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Review

Stress proteins are used by the immune system for cognate interactions with anti-inflammatory regulatory T cells

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ABSTRACT

Since the initial discovery of the protective role of heat shock protein (HSP) 60 in arthritis, T cell recognition of endogenous HSP was found to be one of the possible underlying mechanisms. Recently we have uncovered potent disease-suppressive Tregs (anti-inflammatory immunosuppressive T cells) recognizing HSP70 self-antigens, and enabling selective targeting of such Tregs to inflamed tissues. HSP70 is a major contributor to the major histocompatibility complex (MHC) Class II ligandome and we have shown that a conserved HSP70-epitope (B29) is abundantly present in murine MHC Class II. Upon transfer, B29-induced CD4+CD25+Foxp3+T cells suppressed established proteoglycan-induced arthritis (PGIA) in mice. These self-antigen specific Tregs were activated in vivo and as little as 4.000 cells sufficed to fully inhibit arthritis. Furthermore, in vivo depletion of transferred Tregs abrogated disease suppression. Given that B29 can be presented by most human MHC class II molecules and that B29 inhibited arthritis in HLA-DQ8 (human MHC) transgenic mice, we feel that therapeutic vaccination with selected HSP peptides can be an effective route for induction of anti-inflammatory Tregs as a novel intervention in chronic inflammatory diseases.

1. Introduction

The fundamental problem of autoimmune diseases is faulty regulation of the inflammatory process. In the past we have regarded autoimmune inflammation as a process initiated by the accidental emergence of a forbidden clone of self-reactive effector T cells; however, it is now clear that the immune systems of healthy people are populated with T cells and B cells bearing receptors that can bind self-antigens. Chronic inflammation and autoimmune diseases result from the chronic activation or repeated reactivation of self-reactive lymphocytes that are an intrinsic and normal element of the healthy immune system. Inflammatory disease results from the failure of the immune system to down-regulate these potentially dangerous cells. Thus, the rational goal of therapy in diseases of unregulated inflammatory activation is to reorganize physiological regulation. Current therapies are oriented towards indiscriminate suppression of immune cells and molecules and are therefore less safe, as they create general immunosuppression with risks of losing resistance against for instance infectious diseases or cancer. For these reasons, the relatively recent discovery of a specialized T cell subset with the capacity to regulate (down-modulate) effector T cells has led to a very active area of research aimed at the exploitation of these so-called Tregs for the cure of autoimmune, atopic (allergy) and other inflammatory diseases. In addition Tregs are analyzed for their potential to promote transplant tolerance. Clinical trials with adoptive transfer therapy of autologous polyclonal Tregs are currently carried out for graft-versus-host disease, type 1 diabetes and kidney transplant rejection [1,2]. These involve the isolation and ex-vivo expansion of Tregs and re-infusion into the patient. The logistics of these forms of cellular therapies are however complicated. A much more attractive and straight-forward approach would be the in vivo expansion of Tregs by the immunization with the relevant antigens. Such an approach would resemble the vaccination approach of infectious diseases: in vivo administration of antigen with the purpose of eliciting cognate interactions with antigen specific receptors leading to an adaptive immune response. Like other CD4+ T cells, Tregs are selected in the thymus on the basis of cognate TcR interactions with self-antigens. Therefore, in theory, antigen specific manipulation of Tregs through a vaccine like approach must be possible. Based on recent findings obtained in a mouse model of autoimmune arthritis we now have evidence that stress proteins may constitute an attractive source for antigens that can be used to engage into cognate interactions with Tregs.

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2. Treg, the physiologic inhibitors of inflammatory diseases

The cellular part of the immune system comprises several specialized subsets of T cells, including regulatory T cells (Tregs) that maintain homeostatic self-tolerance and control excessive immune responses (230 Corthay,A. 2009). Dysfunction of Tregs can lead to autoimmunity [3,4], while absence of Tregs results in multi-organ autoimmune diseases [5]. Tregs are characterized by the expression of CD25, the IL-2 receptor alpha chain [5], and the transcription factor forkheadbox P3 (FoxP3), which regulates the expression of Treg associated molecules. Tregs can be subdivided into natural occurring Tregs (nTregs), that come from the thymus and mainly recognize self-antigens in the tissues [6]. Additionally, induced Tregs (iTregs) [7] are another subset which differentiate from CD25− precursors in the periphery and are located at mucosal tissue, while these cells recognize foreign antigens (such as antigens from pathogens and commensals, allergens, alloantigens and tumor antigens, or inflammatory antigens) [8,9].

