Review

Heat shock proteins as regulators of the immune response

A Graham Pockley

Until recently, heat shock proteins (also known as heat stress proteins) have mostly been regarded as intracellular molecules that mediate a range of essential housekeeping and cytoprotective functions. However, interest in their role as intercellular signalling molecules has been fuelled by the observations that these molecules can be released and are present in the extracellular environment under physiological conditions. They can elicit cytokine production by, and adhesion molecule expression of, a range of cell types, and they can deliver maturation signals and peptides to antigen presenting cells through receptor-mediated interactions. These functions suggest that heat shock proteins could be immunoregulatory agents with potent and widely-applicable therapeutic uses. Furthermore, the induction of self heat shock protein immune reactivity can attenuate autoimmunity and delay transplant rejection, and heat shock proteins derived from tumours and pathogens can elicit specific, protective immunity. This review will focus on this rapidly evolving area of heat shock protein biology.

> "Discovery is to see what everyone else has seen and to think what no one else has thought"
>
> Albert von Szent-Györgyi Nagyrapolt
> 1937 Nobel Laureate in Medicine

Heat shock proteins were discovered in 1962 when Ferruccio Ritossa and his co-workers noted that temperature shock had induced odd puffing patterns and an unusual profile of gene expression in the polytene chromosomes of salivary glands in *Drosophila melanogaster* larva.[1] However, not until 1974 were the first products of these genes identified and the term heat shock protein coined.[2] Heat shock proteins are highly conserved molecules that are present, and can be induced, in all eukaryotic and prokaryotic species, including plants.

Heat shock proteins are categorised into several families that are named on the basis of their approximate molecular weight (eg, the 60 kDa Hsp60 family). Heat shock proteins are localised in various intracellular compartments, and in physiological conditions some of these proteins function as intracellular molecular chaperones or proteases (panel). Chaperones take part in the assembly, stabilisation, folding, and translocation of oligomeric proteins, whereas proteases, such as the ubiquitin-dependent proteasome, mediate the degradation of damaged proteins.[3,4]

Heat shock proteins are constitutively expressed (making up to 5–10% of the total protein content in healthy growth conditions), but their intracellular concentrations can be increased two to three times by insults that induce protein unfolding, misfolding, or aggregation, and a flux of newly-synthesised non-native proteins. The term heat shock proteins is something of a misnomer, since in addition to raised temperature, exposure to oxidative stress, nutritional deficiencies, ultraviolet irradiation, chemicals, ethanol, viral infection, and ischaemia-reperfusion injury can also induce the expression of these proteins—including members of the Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small heat shock protein families.[5-12]

Induction and regulation of heat shock protein expression

Regulation of transcription of heat shock protein genes is mediated by the interaction of heat shock factor (HSF) transcription factors with heat shock elements in the heat shock protein gene promoter regions.[13,14] In vertebrates, four HSFs have been identified, of which HSF1 and HSF2 are ubiquitously expressed and conserved.[15,16] The main heat shock factor with a role in vertebrates' response to physiological and environmental stress is HSF1,[17,18] whereas activity of HSF2 is more selective, and is mostly induced during differentiation and early development.[19]

Usually, HSF1 is present in the cytoplasm as a latent monomeric molecule that is unable to bind to DNA. When exposed to stress, an intracellular flux of newly synthesised non-native proteins activates HSF1,[14] which is hyperphosphorylated in a *ras*-dependent manner by mitogen-activated protein kinases.[20,21] HSF1 is converted to phosphorylated trimers that have the capacity to bind DNA, and which translocate from the cytoplasm to the nucleus (figure 1).[11] HSF2 has the characteristics of a temperature-sensitive protein; it is inactivated when exposed to raised temperature, and sequestered to the cytoplasm, and is thereby prevented from interference with HSF1 activity in stressed cells.[22] The consequences of binding of HSF1 to its target, and the events that result

Search strategy and selection criteria

Literature pertinent to this review was identified by current reading in the specialty, and by searches in Medline and PubMed databases. Articles were selected on the basis of their relevance to the immunoregulatory activity of extracellular heat shock proteins, their potential clinical value, and the excellence of methods and the importance of the findings presented therein. There was no positive or negative bias to either the authors of the literature cited, or the journals in which work has been published.

Published online April 29, 2003
http://image.thelancet.com/extras/02art9148web.pdf

Immunobiology Research Group, Division of Clinical Sciences (North), Clinical Sciences Centre (University of Sheffield), Northern General Hospital, Herries Road, Sheffield S5 7AU, UK
(A G Pockley, PhD)
(e-mail: g.pockley@sheffield.ac.uk)

Mammalian heat shock protein families and their intracellular location and function

Major family, and members	Intracellular localisation	Intracellular function
Small Hsps		
αB-crystallin	Cytoplasm	Cytoskeletal stabilisation
Hsp27	Cytoplasm/nucleus	Actin dynamics
Haem oxygenase, Hsp32	Cytoplasm	Haem catabolism, antioxidant of properties
Hsp40		
Hsp40	Cytoplasm/nucleus	Regulates the activity of Hsp70;binds non-native proteins
Hsp47	ER	Processing of pro-collagen; processing and/or secretion of collagen
Hsp60 (or chaperonins)		
Hsp60	Mitochondria	Bind to partly folded polypeptides and assist correct folding. Assembly of multimeric complexes
TCP-1	Cytoplasm	
Hsp70		
Inducible: Hsp70, Hsp70hom	Cytoplasm/nucleus	Bind to extended polypeptides. Prevent aggregation of unfolded peptides. Dissociate some oligomers. ATP binding. ATPase activity. Hsp70 downregulates HSF1 activity
Cognate/constitutive: Hsc70	Cytoplasm/peroxisome	
Grp78/BiP	ER	
mtHsp70/Grp75	Mitochondria	
Hsp90		
Hsp90 (α and β)	Cytoplasm	Bind to other proteins. Regulate protein activity. Prevent aggregation of refolded peptide. Correct assembly and folding of newly synthesised protein. Hsp90 assists the maintenance of the HSF1 monomeric state in non- stressful conditions.
Grp94/gp96/Hsp100	ER	
Hsp110		
Hsp110 (human)	Nucleolus/cytoplasm	Thermal tolerance
Apg-1 (mouse)	Cytoplasm	Protein refolding
Hsp105	Cytoplasm	

ER=endoplasmic reticulum. TCP-1=tailless complex polypeptide. Grp=glucose regulated protein. Hsp70hom=testis-specific Hsp70. BiP=immunoglobulin heavy chain binding protein. mt=mitochondrial; Apg-1=protein kinase essential for autophagy. Adapted from Pockley A G. Heat shock proteins in health and disease: therapeutic targets or therapeutic agents? http://www-ermm.cbcu.cam.ac.uk/01003556h.htm (accessed Jan 22, 2003) by permission of Cambridge University Press.

