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Heat shock protein bystander antigens for peptide immunotherapy in autoimmune disease

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Summary

Mucosal administration of an antigen eliciting bystander suppression at the site of inflammation results in effective antigen-specific immunotherapy for autoimmune diseases. Heat shock proteins are bystander antigens that are effective in peptide-specific immunotherapy in both experimental and human autoimmune disease. The efficacy of preventive peptide immunotherapy is increased by enhancing peptide-specific immune responses with proinflammatory agents. Combining peptide-specific immunotherapy with general suppression of inflammation may improve its therapeutic effect.

Keywords: arthritis, heat shock protein, mucosal, peptide, therapy

Introduction

How to restore the immune balance in a deranged immune system that attacks self tissues in autoimmune diseases is a continuing focus of research. The current treatment of autoimmunity still depends mainly on conventional life-long general immune suppression. Although a step forward has been made in clinical efficacy by the introduction of biologics that block proinflammatory cytokines, inflammation revives as soon as therapy is discontinued. Moreover, the considerable immune suppression evoked by cytokine blockade has been associated with severe side effects such as serious opportunistic infections, and even malignancies such as lymphoma [1–6].

A more specific approach could overcome drawbacks of non-specific immune suppressive therapy. By specific targeting of autoaggressive T cells in autoimmunity, side effects can be reduced. Indeed, such antigen-specific immunotherapy has been shown to be effective in multiple animal models of autoimmunity without severe side effects (reviewed by [7]). Translation of these findings to human therapies showed promising results, but efficacy has been less than expected (Table 1). To improve the effect of antigen-specific therapy in clinical autoimmune diseases, three issues – choice of antigen, route of administration and peptide immunogenicity – need to be addressed.

The choice of antigen in animal models is facilitated by the fact that the disease-inducing antigen is known. The identification of such an antigen in human autoimmunity is more challenging, as the disease-inducing antigen in many autoimmune diseases remains unknown. In addition, it is doubtful whether this single disease-inducing antigen really exists. Therefore new targets for antigen-specific therapy are needed.

The second issue concerns the route of antigen administration. In the majority of clinical trials the antigen is administered by injection, while a more effective option to restore immune tolerance would be the administration of antigen in a tolerogenic environment, such as the gut or nasal mucosa [7,8]. This tolerogenic presentation of the antigen converts the antigen-specific proinflammatory immune response to an antigen-specific regulatory response (reviewed by [9]).

When peptides are administered via the mucosal route the third issue may be a limited immunogenicity, indicating a need for enhancement of peptide recognition [10,11].

When these issues can be addressed, mucosal antigen-specific immunotherapy can be an interesting alternative to

Table 1. Major clinical trials of peptide immunotherapy in autoimmune disease.

	Trial name	Design	Type of therapy	Peptide	Route	Patient group	Immunomodulatory effects	Clinical efficacy	Future	Reference
Diabetes		Phase I	Parenteral peptide	Pro-insulin peptide C19-A3	i.d.	Long-standing DM type I	Increase of IL-10 producing peptide-specific T cells	None measured		Thrower, *Clin Exp Immunol* 2009
		Phase I	Parenteral peptide	Human insulin B chain peptide	i.m.	New-onset DM type I	Insulin-specific B and T cell responses	None measured		Orban, *Autoimmunity* 2010
		Phase I	Parenteral peptide	NBI-6024 (altered peptide of insulin B epitope B9-23)	s.c.	New-onset DM type I	Shift from Th1 to Th2 immunoregulatory phenotype	None measured		Alleva, *Scand J Immunol* 2006
	DIAMYD	Phase II	Parenteral peptide with adjuvant	GAD65-Alum	s.c.	New-onset DM type I	Increased percentage of total T_{reg} and GAD65-specific T_{reg} cells, increased secretion IL-5, IL-10, IL-13	Preservation of residual insulin secretion	Ongoing phase II NCT00529399	Ludvigsson, *N Engl J Med* 2008; Hjorth, *Clin Immunol* 2011
MS	PreCISe	Phase I/II	Parenteral peptide	Copaxone (Glatiramer-acetaat, peptide mixture)	s.c.	Early MS	None measured	Risk reduction for developing clinically definite MS, delayed onset, decreased relapse rate		Comi, *Lancet* 2009; Cohen, *Neurology* 2007
		Phase II	Parenteral peptide	Altered peptide of MBP83-99	s.c.	MS		Increased disease activity		Bielekova & Kappos, *Nat Med* 2000
SLE		Phase II	Parenteral peptide	Spliceosomal peptide p140	s.c.	SLE	Anti-ds DNA antibody levels reduced			Muller, *Arthritis Rheum* 2008
Uveitis		Open therapeutic trial, no control group	Oral peptide	B27PD (self-HLA peptide)	p.o.	Long-lasting therapy refractive uveitis	None measured	Discontinuation of steroids possible because of reduced intraocular inflammation	Ongoing phase I/II NCT01195948	Thureau, *Immunol Lett* 1999, *ANYAS* 2004