Tregs express a variety of effector molecules that enable the suppression of target cells [10]. These mechamisms of suppression can be characterized into four types. First, is the suppression via anti-inflammatory cytokines like transforming growth factor beta (TGF-β), interleukin (IL)-10 and IL-35 [11]. Secondly, Tregs are able to lyse target cells via Granzyme A and B, and perforin [12]. Thirdly, Tregs are able to influence the metabolic pathway of other cells, for instance by depriving them from the growth factor IL-2, due to high IL-2 consumption via CD25 [13]. Finally, the fourth mechanism of suppression involves the action of inhibitory receptors like cytotoxic T lymphocyte antigen-4 (CTLA-4) that binds B7 on antigen presenting cells (APC) [14], which gives an inhibitory signal to the APC. Additionally, lymphocyte activation gene 3 (LAG-3) that binds major histocompatibility complex (MHC)-class II, delivers an inhibitory signal [15].

Tregs are interesting cells to target because they can induce 'bystander suppression': once activated via their TCR, Tregs suppress immune responses to other antigens [16]. Activating Tregs via disease relevant antigens is a suitable approach, however in the case of RA, it is better to select for antigens that are up-regulated during inflammation since the disease inducing antigens are unknown. Not only the antigen-specificity of Tregs makes these cells ideal targets for therapy, also the induction of new suppressor cells via activated Tregs helps to control inflammation. This 'infectious tolerance' induced by Tregs can result in long term suppression of inflammation.

3. Stress proteins and the MHCII ligandome

Being intracellular proteins, textbook immunology would primarily teach that HSP do load MHC class I molecules. And indeed, induction of HSP specific class I restricted CTL responses (cytotoxic T cell responses) have been documented in many different situations of cell stress. One of the first observations was made with CTL raised against mycobacterial HSP65, as these CD8+ class I restricted T cells recognized macrophages subjected to various forms of cells stress. This was a first demonstration of the fact that HSP are processed in stressed host cells and can be presented in the context of class I molecules [17]. Interestingly, besides class I, also class II molecules are loaded with HSP peptides. In fact, HSP70 peptides were prominently represented in the RP-HPLC profile of the content of class II molecules of a human lymphoblastoid cell line [18].

It is of interest to note that the loading of MHCII by intracellular stress proteins occurs especially proficient in cells under stress. In this case the routing of the intracellular cargo into the MHCII compartment is organized through autophagy [19]. Autophagy consists of a collection of intracellular routing pathways and essential homeostatic maneuvers by which cells break down their own components in lysosomes and direct the fragments for presentation on MHCII molecules. Perhaps a primordial function of this lysosomal degradation pathway is adaptation to various forms of stress such as nutrient deprivation. In addition, in complex multicellular organisms, these pathways or autophagy proteins orchestrate diverse aspects of cellular and organismal responses to dangerous stimuli such as infection [20]. Some HSP70 family members are directly involved with one of the molecular machineries that take care of autophagy, such as so-called chaperone mediated autophagy [21]. Chaperone-mediated autophagy (CMA) refers to the chaperone-dependent selection of soluble cytosolic proteins that are then targeted to lysosomes and directly translocated across the lysosome membrane for degradation. This may well explain why fragments of HSP70 have been found to dominate the MHCII ligandome of both human and mouse cells [22,23], especially under conditions of cellular stress. See Table 1 for a rather complete listing of HSP70 peptides eluted from MHC Class II molecules. The B29 sequences are in the boxed area.