in transcription of heat shock protein genes, have been widely reviewed.[23,24]

The induction of heat shock proteins has to be tightly controlled, since their persistent presence would adversely affect protein homoeostasis and intracellular functions, leading to inappropriate growth control and possibly cell death. One mechanism that regulates heat shock protein expression is the binding of Hsp70 to the transactivation domain of HSF1, leading to repression of heat shock gene transcription.[25] The interaction between Hsp70 and HSF1 has no effect on DNA binding or the stress-induced phosphorylation state of HSF1.[25] A second mechanism regulating heat shock protein synthesis is the interaction between heat shock protein binding factor 1 (HSBP1), the active trimeric form of HSF1, and Hsp70, resulting in inhibition of the capacity of HSF1 to bind to DNA.[26] HSBP1 is mainly localised in the nucleus, and HSBP1 mRNA is present at high concentrations in various cell lines and animal tissues that are unaffected by heat shock.[26]

Heat shock proteins as intercellular signalling molecules

The usual view of eukaryotic heat shock proteins is that they are intracellular molecules that are released from necrotic, but not apoptotic cells, and that their release into (and presence in) the extracellular environment indicates non-physiological tissue damage and therefore induces a range of proinflammatory responses. Findings from several studies are consistent with this idea. Human Hsp60 induces the expression of the adhesion molecules E-selectin, intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on vascular endothelial cells, and the secretion of interleukin-6 from vascular endothelial cells, smooth muscle cells, and macrophages.[27,28] Furthermore, bacterial and mycobacterial heat shock proteins induce proinflammatory cytokine expression,[29-31] and the bacterial Hsp60 (termed GroEL) induces expression of ICAM-1 and VCAM-1 on vascular endothelial cells.[29] Chlamydial Hsp60 activates expression of E-selectin, ICAM-1, and VCAM-1 on human vascular endothelial cells, and secretion of interleukin-6 from vascular endothelial cells, smooth muscle cells, and macrophages.[27,28]

The finding that proteins in the Hsp60 family could induce adhesion molecule expression and cytokine secretion from various cell types prompted the search for Hsp60 receptors. The CD14 antigen has been identified as the receptor for this protein on human peripheral blood mononuclear cells and monocytes; thus, Hsp60 uses the signalling pathway also used by lipopolysaccharide.[28] Toll-like receptor 4,[32] which is an important mediator of innate immunity and lipopolysaccharide signalling in mouse cells, is needed for signalling.[33] Data from another study[34] suggest that Toll-like receptor 2 plays a part in human Hsp60 signalling.

The CD14 molecule also has a role in Hsp70-mediated activation of monocytes, the consequences of which are intracellular calcium fluxes and induction of proinflammatory cytokines (interleukin-1β, interleukin-6, tumour necrosis factor [TNF] α).[35] Toll-like receptors 2 and 4[36] and the CD40 molecule[37] are also involved. A CD14-independent, but calcium-dependent response that leads to production of TNF α has also been identified.[35]

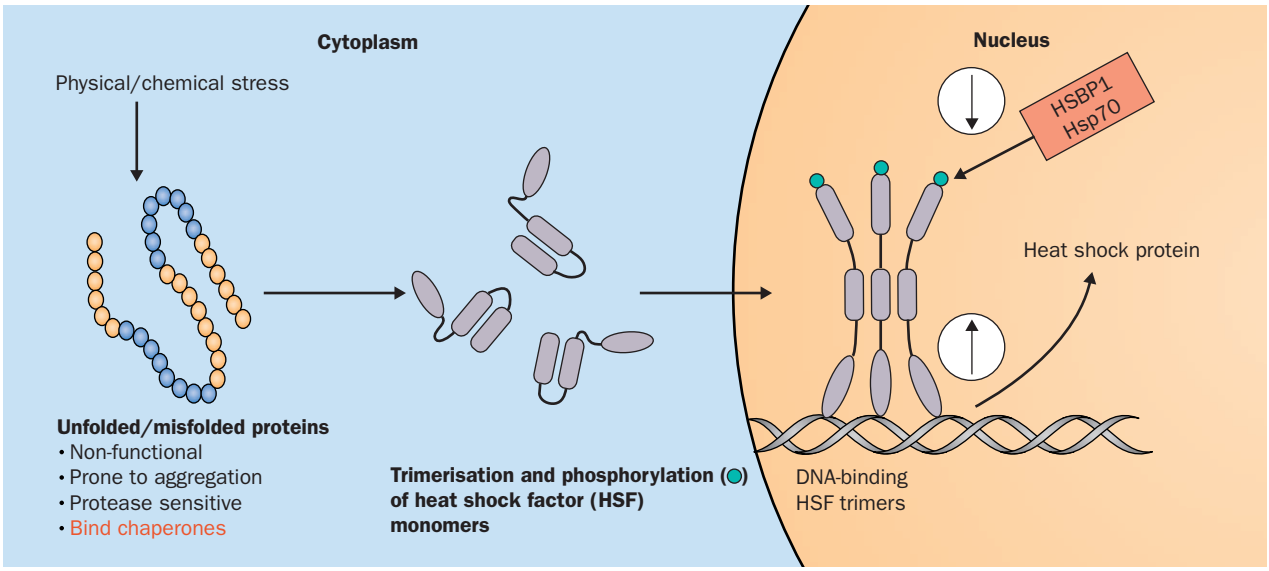

Figure 1: **Induction and regulation of heat shock protein expression**
Physical or chemical stress induces production of unfolded or misfolded proteins. Heat shock factor monomers in the cytoplasm form trimers, are phosphorylated, and translocate into the nucleus. HSF homotrimers bind to heat shock protein gene promoter regions, leading to induction of *Hsp* gene transcription. *Hsp70* gene transcription is downregulated by interaction of Hsp70 or HSBP1 with the HSF trimers.

However, some investigators have expressed concern that the in-vitro responses induced by recombinant heat shock proteins produced in *Escherichia coli* might result from the effects of lipopolysaccharide or other proteins, present either as a contaminant of the preparation or chaperoned by the heat shock protein under investigation,[38,39] especially in view of the similarity in the receptors apparently used by heat shock proteins and lipopolysaccharide. Therefore, controls are essential in such experiments.

The intracellular localisation of eukaryotic heat shock proteins in healthy circumstances, the typical release of heat shock proteins from infectious agents,[40] and the capacity of both human and prokaryotic (bacterial) heat shock proteins to elicit innate and adaptive proinflammatory responses seem to be consistent with the proposed role for these molecules as links between pathogenic processes involving necrotic cell death, and the induction of innate and adaptive immunity. However, further evidence suggests an alternative to this point of view.

