

Summary

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HSP60 and the regulation of inflammation: Physiological and pathological

This is an overview of the role of HSP60 as a regulator of inflammation and cell and body maintenance. As a chaperone, HSP60 participates in intra-cellular maintenance; as an immune signal, HSP60 participates in immune maintenance.

Key Words: HSP60, inflammation; autoimmunity; chaperone; innate receptors; TLR-4; TLR-2; T-cells; B-cells; macrophages; signals; disease; health; healing.

Summary

Willem van Eden, Liesbeth Paul and Ruurd van der Zee

Heat shock proteins and suppression of inflammation

Self-recognition is probably central to the T-cell regulation of immune responsiveness. The recent re-discovery of regulatory T-cells as key elements in the maintenance of self-tolerance has again stimulated interest in the nature of antigens critical to the regulation of immune responsiveness. Now heat shock proteins are recognised as prime target antigens of regulatory T-cells.

The initial observations on heat shock proteins (HSPs) as antigens with the potential to control autoimmune inflammatory disease were made in the model of adjuvant-induced arthritis in rats. From this it appeared that bacterial HSPs had the capacity to trigger T-cells with 'cross-specificity' for mammalian (self) HSP. Subsequent experimentation revealed the recognition of stressed cells by such T-cells and their disease suppressive nature.

Given the disease controlling events induced by HSP and their derivative T-cell epitopes in various model systems of inflammation, not only in autoimmunity, but also in infection, it seems that HSP-specific T-cells have a broad potential to mediate regulatory control in inflammation.

Key Words: Experimental arthritis, regulatory T-cells, inflammation, HSP60, stressed cells.

Summary

Sreyashi Basu and Pramod Srivastava

Heat shock proteins in immune response

Interaction of antigen-presenting cells (APCs) with heat shock proteins (HSPs) leads to the uptake and presentation of the HSP-peptide complexes by antigen presenting molecules through the specific receptors and stimulation of APCs to secrete pro-inflammatory cytokines, chemokines and over-expression of co-stimulatory and antigen presenting molecules on their surface. These unique properties of HSPs suggest their role in inflammation and allow their use in development of new generations of prophylactic and therapeutic vaccines against cancers and infectious diseases.

Key Words: HSP, antigen presentation, receptor, NF- κ B, cancer.

Summary

Clarissa U. I. Prazeres da Costa, Hermann Wagner and Thomas C. Miethke
Heat shock protein-mediated activation of innate immune cells

Heat shock proteins (HSPs) are now recognized to belong to the expanding family of pathogen-associated molecular patterns, i.e., structurally conserved molecules of microorganisms able to stimulate cells of the innate immune system. HSPs are unique in the sense that they are expressed by bacteria and mammalian cells and, e.g., HSP60 from both sources plays an essential role in folding of proteins and in the stimulation of immunocytes. In particular, HSPs are able to activate macrophages and dendritic cells, to increase the expression of immunologically important molecules at the cell surface, to activate NF- κ B and to secrete pro-inflammatory cytokines. Two receptors which bind HSPs at the cell surface are defined, but probably more exist. HSPs trigger a signal cascade initiated at the cell membrane involving the surface molecule CD14 and Toll-like receptors. Intracellular molecules like MyD88 and TRAF6 belonging to the main signal cascade of TLR receptors are crucial to transmit HSP60-induced cellular signals to activate NF- κ B, the stress-induced protein kinases p38 and JNK1/2, and the mitogen-activated protein kinase ERK1/2. Taken together, both endogenous (mammalian) and exogenous (bacterial) HSPs appear to represent a new class of danger signals.

Key Words: Heat shock proteins; endogenous danger signal; PAMP; Toll-like receptors; MyD88; TRAF6; macrophages; dendritic cells.