To activate CD4+ T cells, and thus Treg, peptides should be presented by MHC class II molecules. Cytosolic proteins, like HSP70, are by default loaded on MHC class I molecules while extracellular proteins will be presented on MHC class II. The classical "textbook" distinction between MHC class I and MHC class II loading pathways has been proven not fully correct because cytosolic proteins have been eluted from MHC class II and vice versa. It is known that natural MHC class II ligands are preferentially generated from long-lived cytosolic or nuclear proteins [24], and that long lived proteins are preferentially turned over by autophagy. HSP70 and HSC70 seem to have relatively long half-lives of between 4 and 20 h and are found more frequently in MHCII than in MHCI molecules [25]. Autophagy has been initially found as a process to sustain metabolic fitness during food deprivation through bulk protein degradation [26]. The role of autophagy in the immune system is only now becoming clear [27]. Two pathways can result in loading of intracellular peptides on MHC class II. First, intracellular proteins can be incorporated into autophagosomes that subsequently fuse with lysosomes for degradation of their cargo (macroautophagy). In addition, cytosolic proteins can be transported via LAMP2a directly into the lysosome (chaperone mediated autophagy) [28,29]. Recently, the role of autophagy in loading HSP70 peptides has been described; in human HLA-DR4+ B cells a striking increase of especially HSP70 peptides was eluted from HLA-DR4 upon induction of autophagy by amino acid deprivation [22]. Autophagy induction coincided with elevated HSP70 mRNA levels. In other words, especially under conditions of cell stress, fragments of HSP70 will be presented on antigen presenting cells to T cells, possibly initiating a regulatory T cell response.

A recent paper by Costantino et al. [30] analysed peptides obtained from MHCII molecules of human activated T cells. It was already known that antigens derived from CD4+ T cells injected as a vaccine can activate so called antigen-specific idiotypic and ergotypic responses [31,32], which also can have a regulatory activity. HLA-DR+CD4+ T cells themselves have been hypothesized to present T cell-derived proteins such as CD25 or HSP60 [22]. Costantino et al. were unable to identify any peptides derived from HSP60; interestingly, however, they identified peptides derived from heat shock cognate 71 kDa protein (HSPA8), a ubiquitously expressed chaperone protein.

4. HSP are abundant at sites of inflammation

To understand the pathogenesis of autoimmune diseases an intensive search has been made to delineate specific antigens being involved in the break-down of self-tolerance and leading to these

Table 1
Hsp70 peptides eluted from MHC Class II molecules. (See below-mentioned references for further information.)