Extracellular heat shock proteins

Heat shock proteins can be released from some viable (non-necrotic) mammalian cell types, including cultured rat embryo cells,[41] human islet cells,[42] rat glial cells and a human neuroblastoma cell line,[43] and cultured vascular smooth muscle cells exposed to oxidative stress.[44] These findings have profound implications for the perceived role of these proteins as exclusively proinflammatory intercellular signalling molecules and danger signals. Furthermore, Hsp60 and Hsp70 are present in the peripheral circulation of healthy individuals.[45-51] Hsp60 is also present at high concentrations in people with early atherosclerosis,[45,48] and Hsp70 concentrations are raised in patients with peripheral and renal vascular disease.[52]

The mechanism by which heat shock proteins are released in vitro and in vivo, and their origin in vivo, has received little attention, and is not yet fully understood. However, findings of a seminal study in the late 1980s with cultured rat embryo cells, which showed the release of heat shock proteins from viable cells, suggested that release does not seem to be mediated by the common secretory pathway, since the inhibitors colchicine and monensin do not block secretion.[41] Nor does release seem to be due to cell damage, since Hsp70 was not readily released from cells exposed to low concentrations of nonionic detergents. Rather, the mechanism seems to be selective, since release of Hsp70 is inhibited by the presence of the lysine analogue aminoethyl cysteine, suggesting that an altered structure or function prevents correct interaction with the specific secretory mechanisms.[41]

Since evidence has emerged that heat shock proteins are present in, or can be released into, the extracellular environment in physiological conditions, and that these molecules can elicit a range of biological activities though interactions with specific cell surface receptors, their physiological function in this context should be re-examined.

Sequence versus functional conservation

One of the dogmas of heat shock protein biology is that the high degree of sequence homology between equivalent heat shock protein family members derived from prokaryotes and eukaryotes (about 50%) has led to a high degree of functional conservation. However, the rigidity of this concept is questioned by results of several studies, all of which suggest that heat shock proteins of the same family, but from different species, might have markedly different functions. First, a few bacteria, one of which is *Mycobacterium tuberculosis*, contain multiple genes encoding Hsp60 (chaperonin 60), and despite having greater than 73% aminoacid similarity, mycobacterial chaperonin 60·1 is between ten and 100 times more active in induction of cytokine secretion than is chaperonin 60·2 (otherwise known as Hsp65).[53]

Secondly, chaperonin 60·3 from *Rhizobium leguminosarum* induces the production of a range of cytokines from human monocytes, but chaperonin 60·1, which has a 74% aminoacid sequence homology with chaperonin 60·3, shows no such activity.[54] Thirdly, Hsp60 from the oral bacterium *Actinobacillus actinomycetemcomitans* and from *E coli* are potent stimulators of bone

resorption,[55,56] whereas equivalent molecules from mycobacteria are not.[55,57] Finally, the sequence-sensitivity of the biological function of these molecules is eloquently revealed by the recent observation that a sole aminoacid substitution in the Hsp60 molecule from *E coli* (GroEL) can transform the protein into a potent insecticidal toxin.[58]

Autoreactivity and anti-inflammatory action

Heat shock proteins are immunodominant molecules and a substantial amount of the immune response to pathogenic microorganisms is directed towards peptides derived from these proteins.[59,60] In view of the phylogenetic similarity between microbial and mammalian forms of these molecules, and the consequent potential for crossreactivity, these findings prompted the suggestions that heat shock proteins might act as potentially harmful autoantigens,[59] and that immune recognition of crossreactive epitopes of heat shock proteins provides a link between infection and autoimmunity.[61] Findings that implicate immunity to heat shock proteins (especially Hsp60 and Hsp70) in arthritis,[62-64] multiple sclerosis,[65-67] and diabetes[68-70] lend support to this idea. However, from an evolutionary perspective, why should mammalian responses to bacterial HSP (which presumably evolved as a defence) also occur against mammalian HSP? Several observations question the proposition that self-Hsp60 reactivity has a direct proinflammatory role in inflammatory disease.

First, as shown for many other self peptides, the normal T-cell repertoire includes low affinity T cells reactive against autologous heat shock proteins.[59,71-74] Second, qualitative differences in the phenotype of T cells responding to eukaryotic and prokaryotic Hsp60 and their cytokine secretion profile have been identified. Whereas human Hsp60 activates $CD45RA^{+}RO^{-}$ (naive) human peripheral blood T cells, bacterial-specific peptides activate $CD45RA^{-}RO^{+}$ (memory) T cells, and bacterial Hsp60—which contains both conserved (human) and nonconserved (bacterial) sequences—activates both $CD45RA^{+}RO^{-}$ and $CD45RA^{-}RO^{+}$ T cells.[73] T cells isolated from the synovial fluid of patients with rheumatoid arthritis respond to self (human) Hsp60 mainly by production of regulatory Th2 type cytokine responses, whereas cells stimulated with bacterial Hsp60 produce increased concentrations of interferon gamma; a situation consistent with a proinflammatory Th1 type response.[75] In addition, T-cell lines generated from the synovial fluid of patients with rheumatoid arthritis in response to self-Hsp60 suppress the production of the pro-inflammatory cytokine TNF α by peripheral blood mononuclear cells, whereas cells generated with mycobacterial Hsp65 have no such regulatory effect.[75]

These findings have led to the suggestion that self heat shock protein reactivity might be a physiological mechanism for regulation of proinflammatory disease processes.[76] This notion is further supported by observations that the induction of T-cell reactivity to self-Hsp60 and self-Hsp70 downregulates disease in several experimental models of arthritis, by a mechanism that involves the induction of Th2 type $CD4^{+}$ T cells producing the regulatory cytokines interleukin-4 and interleukin-10.[77-84] The clinical relevance of these findings has been confirmed by data showing an inverse association between the severity of disease and the production of regulatory cytokines such as interleukin-4 and interleukin-10 by T cells stimulated with Hsp60 in patients with rheumatoid arthritis.[74,85,86]

The anti-inflammatory capacity of self-Hsp60 reactivity seems to be the dominant function; the administration of whole mycobacterial Hsp65, which contains the epitope that induces T-cell activation and can induce arthritis in rats when administered alone, does not induce the disease, due to the concomitant presence of conserved (self) epitopes that can dominantly downregulate the arthritogenic capacity of the non-conserved (non-self) epitopes.[79] Although somewhat speculative, this finding might also provide insight into the way in which heat shock proteins derived from infectious agents induce immune responses; antibody responses to non-conserved (non-self) epitopes might be promoted by the concomitant generation of Th2 cytokines, such as interleukin-4, induced by conserved (self) epitopes.