MBP: myelin basic protein; i.d.: intradermal; i.m.: intramuscular; s.c.: subcutaneous; p.o.: per os; MS: multiple sclerosis; SLE: systemic lupus erythematosus; DM: diabetes mellitus; HLA: human leucocyte antigen; IL: interleukin.

generalized immune-suppressive therapy with subsequent unwanted side effects. Results of clinical trials of peptide immunotherapy are promising, but efficiency needs to be enhanced. In this review, we consider ways to improve future antigen-specific immunotherapy with a special focus on heat shock proteins (HSPs).

Heat shock proteins: candidates for immunotherapy

Bystander antigens

At time of diagnosis, human autoimmune diseases are already characterized by a secondary non-specific inflammatory process in which multiple antigens are targets of the immune system (a process known as epitope spreading). The antigens that are specifically up-regulated at the site of inflammation and are immunologically dominant (bystander antigens) are candidates for antigen-specific immunotherapy. Induced tolerogenic immune responses to such bystander antigens could lead to a local down-regulation of the ongoing immune response (bystander suppression).

Heat shock proteins as bystander antigens

HSPs, which are highly conserved intracellular molecular chaperones that are important for cell survival under stressful conditions, fulfil both the above-mentioned criteria for bystander antigens (reviewed in [12]).

First, HSPs are up-regulated upon cell stress and are therefore present exclusively at sites of inflammation. HSPs are indeed abundantly present in muscle cells of juvenile dermatomyositis (JDM) patients [13], in synovial fluid and synovial tissue of juvenile idiopathic arthritis (JIA) [14] and RA patients [15] and in inflamed bowel of Crohn's disease patients [16]. There is also supporting evidence that HSPs are secreted from stressed cells; for example, in blood, free HSP60 is found during various inflammatory conditions [17].

Secondly, in autoimmune disease, HSP-specific responses are immunologically dominant [12,18,19]. For example, HSP60 peptide-specific T cell clones play a significant role in the perpetuation of Crohn's disease and tissue-specific T cell clones from diabetic children recognize human HSP60 as an autoantigen [16,20,21]. Humoral responses to HSPs have also been observed in autoimmune diseases, as antibody responses to multiple HSP families were detected in sera from RA and JIA patients [22,23].

Altogether, HSPs seem to be suitable candidates for the induction of bystander suppression by antigen-specific immunotherapy.

Immunoregulatory properties of HSPs

HSPs are known for their strong evolutionary conservation, resulting in a high level of homology between bacterial and mammalian HSPs [24]. Theoretically, this high homology in combination with their up-regulation during stress and immunodominancy could be dangerous, putting the host at risk for autoimmunity through antigenic mimicry [25]. However, T and B cell responses to self-HSP are present in healthy individuals (and even in cord blood) without widespread inflammation or autoimmunity [26–28]. This could be due to the presence of regulatory immune responses. HSP-specific immune responses have been suggested to have a driving force in the generation of regulatory action via innate and adaptive pathways [12,26,29].

Innate effects of HSP. The innate immune system was thought originally to recognize only pathogen-associated molecular patterns (PAMPs) via their pathogen recognition receptors (PRR), also known as the 'infectious non-self model' [30]. Matzinger proposed rather that the innate immune system responds to endogenous danger signals (danger-associated molecular patterns, DAMPs), released by damaged or stressed cells with the tissue playing an important role in determining the quality of the immune response [31,32]. As HSPs are up-regulated and excreted during stress, these proteins have long been implicated in triggering innate immune responses. There has been a debate as to whether these innate effects of HSP (reviewed in [17]) could have been the result of contamination by other Toll-like receptor (TLR) agonists (reviewed in [33]). Although the use of contaminated HSP has been a problem in several studies, considerable evidence now exists to support the innate effects of HSP. Properly controlled research revealed that self-HSP60 (and not microbial HSP60) has a direct, lipopolysaccharide (LPS)-independent innate effect on T cells mediated through TLR-2 and on monocytes and macrophages through TLR-4 [34].

