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Targeting of tolerogenic dendritic cells towards heat-shock proteins: a novel therapeutic strategy for autoimmune diseases?

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Summary

Tolerogenic dendritic cells (tolDCs) are a promising therapeutic tool to restore immune tolerance in autoimmune diseases. The rationale of using tolDCs is that they can specifically target the pathogenic T-cell response while leaving other, protective, T-cell responses intact. Several ways of generating therapeutic tolDCs have been described, but whether these tolDCs should be loaded with autoantigen(s), and if so, with which autoantigen(s), remains unclear. Autoimmune diseases, such as rheumatoid arthritis, are not commonly defined by a single, universal, autoantigen. A possible solution is to use surrogate autoantigens for loading of tolDCs. We propose that heat-shock proteins may be a relevant surrogate antigen, as they are evolutionarily conserved between species, ubiquitously expressed in inflamed tissues and have been shown to induce regulatory T cells, ameliorating disease in various arthritis mouse models. In this review, we provide an overview on how immune tolerance may be restored by tolDCs, the problem of selecting relevant autoantigens for loading of tolDCs, and why heat-shock proteins could be used as surrogate autoantigens.

Keywords: autoimmune diseases; heat-shock proteins; regulatory T cells; tolerogenic dendritic cells.

Abbreviations: ACPAs, anti-citrullinated peptide antibodies; ER, endoplasmic reticulum; HSP, heat-shock proteins; IDO, indoleamine 2,3-deoxygenase; IL-2, interleukin-2; MITAP, minimal information model for tolerogenic antigen-presenting cells; PD-L1, programmed death ligand 1; RA, rheumatoid arthritis; TGF-β, transforming growth factor-β; TolAPC, tolerogenic antigen-presenting cell; TolDC, tolerogenic dendritic cell; Treg, regulatory T cell

Restoring immune tolerance to 'self' in autoimmune disease: a promising clinical intervention

Immune tolerance is crucial for preventing destructive immune responses to self tissues. In healthy individuals, immune tolerance is maintained at different levels: in the thymus, where T cells that strongly react to self-antigens are deleted, and in the periphery, where self-reactive T cells that escaped negative selection in the thymus are kept in check by regulatory cells. A breach in immune tolerance facilitates immune attacks on self-tissues that, when becoming dysregulated, lead to chronic autoimmune disorders.

Regulatory T (Treg) cells play a pivotal role in maintaining immune tolerance in the periphery. They are a heterogeneous population of cells that can be either derived from the thymus (naturally occurring Treg cells) or induced in the periphery from naive $CD4^+$ T cells (induced Treg cells). They exert their suppressive action on immune effector cells through a number of distinct mechanisms, including inhibition of antigen-presenting cell function, killing of effector cells, secretion of immunosuppressive cytokines and compounds, and interference with metabolic pathways (reviewed in refs 1,2).

Treg cells are critical to prevent autoimmune disease. A total loss of functional Treg cells, as seen in patients with IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome), leads to severe autoimmunity affecting multiple organs.[3] In specific autoimmune diseases, however, it is thought that a more subtle change in the function of Treg cells is involved in the pathogenesis. For example, although patients with type I diabetes have similar numbers of Treg cells to healthy controls, their Treg cells display reduced suppressive activity and defects in interleukin-2 (IL-2) signalling.[4–6] In patients with rheumatoid arthritis (RA), Treg cells have reduced ability to suppress inflammatory cytokine production.[7] Furthermore, enhanced numbers of Treg cells co-expressing IL-17 were found in both the peripheral blood and synovial fluid of patients with RA, suggesting conversion of Treg cells into inflammatory cytokine-producing effector cells.[8]

Restoration of Treg cell function is emerging as a promising clinical intervention for autoimmune diseases. One way of achieving this is by replenishing the Treg cell pool in autoimmune patients with functional Treg cells, either by treating patients with drugs that selectively expand Treg cells *in vivo*, or by generating new Treg cells *ex vivo* before injecting them into the patient (reviewed in refs 2,9). However, a downside of this approach is that expanding Treg cells 'randomly' may give rise to general suppression of the immune response, thereby increasing the risk of infection, and perhaps even cancer. A preferred approach would be to direct the Treg response to defined and relevant antigens that are being expressed in the target tissue. This would not only limit off-target immunosuppression, but would most likely also increase the efficacy of the Treg cell therapy, as was shown in mouse models.[10,11] An outstanding issue is, however, how to achieve the expansion of antigen-specific Treg cells, and how to choose the relevant antigen(s). Here, we propose to use tolerogenic DCs (tolDCs) to induce Treg cells against heat-shock proteins that are ubiquitously expressed in inflamed target tissues, as outlined below.