In addition to arthritis, in which the induction or presence of self-Hsp60 and Hsp70 immune reactivity seems to attenuate the disease process, immunisation of recipient mice with mouse (self)-Hsp60, or Hsp60 peptides that have the capacity to shift Hsp60 reactivity from a proinflammatory Th1 phenotype towards a regulatory Th2 phenotype, can delay mouse skin allograft rejection.[87]

Overall, these findings suggest that, rather than being proinflammatory, self-Hsp60 and self-Hsp70 T-cell reactivity are part of a normal immunoregulatory response that has the potential to dominantly control proinflammatory responses and inflammatory disease.[76,88] Investigations aimed at specifically inducing immuno-regulatory T cells reactive with self-heat shock protein, and monitoring their influence on pro-inflammatory disease processes such as autoimmunity, cardiovascular disease and organ transplant rejection are therefore timely and warranted.

Peptide-specific immunity

Tumours

Work in the 1980s showed that heat shock proteins might be useful for induction of antigen-specific immunity. At this time, Pramod Srivastava and colleagues[89] noted that immunisation of mice with a 96 kDa protein fractionated from a tumour cell lysate induced resistance to the same tumour cell from which the 96 kDa protein had originally been isolated. As intracellular chaperones, heat shock proteins bind to many peptides derived from the cells from which they are isolated[4,90]—the so-called antigenic fingerprint or repertoire of that cell.[91] The protein that induced tumour protection in Srivastava's early work has been shown to be the heat shock protein gp96 (otherwise known as grp94); immunisation of mice with Hsp70, Hsp90, and gp96 isolated from mouse tumour cells induces tumour-specific cytolytic T cells and immunity against tumours.[92-95] This immunity is towards tumour-derived peptides associated with the heat shock protein, rather than the heat shock proteins themselves.[92,93] The heat shock proteins calreticulin, Hsp110, and grp170 have also been shown to elicit similar effects by apparently similar mechanisms.[96,97]

Protection against tumours can also be elicited by Hsp70 and gp96 in amphibians (*Xenopus*),[98] and the evolutionarily conserved nature of these responses strongly suggests that such strategies could be successfully developed for clinical use. Indeed, in preliminary clinical trials, cancer-specific $CD8^{+}$ T-cell responses were induced in six of twelve patients immunised with gp96-peptide complexes prepared from their own tumour.[99] The great advantage of use of tumour-cell-derived heat shock proteins as the immunogen, is that identification of relevant tumour antigens is not necessary.

An interesting and potentially important feature of gp96 is its capacity to both induce and inhibit tumour peptide

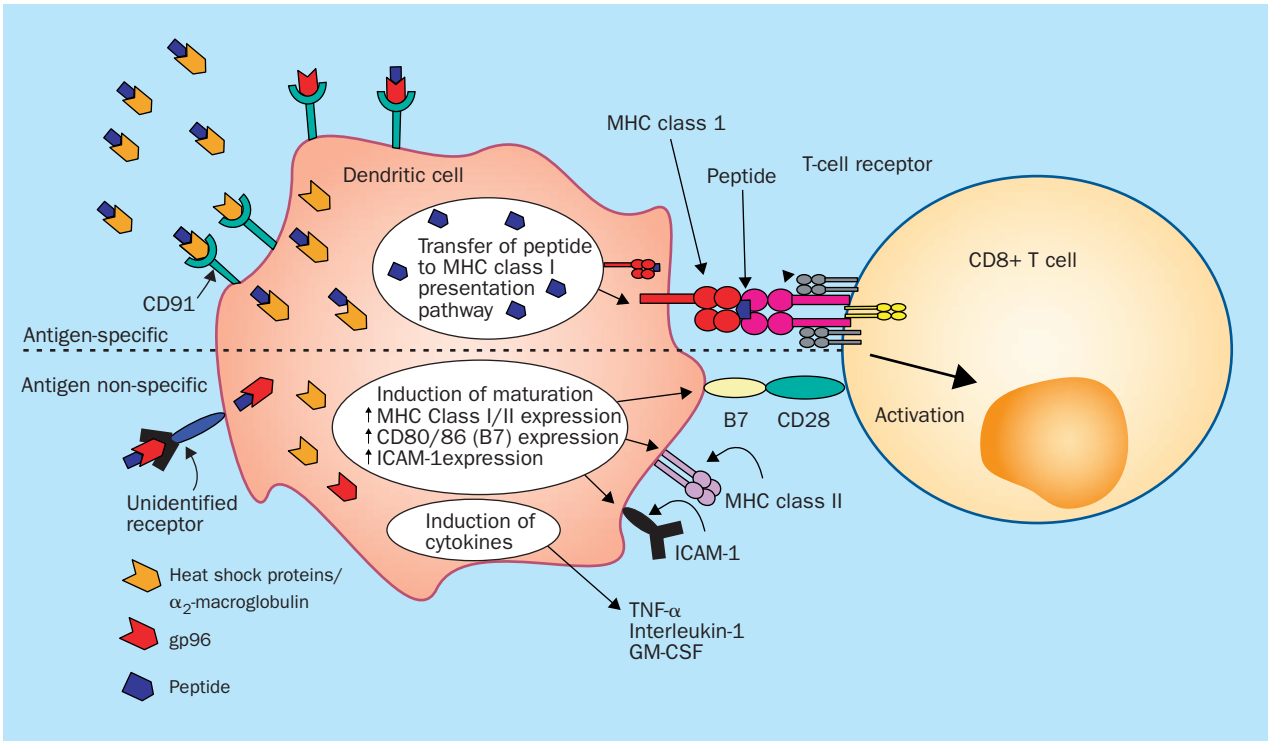

Figure 2: **Effects of heat shock proteins on dendritic cells, and induction of peptide-specific immunity**
GM-CSF=granulocyte-macrophage colony stimulating factor. The heat shock proteins calreticulin, Hsp70, Hsp90, and gp96, and the serum protein α_2-macroglobulin (either complexed with peptide or uncomplexed) are internalised by dendritic cells through the CD91 molecule (α_2-macroglobulin receptor), and/or an as yet unidentified receptor in the case of gp96. Chaperoned peptides are delivered into the MHC class I presentation pathway for subsequent presentation to MHC class I-restricted $CD8^+$ T cells. Hsp70, Hsp90 and gp96 have been shown to concomitantly induce maturation of dendritic cells, as shown by the induction of MHC class II, B7 and ICAM-1 molecule expression, and their release of cytokines.

specific immunity, dependent on the dose of gp96 that is administered. Low doses purified from methylcholanthrene-induced (Meth A) fibrosarcoma given to mice (2×1 μg intradermally) induces protective immunity to the tumour, whereas high doses (2×10 μg intradermally) do not.[94] High doses of tumour-derived gp96 elicit an active, antigen-specific downregulation of tumour-specific immunity that can be adoptively transferred by $CD4^+$ T cells purified from animals treated with high doses of tumour-derived gp96.[94] However, the precise identity and phenotype of these cells has yet to be determined. These findings suggest that immunisation with gp96, either purified from appropriate tissues or chaperoning appropriate peptides, might be an effective strategy for downregulation of several antigen-specific inflammatory conditions. The mechanism by which high doses of gp96 can induce specific immunoregulatory activity is unknown, but is being actively investigated in the author's laboratory.

