REVIEW ARTICLE

The heat shock protein response and its role in inflammatory disease

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The heat shock protein response was first observed in the larvae of the fruit fly *Drosophila busckii*.¹ Under conditions of raised temperature, 'puffs' were seen in salivary gland chromosomes, general protein synthesis was switched off, and a set of specific mRNAs was translated to produce the heat shock proteins (HSPs). The significance of these early observations was soon appreciated by molecular biologists, who used them to study the regulation and mechanisms of gene transcription. Only recently have the biological roles of HSPs been investigated. A number of excellent reviews are available²⁻⁴; this treatise aims at summarising our knowledge of the characterisation, cellular distribution, and function of HSPs, and their possible role in inflammation with specific reference to rheumatology.

Characterisation of heat shock proteins

It is now well established that HSPs occur in all living organisms and are produced after not only heat shock but also other forms of physiological stress.² We may consider this intracellular response complementary and comparable to the slower developing extracellular acute phase response. It has been shown that the genes coding the proteins produced as a result of hyperthermia contain a specific DNA sequence, the 'heat shock consensus element',⁵ which is absent in the genes of proteins produced after some other stress events such as the glucose regulated proteins (GRPs) produced following glucose deprivation; this conserved sequence is located about 20 nucleotides 'upstream' of the 'TATA box' (a common promoter sequence, rich in thymidine and adenine nucleotides, which is found close to the initiation site of eukaryotic genes; an analogous 'box' is found in prokaryotes). As GRPs and HSPs are often produced simultaneously, collectively the proteins are better termed stress proteins. Nevertheless and importantly, the full complement of stress proteins is not induced by every stressor. For example, the human stress protein with a molecular weight of 32 000 daltons (32 kD) can be induced by heavy metals and thiol-reactive agents but not by hyperthermia, the calcium ionophore A23187, or amino acid analogues.⁶ This protein does not contain a heat shock consensus element, but we believe it to be important in rheumatology as many of our disease suppressing therapeutic compounds—for example, gold, D-penicillamine—either contain or affect the inducers of this protein.⁷

Stress proteins are divided into subsets or 'families' according to their molecular weight, which is a source of confusion. Although the genes are remarkably well conserved between species, the proteins vary slightly in molecular weight; thus the 32 kD human stress protein becomes the 34 kD murine protein. The best studied family of proteins is the 70 kD family. In man this family is now known to consist of five structurally and immunologically related proteins (table 1). The proteins are characterised in terms of their molecular weight and isoelectric point (pI) as the method most commonly used to visualise these proteins is that of two dimensional electrophoresis. Firstly, the proteins are separated on a pH gradient gel (isoelectric focusing) and then in the second dimension by molecular weight (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). The pH gradient separates differentially phosphorylated isomers of stress proteins—for example, there are three isomers of the 28 kD protein, which are not all expressed simultaneously in a given cell. The 70 kD family is complicated, however, and there seem to be differences between cell types. Further confusion arises owing to slight variations in gel systems, leading to different workers using different names for the stress proteins. Thus Welch names the most abundant 70 kD proteins as 72 kD and 73 kD while Morimoto uses 70 kD and 72 kD respectively.⁸ Nevertheless, the proteins are biochemically equivalent, the 72/70 kD protein being indicative of and specific to a stress response; the 73 kD (pI 5.5) protein is produced constitutively. Their *drosophila* counterparts have molecular weights of 68 kD and 70 kD. In table 1 alternative names^{8,9} are given, though direct comparison is often impossible. In this review the Welch nomenclature for the 70 kD family will be used.

Another major family of stress proteins is the 90 kD family, and there are low molecular weight stress proteins which have been cate-

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Table 1: Human 70 kD family

Protein	Mol wt (kD)*	pI*	Other names
Constitutive	73	5.5	72,† HSC70‡
Stress inducible	72	5.6	70†
Stress inducible	73	6.3	HSX70/HSP70‡
Constitutive	74-78		GRP78/80 kD‡
Mitochondrial	75		

*After Minota *et al.*⁵³

†After Milarski *et al.*⁸

‡After Pelham.⁹

Table 2: Human families

Mol wt (kD)
90–100, 90
70—See table 1
65–58.6
20–28, 32
—Low molecular weight proteins
Ubiquitin

gorised as a 20 kD family. Table 2 gives the constituent members of the human families. The 65 kD family is very important in bacterial systems; the mycobacterial 65 kD protein seems to be involved in the development of adjuvant arthritis and will be discussed in a later section. Ubiquitin, a 76 amino acid protein, is also an HSP and has been implicated in certain neurological diseases.¹⁰