Tolerogenic dendritic cells as a therapeutic tool

Dendritic cells (DCs) are a heterogeneous family of professional antigen-presenting cells that can be classified on the basis of their ontogeny, surface marker expression profile and anatomical location (reviewed in ref. 12). DCs are as important for the induction of effective immunity against invading pathogens as they are for the maintenance of immune tolerance. Patients with primary immunodeficiency with mutations in *GATA2* have defective DC function, resulting in enhanced susceptibility not only to infection and cancer, but also to autoimmune conditions, most likely due to a reduction in Treg cells.[13]

The role of DCs in instigating immunity versus tolerance is largely determined by their maturation status. Under steady-state conditions, tissue DCs are immature, expressing low levels of MHC class II and co-stimulatory molecules; their 'default' setting is to induce tolerance. These immature DCs can become immunogenic when they sense pathogen-associated molecular patterns and danger-associated molecular patterns via pattern recognition receptors. These include Toll-like receptors, retinoic-acid-inducible gene I-like receptors, and nucleotide-binding oligomerization domain-like receptors. Pattern recognition receptor-mediated signalling plays a central role in the maturation process that DCs need to undergo to acquire potent T-cell stimulatory properties.[14] Fully matured DCs express high levels of MHC class II, co-stimulatory markers (e.g. CD86) and pro-inflammatory cytokines (e.g. IL-12p70, IL-23, tumour necrosis factor), all required for the efficient induction of T effector cell responses. Furthermore, during DC maturation the expression of chemokine receptors is modulated (e.g. CCR5 is down-regulated and CCR7 is up-regulated) enabling DC migration towards lymphoid tissues to present antigen to naive T cells. However, the outcome of maturation of DCs is not always the generation of DCs with immunogenic properties. Certain danger-associated molecular patterns and immune suppressive compounds have been shown to drive the maturation of DCs with tolerogenic properties (i.e. tolDCs).[15–18] These tolDCs may be phenotypically mature (i.e. high levels of MHC class II and co-stimulatory molecules), but may express co-inhibitory molecules [e.g. programmed death ligand 1 (PD-L1), PD-L2, immunoglobulin-like transcript 3], lack

expression of pro-inflammatory cytokines and instead produce immunosuppressive cytokines and compounds [e.g. IL-10, transforming growth factor-β (TGF-β), indoleamine 2,3-dioxygenase (IDO)]. The maturation status of these DCs has been referred to as 'semi-mature'. Hence, there is plasticity with regard to the functional maturation of DC, and the environmental cues that DCs receive during the maturation process determine whether they become immunogenic or tolerogenic.

Dendritic cells are able to mediate tolerance through several mechanisms. They can induce iTreg cells through, for example, membrane-bound PD-L1, which blocks the Akt/mTOR pathway to preferentially stimulate naive T cells to become iTreg cells.[19] Furthermore, PD-L1 and PD-L2 provide inhibitory signals to both $CD8^+$ and $CD4^+$ T cells, which drives the T cell into a state of tolerance.[19] Secreted compounds such as IL-10, IL-27, TGF-β, retinoic acid and IDO, can convert naive T cells into iTreg cells. DCs can also promote T-cell tolerance through T-cell killing, and the induction of T-cell hyporesponsiveness (anergy).[20,21]

