease activity [61].

It seems that by recognizing self-HSPs in the inflamed joint, Tregs are activated, producing immunomodulating cytokines, which may diminish inflammation and thus local joint damage (Fig. 1). This makes immunotherapy with HSPs a potential new strategy for Treg-promoting interventions.

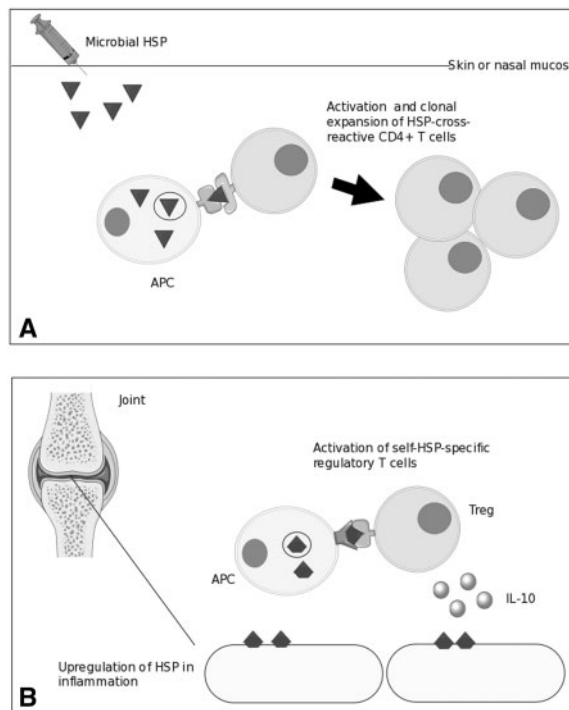
### HSP as a therapeutic strategy in arthritis

The administration of several HSP family members, HSP-derived peptides or altered peptide ligands has been studied in relation to arthritis therapy, both in animals or *in vitro* using cells of JIA and RA patients, and some were even tested *in vivo* in clinical trials for RA (Tables 2 and 3). We will now summarize the results of these studies.

*In vitro* studies with cells from RA patients show a decrease in pro-inflammatory and an increase in anti-inflammatory cytokine production when peripheral blood mononuclear cells (PBMCs) were stimulated with HSP60 [89]. Remarkably, in most cases this effect was not seen in healthy controls.

Another *in vitro* study tested altered peptide ligands derived from a CD4<sup>+</sup> T cell epitope of human HSP60 (APL-1) on PBMCs, which showed an immunomodulating effect [90]. APL-2 showed similar results in PBMCs from RA patients [73]. APLs are peptides similar to the original epitopes but with alterations in the contact positions with the T cell receptor necessary for activation of these cells [92]. This results in different phenotypes of T cell activation. The effect of APLs on T cells may range from partial activation to complete turn-off of their functional capacity (anergy). As was also demonstrated in models of arthritis, the effects of APL, including HSP60-based APL, can be a combination of competition for MHC binding or T cell inhibition, but also induction of immune regulation, since APL immunization sometimes led to prevention of subsequently induced disease [93].

**Fig. 1** HSP-based vaccines enhance regulatory activity through expansion of HSP-cross-reactive T cells and activation of self-HSP-specific Tregs



(A) Immunization with microbial HSP. (B) Immunoregulatory response at the site of inflammation after immunization.

In studies with adjuvant-induced arthritis (AIA) in rats, it was recognized that priming of self-HSP60-directed T cells led to protection against arthritis instead of disease induction or aggravation. Furthermore, immunization of rats with synthetic peptides derived from human or micro-biological HSP60 (which are cross-reactive) led to protection against AIA, suppression of pro-inflammatory cytokines and an increase of anti-inflammatory cytokines [74, 89]. The effects of a combination therapy with HSP60 and a low-dose anti-TNF- $\alpha$  were investigated as well. A single low dose of anti-TNF- $\alpha$ , which in itself did not suppress disease, was combined with intranasal HSP60 peptide from heat-killed *Mycobacterium tuberculosis* in AIA rats and was therapeutically effective [77]. Similar therapeutic effects were accomplished with administration of mycobacterial or self-HSP70 in several experimental disease models [29, 83, 84, 86, 98, 99]. Some of these effects were thought to be facilitated by HSP modulation of the APCs, which influences the nature of the cytokine response and supports the anti-inflammatory role of this protein [99].