Because of the high degree of homology between species¹¹ and because the proteins are highly antigenic, antibodies raised against HSPs of one species often cross react with another. This is not absolute, however, as a monoclonal antibody (TB78) which recognises the 65 kD protein of *Mycobacterium tuberculosis* does not cross react with the *Escherichia coli* GroEL protein or the human 65 kD mitochondrial protein, whereas ML30, raised against *Mycobacterium leprae*, is broadly cross reactive (J Ivanyi, personal communication). GroEL is the 'common antigen' detected in about 50 species of bacteria and shows considerable homology to the 65 kD mycobacterial protein.¹² Table 3 lists some bacterial and human homologous proteins.

Function and cellular localisation of stress proteins

Until recently, the functions of HSPs remained a matter of speculation,⁹ clear evidence being available for the 70 kD family only. Transfection of cells with a plasmid which overproduces HSP70 accelerates their recovery from heat shock,¹³ and more recently it was shown that the microinjection of monoclonal antibodies to HSP70 before heat shock was lethal to fibroblasts.¹⁴ Thus these proteins are clearly necessary in helping cells survive physiological stress. The effect is fully reversible, total protein synthesis recovering within a few hours of the return to normal temperatures.^{2 15} In addition, cells can be rendered 'tolerant' by repeated stress events and subsequently require more severe conditions to reinitiate HSP synthesis.^{2 4 16}

The 70 kD proteins show the highest degree of interspecies homology at the NH₂ terminus of the protein, and it has been shown that this region has an ATPase activity. This activity is responsible for the disruption of the clathrin

cages covering coated vesicles, which are intermediates in the pathway of receptor mediated endocytosis.¹⁷ In the human this activity is ascribed to the 72/73 kD proteins.³ The 70 kD proteins are localised in the cytoplasm and nucleus but after heat shock are concentrated in the nucleus and nucleoli, where they bind to partially assembled ribosomes; the human 72 kD protein co-localises with ribosomes.¹⁸ Heat shock and other stressors cause a rapid collapse of the intermediate filaments, particularly vimentin, in and around the nucleus and the mitochondria, and ribosomes become trapped within this network. Again, it is the human 73 kD and 72 kD proteins which are found associated with microtubules and vimentin.^{3 19 20} The GRP78 member of this family (unaffected by heat) is always found in the lumen of the endoplasmic reticulum.

The HSP70 family of proteins has been referred to as ATP dependent 'unfoldases', 'molecular chaperones', or 'chaperonins'. This implies that they function in recognising nascent proteins and incorrectly folded or glycosylated proteins, and correct problems incurred during their association into oligomeric structures. Indeed it has recently been shown that a protein which binds to immunoglobulin heavy chain (BiP—immunoglobulin binding protein) is homologous to GRP78,²¹ and also that in yeast, a eukaryote, HSP70 aids in the translocation of precursor protein into microsomes,²² mitochondria, and the lumen of the endoplasmic reticulum²³ (ATP-dependent unfoldases), and subsequently HSP60 assembles protein subunits within the yeast mitochondria.²⁴

A number of workers have implicated HSPs in developmental control and in infection. Heat stress is often used to produce developmental defects in drosophila.²⁵ It may be important in the life cycle of certain parasites which use insect vectors, where they pass from the 25°C poikilothermic host to 37°C in a mammalian host. Thus *Trypanosoma cruzi*, the aetiological agent in Chagas' disease, expresses HSP70 antigens,²⁶ and *Plasmodium falciparum*, the causative agent of human malaria, bears an HSP70 related protein in its merozoite form in human blood.²⁷ Leishmania protozoa and schistosoma worms also express HSP70-like antigens (Young D B, Mehler A, Smith D F, unpublished data). As has been stated, mycobacteria bear multiple HSP antigens. This has led to the idea that these antigens may be implicated in autoimmune disease by the process of 'molecular mimicry'.^{28 29} Interestingly, many of the proteins bound by HSPs (immunoglobulin, vimentin, histones) are common targets of autoantibody formation in rheumatic disease.