Sequence of eluted peptide	Peptide originating from [(a)]	MHC Class II type	Ref.
QQYLPLPTPKVIGID	human HSPA13 (23-37)	HLA–DR10 (DRB1*1001)	54
IIANDQGNRTTPSY	mouse HSPA1A (28-41), HSPA1B (28-41), HSPA1L (30-43), HSPA2 (29-42), HSPA8 (28-41)	I-Ak	55
ITPSYVAFTPEGERL	mouse HSPA5 (62-76)	I-Ab	56
TPSYVAFTDTERLIG(DA)	human HSPA1A (38-52), HSPA1L (40-54), HSPA2 (39-53), HSPA8 (38-52)	HLA-DR7	57
TPSYVAFTDTERLIGD	human HSPA1A (38-52), HSPA1L (40-54), HSPA2 (39-53), HSPA8 (38-52)	HLA-DQ2	58
DVYVGYESVELADSNPQ	human HSPA13 (77-93)	HLA-DQ2	58
DAAKNQLTSNPEN	mouse HSPA5 (79-91)	I-Ag7	59
PFVEAEKSNLAYD	mouse HSPH2 (78-90)	I-Ab	60
DAAKNQVAMNPTNTVFDAK	human HSPA8 (53-71)	HLA-DRB1*1301; *1501; DRB3*0202; DRB5*0101	61
NPTNTVFDAKRLIGRRFD	human HSPA8 (62-79)	HLA-DRB1*1104	62
LIGRTWNDPSVQQDIKFLP	human HSPA5 (98-116)	HLA-DR7	63
QDIKFLPFKVVEKKTKPY	mouse HSPA5 (111-128)	BoLA-DRB3*1201(in mus line)	64
LTKMKEIAEAYLGKTVTNAV	human HSPA8 (124-143)	HLA-DR7	63
AVVTVPAYFNDSQRQATKDAGTIAGLN	human HSPA8 (142-168)	HLA-DR7	63
LNVLRIINEPTAAAIAYG (NVLRIINEPTAAAIAYG)	rat HSPA1A (167-184), HSPA1L (169-186), HSPA2 (168-185), HSPA8 (167-184)	HLA-DRB1*0401 (in rat line)	65
NVLRIINEPTAAAIAYG	human HSPA1A (168-184), HSPA1L (170-186), HSPA2 (169-185), HSPA6 (170-186), HSPA8 (168-184)	HLA-DRB1*0401/DRB4*0101	66
NVLRIINEPTAAAIA	human HSPA1A (168-184), HSPA1L (170-186), HSPA2 (169-185), HSPA6 (170-186), HSPA8 (168-184)	HLA-DRB1*0401/*02x/DRB5*0101	67
NVMRIINEPTAAAIAYG	human HSPA5 (194-210)	HLA-DRB1*0401/*02x/DRB5*0101	67
VMRIINEPTAAAIAYG	human HSPA5 (195-210)	HLA-DRB1*0401/DRB4*0101	66
IINEPTAAAIAYGLD	human HSPA1A (172-186), HSPA1L (174-188), HSPA2 (173-187), HSPA5 (198-212), HSPA8 (172-186)	HLA-DQ6 (B*602)	68
KREGEKNILVFDLGGGTFD	mouse HSPA5 (214-232)	I-Ab	60
FDVSILTIEDGIFE	human HSPA8 (205-218)	HLA-DQ2	58
GIFEVKSTAGDTHLGGEDFD	mouse HSPA2 (218-237), HSPA8 (215-234)	I-Ab	60
NRMVNHFIAEFKRK	mouse HSPA8 (236-249)	I-Ek	69
RMVNHFIAEFKRKH	mouse HSPA8 (236-249)	I-Ek	70
VNHFIAEFKRKHKKD	human HSPA8 (238-252)	HLA-DR11/w52	18
XDFYTSITRAXFEE	human HSPA1A (291-304), HSPA1L (293-306), HSPA2 (294-307), HSPA6 (294-306), HSPA8 (291-304)	HLA-DR11/w52	18
EGEDFSETLTRAKFEEL	mouse HSPA5 (315-331)	BoLA-DRB3*1201(in mus line)	64
ADLFRGTLDPVEK	human HSPA8 (307-319)	HLA-DQ6 (B*0604)	68
PVEKALRDAKLDKSQIHD	mouse HSPA8 (316-333)	I-Ab	60
DLNKSINPDEAVAYGA	human HSPA1A (358-373), HSPA1L (360-375)	HLA-DRB1*0101; 0301; DRB3*0101	61
KSINPDEAVAYG	human HSPA1A (361-372), HSPA1L (363-374), HSPA2 (364-375), HSPA6 (363-374), HSPA8 (361-372)	HLA-DQ2	58
TIPTKQTQTFTTYSDNQP	rat HSPA1A (419-436), HSPA8 (419-436)	RT1.Bl	71
VPTKKSQIFSTASDNQPTVT	human HSPA5 (443-462)	HLA-DRB1*0401/DRB4*0101	66
GERAMTKDNNLLG	human HSPA1A (445-457), HSPA1L (447-459), HSPA2 (448-460), HSPA6 (447-459), HSPA8 (445-457)	HLA-DR4Dw4	72
GERAMTKDNNLLGKFE	human HSPA1A (445-460), HSPA8 (445-460)	HLA-DRB1*0401/DRB4*0101	66
GERAMTKDNNLLGRFE	human HSPA6 (447-462)	HLA-DRB1*0401/DRB4*0101	66
ANGILNVSAVDKSTGKE	human HSPA8 (482-499)	HLA-DRB*0401	73
GILNVSAVDKSTGK	human HSPA8 (484-497)	HLA-DRB*0401	73
GILNVSAVDKSTGKE	human HSPA8 (484-498)	HLA-DRB1*0401/DRB4*0101	66
CNEIINWLDKNQ	human HSPA8 (574-585)	HLA-DR4Dw10	72
EIINWLDKNQTA	human HSPA8 (576-587)	HLA-DRB1*0402 and -1104	74
ISWLDKNQTAEKEEFE	human HSPA8 (578-593)	HLA-DQ8 (transgenic in NOD)	59
YGSGGPPPTGEEDTSEKDEL	mouse HSPA5 (636-655)	I-Ag7	59