BiP, an HSP70 family member, was studied *in vitro* in PBMCs of patients with RA. The cells were found to proliferate and produce mostly pro-inflammatory mediators such as IL-1 when stimulated with BiP. On the other hand, incubation of monocytes with BiP downregulated

TABLE 2 Overview of experimental inflammatory arthritis trials with HSPs

Type of study	Type of HSP product	Disease/model	Route	Effect	References
Animal model	HSP60 or derived peptide	AIA (rat) or PIA (mice)	i.p.	Protection against induced arthritis. No exacerbation of arthritis. Less severe arthritis Suppression of IL-17, enhanced production of anti-mycobacterial HSP65 antibodies	[30, 62–67]
Animal model	HSP60 or derived peptide	AIA rat	i.c.	Protection against induced arthritis	[28, 68, 69]
Animal model	HSP60	AIA rat or CIA mouse	Oral	Less severe arthritis	[70–72]
Animal model	HSP60 or derived peptide in combination with low-dose TNF- $\alpha$ therapy	AIA rat	i.n. or s.c.	Inhibition of arthritis for certain epitopes Inhibition of arthritis Inhibition of arthritis with microbial HSP60, but not with self-HSP60	[73–78]
Animal model	DNA-HSP60 and DNA-HSP65	AIA rat PIA mice	i.m.	Inhibition and prevention of arthritis	[79–81]
Animal model	HSP10	AIA rat	i.c.	Less severe arthritis, delay onset arthritis	[82]
Animal model	HSP70	AIA, aviridine, CIA rat	s.c.	Less severe arthritis, protection against arthritis	[83, 84]
Animal model	HSP70-derived peptide	AIA rat	i.n.	Inhibition of arthritis	[98]
Animal model	HSP70-derived peptide	AIA rat	i.c.	Suppression of arthritis, higher IL-10 production	[86]
Animal model	HSP70 and HSP90	AIA rat	i.m.	Inhibition arthritis	[85]
Animal model	BiP	CIA mice	Oral	Less severe arthritis and decreased T cell proliferation, increased secretion of IL-10, increased number of Tregs	[88]
Animal model	BiP	CIA or PIA in mice and Lewis rats	i.v.	Inhibition of arthritis, prevention of arthritis	[87]
<i>In vitro</i> model	HSP65 and APL-1	PBMCs from RA patients		No effect on healthy controls. Inhibition of PBMCs from RA patients, less production of TNF- $\alpha$ and IFN- $\gamma$ . Increased secretion of IL-4 Induction Tregs and apoptosis in activated CD4 <sup>+</sup> T cells	[89, 90]
<i>In vitro</i> model	APL-2	PBMCs from JIA patients		Increased IL-10 level without affecting IFN- $\gamma$ and TNF- $\alpha$ levels	[73]
<i>In vitro</i> model	Exogenous recombinant human BiP	PBMCs from RA patients		Secretion of anti-inflammatory cytokines	[91]

CIA: collagen-induced arthritis; i.c.: intracutaneous; i.n.: intranasal; i.p.: intraperitoneal; PIA: pristane-induced arthritis.

CD86 and HLA-DR expression. Similar responses were found in a study using RA synovial membrane transplants in severe combined immunodeficiency mice [91, 100]. The underlying mechanism for the latter effect seems to be based on the activation of tolerogenic dendritic cells (tolDCs) and subsequent generation of Tregs [101].

Similar versatile effects of BiP occurred in a recent study, which showed that effector and Treg cells recognize different BiP epitopes. *In vitro*, two BiP epitopes were tested on PBMCs of RA patients. One epitope (BiP 336–355) led to strong proliferation of the PBMCs, correlating with disease activity, while the other epitope (BiP

**TABLE 3** Overview of clinical trials in inflammatory arthritis with HSPs

Type of HSP/ product	Patients	Administration	Effect	References
dnaJ dnaJP1	RA, n = 15	Oral, 6 months	Increased production of IL-4 and IL-10. Decreased T cell proliferation, and production of IL-2, IFN- $\gamma$ and TNF- $\alpha$	[94]
dnaJ dnaJP1	RA, n = 160	Oral, 6 months	Clinical response, ACR20. Fewer T cells producing TNF- $\alpha$	[95]
Chaperonin 10	RA, n = 23	Intravenous, 12 weeks	Clinical improvement of disease activity	[96]

456–475) had an anti-inflammatory effect. This last epitope was further examined by oral administration in mice with CIA. A reduction in T cell proliferation and arthritis was found in this group, as well as increased secretion of IL-10 from T cells [88].

We described the immunomodulating effects of different HSP members in arthritis animal models or in *in vitro* studies with cells from patients with RA or JIA. These proteins could be a potential therapeutic target for tolerance-promoting interventions in rheumatic diseases. Given the physiological role of HSPs in healthy immune regulation, the effects of HSPs on inflammation reported in several studies could be a manifestation of a state of permanent immunotolerance rather than temporary immunological control. An attractive benefit of targeting HSP-specific Tregs is that effects are guided towards sites of inflammation where HSPs are expressed and thus induction of systemic immunosuppression may be avoided.