The 90 kD HSP is a cytoplasmic protein with many isoelectric forms. It can associate with tyrosine kinases.³⁰ and is also a constituent of the steroid hormone receptor complex.^{30 31} The 100 kD stress protein is glucose regulated and is present in the Golgi. High molecular weight stress proteins occur; little is known of the nucleolar 110 kD protein.³² There is no 100 kD or 110 kD counterpart in drosophila.²

Although there is a vast number of publications about the 7–8 kD protein, ubiquitin, less

Table 3: Homologous interspecies stress proteins

Molecular weights (kD)			
<i>M leprae</i>	<i>M tuberculosis</i>	<i>E coli</i>	Human
65	65	GroEL	58.6
70	71	dnaK	See table 1

is known of the functions of other low molecular weight stress proteins. Ubiquitin, the most highly conserved HSP, performs a number of essential cellular functions, attaching to cytosolic proteins and marking them for degradation when beyond salvage by the 70 kD family. Also, it attaches to certain histones in chromatin,³³ and a role in HSP transcription has been suggested.²⁵⁻³⁴ The mammalian (rat) 28 kD protein consists of at least four isoforms, three of which are differentially phosphorylated; the degree of phosphorylation of the 28 kD protein, but not the synthesis, is dramatically increased after exposure to mitogens or phorbol esters—that is, cell activation.³⁵

Recently, it was shown that the major 32 kD stress protein produced by a human skin fibroblast cell line after ultraviolet irradiation or exposure to hydrogen peroxide is the enzyme haem oxygenase.³⁶ This is the protein induced by metals and thiol reactive agents. Speculations on its role in disease are given below.

Stress proteins in inflammatory rheumatic disease

What is the relevance of these proteins to rheumatology? Perhaps the most direct answer is simply that we do not know. Two independent pieces of evidence attach unequivocal importance to HSPs in rheumatic disease, however. The first is the observation of Lakomek *et al*, who, while screening patients' sera for antinuclear antibodies, exploiting the large polytene chromosomes from the salivary glands of heat shocked *Drosophila* larvae, showed that 39% of patients with ankylosing spondylitis produce antibodies reactive only with a certain (93 D) heat shock puff; serum samples from patients with other forms of rheumatic disease or from healthy controls showed no reactivity at this chromosome locus.³⁷ More recently, Brand *et al* have shown that 49% of patients with ankylosing spondylitis have antibodies against the 63 kD HSP—again with exquisite disease specificity.³⁸ The association between ankylosing spondylitis and the major histocompatibility antigen HLA-B27 is well reported³⁹; associations of ankylosing spondylitis or B27 with *Klebsiella* and other Gram negative organisms have been the subject of much controversy.⁴⁰⁻⁴³ It can be no accident that the organisms triggering B27 associated reactive arthritis are all intracellular parasites, and can be expected to express ample 65 kD HSP when inside host phagocytes. Stress proteins may prove to be the 'missing link'.

The second direct link is through the rat model of adjuvant induced arthritis. Arthritis is induced by injection of Freund's adjuvant, a suspension of heat killed *M. tuberculosis* in oil, the arthritis appearing 11–13 days later. It was shown that transfer of an autoreactive T cell clone recognising a determinant on the mycobacterial 65 kD antigen to a heavily irradiated syngeneic rat was arthritogenic; prior immunisation with the purified 65 kD antigen abrogated this effect.⁴⁴ Interestingly, these clones also recognise the core protein of cartilage proteoglycan, implying some degree of joint specificity.⁴⁵ In addition, peripheral blood and synovial

fluid lymphocytes of patients with rheumatoid arthritis showed this cross reactivity, the T cells recognising both the 65 kD antigen and cartilage proteoglycan.⁴⁶ In reactive arthritis, known to be induced by an infectious agent, synovial fluid T cells reactive with salmonella antigens demonstrated reactivity with a recombinant 65 kD protein of *M. leprae*; this cross reactivity was not seen with peripheral blood T cells, again suggesting joint specificity.⁴⁷

Further supporting evidence for the role of HSPs in rheumatology is circumstantial. This includes the observation that chondrocytes from patients with osteoarthritis constitutively produce 70 kD and 90 kD proteins,⁴⁸ and sera from patients with rheumatoid arthritis recognise 70 kD and 28 kD antigens in extracts of synoviocytes.⁴⁹ Our own immunohistological studies have shown the presence of the highly inducible (stress specific) human 72 kD protein in synovium from patients with rheumatoid arthritis and osteoarthritis,⁵⁰ while others have detected raised serum IgG and IgA antibodies to human 70 kD and mycobacterial 65 kD HSPs; the levels were not related to age, sex, duration or activity of disease and were not associated with any particular HLA-DR haplotype.⁵¹ Patients with systemic lupus erythematosus have serum IgG antibodies to HSP90⁵² and both IgM and IgG antibodies to a constitutively produced member of the 70 kD family (73 kD/pI 5.5).⁵³ Also, peripheral blood cells from patients with systemic lupus erythematosus produce raised levels of HSPs.⁵⁴ In a recent communication raised IgG (but not IgM) antibodies to the Golgi complex were reported in the sera of patients with primary Sjögren's syndrome⁵⁵; this is the site of the 100 kD stress protein.