Summary

Arne von Bonin, Minka Breloer and Solveig H. Moré
Eukaryotic HSP60: A "danger signal" for T- and natural killer cells

Heat shock proteins (HSP) are intracellular proteins and their functions as signaling molecules in the immune system are not directly obvious. However, one can envisage situations, e.g. necrotic cell death, in which intracellular proteins are delivered from inside the cell and are able to influence cellular responses in the close microenvironment. In this respect it could be shown that Hsp60 directly acts on antigen presenting cells (APC) by inducing release of inflammatory cytokines or by changing the pattern of molecules expressed on the cell surface. The latter effects require the presence of toll-like receptor (TLR) 4. As a consequence of Hsp60-induced APC stimulation, especially naive CD4⁺ and CD8⁺ T cells are activated, release IFN- γ and increase the expression of the T cell activation marker CD25 and CD69. In contrast, "effector" T cells displayed almost unchanged activation kinetics in the presence of Hsp60. Hsp60 induced IFN- γ in cocultures of naive T cells and PEC even in the absence of antigenic peptide. The Hsp60 mediated stimulation of immune cells is specific in the sense that IFN- γ levels are elevated whereas other cytokines and cellular proliferation remain largely unchanged. Moreover, the IL-12 dependent release of IFN- γ is strongly enhanced by the addition of Hsp60 to Rag^{-/-} spleen cells and purified DX5-positive cells cocultured with peritoneal exudate cells (PEC) as APC. Taken together, Hsp60, which can be released from dying cells in vivo, leads to significant production of IFN- γ by NK cells and T cells in the presence of professional APC. On one hand, thus, the early activation of innate immune cells like NK cells might help to prime the immune response, e.g. under conditions involving tissue destruction. On the other hand, the presence of eukaryotic Hsp60 allows antigen specific IFN- γ secretion under conditions where the antigenic stimulus alone is not sufficient to activate naive T cells.

Key Words: Danger-signal, NK cell activation, toll-like receptor, naive T-cell, effector cell, CD14, HSP60 receptor.

Summary

Rebecca J. Brownlie and Stephen J. Thompson

Heat shock proteins and experimental arthritis

Many animal models of arthritis have been developed over the years in order to help gain a better understanding of rheumatoid arthritis (RA). Initial studies in rat adjuvant arthritis, then latterly in several other models, suggested that heat shock proteins contributed to the pathogenesis of disease and that these molecules could be important target auto-antigens under immunological attack. However, attempts to induce disease with these proteins failed. By contrast, it was shown that the administration of bacterial, and in some cases endogenous heat shock proteins (or peptides), would protect rodents from subsequent disease development in models such as adjuvant arthritis, streptococcal cell wall-induced arthritis and pristane-induced arthritis. The mechanism(s) behind disease amelioration is an area of intense research and this chapter attempts to give an overview highlighting recent research developments.

Keywords: Experimental arthritis, heat shock proteins, T-cells, cytokines, bacteria, inflammation, immune regulation, immunotherapy, immune deviation, cross-reactivity, autoimmunity.

Summary

J. S. Hill Gaston, Richard C. Duggleby, Jane C. Goodall, Roberto Raggiaschi and Mark S. Lillierap

Heat shock proteins and reactive arthritis

Recognition of bacterial heat shock proteins (HSP) is part of the normal immune response to many bacteria, including those which initiate reactive arthritis, and organism-specific T-cells, both CD4+ and CD8+, which recognize bacterial HSP (especially HSP60) have been isolated from affected joints. To determine whether these cells play a part in the pathogenesis of the arthritis their properties, particularly the peptide epitopes which they recognize, have been studied in detail. This has shown that epitopes are often highly conserved in other bacteria which the patient is likely to have encountered, so that there are opportunities for priming the response to reactive arthritis associated bacteria and altering its quality. This could result in hypersensitivity responses, or alternatively in responses which fail to clear infection efficiently. Whilst pathologic autoimmune responses to HSP have not been clearly demonstrated, self HSP may be a source of altered peptide ligands which modulate the anti-bacterial response.

Keywords: Reactive arthritis, heat shock protein, immune responses, epitope, CD4+ T lymphocytes, CD8+ T lymphocytes, cross-reactivity, cytokines.