### Clinical trials with HSP-based immunotherapy

Until now, only a few clinical trials with HSPs have been reported. The first HSP tested in the clinic in a phase I trial was with dnaJP1 in 15 patients with early RA. Mucosal administration of dnaJP1 during 6 months led to a shift in T cells from a pro-inflammatory to a regulatory phenotype. Three different dosages were tested: 0.25, 2.5 and 25 mg/day. There were no important side effects reported [94]. Koffeman *et al.* [95] performed a placebo-controlled phase II/III trial with dnaJP1 in 160 RA patients. After daily oral administration with 25 mg over 6 months, a trend towards clinical improvement was seen. The treatment was reported as safe and well tolerated. The only side effect reported was a self-remitting leukopenia in six patients. The short-term effects of intravenous administration of HSP10 were investigated in a randomized, double-blind, multicentre study. In this study with 23 treatment-resistant RA patients, chaperonin 10 was given twice weekly for 12 weeks at doses of 5, 7.5 or 10 mg. Disease activity scores improved significantly during the 12 weeks of therapy in the high-dose group. Most adverse events were reported to be mild or moderate; seven patients recorded an RA flare and recurrent upper

respiratory tract infections were common. One patient had severe myalgia [96].

Currently a first phase I/IIa clinical trial with BiP in RA patients is being carried out and the results will be made public soon (Corrigall V, personal communication). But more trials are needed, especially with regard to long-term efficacy and safety and use in combination with current therapeutic approaches.

### Targeting Tregs in inflammatory arthritis and Treg resistance

A point of concern about the expected efficacy of tolerance induction therapy by targeting Tregs in the clinical practice is the fact that these cells may have altered functions in patients with inflammatory arthritis. Besides the disturbed activation of T effector cells and pro-inflammatory stimuli, several studies point out that Treg function is impaired and that they are unable to suppress pro-inflammatory cytokine production [1, 4, 102]. One underlying mechanism that explains the altered functioning of Tregs in inflammatory arthritis involves the canonical Wnt pathway. T cell factor 1 is a Foxp3 interaction partner and modulates transcriptional activity of Foxp3. T cell factor 1 is regulated by canonical Wnt signalling. Activation of the Wnt pathway reduced Treg-mediated suppression in *in vitro* and *in vivo* experiments. Activation of effector T cells increased Wnt3a production and Wnt3a levels were found to be elevated in mononuclear cells isolated from the SF of arthritis patients. Therefore, Wnt signalling directly modulates FoxP3 activity and thereby Treg cell function [103]. Furthermore, the inflammatory environment may lead to Treg dysfunction in these patients [104]. Altered Treg functions caused by pro-inflammatory stimuli might indicate the need for a pretreatment or a combination of medication to optimize the effective targeting of Tregs, as we will discuss below.

### Considerations for therapy in the clinic

In general, long-lasting peripheral immune tolerance could be established by repetitive administration of a specific self-antigen. Although such a specific disease-inducing antigen is not known for diseases such as RA and JIA, the studies discussed in this review suggest that several

HSP members could be used as antigens to induce such an effect. In fact, since this immune response is achieved by activating elements of a self-reactive repertoire of T cells that is part of the adaptive immune system, immunological memory could be established, similar to what happens in vaccination. Given the versatile effects of whole protein, one should consider the use of selected peptides derived from the whole proteins to reach a more specific immunomodulation. Several studies have indicated how a translational path from animal models to the clinic has led to the proper selection of peptides for human application [105–107].

The route of administration to induce immune tolerance with HSP is a point of debate. To our knowledge no studies have been performed investigating the superiority of oral administration to other routes. Theoretically, mucosal delivery would be a more appropriate way because it mimics the natural routes of tolerance induction [108]. Furthermore, mucosal administration of antigen might be a safer route compared with subcutaneous delivery since subcutaneous administration of antigens in general could lead to hypersensitivity reactions [109].

A recent addition to the immunotherapeutic armamentarium directed at restoring immune tolerance in RA is treatment with autologous tIDCs. Their therapeutic potential has been demonstrated in experimental animal models of autoimmune disease, including RA [110–112]. Results from the first clinical trials were published recently. In a small, phase I, open-label trial in patients with RA, intradermal administration of autologous modified DCs exposed to citrullinated peptides appeared to be safe and biologically active [113]. Another phase I trial in RA patients, the results of which have not yet been published, is using tIDCs loaded with autologous SF [114].

With regard to the choice and source of the antigen used to modify DCs to target autoantigen-specific T cells, several remarks can be made. As mentioned before, no universal autoantigen has been identified in RA. In both studies mentioned above, similar to HSPs, the target antigens are specific for the inflammation itself and mainly present at the site of inflammation (the synovium). However, when using citrullinated peptides as an autoantigen, only RA patients with ACPAs are qualified to receive this treatment. Using autologous SF might cover a broad spectrum of antigens, but has the disadvantage that monitoring of the immunological effect with specific antigens would be challenging. Furthermore, SF is not available from early RA cases, the group that would qualify best for tolerance-promoting interventions. Here, HSP would offer obvious advantages, including the possible standardized production of its derivative peptides.