Adaptive effects of HSP. The induction of regulation by HSPs has also been described via adaptive immune responses. Presumably as a result of stimulation by homologous HSPs from commensal bacteria in the gut, self-HSP-specific T and B cell responses are present in healthy individuals [18]. To contain these autoreactive T cells that escaped central tolerance safely, peripheral tolerance mechanisms are needed. It has been shown that self-HSP reactive T cells evoke regulatory immune responses. Data from animal models indicate that cross-reactive immunoregulatory T cell responses to self-HSP play a role in disease protection [29,35–37]. In line with these findings, the presence of self-HSP60-specific T cell responses in JIA patients correlates with a benign disease course [38–40]. Self-HSP-specific T cell responses have also been reported to be immunoregulatory in various other autoimmune diseases, such as RA [41] and JDM [13], by the production of anti-inflammatory cytokines such as interleukin (IL)-10, IL-4 and transforming growth factor (TGF)-β [38,42]. A recent study revealed that self-HSP60 could directly induce highly suppressive forkhead box protein 3 ($FoxP3^+$) T_{reg} in vitro [43]. Finally, low concentrations of

human HSP60 or p277 (a synthetic human HSP60-derived peptide) have been shown to be able to enhance the regulatory function of $CD25^+$ T_{reg} from human peripheral blood mononuclear cells (PBMC) [19,44].

In conclusion, HSPs are bystander antigens that can elicit regulatory responses in human autoimmune disease, and are therefore interesting targets for antigen-specific immunotherapy.

Peptide immunotherapy

The development of antigen-specific immunotherapy with proteins has been hampered by side effects such as mast cell activation or cytotoxic T cell responses [45–47]. Peptide immunotherapy can be an attractive alternative, as it increases specificity and thereby reduces side effects. Moreover, synthetic peptides are free of microbial products.

Peptide selection. In human disease, selection of appropriate peptides for immunotherapy is a major challenge. The selection process is helped by focusing on desirable characteristics of the peptide.

First, the peptide should be recognized by the human immune system and thus be able to bind disease-associated human leucocyte antigen (HLA) molecules. For this purpose, multiple prediction models of peptide binding to HLA have been shown to be helpful [48–51]. Secondly, the peptide should mimic the naturally processed epitope, as altered peptides may behave unpredictably [52,53]. To fulfil this criterion, selection can be based on elution studies of HLA-peptide complexes. Thirdly, as self-cross-reactive responses have been shown to be important for the disease-protective effect of peptides [29], a peptide needs to have high homology to self and still be immunogenic.

HSP-peptide immunotherapy. HSP-peptides prevent autoimmune disease in multiple experimental animal models (Table 2). However, most peptides used in these models were not selected primarily on their binding capacity of disease-associated human HLA molecules, a feature

Table 2. Protective heat shock protein (HSP) peptide treatment in experimental models of autoimmunity.