Summary

Ismé M. de Kleer, Berent P. Prakken, Salvatore Albani, Wietse Kuis

The development of immune therapy with HSP60 for juvenile idiopathic arthritis

Multiple populations of T-cells with specialized regulatory capacity have been identified to contribute to the remitting character of oligoarticular Juvenile Idiopathic Arthritis (JIA). One of the most interesting regulatory T-cell population that seems to contribute to disease remission in oligoarticular JIA patients consists of T-cells recognizing self-heat shock protein 60. As a consequence of the identification of human HSP60 as an antigen triggering regulatory T-cells new ways for immunotherapy aimed at restoring the natural regulatory responses in patients with chronic arthritis, such as polyarticular JIA and rheumatoid arthritis, are under development. Mucosal administration of or immunization with heat shock proteins might be a promising way to re-activate the self-HSP reactive T-cells and restore the balance between immunity and regulation. With the identification of self HSP60 epitopes that are recognized in the majority of JIA patients a phase I clinical trial has come near.

Key Words: Juvenile idiopathic arthritis (JIA), heat shock protein 60 (HSP60), immunotherapy, adjuvant arthritis (AA), regulatory cells, CD30.

Summary

Gabriel S. Panayi and Valerie M. Corrigan

Heat shock proteins and rheumatoid arthritis

Heat shock proteins have been considered to be involved in the pathogenesis of rheumatoid arthritis under three distinct headings: T- and B-cell responses, protecting the synovium from apoptosis under conditions of stress, when released extra-cellularly to induce a pro-inflammatory response, or, conversely to down-modulate inflammation. HSP and molecular chaperones are over-expressed in the rheumatoid arthritis synovial membrane so that all these properties could potentially be operating. However, no evidence for an antibody or a T-cell mediated immune response to HSP in maintaining rheumatoid inflammation has been satisfactorily demonstrated. There is evidence that extra-cellular heat shock proteins may stimulate pro-inflammatory response through the release of cytokines such as tumour necrosis factor α and interleukin 1. Finally, the 78kD glucose regulated protein BiP, an endoplasmic reticulum chaperone, may down-modulate inflammation by stimulating the secretion of IL-10. All these putative functions of HSP and molecular chaperones in rheumatoid arthritis will only be satisfactorily analysed as the result of appropriate therapeutic studies in patients with the disease.

Key Words: Rheumatoid arthritis, synovial membrane, heat shock proteins, HSP60, DnaK, DnaJ, BiP, pathogenesis, T cell responses, antibody responses, molecular mimicry, shared epitope.

Summary

Gisella L. Puga Yung, Tho D. Le, Sarah Roord, Berent Prakken, Salvatore Albani
Heat shock proteins for immunotherapy of rheumatoid arthritis

This chapter presents a short view of recent experiences from us and others in modulation of antigen specific responses as a tool for manipulating autoimmune inflammation. Particular emphasis will be given to the concept of exploiting for therapeutic purposes a natural mechanism of immune regulation. *E. coli* heat shock protein (HSP) derived peptides are one of several possible antigens able to modulate the immune system in rheumatoid arthritis (RA). We will lead through the rationale that we and others followed to reach this goal. We will start with a brief introduction of RA; the actual therapies used nowadays and the important role of use a specific treatment instead of traditional therapy. We explain the immune basis of physiologic tolerance and the strategy behind the therapy that we state introducing our model of molecular dimmer. Finally we present data of modulation of immune response to HSP in RA in humans and combination of epitope specific and anti-cytokine therapy in a rat RA model.

Key Words: Immune regulation, inflammation, molecular dimmer model, rheumatoid arthritis, dnaJp heat shock protein, HSP60, therapeutic new approaches, pro-inflammatory/tolerogenic pattern, phase I clinical trial, T-cell capture (TCC), T reg cells.

Summary

Michael Knoflach, Bruno Mayrl, Mahavir Singh, Georg Wick
Immunology to heat shock proteins and atherosclerosis

As documented elsewhere in this book, heat shock proteins (HSPs) clearly play a central role in activation and modulation of unspecific as well as specific immune responses. In this chapter, we will describe early atherosclerosis as an autoimmune disorder and heat shock protein 60 (HSP60) as the culprit-autoantigen. After a brief review of experimental *in vitro* data and results from animal experiments, we will focus on clinical data confirming our pathophysiological concept that early atherosclerosis is the price we pay for protective immunity against microbial HSP60 or *bona fide* autoimmunity to eliminate biochemically-altered autologous HSP60.

Key Words: Aging, atherosclerosis, clinical study, animal study, T-cell, lymphocyte, immunity, inflammation, antibody, HSP60, HSP65.