HSP treatments in inflammatory diseases may potentially be safe and effective since their recognition as modulators of inflammation is built into the immune system—HSP treatment is not an artificial immune processor but a natural regulator selected by evolution. Moreover, the immune system is primed to respond to HSP molecules and their epitopes by combined adaptive and innate receptor systems; the adaptive and innate

effects are synergistic for enhanced recognition. It may well be that we are seeing the beginning of a very novel approach of immunomodulatory vaccination in autoimmunity, which may lead to novel treatments as well prophylactic modalities. More clinical trials will be necessary to reveal further aspects, such as long-term efficacy and safety, as well as to examine the optimal dose, route and time point of administration and possible combination with other immunosuppressive or immunomodulatory agents.

## Conclusion

In this review we provided an overview of the immunomodulatory effects of several members of the HSP family in general and in inflammatory arthritis. These effects can be attributed to the activation of Tregs. HSPs can induce an immunomodulatory effect *in vitro* in PBMCs from patients with RA and JIA and *in vivo* in experimental animal models with AIA. There are a few clinical trials that show some first promising effects of HSP administration in patients with RA. The effect of HSP-specific therapies should be explored further, especially with regard to long-term efficacy and safety and their use in combination with current therapeutic approaches.

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## References

- 1 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011;365:2205–19.
- 2 Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003;423:356–61.
- 3 Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3<sup>+</sup> regulatory T cells in the human immune system. *Nat Rev Immunol* 2010;10:490–500.
- 4 Lawson C. a, Brown a. K, Bejarano V *et al.* Early rheumatoid arthritis is associated with a deficit in the CD4<sup>+</sup>CD25<sup>high</sup> regulatory T cell population in peripheral blood. *Rheumatology* 2006;45:1210–7.
- 5 Tang Q, Bluestone JA. The Foxp3<sup>+</sup> regulatory T cell: a jack of all trades, master of regulation. *Nat Immunol* 2008;9:239–44.
- 6 Zhang ZX, Yang L, Young KJ, DuTemple B, Zhang L. Identification of a previously unknown antigen-specific