Speculations on the role of stress proteins in synovitis

As heat is one of the five cardinal signs of inflammation and as HSPs can be induced by a number of stressors, including hypoxia, reactive oxygen species, and cytokines, it is perhaps not surprising that such proteins are found at sites of inflammation. Although the benefit of heat treatment in arthritis remains controversial, recent evidence suggests that selective application of deep heat to the synovium ('thermal synovectomy') is therapeutic.⁵⁶ Such targeting, leading to localised HSP production, may be important as similar cells from different sites⁵⁷ and different cells from adjacent tissues⁵⁸ appear to show differential expression of HSPs. Whether these differences are related to maturation or site and whether changes occur in disease states remains to be elucidated. Repeated heat shock can induce a state of thermotolerance to cells in vitro and this tolerance also aids the cell in overcoming further stressors.¹⁶ It is tempting to speculate that this is one of the mechanisms which downregulates acute episodic 'flares' in palindromic and early rheumatoid arthritis. Clearly many questions remain unanswered!

We have postulated that reactive oxygen metabolites, including hydrogen peroxide

(H_2O_2), superoxide ($\text{O}_2^{\bullet-}$), and the hydroxyl radical ($\bullet\text{OH}$), are released, probably from endothelial cells, as a consequence of hypoxic reperfusion injury in a mobile inflamed joint.⁵⁹ Polla has suggested that stress proteins may have antioxidant effects as they show increased synthesis in the presence of reactive oxygen metabolites⁶⁰; Burdon (personal communication) has found that hydrogen peroxide (possibly $\bullet\text{OH}$ radical) but not superoxide induces HSP synthesis. The recent work by Keyse and Tyrrell defines the major 32 kD stress protein induced by H_2O_2 as the enzyme haem oxygenase.³⁶ This enzyme cleaves haem to form biliverdin, which is subsequently converted to bilirubin. Bilirubin, whether unconjugated or albumin bound, is an efficient free radical scavenger.

Normal movement of an inflamed joint generates pressure such that transient synovial capillary ischaemia inevitably follows. Transient anaerobic exposure leads to the induction of GRPs.⁶¹ As the pressure falls, reperfusion occurs, leading to the release of oxidants which have the capacity to denature proteins.⁶²⁻⁶⁴ The damage to the intermediate filament protein, vimentin, is of particular interest as collapse of the cytoskeleton is a prerequisite of HSP release. Our previous work showed that synovial cells have abnormal intermediate filaments⁶⁵ and also that these are very susceptible to damage induced by reactive oxygen species.⁶³ Recently, there have been a number of reports of reduced glycosylation of IgG in rheumatoid arthritis and it is known that glycosylation of cellular proteins is inhibited during heat shock while hypoglycosylated proteins accumulate intracellularly.⁶⁶ Release of cytoplasmic contents to the local environment is an inevitable consequence of cell destruction in inflamed tissue and thus immunological reactivity against these intracellular proteins may arise. Alternatively, the propensity of certain HSPs to bind IgG and related proteins destined for export raises the possibility that some HSPs may indeed reach the cell membrane in immunogenic form. Both reactive oxygen species and abnormal proteins serve as stress signals and trigger the activation of heat shock genes.⁶⁷ In particular, H_2O_2 specifically induces the synthesis of haem oxygenase, which theoretically would abrogate further protein damage by haem iron catalysed $\bullet\text{OH}$ radical formation. Thus there exists in the joint a highly regulated but complex defence system for limiting hypoxic-reperfusion injury. Interestingly, it has been shown that penicillamine acts in suppressing endothelial cell proliferation by generating low levels of H_2O_2 .⁶⁸

Concluding remarks

Heat shock proteins are both highly conserved and immunogenic. The latter property has suggested to some a role in the maintenance of synovitis by an autoimmune reaction. Conversely, their induction may limit the inflammatory response, protecting the cell in various ways from a variety of insults. Are stress proteins friend or foe to the rheumatic patient? The answer, like so many answers to such

fundamental questions in rheumatology, appears to be yes!

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