	Model	Route	Adjuvant	Regimen	Peptides	HSP	References
Arthritis	DIA	i.d.	IFA	p	Mixture of 120–134 and 213–277	Self	Moudgil, *J Immunol* 2005
	PIA	i.p.	None	p and t	261–271	Non-self	Thompson, *J Immunol* 1998; Francis, *Immunology* 2000
	AA and CPIA	i.d.	DDA	p	256–270	Non-self	Anderton, *J Exp Med* 1995
	AA and AIA	i.n.	None	p	176–190	Non-self	Prakken, *Proc Natl Acad Sci* 1997
	AA	i.d.	IFA	p	180–188	Non-self	Golden, *Agents Actions* 1991
	AA	i.n.	None	t	180–188	Non-self	Roord, *PLoS ONE* 2006
	AA	i.d.	IFA	p	234–252	Non-self	Tanaka, *J Immunol* 1999
	AA	i.n.	None	p	111–125	Non-self	Wendling, *J Immunol* 2000
	AA	i.p.	None	p	61–80 (mHSP65), 31–46, 37–52 (self)HSP60	Both	Ulmansky, *J Immunol* 2002
	AA	i.n.	None	p	254–268	Non-self	Zonneveld-Huijssoon, *Ann Rheum Dis* 2011
	AA	s.c.	DDA	p	Mixture of 417–431, 441–455, 465–479, 513–527, 521–535 (BCTD)	Non-self	Moudgil, *J Exp Med* 1997; Durai, *J Immunol* 2004
	AA	s.c.	IFA	p	177–191	Non-self	Durai, *J Rheumatol* 2007
DM	NOD	s.c.	IFA	t	437–460 (p277)	Self	Cohen, *Lancet* 1994
	NOD	i.p.	IFA	p and t	437–460 (p277)	Self	Elias, *Diabetes* 1995, 1997; Ablamunits, *J Autoimmun* 1998; Tian, *J Immunol* 1998; Elia, *Proc Natl Acad Sci* 1991
	STZ	i.p.	Mineral oil	t	437–460 (p277)	Self	Elias, *Diabetes* 1996
	NOD	s.c.	IFA	p	166–185 (p12)	Self	Elias, *J Autoimmun* 1997
	BB-DP	p.o.	None	p	Peptide analogue of p277 (Diapep277)	Self	Brugman, *Diabetologia* 2004
Sjögren	SS	s.c.	IFA	p	437–460	Self	Delaleu, *Arthritis Rheum* 2008

Adapted from [12] and [22]. AA: adjuvant arthritis; DIA: dimethyl dioctadecyl ammoniumbromide-induced arthritis; PIA: pristine-induced arthritis; AIA: avridine-induced arthritis; CPIA: CP20961-induced arthritis; STZ: STZ toxin-induced diabetes; BB-DP: BioBreeding-Diabetes Prone rat; SS: spontaneous Sjögren syndrome; i.d.: intradermal; i.p.: intraperitoneal; i.n.: intranasal; s.c.: subcutaneous; p.o.: per os, oral; IFA: incomplete Freund's adjuvant; DDA: dimethyl dioctadecyl ammoniumbromide; p: preventive regimen; t: therapeutic regimen.

desired for translation of the experimental results into humans.

In two recent studies, HSP60-derived HLA-binding peptides were tested in an experimental arthritis model [54,55]. In one of the two studies, the identified human HSP60 epitope was modified artificially to increase the HLA binding affinity and to skew towards a regulatory T cell response [54]. Intradermal administration of this altered peptide suppressed experimental arthritis (AA) *in vivo* by the induction of regulatory T cells (T_{reg}) and increased T_{reg} frequency in *ex-vivo* assays with PBMC from RA patients in contrast to the native peptide [54]. However, as mentioned previously, native peptides that mimic the naturally processed epitope are preferred for safe antigen-specific immunotherapy, and intradermal administration is not the optimal route for tolerance induction.

The other study performed by our group used a native HLA-binding T cell epitope of mycobacterial HSP60 that evoked a tolerogenic immune response in PBMCs of arthritis patients [41,42]. Nasal administration of this peptide was effective in experimental arthritis and induced a $CD4^+$ T cell population with reduced tumour necrosis factor (TNF)-α production at the site of inflammation. This induced T cell population also expressed FoxP3 and had potent suppressive capacity which, upon transfer, protected against arthritis [55].

These specific experimental results have not yet been translated into clinical trials. So far, clinical trials have been performed with two other interesting HSP-epitopes (Table 3).

DnaJP1. The first clinical trial with an HSP-peptide in human arthritis was performed with dnaJP1. DnaJP1 is a peptide derived from Escherichia coli HSP40, containing a sequence of five amino acids found in the majority of HLA-DR alleles linked with RA ('shared epitope'). In a Phase I trial, patients with early active RA received oral dnaJP1 during 6 months. After treatment, in-vitro responses to dnaJP1 changed from proinflammatory to anti-inflammatory, with increased IL-10 production and augmented FoxP3 expression in T_{reg} cells [56].

In a following Phase II trial, patients with active RA with proven immunological reactivity to dnaJP1 received the same mucosal dnaJ treatment. Clinical improvement was achieved at multiple time-points and was accompanied again by anti-inflammatory *in-vitro* responses to dnaJP1 with reduced production of TNF-α and a trend towards increased production of IL-10 [57].