- regulatory T cell and its mechanism of suppression. *Nat Med* 2000;6:782–9.
- 7 Sakaguchi S. Naturally arising CD4<sup>+</sup> regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004;22:531–62.
  - 8 Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol Rev* 2008;223:371–90.
  - 9 Fink AL. Chaperone-mediated protein folding. *Physiol Rev* 1999;79:425–49.
  - 10 van Eden W, van der Zee R, Prakken B. Heat-shock proteins induce T-cell regulation of chronic inflammation. *Nat Rev Immunol* 2005;5:318–30.
  - 11 Daugaard M, Rohde M, Jäättelä M. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett* 2007;581:3702–10.
  - 12 Todryk SM, Gough MJ, Pockley AG. Facets of heat shock protein 70 show immunotherapeutic potential. *Immunology*. 2003;110:1–9.
  - 13 Calderwood SK, Mambula SS, Gray PJ, Theriault JR. Extracellular heat shock proteins in cell signaling. *FEBS Lett* 2007;581:3689–94.
  - 14 Todryk S, Melcher A, Hardwick N *et al*. Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. *J Immunol* 1999;163:1398–408.
  - 15 Chen W, Syldath U, Bellmann K, Burkart V, Kolb H. Human 60-kDa heat-shock protein: a danger signal to the innate immune system. *J Immunol* 1999;162:3212–9.
  - 16 Kampinga HH, Hageman J, Vos MJ *et al*. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* 2009;14:105–11.
  - 17 Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994;12:991–1045.
  - 18 Zanin-Zhorov A, Cahalon L, Tal G *et al*. Heat shock protein 60 enhances CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cell function via innate TLR2 signaling. *J Clin Invest* 2006;116:2022–32.
  - 19 Borges TJ, Wieten L, Van Herwijnen MJC *et al*. The anti-inflammatory mechanisms of Hsp70. *Front Immunol* 2012;3:1–12.
  - 20 Asea A, Rehli M, Kabingu E *et al*. Novel signal transduction pathway utilized by extracellular HSP70. Role of Toll-like receptor (TLR) 2 and TLR4. *J Biol Chem* 2002;277:15028–34.
  - 21 Pockley AG, Muthana M, Calderwood SK. The dual immunoregulatory roles of stress proteins. *Trends Biochem Sci* 2008;33:71–9.
  - 22 Wieten L, Broere F, van der Zee R *et al*. Cell stress induced HSP are targets of regulatory T cells: a role for HSP inducing compounds as anti-inflammatory immunomodulators? *FEBS Lett* 2007;581:3716–22.
  - 23 Van Eden W, Van Herwijnen M, Wagenaar J *et al*. Stress proteins are used by the immune system for cognate interactions with anti-inflammatory regulatory T cells. *FEBS Lett* 2013;587:1951–8.
  - 24 Cohen IR, Lohse AW. Physiology and pathophysiology of autoimmunity. *Semin Liver Dis* 1991;11:183–6.
  - 25 Cohen IR. Autoimmunity to hsp65 and the immunologic paradigm. *Adv Intern Med* 1992;37:295–311.
  - 26 Coelho V, Faria AMC. HSP60: issues and insights on its therapeutic use as an immunoregulatory agent. *Front Immunol* 2012;2:97.
  - 27 Breloer M, Moré SH, Osterloh A *et al*. Macrophages as main inducers of IFN- $\gamma$  in T cells following administration of human and mouse heat shock protein 60. *Int Immunol* 2002;14:1247–53.
  - 28 Anderton SM, van der Zee R, Prakken B, Noordzij A, van Eden W. Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. *J Exp Med* 1995;181:943–52.
  - 29 Spiering R, Van Der Zee R, Wagenaar J, Van Eden W, Broere F. Mycobacterial and mouse HSP70 have immunomodulatory effects on dendritic cells. *Cell Stress Chaperones* 2013;18:439–46.
  - 30 van Eden W, Thole JE, van der Zee R *et al*. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* 1988;331:171–3.
  - 31 van Eden W, Wendling U, Paul L *et al*. Arthritis protective regulatory potential of self-heat shock protein cross-reactive T cells. *Cell Stress Chaperones* 2000;5:452–7.
  - 32 Kaufmann SH. Heat shock proteins and the immune response. *Immunol Today* 1990;11:129–36.
  - 33 Celis L, Vandevyver C, Geusens P *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;40:510–9.
  - 34 Bausinger H, Lipsker D, Ziylan U *et al*. Endotoxin-free heat-shock protein 70 fails to induce APC activation. *Eur J Immunol* 2002;32:3708–13.
  - 35 Wallin RPA, Lundqvist A, Moré SH *et al*. Heat-shock proteins as activators of the innate immune system. *Trends Immunol* 2002;23:130–5.
  - 36 Bendz H, Marincek BC, Momburg F *et al*. Calcium signaling in dendritic cells by human or mycobacterial Hsp70 is caused by contamination and is not required for Hsp70-mediated enhancement of cross-presentation. *J Biol Chem* 2008;283:26477–83.
  - 37 Stocki P, Wang XN, Dickinson AM. Inducible heat shock protein 70 reduces T cell responses and stimulatory capacity of monocyte-derived dendritic cells. *J Biol Chem* 2012;287:12387–94.
  - 38 Broere F, van der Zee R, van Eden W. Heat shock proteins are no DAMPs, rather 'DAMPERS'. *Nat Rev Immunol* 2011;11:565;author reply 565.
  - 39 Van Eden W, Spiering R, Broere F, Van Der Zee R. A case of mistaken identity: HSPs are no DAMPs but DAMPERS. *Cell Stress Chaperones* 2012;17:281–92.
  - 40 Nguyen TTH, Gehrmann M, Zlacka D *et al*. Heat shock protein 70 membrane expression on fibroblast-like synovial cells derived from synovial tissue of patients with rheumatoid and juvenile idiopathic arthritis. *Scand J Rheumatol* 2006;35:447–53.
  - 41 Boog CJ, de Graeff-Meeder ER, Lucassen MA *et al*. Two monoclonal antibodies generated against human hsp60 show reactivity with synovial membranes of patients with juvenile chronic arthritis. *J Exp Med* 1992;175:1805.
  - 42 Sedlackova L, Nguyen TTH, Zlacka D, Sosna A, Hromadnikova I. Cell surface and relative mRNA