(a) Identification of the Hsp70 proteins with the Entrez Gene ID between brackets:
human HSPA1A (3303), HSPA1L (3305), HSPA2 (3306), HSPA5 (3309), HSPA6 (3310), HSPA8 (3312), HSPA13 (6782),
mouse HSPA1A (193740), HSPA1B (15511), HSPA1L (15482), HSPA2 (15512), HSPA5 (14828), HSPA8 (15481), HSPH2 (15525),
bovine HSPA5 (14828) and rat HSPA1A (24472), HSPA1L (24963), HSPA2 (60460), HSPA8 (24468).
For the Hsp70 nomenclature (including the traditional names) see Kampinga et al., Cell Stress and Chaperones, Volume 14, Number 1, 105-111, DOI: 10.1007/s12192-008-0068-7

diseases. This has resulted in a broad range of distinct antigens possibly causally involved. On theoretical grounds however one would have doubts whether low numbers of autoantigen specific Tregs in polyclonal T cell populations would be able to exert sufficient effect to suppress ongoing inflammation. For this process to be effective the likely scenario is that only sufficiently prevalent Tregs with the ability to engage into cognate interactions with an abundantly produced autoantigen would be able to have significant impact on such a process.

And here heat shock proteins are interesting candidate antigens to serve as targets for Tregs at sites of inflammation. Inflammation goes together with the production of various mediators that trigger the up-regulation of HSP. Most prominent in this can be the reactive oxygen species (ROS), toxic factors that lead to tissue damage and amplification of the inflammatory reaction. In addition to this the lipid mediators of inflammation (products of the arachidonic acid cascade) and pro-inflammatory cytokines (IL1, IL6, TNFα) are known inducers of the heat shock response [33]. It is probably this up-regulated presence of HSP that induces the production of HSP specific antibodies in patients with chronic inflammatory conditions. Local presence of up-regulated HSP at sites of inflammation was already shown by HSP60 antibody staining in light microscopy immunohistochemistry of synovial membranes of patients with juvenile chronic arthritis. The increased staining for LK1, with a unique specificity for mammalian HSP60, the mitochondrial chaperone, thus unequivocally demonstrated that this is due to a raised expression level of endogenously produced host HSP60 and not to deposition of bacterial antigens [34].

Aging is known to impact the capacity of cells to upregulate stress proteins. A decrease in HSF1 (the transcription factor which organizes HSP expression) activity has been seen in normally aging individuals, and in aging fruit flies and worms, leading to relative loss of the heat shock system [35,36].The reason for the decrease in the HSF1 activity is unknown but it results in an attenuated heat shock response and a decrease in the ability of an individual to cope with stress. Possibly, along similar lines, the relative loss of expressed HSPs can contribute to a loss of Treg activity, resulting in a relative loss of self-tolerance. If so, this may be an explanation for the increased occurrence of chronic inflammatory diseases in aged individuals.

Besides reduced expression, also structural changes in HSPs might influence the maintenance of self-tolerance. HSP70 polymorphisms have been associated with inflammatory or autoimmune diseases such as Crohn's disease [37], Alzheimer's disease [38], pancreatitis [39] and with development of graft versus host disease upon allogeneic haematopoietic stem cell transplantation [40].

Interestingly, decreased HSP expression has been observed in some immune disorders. A low HSP70 response has also been described in a subtype of Biobreeding (BB) rats with a high susceptibility for development of autoimmune diabetes [41]. Similar results have been found in human PBMC from patients with newly diagnosed type-1 diabetes. In that study, stress responses were found to become re-established again in patients with longstanding diabetes, more than eight months after disease manifestation. So, defective HSP70 induction coincided with beta cell directed inflammatory activity, and seemed modulated by pro-inflammatory cytokines rather than metabolic factors [42].

Additional evidence for the role of HSPs as targets for Tregs may have come from studies of in vivo manipulation of HSP expression levels. In one of our previous studies we have identified carvacrol, one of the main essential oils of many oregano species, as an effective co-inducer for HSP70. Oral administration of carvacrol in mice was found to up-regulate the expression of HSP70 in Peyer's patches, the secondary lymphoid organs of the gut. When lymphocytes were collected from carvacrol treated animals, raised T cell responses to HSP70 were observed. Moreover, the induction of arthritis in carvacrol treated animals was almost fully impossible. The inhibitory effect on arthritis turned out to be transferable with CD4+ T cells obtained from carvacrol fed mice. Altogether, the effects seen with the HSP co-inducer carvacrol were fully compatible with the induction of HSP specific anti-inflammatory Tregs that merely resulted from the upregulated HSP70 in gut lymphoid tissues[43].

Also by other means, such as serological identification of antigens by recombinant expression cloning (SEREX), stress proteins such as DNAJA1 (a HSP70 associated co-chaperone), were defined as targets for naturally occurring Tregs [75].