The importance of DCs in maintaining immune tolerance has led to exploring the therapeutic use of DCs. Various ways have been described to create DCs with stable tolerogenic properties (tolDCs). The tolerogenic properties of these *in vitro* generated tolDCs depend on the specific method used (reviewed in ref. 22). For example, tolDCs generated with the immunosuppressive agents dexamethasone and/or the active form of Vitamin D3 (1α,25-dihydroxyvitamin D3) are characterized by a semi-mature phenotype, with high levels of MHC class II, intermediate levels of co-stimulatory molecules, low levels of pro-inflammatory cytokines and high levels of the immunosuppressive cytokines IL-10 and TGF-β.[23–27] TolDCs can also be genetically engineered, for example through the transduction of immunosuppressive or pro-apoptotic molecules (e.g. IL-10, CTLA-4, FASL) or silencing of immunostimulatory molecules (e.g. CD80/CD86, IL-12) (reviewed in ref. 28). These different types of tolDCs have been shown to reduce or prevent autoimmune diseases or transplant rejection in animal models, providing important proof of principle evidence that these cells can be applied therapeutically.[27,29–33] Their therapeutic benefit is associated with a reduction of pro-inflammatory effector T cells and natural killer cells, and the induction of Treg cells or IL-10-producing T cells.[27,29,34–36]

Efforts have been made to translate these findings from animal studies to the clinical setting. Good Manufacturing Protocols to generate tolDCs from human donor cells have been developed,[26,37] and methods to preserve the tolDCs and reduce the production costs are being explored.[29] As there are diverse methods of generating tolDCs and other types of tolerogenic APC (tolAPCs), a minimum information model for tolAPC (MITAP) was generated. MITAP enables researchers to report their data in a standardized and more transparent manner, facilitating data comparison and interpretation, ultimately paving the way for the development of standardized protocols for the production of tolDCs and other tolAPCs for therapeutic application.[38] A number of tolDCs have been tested in phase I clinical trials, including for type I diabetes,[30] Crohn's disease[39] and RA.[40,41] Encouragingly, tolDC therapy in all these studies was found to be feasible and safe, providing rationale to conduct further studies into their efficacy.

The problem of targeting autoantigen(s) – which ones?

One of the main advantages of tolDC therapy is the specific targeting of pathogenic immune responses. Many of the drugs that are currently used to treat autoimmune diseases are non-antigen-specific, leading to general immunosuppression. With tolDCs, autoreactive T cells can, theoretically, be exclusively targeted. But how to achieve this is still a debate. A number of studies have provided clear evidence that tolDCs need to be loaded with a disease-relevant antigen to exert their beneficial immune modulatory action. Loading of tolDCs with type II collagen was required, for example, for antigen-specific disease remission in the collagen-induced arthritis model.[27,42,43] More recent research shows that this is also applicable in other autoimmune diseases.[44] Furthermore, when comparing the therapeutic action of unloaded tolDCs and tolDCs loaded with a disease relevant peptide ($MOG_{40–55}$) in the experimental autoimmune encephalomyelitis model, Mansilla *et al.*[45] showed that although the unloaded tolDCs inhibited disease symptoms, the $MOG_{40–55}$-loaded tolDCs diminished disease even more.

In contrast, other studies have shown that disease remission can be established when administering unloaded tolDCs.[46,47] This may suggest that tolDCs are able to take up the relevant antigen *in vivo*. It has been hypothesized that unloaded tolDCs induce T-cell anergy rather than promoting Treg cells. These anergic T cells might be capable of suppressing excessive T helper type 17 and type 1 responses.[48] Non-antigen-pulsed tolDCs might also induce regulatory populations that do not require an antigen. For instance, B cells can be converted into regulatory B cells partly through the production of retinoic acid by the tolDCs.[49] However, if these non-antigen-pulsed tolDCs are able to take up antigen *in vivo*, one has to consider the safety of these tolDCs, as it is possible that the non-antigen-pulsed tolDCs also take up other antigens that should not be targeted.

Nonetheless, if tolDCs need to be loaded with antigen (s) before infusion, a remaining problem is the question

of which antigen to use, and in what form. In many autoimmune diseases, including RA, the knowledge about the relevant autoantigen(s) involved is insufficient. Moreover, even if some of the relevant autoantigens are known, as is the case for multiple sclerosis, the problem of HLA diversity remains.[44] Some peptides (e.g. proteolipid protein) that have been shown to be involved in the pathogenesis of multiple sclerosis are restricted to a specific HLA-class (e.g. HLA-DQB1*0602), making it more difficult to standardize the peptides used for all patients with multiple sclerosis.[50]