Diapep277. A vaccination strategy based on HSP60 as a diabetes autoantigen was performed with p277 (DiaPep277), a 24-amino-acid peptide of mouse HSP60 (437–460) that has been shown to have preventive and therapeutic effects in experimental diabetes [58,59].

Multiple Phase II trials have been performed with subcutaneous p277 [60–63]. In adults newly diagnosed with type 1

Table 3. Clinical trials of peptide immunotherapy with heat shock protein (HSP) peptides in autoimmune diseases.

	Trial name	Design	Type of therapy	Peptide	Route	Patient group	Immunomodulatory effects	Clinical efficacy[†]	Reference
Diabetes	DiaPep277	Phase II	Parenteral peptide	p277 (HSP60 437–460)	s.c.	New-onset T1D	Increased IL-10 production by T cells is associated with preservation of C-peptide up to 12–18 months	Lower need for exogenous insulin	Huurma, *Clin Exp Immunol* 2008
RA		Phase II	Oral peptide	DnaJP1 peptide (HSP40)	Oral	RA	Immune diviation from TNF-α to IL-10, peptide-induced FoxP3 expression on $CD25^{bright}$ cells	ACR20 and ACR50 score reduced	Koffeman, *Arthritis Rheum* 2009

[†]ACR 20, ACR50, measurements of improvement in rheumatoid arthritis. T1D: type 1 diabetes; RA: rheumatoid arthritis; IL: interleukin; TNF: tumour necrosis factor.

diabetes (T1D), residual C-peptide levels (reflecting the amount of insulin production) could be preserved [63,64]. A recent immunological study revealed that the preservation of C-peptide was associated with peptide specific tolerance [61]. Phase II trials are currently ongoing [65].

Good candidates for immunotherapy have now been proved to be effective in multiple animal models of autoimmune diseases, and translation into humans has provided encouraging results; the next step will be to improve therapeutic efficiency in human autoimmune disease. Enhancement of peptide immunogenicity when delivered via the mucosal route and combination of peptide therapy with immune modulating agents could be interesting options.

Improving antigen-specific therapy

Enhancing peptide immunogenicity

As regulation of effector T cells is an active process, immune activation is needed for optimal control [32]. A peptide signal delivered via the tolerogenic mucosal route may be too small to induce the desired immune deviation due to the intrinsic weakness of the peptide signal alone, triggering only the adaptive immune system [10,11]. In a healthy immune system, activation of the innate immune system leads to better presentation of the peptide and thereby enhances peptide-triggered adaptive immune responses. The combination of enhancing both adaptive and innate immunity may therefore be an attractive option for the enhancement of mucosal immunotherapy in autoimmune disease.

Adjuvant. Combination of an adjuvant triggering innate immunity with a T cell-epitope triggering adaptive immunity could also enhance peptide immunogenicity. Although adjuvants have been used in non-mucosal vaccination strategies in autoimmunity, the concept of enhancing mucosal vaccination with an adjuvant for preventive peptide therapy in autoimmune disease is somewhat new (Fig. 1). Prerequisites for such an innate triggering mucosal adjuvant would be applicability at mucosal sites, activation of antigen-presenting cells (APCs) and skewing towards T_{reg} cell responses. Co-administration of such an adjuvant would result in more efficient antigen presentation by mucosal APCs and enlarge the beneficial effect of mucosal peptide treatment.

In clinical diabetes, alum as an adjuvant for subcutaneous antigen-specific immunotherapy led to preservation of residual insulin secretion in adults and children with early-onset T1D [66–69]. The increased effectiveness of alum-adjuvanted peptide immunotherapy has been shown to depend on the activation of innate immunity by DNA released from dying cells [70]. However, alum as an adjuvant could not restore euglycaemia in T1D patients and was not tested mucosally, indicating a need to explore other adjuvants.