5. Peptide B29: a conserved Treg inducing HSP70 epitope

As mentioned above, fragments of stress proteins, such as HSP70 family members, are frequent and relatively abundant in the MHCII ligandome of cells and stressed cells in particular. This means that HSP epitopes are well represented on cells poised for presenting their internal cargo to T cells. Regulatory T cells are for their function dependent on triggering through their TcR (T cell receptor). That such stress protein fragments can be targeted by Treg was demonstrated recently by van Herwijnen et al. (159 [23]). A conserved mycobacterial HSP70 peptide (B29) (see Fig. 1 for its structure, conservation and position in the HSP70 sequence) was found to have the capacity to induce a very potent regulatory T cell response. Due to its conservation, various self-homologs with amino acid sequences almost identical with B29, were identified in the mammalian HSP70 family members. Interestingly, the mammalian B29 homologs were also found to be present in human HLA-DR4 molecules obtained from stressed B cells (130 [22]). Following immunization with B29, responding spleen lymphocytes were selected by cell-sorting for regulatory T cells on the basis of CD4+CD25+Foxp3+expression. By adoptive transfer of these sorted Tregs we found that these cells had a remarkable capacity to suppress (ongoing) disease. The latter was shown in an experimental model of autoimmunity, proteoglycan induced arthritis. By the in vitro re-stimulation of the B29 induced Treg with the mammalian homolog's, the up-regulation of Treg associated activation markers was seen, indicating that indeed the mammalian homologs were in vivo targets of these Tregs.

By using a congenic cell marker (CD90.1) is was possible to trace back transferred CD90.1.2 positive T cells in the CD90.2 recipient animals. By doing this the Treg phenotype (Foxp3+, CD25+) was found to remain stable, even until day 50 after the transfer. The cells were found in peripheral blood, bone-marrow, draining lymph-nodes, spleen and also the joint synovium. In addition, by infusing anti-CD90.1 specific antibodies to deplete transferred disease suppressive CD90.1.2+ cells in vivo, it was shown that the disease returned, providing direct proof of the disease suppressive nature of the transferred Treg.

Therefore, as it seems, we have identified peptide B29 as an evolutionary conserved HSP70 epitope with homologues abundantly present in human and mouse MHC class II molecules. T cells recognizing B29 and these homologues were found to be strongly disease suppressive and characterized by CD25+Foxp3+LAG3+expression. Exceptionally low numbers of these cells, up to as few as 4000 cells, were capable of preventing induction of disease and of suppressing already established disease. These cells were long-lived and found to reside in the joints and draining lymph-nodes.

The findings made with the B29 epitope of HSP70 in Balb/c mice concur with earlier findings of disease inhibition with a conserved