For RA, no universal autoantigen exists. Several candidate self-proteins have been described in relation to the pathogenesis of this disease. Epitopes from joint-derived antigens such as collagen type II and human cartilage-derived glycoprotein HCgp39 are presented by DCs and macrophages to T cells in inflamed joints of patients with RA.[51] Furthermore, the endoplasmic reticulum (ER) stress-associated protein GRP78/BiP is described as a potential autoantigen. The ER stress response is increased in RA synovial tissue and fluid and the ER chaperone, GRP78, is important for synoviocyte proliferation and angiogenesis, which are substantial indicators of RA.[52]

Post-translational modifications may also be important in generating novel epitopes that trigger autoimmunity. Anti-citrullinated peptide antibodies (ACPAs) are found in the sera of 70–80% of patients with RA.[53] Immunogenetic studies have shown that more than 90% of patients with RA share an HLA-II epitope in the DRB1 chain (HLA-DRB1 *0101, *0401, *0404). This so-called shared epitope is also associated with ACPAs; shared epitope-positive patients are predisposed to having ACPAs.[54,55] Feitsma *et al.* identified two HLA-DRB1-restricted $CD4^+$ T-cell clones that recognized citrullinated vimentin and were also present in the inflamed joints of patients with RA. This indicates that $CD4^+$ T cells can respond to naturally processed epitopes from an autoantigen.[54] The finding that ACPAs were present in the inflamed joints of patients but not in the joints of healthy individuals, together with the discovery that citrullinated autoantigen-specific $CD4^+$ T cells were only found in the peripheral blood mononuclear cells from patients with RA, suggests that both the ACPAs and these $CD4^+$ T cells play a significant role in the pathogenesis of RA.[55,56] Scally *et al.* (and others) provide molecular evidence on how $CD4^+$ T cells are able to recognize citrullinated antigens.[57–59] They also showed that in the autoantigen recognizing $CD4^+$ T-cell population of *HLA-DRB1*04:01*$^+$ RA patients, the percentage Treg cells (both activated and resting) was reduced, whereas the populations of naive and effector memory $CD4^+$ T cells were increased compared with healthy subjects.[57] This indicates that citrullinated peptides are plausible autoantigens in RA.

To test if citrullinated antigens are good candidates for an immunomodulatory therapy, a phase I clinical trial was performed. In this study autologous *in vitro* generated tolDCs were exposed to citrullinated autoantigenic epitopes and administered intradermally into patients.[40] The trial showed that the DC vaccination was safe and indicated an anti-inflammatory effect after DC administration. However, using citrullinated peptides has the consequence that therapy is limited to patients with HLA-DRB1 (*0101, *0401, *0404) and it is unknown if the reactivity in these patients is similar. We took a different approach in our recent phase I safety trial in patients with rheumatoid and inflammatory arthritis.[41] TolDCs were loaded with autologous synovial fluid; the rationale being that this fluid contains relevant joint-associated antigens. The downside of this approach is that it is not always possible to obtain sufficient synovial fluid from patients with RA for tolDC loading. Furthermore, as the antigens are unknown, it is difficult to monitor changes in the antigen-specifc T-cell response after tolDC administration.

The use of surrogate autoantigens could be a preferred option for the loading of tolDCs. Possible candidates are heat-shock proteins (HSPs). HSPs are typically intracellular proteins with no peptide leader sequences that can target secretion. However, there is evidence that HSPs can have access to the extracellular milieu, either by passive or active mechanisms. Both the endogenous up-regulation of HSPs with so-called HSP co-inducers and the exogenous administration of (recombinant) HSPs have led to immunomodulatory effects in various models of experimental autoimmunity.[60–62] Therefore, HSPs could be used as surrogate autoantigens not only for RA but also for other autoimmune diseases. This will be discussed in further detail in the next section (Figure 1).

HSPs as surrogate autoantigens for autoimmunity

The main function of HSPs is to support folding and transport of a large variety of (misfolded) proteins as intracellular molecular chaperones. Their expression can be significantly up-regulated under conditions of stress like fever, viral infection, nutritional deficiency, cold and exposure to the pro-inflammatory cytokines interferon-γ and tumour necrosis factor.[63–65] Generally, HSPs can be classified into different families based on their monomeric molecular weight (HSP 10, HSP 20–30, HSP 40, HSP 60, HSP 70, HSP 90 and HSP 100 families). Some HSP family members (e.g. HSP 60 and HSP 70) are highly conserved throughout evolution, resulting in immunological cross-recognition of certain mammalian and microbial HSP homologues.