- expression of heat shock protein 70 in human synovial cells. *Autoimmunity* 2009;42:17–24.
- 43 Luo X, Zuo X, Zhang B *et al.* Release of heat shock protein 70 and the effects of extracellular heat shock protein 70 on the production of IL-10 in fibroblast-like synoviocytes. *Cell Stress Chaperones* 2008;13:365–73.
- 44 Luo X, Zuo X, Zhou Y *et al.* Extracellular heat shock protein 70 inhibits tumour necrosis factor- $\alpha$  induced proinflammatory mediator production in fibroblast-like synoviocytes. *Arthritis Res Ther* 2008;10:R41.
- 45 Sedlackova L, Sedlacek P, Hromadnikova I. Peripheral blood mononuclear cell responses to heat shock proteins in patients undergoing stem cell transplantation. *Pediatr Transplant* 2006;10:178–86.
- 46 Albani S, Ravelli A, Massa M *et al.* Immune responses to the *Escherichia coli* dnaJ heat shock protein in juvenile rheumatoid arthritis and their correlation with disease activity. *J Pediatr* 1994;124:561–5.
- 47 Life P, Hassell A, Williams K *et al.* Responses to gram negative enteric bacterial antigens by synovial T cells from patients with juvenile chronic arthritis: recognition of heat shock protein HSP60. *J Rheumatol* 1993;20:1388–96.
- 48 Danielli MG, Markovits D, Gabrielli A *et al.* Juvenile rheumatoid arthritis patients manifest immune reactivity to the mycobacterial 65-kDa heat shock protein, to its 180–188 peptide, and to a partially homologous peptide of the proteoglycan link protein. *Clin Immunol Immunopathol* 1992;64:121–8.
- 49 Wu CTC, Ou LS, Yeh KW, Lee WI, Huang JL. Serum heat shock protein 60 can predict remission of flare-up in juvenile idiopathic arthritis. *Clin Rheumatol* 2011;30:959–65.
- 50 Prakken ABJ, Van Eden W, Rijkers GT *et al.* Autoreactivity to human heat-shock protein 60 predicts disease remission in oligoarticular juvenile rheumatoid arthritis. *Arthritis Rheum* 1996;39:1826–32.
- 51 Massa M, Passalia M, Manzoni SM *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;56:1648–57.
- 52 De Kleer IM, Kamphuis SM, Rijkers GT *et al.* The spontaneous remission of juvenile idiopathic arthritis is characterized by CD30<sup>+</sup> T cells directed to human heat-shock protein 60 capable of producing the regulatory cytokine interleukin-10. *Arthritis Rheum* 2003;48:2001–10.
- 53 van Roon JA, van Eden W, van Roy JL, Lafeber FJ, Bijlsma JW. 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;100:459–63.
- 54 Tukaj S, Kotlarz A, Jozwik A *et al.* Hsp40 proteins modulate humoral and cellular immune response in rheumatoid arthritis patients. *Cell Stress Chaperones* 2010;15:555–66.
- 55 Kotlarz A, Tukaj S, Krzewski K, Brycka E, Lipinska B. Human Hsp40 proteins, DNAJA1 and DNAJA2, as potential targets of the immune response triggered by bacterial DnaJ in rheumatoid arthritis. *Cell Stress Chaperones* 2013;18:653–9.
- 56 van Herwijnen MJC, Wieten L, van der Zee R *et al.* Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis. *Proc Natl Acad Sci USA* 2012;109:14134–9.
- 57 de Wolf C, van der Zee R, den Braber I *et al.* An arthritis suppressive and regulatory T cell-inducing CD4<sup>+</sup> T cell epitope is functional in the context of HLA-restricted T cell responses. *Arthritis Rheumatol* 2016;68:639–47.
- 58 Shoda H, Fujio K, Shibuya M *et al.* Detection of autoantibodies to citrullinated BiP in rheumatoid arthritis patients and pro-inflammatory role of citrullinated BiP in collagen-induced arthritis. *Arthritis Res Ther* 2011;13:R191.
- 59 Bläß S, Union A, Raymackers J *et al.* The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. *Arthritis Rheum* 2001;44:761–71.
- 60 Bodman-Smith MD, Corrigan VM, Kemeny DM, Panayi GS. BiP, a putative autoantigen in rheumatoid arthritis, stimulates IL-10-producing CD8-positive T cells from normal individuals. *Rheumatology* 2003;42:637–44.
- 61 Najafizadeh SR, Ghazizadeh Z, Nargesi AA *et al.* Analysis of serum heat shock protein 70 (HSPA1A) concentrations for diagnosis and disease activity monitoring in patients with rheumatoid arthritis. *Cell Stress Chaperones* 2015;20:537–43.
- 62 van den Broek MF, Hogervorst EJ, Van Bruggen MC *et al.* Protection against streptococcal cell wall-induced arthritis by pretreatment with the 65-kD mycobacterial heat shock protein. *J Exp Med* 1989;170:449–66.
- 63 Thompson SJ, Francis JN, Siew LK *et al.* An immunodominant epitope from mycobacterial 65-kDa heat shock protein protects against pristane-induced arthritis. *J Immunol* 1998;160:4628–34.
- 64 Ulmansky R, Cohen CJ, Szafer F *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;168:6463–9.
- 65 Thompson SJ, Butcher PD, Patel VK *et al.* Modulation of pristane-induced arthritis by mycobacterial antigens. *Autoimmunity* 1991;11:35–43.
- 66 Satpute SR, Soukhareva N, Scott DW, Moudgil KD. Mycobacterial Hsp65-IgG-expressing tolerogenic B cells confer protection against adjuvant-induced arthritis in Lewis rats. *Arthritis Rheum* 2007;56:1490–6.
- 67 Satpute SR, Rajaiah R, Polumuri SK, Moudgil KD. Tolerization with Hsp65 induces protection against adjuvant-induced arthritis by modulating the antigen-directed interferon- $\gamma$ , interleukin-17, and antibody responses. *Arthritis Rheum* 2009;60:103–13.
- 68 Billingham ME, Carney S, Butler R, Colston MJ. A mycobacterial 65-kD heat shock protein induces antigen-specific suppression of adjuvant arthritis, but is not itself arthritogenic. *J Exp Med* 1990;171:339–44.
- 69 Moudgil KD, Chang TT, Eradat H *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;185:1307–16.
- 70 Cobelens PM, Heijnen CJ, Nieuwenhuis EES *et al.* Treatment of adjuvant-induced arthritis by oral administration of mycobacterial Hsp65 during disease. *Arthritis Rheum* 2000;43:2694–702.
- 71 Jorgensen C, Gedon E, Jaquet C, Sany J. Gastric administration of recombinant 65 kDa heat shock protein