By contrast, the mechanism by which tumour-derived gp96 can induce tumour-specific immunity has, at least in part, been elucidated. Antigen-presenting cells, such as dendritic cells, spontaneously internalise gp96 by receptor-mediated endocytosis[100-102] through the α_2-macroglobulin receptor (CD91 molecule)[103] or a CD91-independent mechanism,[104] or both. Chaperoned proteins and peptides are directed into the intracellular pathway for MHC class I-restricted presentation to $CD8^+$ T cells.[100-104] For dendritic cells to fully activate T cells, they must be mature and express essential costimulatory molecules such as CD80 and CD86, and gp96 also induces the maturation of, and cytokine secretion from, these cells (figure 2).[105]

The CD91 molecule is also common to other heat shock proteins that have the capacity to induce tumour-specific immunity (such as calreticulin, Hsp70, Hsp90).[106] α_2-macroglobulin, which is a serum protein and was originally described as the ligand for CD91, is also able to direct exogenous antigens into the endogenous pathway of antigen presentation through the same receptor; such a mechanism could allow continual sampling of the antigenic profile of the organism, and hence appropriate regulation of immune responses.[107] Hsp60 does not bind to CD91; nor does tumour-derived Hsp60 induce tumour-specific immunity. The identification on macrophages of an Hsp60 binding site that is unique to this receptor suggests that Hsp60 has a role in immunoregulation that is distinct from that of the other heat shock proteins.[108]

Pathogens

The capacity of heat shock proteins to elicit specific immunity to infectious agents is also being investigated.[109] Treatment of mice with a fusion protein (HspE7) incorporating an early viral protein (E7) of the human papillomavirus 16 has been shown to induce specific cytotoxic T-cell activity, and to confer protection against a mouse tumour cell expressing E7.[110,111] Heat shock proteins isolated from SV40-transformed and influenza-infected cells, or a mixture of gp96 or Hsp70 reconstituted with specific cytotoxic T lymphocyte epitopes from SV40 and influenza virus, have been shown to elicit peptide-specific cytolytic T cells and protective antiviral immunity in mice.[112-115] Similar results have been obtained with lymphocytic choriomeningitis virus.[116] Immunity can also be induced to proteins covalently linked to appropriate heat shock proteins; immunisation of mice with the HIV-1 p24 protein covalently linked to mycobacterial Hsp70 elicits antibody, cytokine, and lymphocyte proliferative responses.[117] Furthermore,

administration of recombinant fusion protein, consisting of mycobacterial Hsp65 and portions of the nucleoprotein antigen of influenza virus, elicits MHC class I restricted, nucleoprotein-specific cytotoxic T cells.[118]

Clearly, in view of this evidence, heat shock proteins chaperone intracellular peptides and can deliver these peptides, concomitant with a proinflammatory stimulus, to antigen presenting cells for subsequent activation of peptide-specific T cells. This response provides an adaptive advantage, in that the release of heat shock proteins from cells undergoing necrosis promotes the activation of innate and adaptive immunity, and the crosspresentation of cytoplasmic protein-derived peptides. This method allows the organism to respond to cell death, and is especially advantageous in viral infections.[119]

Summary

Much remains to be learnt about heat shock protein biology. In the past, reactivity to heat shock proteins has been associated with several kinds of disease, yet evidence now suggests that immune reactivity to self-derived molecules is anti-inflammatory, and attenuates proinflammatory conditions such as arthritis and organ transplant rejection. The physiological and immunological role of extracellular heat shock proteins and their intercellular signalling capacity should also be reconsidered, since these proteins can be released from viable cells, and are present in the peripheral circulation of healthy individuals.

Heat shock proteins are extremely potent molecules, the importance of which to physiological and immunological processes is indicated by the high degree to which their structure and function are phylogenetically conserved. Enhanced understanding of the various immunoregulatory mechanisms in which heat shock proteins are involved could help us to harness the power of these molecules for the control of inflammatory processes and the treatment of human disease.

Conflict of interest statement

None declared.

Acknowledgments

Work in the author's laboratory is supported by the US National Institutes of Health/National Heart, Lung and Blood Institute (grant HL69726), the Association for International Cancer Research (grant 03-078) and the British Heart Foundation (grant PG/2000042). There was no specific funding source for this article.