Initial observations that ignited studies on the role of HSPs in autoimmunity were made in the mycobacteria-

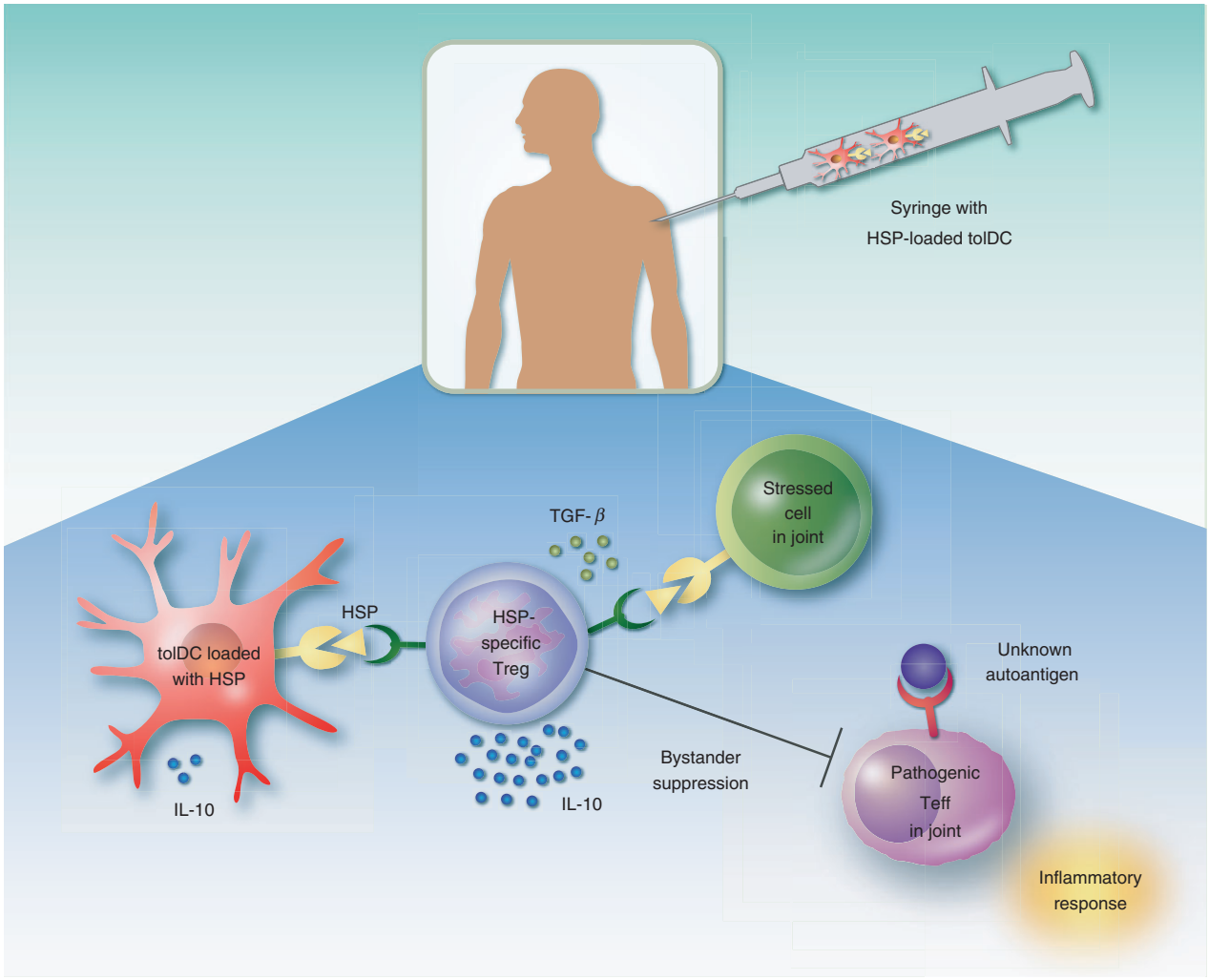

Figure 1. Heat-shock protein (HSP) loaded tolerogenic dendritic cell (tolDC) vaccination in rheumatoid arthritis (RA). This figure depicts the potential process that takes place in the patient's joint after injection with HSP loaded tolDCs. TolDCs produce anti-inflammatory cytokines [e.g. interleukin-10 (IL-10)] and present epitopes of HSP to naive $CD4^+$ T cells. These $CD4^+$ T cells differentiate into HSP-specific regulatory T (Treg) cells and suppress stressed (HSP expressing) cells via immunomodulatory cytokines like IL-10 and transforming growth factor-β (TGF-β). Furthermore, bystander suppression could lead to suppression of pathogenic effector T (Teff) cells recognizing the unknown autoantigen, thereby inhibiting inflammatory symptoms. The presence of self HSP in the synovial fluid of RA patients might favour the selection of the generation of Treg cells and their function.

induced adjuvant arthritis model in rats. Generated mycobacteria-specific T-cell lines were shown to have arthritogenic potential[66] and it was later discovered that HSP 60 was the antigen recognized by the mycobacteria-specific T-cell lines.[67] Further studies followed showing that synovial fluid cells and peripheral blood mononuclear cells of patients with chronic inflammatory arthritis could also respond to mycobacterial HSP 60. In contrast, HSP 60 responses were absent in control subjects.[68] Moreover, monoclonal antibodies recognizing mammalian HSP 60 were produced and it was found that HSP 60 was expressed in the synovial membranes of patients with chronic arthritis.[69,70] Similar results were found for the HSP family members HSP 40 and HSP 70. Synovial fluid and peripheral blood T cells of patients with RA could recognize a bacterial variant of HSP 40, but those from healthy subjects or disease controls could not.[71] In addition, the human homologues of HSP 40 and HSP 70 were found to be over-expressed in the synovial lining of the joints of patients with RA.[72,73]