- delays the severity of type II collagen induced arthritis in mice. *J Rheumatol* 1998;25:763-7.
- 72 Haque MA, Yoshino S, Inada S *et al*. Suppression of adjuvant arthritis in rats by induction of oral tolerance to mycobacterial 65-kDa heat shock protein. *Eur J Immunol* 1996;26:2650-6.
- 73 Lorenzo N, Barberá A, Domínguez MC *et al*. Therapeutic effect of an altered peptide ligand derived from heat-shock protein 60 by suppressing of inflammatory cytokines secretion in two animal models of rheumatoid arthritis. *Autoimmunity* 2012;45:449-59.
- 74 Shi X-L, Wang L-P, Feng X *et al*. Inhibition of adjuvant-induced arthritis by nasal administration of novel synthetic peptides from heat shock protein 65. *BMC Musculoskeletal Disord* 2014;15:253.
- 75 Prakken BJ, van der Zee R, Anderton SM *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 USA* 1997;94:3284-9.
- 76 Dominguez MDC, Lorenzo N, Barbera A *et al*. An altered peptide ligand corresponding to a novel epitope from heat-shock protein 60 induces regulatory T cells and suppresses pathogenic response in an animal model of adjuvant-induced arthritis. *Autoimmunity* 2011;44:471-82.
- 77 Roord STA, Zonneveld-Huijssoon E, Le T *et al*. Modulation of T cell function by combination epitope specific and low dose anticytokine therapy controls autoimmune arthritis. *PLoS One* 2006;1:e87.
- 78 Zonneveld-Huijssoon E, Roord STA, de Jager W *et al*. Bystander suppression of experimental arthritis by nasal administration of a heat shock protein peptide. *Ann Rheum Dis* 2011;70:2199-206.
- 79 Quintana FJ, Carmi P, Mor F, Cohen IR. Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J Immunol* 2002;169:3422-8.
- 80 Santos-Junior RR, Sartori A, De Franco M *et al*. Immunomodulation and protection induced by DNA-hsp65 vaccination in an animal model of arthritis. *Hum Gene Ther* 2005;16:1338-45.
- 81 Ragno S, Colston MJ, Lowrie DB *et al*. Protection of rats from adjuvant arthritis by immunization with naked DNA encoding for mycobacterial heat shock protein 65. *Arthritis Rheum* 1997;40:277-83.
- 82 Ragno S, Winrow VR, Mascagni P *et al*. A synthetic 10-kD heat shock protein (hsp10) from *Mycobacterium tuberculosis* modulates adjuvant arthritis. *Clin Exp Immunol* 1996;103:384-90.
- 83 Kingston AE, Hicks CA, Colston MJ, Billingham ME. A 71-kD heat shock protein (hsp) from *Mycobacterium tuberculosis* has modulatory effects on experimental rat arthritis. *Clin Exp Immunol* 1996;103:77-82.
- 84 Prakken BJ, Wendling U, van der Zee R *et al*. Induction of IL-10 and inhibition of experimental arthritis are specific features of microbial heat shock proteins that are absent for other evolutionarily conserved immunodominant proteins. *J Immunol* 2001;167:4147.
- 85 Quintana FJ, Carmi P, Mor F, Cohen IR. Inhibition of adjuvant-induced arthritis by DNA vaccination with the 70-kd or the 90-kd human heat-shock protein: immune cross-regulation with the 60-kd heat-shock protein. *Arthritis Rheum* 2004;50:3712-20.
- 86 Tanaka S, Kimura Y, Mitani A *et al*. Activation of T cells recognizing an epitope of heat-shock protein 70 can protect against rat adjuvant arthritis. *J Immunol* 1999;163:5560-5.
- 87 Corrigan VM, Bodman-Smith MD, Fife MS *et al*. The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. *J Immunol* 2001;166:1492.
- 88 Shoda H, Fujio K, Sakurai K *et al*. Autoantigen BiP-derived HLA-DR4 epitopes differentially recognized by effector and regulatory T cells in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:1171-81.
- 89 Zhou J, Wang L-P, Feng X *et al*. Synthetic peptides from heat-shock protein 65 inhibit proinflammatory cytokine secretion by peripheral blood mononuclear cells from rheumatoid arthritis patients. *Clin Exp Pharmacol Physiol* 2014;41:67-72.
- 90 Barbera A, Lorenzo N, Garrido G *et al*. APL-1, an altered peptide ligand derived from human heat-shock protein 60, selectively induces apoptosis in activated CD4<sup>+</sup> CD25<sup>+</sup> T cells from peripheral blood of rheumatoid arthritis patients. *Int Immunopharmacol* 2013;17:1075-83.
- 91 Corrigan VM, Bodman-Smith MD, Brunst M, Cornell H, Panayi GS. Inhibition of antigen-presenting cell function and stimulation of human peripheral blood mononuclear cells to express an antiinflammatory cytokine profile by the stress protein BiP: relevance to the treatment of inflammatory arthritis. *Arthritis Rheum* 2004;50:1164-71.
- 92 Anderton SM, Wraith DC. Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat Rev Immunol* 2002;2:487-98.
- 93 Wauben MH, Boog CJ, van der Zee R *et al*. Disease inhibition by major histocompatibility complex binding peptide analogues of disease-associated epitopes: more than blocking alone. *J Exp Med* 1992;176:667-77.
- 94 Prakken BJ, Samodal R, Le TD *et al*. Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2004;101:4228-33.
- 95 Koffeman EC, Genovese M, Amox D *et al*. Epitope-specific immunotherapy of rheumatoid arthritis: Clinical responsiveness occurs with immune deviation and relies on the expression of a cluster of molecules associated with T cell tolerance in a double-blind, placebo-controlled, pilot phase II trial. *Arthritis Rheum* 2009;60:3207-16.
- 96 Vanags D, Williams B, Johnson B *et al*. Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. *Lancet* 2006;368:855-63. Elsevier Ltd;
- 97 Wieten L, Berlo SE, ten Brink CB *et al*. IL-10 is critically involved in mycobacterial HSP70 induced suppression of proteoglycan-induced arthritis. *PLoS One* 2009;4:e4186.
- 98 Wendling U, Paul L, van der Zee R *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;164:2711-7.