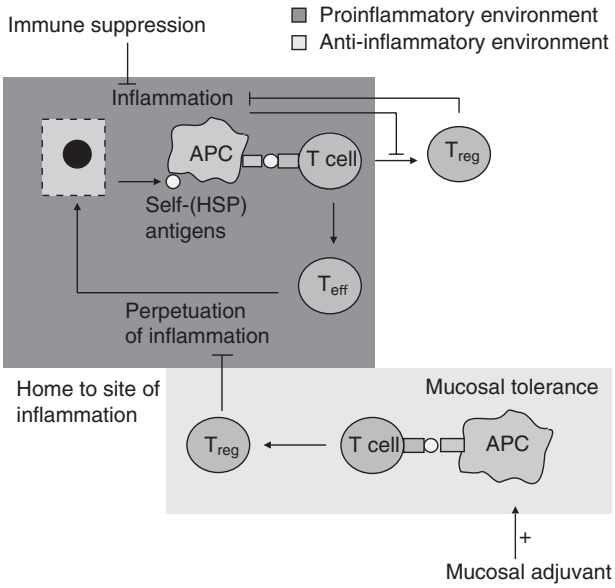

Fig. 1. The dual role of heat shock proteins (HSPs), inducing proinflammatory effector T cells (T_{eff}) or tolerogenic/regulatory T cells (T_{reg}), is influenced by the inflammation status of the tissues. At the site of inflammation, self-HSP antigens are released from damaged cells. These antigens are presented by activated antigen-presenting cells (APC) to T cells. In a proinflammatory environment this results in predominantly T_{eff} cells, contributing to perpetuation of inflammation. T cells induced via the mucosal route are directed by the anti-inflammatory environment towards a predominantly tolerogenic response (T_{reg}) (mucosal tolerance induction). The mucosally induced antigen-specific T cells consist of multiple kinds of regulatory cells and which are thought to migrate to the site of inflammation as their cognate antigen (e.g. HSP) is expressed there. At the site of inflammation, these antigen-specific T_{regs} skew the proinflammatory T cell response towards an anti-inflammatory phenotype by cytokine release-like interleukin (IL)-10 or cell–cell contact. Mucosal adjuvant can enhance peptide presentation by APCs at the site of tolerance induction, enlarging the pool of T_{reg} formed after mucosal tolerance induction. The inflammatory environment hampers the development of (self-HSP-specific) T cells. Generalized immune suppressive therapy reduces inflammation, creating a more favourable environment for the development of T_{reg}. Combination therapy of antigen-specific mucosal tolerance induction with immune suppressive therapy could therefore enhance efficacy of peptide specific immunotherapy.

An adjuvant with promising results in non-obese diabetic (NOD) mice is the non-toxic B subunit of the cholera enterotoxin (CTB). Oral administration of islet autoantigens linked to CTB improved significantly suppression of hyperglycaemia and pancreatic inflammation [71,72]. CTB has been shown more recently to induce enhanced antigen capture by dendritic cells and migration of the dendritic cells towards the site of antigen administration (the Peyer's patches) [73,74]. Another agent that can be considered an innate-activating adjuvant for mucosal peptide therapy is HSP itself. HSP can up-regulate adaptive immune responses

by stimulating innate receptors such as TLR-2 and TLR-4 [34]. Indeed, HSP60 enhanced immunogenicity of CMV peptide vaccines [75] and increased the efficacy of p277 therapy in diabetic mice [76]. HSP60 seems to function as the body's natural adjuvant as a result of its ability to activate both the innate and adaptive responses [19].

Adjuvants of particular interest that have been administered mucosally are cytosine–guanine–oligodeoxynucleotides (CpG-ODN), which consist of a nucleotide sequence common in bacterial DNA. CpG-ODN stimulate TLR-9 on antigen-presenting cells and have been used successfully as nasal vaccine adjuvant in anthrax (AVA) vaccination in mice [77–79]. Data from our group indicate that CpG-ODN enhance antigen-specific immunotherapy in an experimental arthritis model (ARD, in press).

In conclusion, enhancing immunogenicity of a peptide in a preventive regimen seems very efficient in improving peptide-specific immunotherapy. However, caution should be taken in the addition of proinflammatory agents to a peptide in a therapeutic setting as it could, in theory, lead to over-activation of an already deranged immune system.

Combination therapy with general immune modulators

Although boosting the antigen-specific immune response seems effective in a preventive regimen, the ongoing widespread inflammation present in established autoimmune disease probably hampers antigen-specific immune modulation in a therapeutic setting. Short-term non-specific dampening of inflammation before administration of antigen could create an environment in which the antigen-specific response can be detected and modulated, thereby improving therapeutic efficacy of antigen-specific immunotherapy (Fig. 1).