Interestingly, numerous experimental animal models and even a few clinical trials have shown that treatment with (myco)bacterial HSPs can induce HSP-specific anti-inflammatory T-cell responses. Experimental autoimmune disease models in both rat and mouse showed significantly reduced arthritis severity after prophylactic immunization with mycobacterial HSP 60 or HSP 70.[74,75] Although the exact mechanism for disease amelioration is still not completely understood, suppression of arthritis is probably induced by IL-10-producing

Treg cells.[75–78] One possible explanation for the propagation and/or induction of a regulatory phenotype in HSP 60/70-specific T cells lies in the high homology between the bacterial and mammalian variants of the HSPs. Even though HSPs are considered immunogenic – microbial HSP 60, for example, was already known as the so-called 'common antigen of Gram negatives' before its molecular definition[79] – the highly conserved parts of the proteins could induce a tolerogenic response as these can be recognized as self-antigens by the body's own immune system.[80] Moreover, since bacterial HSPs are mostly encountered in the tolerizing gut or lung mucosa, conserved and hence repeatedly encountered HSP antigens are more likely to obtain a regulatory phenotype. In addition to conservation and microbial-self cross-recognition, HSP 70 family members are directly involved with antigen processing and consequently, HSP 70 fragments were found to be one of the most frequent cytosolic MHC class II natural ligand sources.[81–83] Presentation of HSP 70 peptides may therefore be part of the earlier mentioned default tolerant state of the immune system, where MHC class II presented HSP peptides are part of a continuous and credible target for Treg cells. It is, however, important to keep in mind that in a dysregulated immune system, as is seen in patients with autoimmune diseases, antigens that would normally induce an anti-inflammatory immune response could now potentially induce a pro-inflammatory response.