- 99 Detanico T, Rodrigues L, Sabritto AC *et al.* Mycobacterial heat shock protein 70 induces interleukin-10 production: immunomodulation of synovial cell cytokine profile and dendritic cell maturation. *Clin Exp Immunol* 2004;135:336-42.
- 100 Yoshida K, Ochiai A, Matsuno H, Panayi GS, Corrigan VM. Binding immunoglobulin protein resolves rheumatoid synovitis: a xenogeneic study using rheumatoid arthritis synovial membrane transplants in SCID mice. *Arthritis Res Ther* 2011;13:R149.
- 101 Corrigan VM, Vittecoq O, Panayi GS. Binding immunoglobulin protein-treated peripheral blood monocyte-derived dendritic cells are refractory to maturation and induce regulatory T-cell development. *Immunology* 2009;128:218-26.
- 102 Wei C-M, Lee J-H, Wang L-C *et al.* Frequency and phenotypic analysis of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in children with juvenile idiopathic arthritis. *J Microbiol Immunol Infect* 2008;41:78-87.
- 103 Loosdregt J, Van Fleskens V *et al.* Canonical Wnt signaling negatively modulates regulatory T cell function. *Immunity* 2013;1:1-13.
- 104 Herrath J, Muller M, Amoudruz P *et al.* The inflammatory milieu in the rheumatic joint reduces regulatory T-cell function. *Eur J Immunol* 2011;41:2279-90.
- 105 Kamphuis S, Kuis W, De Jager W *et al.* Tolerogenic immune responses to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. *Lancet* 2005;366:50-6.
- 106 Vercoulen Y, van Teijlingen NH, de Kleer IM *et al.* Heat shock protein 60 reactive T cells in juvenile idiopathic arthritis: what is new? *Arthritis Res Ther* 2009;11:231.
- 107 van Eden W, Lisse J, Prakken B, Albani S. Biologics and postbiologics: novel immunotherapeutics for the induction and maintenance of remission. *Drug Discov Today* 2010;15:71-7.
- 108 Weiner HL. Current issues in the treatment of human diseases by mucosal tolerance. *Ann N Y Acad Sci* 2004;1029:211-24.
- 109 Kamphuis S, Albani S, Prakken BJ. Heat-shock protein 60 as a tool for novel therapeutic strategies that target the induction of regulatory T cells in human arthritis. *Expert Opin Biol Ther* 2006;6:579-89.
- 110 Jaen O, Rullé S, Bessis N *et al.* Dendritic cells modulated by innate immunity improve collagen-induced arthritis and induce regulatory T cells in vivo. *Immunology* 2009;126:35-44.
- 111 van Duivenvoorde LM, Han WGH, Bakker AM *et al.* Immunomodulatory dendritic cells inhibit Th1 responses and arthritis via different mechanisms. *J Immunol* 2007;179:1506-15.
- 112 Healy LJ, Collins HL, Thompson SJ. Systemic administration of tolerogenic dendritic cells ameliorates murine inflammatory arthritis. *Open Rheumatol J* 2009;2:71-80.
- 113 Benham H, Nel HJ, Law SC *et al.* Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. *Sci Transl Med* 2015;7:290ra87.
- 114 Hilkens CMU, Isaacs JD. Tolerogenic dendritic cell therapy for rheumatoid arthritis: where are we now? *Clin Exp Immunol* 2013;172:148-57.