References

1 Ritossa FA. A new puffing pattern induced by temperature shock and DNP in *Drosophila*. *Experientia* 1962; **18:** 571–73.
2 Tissières A, Mitchell HK, Tracy U. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. *J Mol Biol* 1974; **84:** 389–98.
3 Hightower LE. Heat shock, stress proteins, chaperones and proteotoxicity. *Cell* 1991; **66:** 191–97.
4 Gething MJ, Sambrook J. Protein folding in the cell. *Nature* 1992; **355:** 33–45.
5 Nover I. Heat Shock Response. Boca Raton: CRC, 1991.
6 Welch WJ. How cells respond to stress. *Scientific American* 1993; **268:** 56–64.
7 Chouchane L, Bowers S, Sawasdikosol S, Simpson RM, Kindt TJ. Heat shock proteins expressed on the surface of human T cell leukemia virus type I–infected cell lines induce autoantibodies in rabbits. *J Infect Dis* 1994; **169:** 253–59.
8 Lathangue NB, Latchman DS. A cellular protein related to heat shock protein 90 accumulates during herpes-simplex-virus infection and is overexpressed in transformed cells. *Exp Cell Res* 1988; **178:** 169–79.
9 Donati YRA, Slosman DO, Polla BS. Oxidative injury and the heat shock response. *Biochem Pharmacol* 1990; **40:** 2571–77.
10 Fincato G, Polentarutti N, Sica A, Mantovab A, Collotti F. Expression of a heat inducible gene of the Hsp70 family in human myelomonocytic cells: regulation by bacterial products and cytokines. *Blood* 1991; **77:** 579–86.
11 Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Develop* 1998; **12:** 3788–96.
12 Parsell DA, Lindquist S. The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Ann Rev Genetics* 1993; **27:** 437–96.
13 Voellmy R. Transduction of the stress signal and mechanisms of transcriptional reguilation of heat shock/stress protein gene expression in higher eukaryotes. *Crit Rev Eukaryot Gene Expr* 1994; **4:** 357–401.
14 Morimoto RI, Jurivich DA, Kroger PE, et al. Regulation of heat shock gene transcription by a family of heat shock factors. In: Morimoto RI, Tissières A, Georgopoulos C, eds. The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1994: 417–55.
15 Nakai A, Morimoto RI. Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. *Mol Cell Biol* 1993; **13:** 1983–97.
16 Sarge KD, Zimarino V, Holm K, Wu C, Morimoto RI. Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. *Genes Develop* 1991; **5:** 1902–11.
17 Sarge KD, Murphy SP, Morimoto RI. Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. *Mol Cell Biol* 1993; **13:** 1392–407.
18 Zuo J, Baler R, Dahl G, Voellmy R. Activation of the DNA-binding ability of human heat shock transcription factor 1 may involve the transition from an intramolecular triple-stranded coiled-coil structure. *Mol Cell Biol* 1994; **14:** 7447–68.
19 Pirkkala L, Nykanen P, Sistonen L. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *FASEB J* 2001; **15:** 1118–31.
20 Knauf U, Newton EM, Kyriakis J, Kingston RE. Repression of human heat shock factor 1 activity at control temperature by phosphorylation. *Genes Develop* 1996; **10:** 2782–93.
21 Kim J, Nueda A, Meng YH, Dynan WS, Mivechi NF. Analysis of the phosphorylation of human heat shock transcription factor-1 by MAP kinase family members. *J Cell Biochem* 1997; **67:** 43–54.
22 Mathew A, Mathur SK, Jolly C, Fox SG, Kim S, Morimoto RI. Stress-specific activation and repression of heat shock factors 1 and 2. *Mol Cell Biol* 2001;**21:** 7163–71.
23 Wu C. Heat shock transcription factors: structure and regulation. *Ann Rev Cell Dev Biol* 1995; **11:** 441–69.
24 Nollen EA, Morimoto RI. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci* 2002; **115:** 2809–16.
25 Shi Y, Mosser DD, Morimoto RI. Molecular chaperones as HSF1-specific transcriptional repressors. *Genes Develop* 1998; **12:** 654–66.
26 Satyal SH, Chen D, Fox SG, Kramer JM, Morimoto RI. Negative regulation of the heat shock transcriptional response by HSBP1. *Genes Develop* 1998; **12:** 1962–74.
27 Kol A, Bourcier T, Lichtman A, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J Clin Invest* 1999; **103:** 571–77.
28 Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. *J Immunol* 2000; **164:** 13–17.
29 Galdiero M, de l'Ero GC, Marcatili A. Cytokine and adhesion molecule expression in human monocytes and endothelial cells stimulated with bacterial heat shock proteins. *Infect Immun* 1997; **65:** 699–707.
30 Retzlaff C, Yamamoto Y, Hoffman PS, Friedman H, Klein TW. Bacterial heat shock proteins directly induce cytokine mRNA and interleukin-1 secretion in macrophage cultures. *Infect Immun* 1994; **62:** 5689–93.
31 Peetermans WE, Raats CJ, Langermans JA, van Furth R. Mycobacterial heat shock protein 65 induces proinflammatory cytokines but does not activate human mononuclear phagocytes. *Scand J Immunol* 1994; **39:** 613–17.
32 Ohashi K, Burkart V, Flohé S, Kolb H. Heat shock protein 60 is a putative endogenous ligand of the Toll-like receptor-4 complex. *J Immunol* 2000; **164:** 558–61.
33 Hoshino K, Takeuchi O, Kawai T, et al. Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999; **162:** 3749–52.
34 Vabulas RM, Ahmad-Nejad P, da Costa C, et al. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the

toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 2001; **276:** 31332–39.
35 Asea A, Kraeft S-K, Kurt-Jones EA, et al. Hsp70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 2000; **6:** 435–442.
36 Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H. HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *J Biol Chem* **2002:** 15107–12.
37 Becker T, Hartl FU, Wieland F. CD40, an extracellular receptor for binding and uptake of Hsp70-peptide complexes. *J Cell Biol* 2002; **158:** 1277–85.
38 Wallin RPA, Lundqvist A, Moré SH, von Bonin A, Kiessling R, Ljunggren H-G. Heat-shock proteins as activators of the innate immune system. *Trends Immunol* 2002; **23:** 130–35.
39 Gaston JSH. Heat shock proteins and innate immunity. *Clin Exp Immunol* 2002; **127:** 1–3.
40 Ranford JC, Coates ARM, Henderson B. Chaperonins are cell-signalling proteins: the unfolding biology of molecular chaperones. *Exp Rev Mol Med* 2000.
41 Hightower LE, Guidon PT. Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. *J Cell Physiol* 1989; **138:** 257–66.
42 Child DF, Williams CP, Jones RP, Hudson PR, Jones M, Smith CJ. Heat shock protein studies in Type 1 and Type 2 diabetes and human islet cell culture. *Diabet Med* 1995; **12:** 595–99.
43 Bassan M, Zamostiano R, Giladi E, et al. The identification of secreted heat shock 60-like protein from rat glial cells and a human neuroblastoma cell line. *Neurosci Lett* 1998; **250:** 37–40.
44 Liao D-F, Jin Z-G, Baas AS, et al. Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells. *J Biol Chem* 2000; **275:** 189–96.
45 Pockley AG, Wu R, Lemne C, Kiessling R, de Faire U, Frostegård J. Circulating heat shock protein 60 is associated with early cardiovascular disease. *Hypertension* 2000; **36:** 303–07.
46 Pockley AG, Bulmer J, Hanks BM, Wright BH. Identification of human heat shock protein 60 (Hsp60) and anti-Hsp60 antibodies in the peripheral circulation of normal individuals. *Cell Stress Chaperones* 1999; **4:** 29–35.
47 Pockley AG, Shepherd J, Corton J. Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. *Immunol Invest* 1998; **27:** 367–77.
48 Xu Q, Schett G, Perschinka H, et al. Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation* 2000; **102:** 14–20.
49 Rea IM, McNerlan S, Pockley AG. Serum heat shock protein and anti-heat shock protein antibody levels in aging. *Exp Gerontol* 2001; **36:** 341–52.
50 Lewthwaite J, Owen N, Coates A, Henderson B, Steptoe A. Circulating human heat shock protein 60 in the plasma of British civil servants. *Circulation* 2002; **106:** 196–201.
51 Pockley AG, de Faire U, Kiessling R, Lemne C, Thulin T, Frostegård J. Circulating heat shock protein and heat shock protein antibody levels in established hypertension. *J Hypertens* 2002; **20:** 1815–20.
52 Wright BH, Corton J, El-Nahas AM, Wood RFM, Pockley AG. Elevated levels of circulating heat shock protein 70 (Hsp70) in peripheral and renal vascular disease. *Heart Vessels* 2000; **15:** 18–22.
53 Lewthwaite JC, Coates ARM, Tormay P, et al. Mycobacterium tuberculosis chaperonin 60.1 is a more potent cytokine stimulator than chaperonin 60.2 (hsp 65) and contains a CD14-binding domain. *Infect Immun* 2001; **69:** 7349–55.
54 Lewthwaite J, George R, Lund PA, et al. *Rhizobium leguminosarum* chaperonin 60.3, but not chaperonin 60.1, induces cytokine production by human monocytes: activity is dependent on interaction with cell surface CD14. *Cell Stress Chaperones* 2002; **7:** 130–36.
55 Kirby AC, Meghji S, Nair SP, et al. The potent bone-resorbing mediator of *Actinobacillus actinomycetemcomitans* is homologous to the molecular chaperone GroEL. *J Clin Invest* 1995; **96:** 1185–94.
56 Reddi K, Meghji S, Nair SP, et al. The *Escherichia coli* chaperonin 60 (groEL) is a potent stimulator of osteoclast formation. *J Bone Miner Res* 1998; **13:** 1260–66.
57 Meghji S, White P, Nair SP, et al. *Mycobacterium tuberculosis* chaperonin 10 stimulates bone resorption: a potential contributory factor in Pott's disease. *J Exp Med* 1997; **186:** 1241–46.
58 Yoshida N, Oeda K, Watanabe E, et al. Protein function. Chaperonin turned insect toxin. *Nature* 2001; **411:** 44.
59 Kaufmann SHE. Heat shock proteins and the immune response. *Immunol Today* 1990; **11:** 129–36.
60 Young RA. Stress proteins and immunology. *Annu Rev Immunol* 1990; **8:** 401–20.
61 Lamb JR, Bal V, Mendez-Samperio A, et al. Stress proteins may provide a link between the immune response to infection and autoimmunity. *Int Immunol* 1989; **1:** 191–96.
62 Res PC, Schaar CG, Breedveld FC, et al. Synovial fluid T cell reactivity against 65 kDa heat shock protein of mycobacteria in early chronic arthritis. *Lancet* 1988; **2:** 478–80.
63 Gaston JSH, Life PF, Jenner PJ, Colston MJ, Bacon PA. Recognition of a mycobacteria-specific epitope in the 65kD heat shock protein by synovial fluid derived T cell clones. *J Exp Med* 1990; **171:** 831–41.
64 de Graeff-Meeder ER, van der Zee R, Rijkers GT, et al. Recognition of human 60 kD heat shock protein by mononuclear cells from patients with juvenile chronic arthritis. *Lancet* 1991; **337:** 1368–72.
65 Wucherpfennig K, Newcombe J, Li H, Keddy C, Cuzner ML. γδ T cell receptor repertoire in acute multiple sclerosis lesions. *Proc Natl Acad Sci USA* 1992; **89:** 4588–92.
66 Georgopoulos C, McFarland H. Heat shock proteins in multiple sclerosis and other autoimmune diseases. *Immunol Today* 1993; **14:** 373–75.
67 Stinissen P, Vandevyver C, Medaer R, et al. Increased frequency of γδ T cells in cerebrospinal fluid and peripheral blood of patients with multiple sclerosis: Reactivity, cytotoxicity, and T cell receptor V gene rearrangements. *J Immunol* 1995; **154:** 4883–94.
68 Elias D, Markovits D, Reshef T, van der Zee R, Cohen IR. Induction and therapy of autoimmune diabetes in the non-obese diabetic mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci USA* 1990; **87:** 1576–80.
69 Child D, Smith C, Williams C. Heat shock protein and the double insult theory for the development of insulin-dependent diabetes. *J Royal Soc Med (Eng)* 1993; **86:** 217–19.
70 Tun RYM, Smith MD, Lo SSM, Rook GAW, Lydyard P, Leslie RDG. Antibodies to heat shock protein 65 kD in Type 1 diabetes mellitus. *Diabet Med* 1994; **11:** 66–70.
71 Munk ME, Schoel B, Modrow S, Karr RW, Young RA, Kaufmann SHE. T lymphocytes from healthy individuals with specificity to self-epitopes shared by the mycobacterial and human 65-kilodalton heat shock protein. *J Immunol* 1989; **143:** 2844–49.
72 Cohen IR. Heat shock protein 60 and the regulation of autoimmunity. In: van Eden W, Young DB, eds. Stress Proteins in Medicine. New York: Marcel Dekker, 1996: 93–102.
73 Ramage JM, Young JL, Goodall JC, Hill Gaston JS. T cell responses to heat shock protein 60: differential responses by $CD4^+$ T cell subsets according to their expression of CD45 isotypes. *J Immunol* 1999; **162:** 704–10.
74 Macht LM, Elson CJ, Kirwan JR, et al. Relationship between disease severity and responses by blood mononuclear cells from patients with rheumatoid arthritis to human heat-shock protein 60. *Immunology* 2000; **99:** 208–14.
75 van Roon JAG, van Eden W, van Roy JLAM, Lafeber FJPG, Bijlsma JWJ. 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.
76 van Eden W, van der Zee R, Paul AGA, et al. Do heat shock proteins control the balance of T-cell regulation in inflammatory diseases? *Immunol Today* 1998; **19:** 303–307.
77 van den Broek MF, Hogervorst EJM, van Bruggen MCJ, van Eden W, van der Zee R, van den Berg W. Protection against streptococcal cell wall induced arthritis by pretreatment with the 65kD heat shock protein. *J Exp Med* 1989; **170:** 449–66.
78 Thompson SJ, Rook GAW, Brealey RJ, van der Zee R, Elson CJ. Autoimmune reactions to heat shock proteins in pristane induced arthritis. *Eur J Immunol* 1990; **20:** 2479–84.
79 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.
80 Kingston AE, Hicks CA, Colston MJ, Billingham MEJ. A 71-kD heat shock protein (hsp) from *Mycobacterium tuberculosis* has modulatory effects on experimental rat arthritis. *Clin Exp Immunol* 1996; **103:** 77–82.
81 Anderton SM, van Eden W. T lymphocyte recognition of hsp60 in experimental arthritis. In: van Eden W, Young D, eds. Stress Proteins in Medicine. New York: Marcel Dekker, 1996: 73–91.
82 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:** 5560–65.
83 Paul AGA, van Kooten PJS, van Eden W, van der Zee R. Highly autoproliferative T cells specific for 60-kDa heat shock protein produce IL-4/IL-10 and IFN-γ and are protective in adjuvant arthritis. *J Immunol* 2000; **165:** 7270–77.
84 Wendling U, Paul L, van der Zee R, Prakken B, Singh M, van Eden W. 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:** 2711–17.

85 de Graeff-Meeder ER, van Eden W, Rijkers GT, et al. Juvenile chronic arthritis: T cell reactivity to human HSP60 in patients with a favorable course of arthritis. *J Clin Invest* 1995; **95:** 934–40.