As the HSPs used for these experiments are from bacterial origin and can potentially induce an unwanted anti-inflammatory response towards these bacteria, a safer form of the HSPs is needed. One way to accomplish this is to use bacterial HSP-derived peptides that show high homology with the mammalian variant. The high homology to the self-antigen will prevent unwanted responses towards the bacteria and at the same time ensure cross-reactivity with the mammalian HSPs presented in the inflamed joint. Indeed, two of the three clinical trials using HSPs as therapy were performed with HSP-derived peptides (Table 1). A pilot phase II trial using an HSP 40-derived peptide, dnaJP1; which also contains the 'shared epitope',[84] was tested in patients with juvenile idiopathic arthritis. After oral administration of the dnaJP1, a change from a pro-inflammatory to a tolerogenic T-cell response to dnaJP1 could be observed.[85,86] In a second phase II trial, an HSP 60-derived peptide, DiaPep277, was used to treat patients with type I diabetes. It was found that DiaPep277 was safe and showed a trend towards a greater preservation of beta-cell function compared with controls.[87,88] In a third recent trial, a mammalian HSP 70 family member, BiP, was tested in patients with RA. In this case, whole protein was administered intravenously. The results of this phase I/II safety trial showed no serious adverse drug reactions. Moreover, at the higher treatment doses disease remissions were seen in some cases.[89]

Table 1. Heat-shock proteins (HSPs) and peptides associated with therapeutic interventions in chronic inflammatory diseases. dnaJP1 and DiaPep277 were tested in phase II clinical trials in juvenile RA and diabetes (refs. 85,87). mB29a is now explored for the loading of tolDCs in RA (refs. 83,92). The peptides are based on human Hsp sequences

HSP	Peptide	Sequence
HSP 40 (dnaJB1)	dnaJP1	QKRAAYDQYGHAAFE
HSP 60 (HspD1)	DiaPep277	VLGGGVALLRVIPALDSLTPANED
HSP 70 (HspA9)	mB29a	VLRVINEPTAAALAY

As discussed earlier, one potential disadvantage of using peptides is HLA diversity in patients. Consequently, HSP peptides need to either (i) be able to bind multiple HLA-DR molecules, including the RA-associated HLA-DRB1 *0101, *0401, *0404 molecules, or (ii) a peptide pool of several HSP peptides able to bind one or more of the RA-associated HLA-DR molecules needs to be administered. For HSP 60 and HSP 70 several pan-DR peptides have been discovered. Kamphuis *et al.* used a computer algorithm to identify both self and bacterial HSP 60 peptides able to bind a number of distinct HLA-DR haplotypes. They found several peptides that were able to bind the major RA/juvenile idiopathic arthritis-associated HLA-DR molecules and T cells from both juvenile idiopathic arthritis and RA patients were able to respond to five out of eight peptides.[90,91] In addition, de Wolf *et al.* showed that an HSP 70 peptide, B29, also binds multiple HLA-DR molecules. They concluded that more than 80% of human individuals can present B29 to their T cells (and among patients with RA possibly even more due to the high presence of HLA-DRB1 *0401). In subsequent cultures they showed that 10 out of 14 healthy individuals could respond to the peptide.[92] The B29 peptide was earlier tested in a mouse model of arthritis and it was found that prophylactic intranasal administration of B29 could suppress disease. Moreover, $CD25^+$ $CD4^+$ T cells from B29 immunized mice could decrease disease severity in recipient arthritic mice, indicating that B29-specific Treg cells are effective in diminishing autoimmune arthritis.[83]

Next to the Treg cell inducing potential of B29, bone-marrow-derived DCs pulsed with *Mycobacterium tuberculosis* or mouse HSP 70 induced IL-10 production in antigen-specific T cells and suppressed arthritis, showing that HSP 70 loading of DCs by itself is tolerizing.[93]

In order to make both tolDC therapy and HSP peptide treatment in autoimmune diseases (e.g. RA) as potent as possible, a combination therapy could be the solution. Pulsing tolDCs with HSP peptides could (i) solve the autoantigen problem and (ii) the HSP peptides will be

targeted to the HSP-specific T cells by DCs with stable tolerogenic function, making sure a regulatory response towards the antigen is induced.

Conclusion

The fundamental problem in autoimmune diseases is the failure of the immune system to down-regulate its own potentially dangerous cells, leading to destruction of tissue expressing the autoantigen. In the case of RA, currently available immunosuppressive therapies offer relief but fail to induce long-term physiological regulation resulting in medication-free remission.

As argued here, to restore immune tolerance, autologous tolDCs loaded with an HSP-derived peptide antigen could be used. Such a therapy could, potentially, both tolerize arthritogenic T cells and induce disease-suppressive regulatory T cells. Targeting the physiological mechanism of re-establishing tolerance for self-antigens offers the opportunity to inhibit joint-destroying immune responses long-term.

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