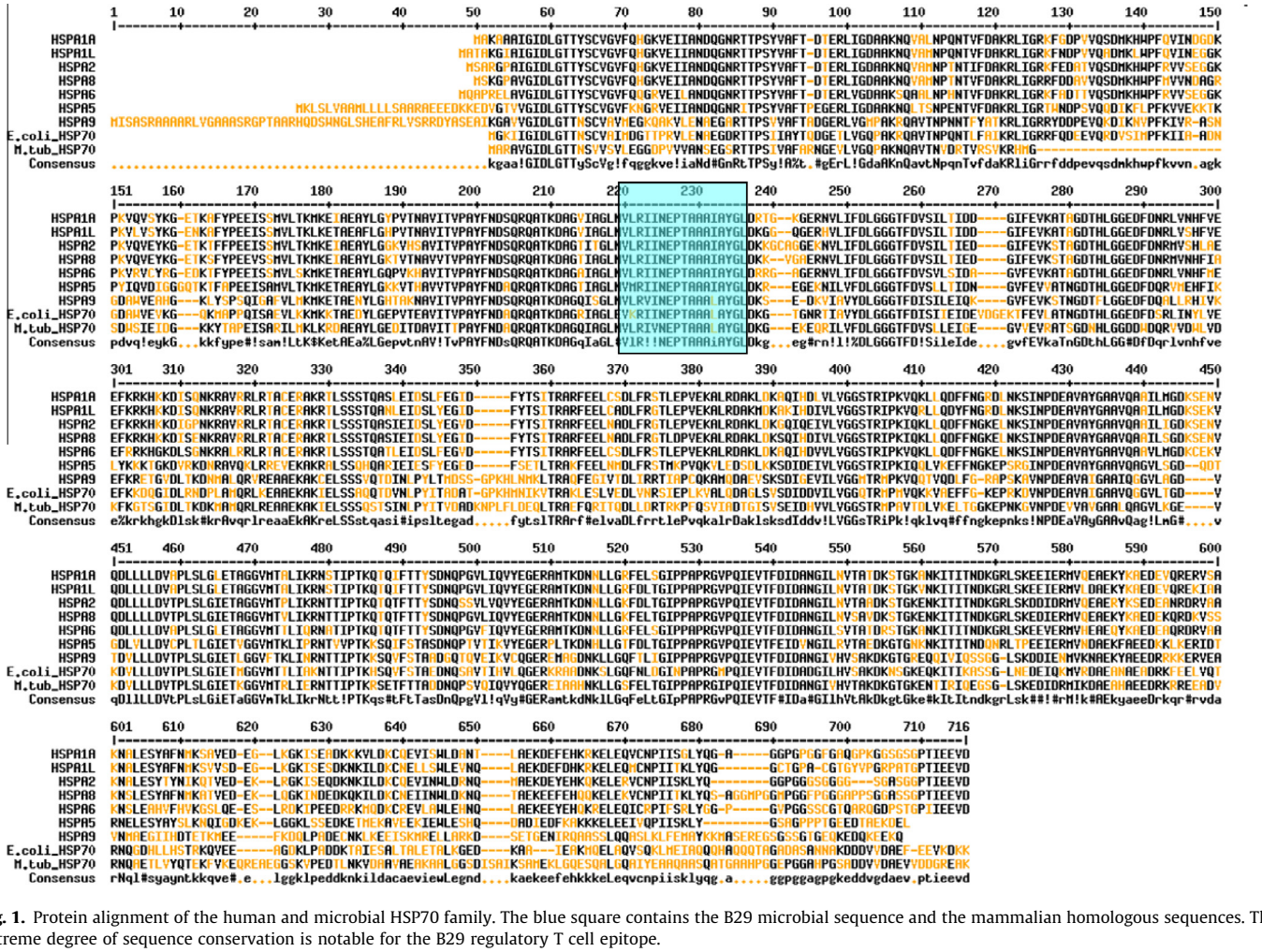

Fig. 1. Protein alignment of the human and microbial HSP70 family. The blue square contains the B29 microbial sequence and the mammalian homologous sequences. The extreme degree of sequence conservation is notable for the B29 regulatory T cell epitope.

mycobacterial HSP70 derived peptide in rats with mycobacteria induced adjuvant arthritis [76]. In this case the peptide, p111, was found to be effective upon nasal and not upon parenteral administration. The analysis of responding T cells following activation with p111, revealed the production of IL10 as the possible disease suppressive mechanism of the p111 specific T cells.

6. Other examples of HSP specific Treg that inhibit inflammatory diseases

In the model of atherosclerosis induced by a, cholesterol rich, western type diet in LDL receptor knock-out mice, oral administration of HSP60 was found to inhibit plaque formation in the clipped (partially obstructed) carotid artery and in the aortic root. Reduction in plaque size correlated with an increase in CD4(+)CD25(+)Foxp3(+) regulatory T cells in several organs and in an increased expression of Foxp3, CD25, and CTLA-4 in atherosclerotic lesions of HSP60-treated mice. The production of interleukin (IL)-10 and transforming growth factor (TGF)-beta by lymph node cells in response to HSP60 was observed after tolerance induction [44]. Similar observations with oral administration of whole (mycobacterial) HSP60 had been made by others earlier, very much in line with the accumulating evidence for a pivotal role of HSP60 in atherosclerosis [45–48]. In the study of van Puijvelde [44], however, the same disease inhibitory effect was seen with both whole HSP60 and a defined HSP60 derived T cell epitope: 253–268. Therefore, the latter study provides additional evidence for a disease suppressive mechanism mediated by HSP specific Tregs.