86 van Roon J, van Eden W, Gmelig-Meylig E, Lafeber F, Bijlsma J. Reactivity of T cells from patients with rheumatoid arthritis towards human and mycobacterial hsp60. *FASEB J* 1996; **10:** A1312.

87 Birk OS, Gur SL, Elias D, et al. The 60-kDa heat shock protein modulates allograft rejection. *Proc Natl Acad Sci USA* 1999; **96:** 5159–63.

88 van Eden W. Stress proteins as targets for anti-inflammatory therapies. *Drug Discovery Today* 2000; **5:** 115–20.

89 Srivastava PK, DeLeo AB, Old LJ. Tumor rejection antigens of chemically induced sarcomas of inbred mice. *Proc Natl Acad Sci USA* 1986; **83:** 3407–11.

90 Young D, Romain E, Moreno C, O'Brien R, Born W. Molecular chaperones and the immune system response. *Phil Trans R Soc Lond* 1993; **339:** 363–67.

91 Srivastava PK, Udono H. Heat shock protein-peptide complexes in cancer immunotherapy. *Curr Opin Immunol* 1994; **6:** 728–32.

92 Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. *J Exp Med* 1993; **178:** 1391–96.

93 Udono H, Srivastava PK. Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90 and hsp70. *J Immunol* 1994; **152:** 5398–5403.

94 Chandawarkar RY, Wagh MS, Srivastava PK. The dual nature of specific immunological activity of tumour-derived gp96 preparations. *J Exp Med* 1999; **189:** 1437–42.

95 Ciupitu AM, Petersson M, Kono K, Charo J, Kiessling R. Immunization with heat shock protein 70 from methylcholanthrene-induced sarcomas induces tumor protection correlating with in vitro T cell responses. *Canc Immunol Immunother* 2002; **51:** 163–70.

96 Basu S, Srivastava P. Calreticulin, a peptide-binding chaperone of the endoplasmic reticulum, elicits tumor- and peptide-specific immunity. *J Exp Med* 1999; **189:** 797–802.

97 Wang X-Y, Kazim L, Repasky EA, Subjeck JR. Characterization of heat shock protein 110 and glucose-regulated protein 170 as cancer vaccines and the effect of fever-range hyperthermia on vaccine activity. *J Immunol* 2001; **165:** 490–97.

98 Robert J, Ménoret A, Basu S, Cohen N, Srivastava PK. Phylogenetic conservation of the molecular and immunological properties of the chaperone gp96 and hsp70. *Eur J Immunol* 2001; **31:** 186–95.

99 Janetzki S, Palla D, Rosenhauer V, Lochs H, Lewis JJ, Srivastava PK. Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: a pilot study. *Int J Cancer* 2000; **88:** 232–38.

100 Arnold-Schild D, Hanau D, Spehner D, et al. Receptor-mediated endocytosis of heat shock proteins by professional antigen-presenting cells. *J Immunol* 1999; **162:** 3757–60.

101 Singh-Jasuja H, Toes REM, Spee P, et al. Cross-presentation of glycoprotein 96-associated antigens on major histocompatibility complex molecules requires receptor-mediated endocytosis. *J Exp Med* 2000; **191:** 1965–74.

102 Castellino F, Boucher PE, Eichelberg K, et al. Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct pathways. *J Exp Med* 2000; **191:** 1957–64.

103 Binder RJ, Han DK, Srivastava PK. CD91: a receptor for heat shock protein gp96. *Nat Immunol* 2000; **1:** 151–55.

104 Berwin B, Hart JP, Pizzo SV, Nicchitta CV. CD91-independent cross-presentation of grp94(gp96)-associated peptides. *J Immunol* 2002; **168:** 4282–86.

105 Singh-Jasuja H, Scherer HU, Hilf N, et al. The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. *Eur J Immunol* 2000; **30:** 2211–15.

106 Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70 and calreticulin. *Immunity* 2001; **14:** 303–13.

107 Binder RJ, Karimeddini D, Srivastava PK. Adjuvanticity of α_2-macroglobulin, an independent ligand for the heat shock protein receptor CD91. *J Immunol* 2001; **166:** 4968–72.

108 Habich C, Baumgart K, Kolb H, Burkart V. The receptor for heat shock protein 60 on macrophages is saturable, specific, and distinct from receptors for other heat shock proteins. *J Immunol* 2002; **168:** 569–76.

109 Mizzen L. Immune responses to stress proteins: applications to infectious disease and cancer. *Biotherapy* 1998; **10:** 173–89.

110 Chu NR, Wu HB, Wu TC, Boux LJ, Mizzen LA, Siegel MI. Immunotherapy of a human papillomavirus type 16 E7-expressing tumor by administration of fusion protein comprised of Mycobacterium bovis BCG Hsp65 and HPV16 E7. *Cell Stress Chaperones* 2000; **5:** 401–05.

111 Chu NR, Wu HB, Wu T, Boux LJ, Siegel MI, Mizzen LA. Immunotherapy of a human papillomavirus (HPV) type 16 E7-expressing tumour by administration of fusion protein comprising Mycobacterium bovis bacille Calmette-Guerin (BCG) hsp65 and HPV16 E7. *Clin Exp Immunol* 2000; **121:** 216–25.

112 Blachere NE, Udono H, Janetzki S, Li Z, Heike M, Srivastava PK. Heat shock protein vaccines against cancer. *J Immunother* 1993; **14:** 352–56.

113 Heikema A, Agsteribbe E, Wiscjut J, Huckriede A. Generation of heat shock protein-based vaccines by intracellular loading of gp96 with antigenic peptides. *Immunol Lett* 1997; **57:** 69–74.

114 Suto R, Srivastava PK. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. *Science* 1995; **269:** 1585–88.

115 Blachere NE, Li ZL, Chandawarkar RY, et al. Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and tumor immunity. *J Exp Med* 1997; **186:** 1315–22.

116 Ciupitu AT, Petersson M, O'Donnell CL, et al. Immunization with a lymphocytic choriomeningitis virus peptide mixed with heat shock protein 70 results in protective antiviral immunity and specific cytotoxic T lymphocytes. *J Exp Med* 1998; **187:** 685–91.

117 Suzue K, Young RA. Adjuvant-free hsp70 fusion protein system elicits humoral and cellular immune responses to HIV-1 p24. *J Immunol* 1996; **156:** 873–79.

118 Anthony LS, Wu H, Sweet H, Turnnir C, Boux LJ, Mizzen LA. Priming of $CD8^+$ CTL effector cells in mice by immunization with a stress protein-influenza virus nucleoprotein fusion molecule. *Vaccine* 1999; **17:** 378–83.

119 Anderson KM, Srivastava PK. Heat, heat shock, heat shock proteins and death: a central link in innate and adaptive immune responses. *Immunol Lett* 2000; **74:** 35–39.