In addition to the reduction of inflammatory background 'noise', dampening inflammation is crucial for adequate functioning of T_{reg} (reviewed by [80]). For example, a chronic inflammatory environment causes local dysfunction of T_{reg} or converts them into proinflammatory T helper type 17 (Th17) cells [81–86]. In line with these observations, generalized immune suppression by TNF-α blockade [87,88] or immune modulation by anti-CD3 [89–91] favours the development of T_{reg} cells. However, it is conceivable that due to non-antigen-specific immune therapies only a small number of induced T_{reg} will be specific for antigens expressed in the target autoimmune organ. Combining generalized immune suppression with antigen-specific peptide therapy could therefore expand antigen-specific T_{reg} that are able to migrate to the tissue where their cognate antigen is expressed: the site of inflammation.

Some successful combination therapy strategies in autoimmune diseases have been reported in the literature. For example, combination therapy of anti-CD3 with disease-related peptides has been shown to be effective in experimental models of new-onset diabetes. The combined approach was more efficient than peptide or anti-CD3 alone and induced antigen-specific T_{reg} that could transfer protection [92,93]. Combined anti-CD3 therapy with disease-related peptides has not been tested so far in human T1D, but perhaps such a combined approach could improve recent results of anti-CD3 monotherapy in human T1D [91].

Another proven effective strategy in experimental models is the combination of antigen-specific immunotherapy with TNF-α blockade. In the rat adjuvant arthritis model, low-dose anti-TNF-α (Etanercept) combined with nasally administered HSP60 peptide induced clinical control in a therapeutic setting to a larger extent than peptide treatment alone. The clinical response was accompanied by an increase in peptide-specific FoxP3-expressing T cells to a degree comparable to full-dose Etanercept. Finally, the combination treatment induced more peptide-specific IL-10 production than did Etanercept alone [94].

An interesting finding regarding combination therapy of a peptide with immune modulation in humans has been reported in the earlier-described dnaJP1 clinical trial in RA. *Post-hoc* analysis revealed that the best clinical results were obtained in a subgroup of patients taking hydroxychloroquine (HCQ), an immune modulating agent [95]. In addition to the earlier-mentioned combination strategies in animal models, this finding indicates the potential therapeutic efficacy of combination treatment in humans as well [57].

In summary, boosting the peptide-specific immune response, on one hand, and short-term dampening of the ongoing systemic inflammation, on the other hand, could improve the therapeutic efficiency of antigen-specific immunotherapy. Combination therapy shows promising results in experimental autoimmunity, but evidence is limited as yet for human autoimmune disease. Enlarging the efficacy of antigen-specific therapy is worth exploring, while the possibility of lowering the dose of immune-suppressive medication reduces side effects associated with life-long drug administration.

Conclusion

In this review, we have discussed strategies to improve the clinical outcome of antigen-specific immunotherapy in human autoimmune disease. Three major issues concerning the choice of antigen, route of administration and peptide immunogenicity were dealt with.

Some issues remain to further optimize antigen-specific immunotherapy for human autoimmune disease. In this regard, dosing is important for mucosal tolerance induction to be effective [8] and dose-finding studies are needed to improve therapeutic results further.

Furthermore, the selection of patients in clinical trials of peptide-specific immunotherapy is crucial. Some prerequisites for treatment response have been identified in experimental and human studies. First, genetic factors play a role as

the availability of beneficial antigen-specific T cells varies between different genetic backgrounds in mice models of diabetes and correlates with treatment outcome [93]. Secondly, a high representation of tolerogenic and anergic immune pathways at baseline is associated with clinical responsiveness to peptide immunotherapy [57]. Thirdly, the presence and quality of peptide-specific responses before start of treatment play a role in the eventual efficacy of peptide immunotherapy [57,61]. Selecting patients on these criteria could be of help in further optimization of antigen-specific immunotherapy.

In conclusion, peptide immunotherapy with bystander antigens such as HSPs shows promising results in experimental models, and the first positive results from clinical trials are currently emerging. New approaches aiming for enhanced peptide recognition in a controlled immune environment by the use of adjuvant and/or combining peptide treatment with short-term immune suppressive medication may hold promise for a successful future for peptide-specific immunotherapy in autoimmune diseases.

Disclosure

The authors have no conflicts of interest to declare.

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