HSP90 was also found to inhibit spontaneous diabetes in NOD mice, although in this case mechanisms may have remained to be solved [49]. A more exciting set of findings was made with an HSP60 peptide, HSP60 peptide 277, in type I diabetes. This peptide, also known as DiaPep277 may well be the first therapeutic vaccine with the capacity to reinstall the HSP-mediated immune regulation in this important clinical entity [50]. The Cohen group (Weizmann Institute) has done pre-clinical studies of HSP60 peptides in NOD mice, the model of spontaneous type I diabetes [51,52] and has gone onto develop DiaPep277 in particular for the treatment of developing diabetes mellitus in humans. DiaPep277 performed very well in phase II ([53] and recently in phase III clinical studies: Newly diagnosed patients were randomized to receive injections of 1 mg DiaPep277® or placebo subcutaneously for 2 years at quarterly intervals. Insulin treatment was administered by the patients' physicians as needed. The primary efficacy endpoint was the change from baseline to study end in glucagon-stimulated C-peptide. The study was carried out in 40 centers in Europe, Israel and South Africa. Excitingly enough, DiaPep277®-treatment was safe, well tolerated and significant preservation of C-peptide levels was observed. Treated patients experienced fewer hypoglycemic events with a significant difference in the rate of decline in the hypoglycemic events/month. More patients in the treated group

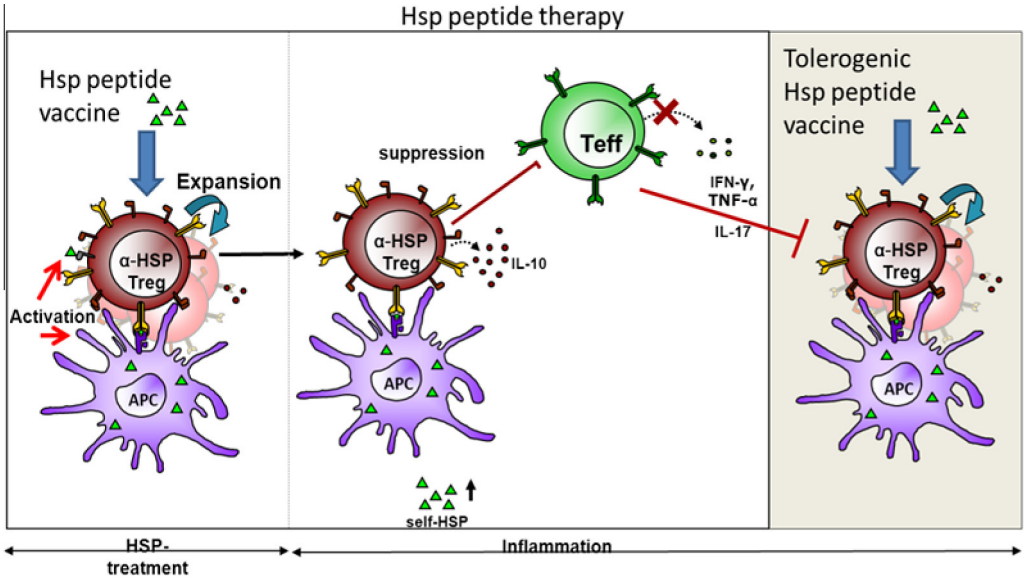

Fig. 2. HSP peptide based vaccines have been shown to enhance regulatory activity in chronic inflammatory disorders through expansion of Tregs amongst others via peptide MHC complex activation. Direct expansion of Tregs is hampered by the pro-inflammatory cytokines produced by the inflammatory T effector cells. To enhance the Treg inducing capacity of the vaccines, tolerogenic adjuvants and routes of administration will be exploited.

were in partial remission maintaining target HbA1c levels while requiring less insulin. Thus, HSP60 peptide treatment preserved beta-cell function and improved clinical outcomes over 2 years in newly diagnosed type 1 diabetes patients).

7. Conclusion

Findings on the role of HSP in the induction of anti-inflammatory T cell responses have led to a concept wherein the regulatory T cells of the immune system exploit the abundant presence of stress proteins for their default suppressive activity. The cellular interaction in this concept are presented in Fig. 2. In the occasion of inflammatory stress the further up-regulation of stress proteins by antigen presenting cells leads to a further enforcement of the regulatory T cell activity. By artificial immunization with stress proteins such as HSP60 and HSP70 the repertoire of HSP specific Tregs is increased leading to a raised resistance against inflammatory diseases. The first clinical trials with HSP peptides in autoimmune diseases have been promising and therefore lead to the expectation that further development of immuno-modulatory vaccines will be possible with the use of HSP proteins and peptides.

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