Summary

Brian Henderson
Chaperonins: Chameleon proteins that influence myeloid cells

Thomas Kuhn's concept of paradigm revolutions has had a field day in chaperonin research. Just as these proteins were becoming established as essential intracellular factors in protein folding, and as their mechanism was being defined, it became clear that they also had intercellular signalling capacity. A growing range of cells and cellular functions are modulated by recombinant chaperonins, including activation of monocytes, dendritic cells, vascular endothelial cells, epithelial cells and neurones. The enormous sequence conservation of chaperonin 60 forms part of yet another paradigm. Yet it is surprising to find that highly homologous chaperonins can have divergent actions. Perhaps the most profound example of

this is the capacity to turn the *E. coli* chaperonin 60 from an inactive protein to an active insect neurotoxin by single residue mutations. It is for this reason that it is proposed that these proteins are chameleons of the protein world, having a range of guises including that of endocrine hormones.

Key Words: Chaperonin 60, chaperonin 10, myeloid cells, bone, bone remodelling, osteoclasts, osteoblasts, cytokines.

Summary

Thomas Lehner, Yufei Wang and Charles Kelly

Heat shock protein receptors, functions and their effect on monocytes and dendritic cells

Significant progress has been made recently defining the receptors which interact with different heat shock proteins (HSP). Mammalian HSP90, HSP70 and HSP60 interact with CD14, CD91, TLR2 and TLR4. However, both microbial and human HSP70 bind CD40, though at two distinct sites. TLR2 or TLR4 expressed by the innate immune system might be used as a signalling pathway for the HSPs. The peptide binding motifs of HSPs have been defined and ATP-treated (peptide-free) HSP can be loaded *in vitro* non-covalently with peptides. Alternatively, HSPs can be covalently linked for which we favour a bifunctional reagent (SPDP). An important property of HSP70 and HSPgp96 is that they can deliver exogenous antigen into the MHC Class I, as well as Class II pathway. This carrier function of HSPs is coupled with potent adjuvanticity, on account of stimulating the production of CC chemokines which attract the immunological repertoire of cells. Stimulation of IL-12 and TNF- α production may induce TH1 polarization of the adjuvant function of HSPs. Maturation of DC, especially by the C-terminal fragment of HSP70 is as effective as that induced by CD40L. The remarkable biological properties of HSP70 and HSP90 have intensified interest in the immuno-modulating, therapeutic and preventive applications of HSPs to a variety of immune, microbial and neoplastic conditions.

Key Words: Heat shock proteins, CD40 receptor, CC chemokines, adjuvant.

Summary

Klemens Trieb

Heat shock protein expression in transplanted kidney

Kidney transplantation is the standard treatment for end-stage renal failure and improves quality of life. In 1954 the first successful kidney transplantation between identical teens was performed, which was done then in several cases. Despite this success, the problem of allograft rejection remained due to the lack of immunosuppression. In the early 1960s azathioprine, a less toxic derivate of the anti-cancer agent 6-mercaptopurine, was introduced. In combination with steroids, this standard immunosuppressive therapy enabled a breakthrough of kidney transplantation. The introduction of Cyclosporine A led to the next breakthrough in kidney transplantation. Although the one year survival rates increased in the last years due to a decrease in acute allograft rejection, chronic allograft failure still remains a main problem limiting long-term survival. The mechanisms underlying long-term allograft failure are still not fully understood. The one year allograft survival increased to over 80 % for cadaveric and about 95 % for living related donors due the introduction of potent immunosuppressants, such as cyclosporin and tacrolimus, and the consequent treatment of rejection episodes. The cause of renal allograft failure is still not fully understood, although

both immunological and non-immunological factors play a role. Deterioration of renal function by chronic graft nephropathy results in an increased serum creatinine level, proteinuria and increased diastolic blood pressure. Graft rejection depends on the recognition of the grafted tissue by the host as foreign; the antigens responsible for this recognition are the histocompatibility complex antigens. Graft rejection is a complex immunological process of cellular and humoral contribution. T lymphocyte-mediated reactions are based on direct recognition of allogeneic molecules on antigen presenting cells of the graft or antigens from the donor are presented by the recipients own antigen-presenting cells in the same way as physiologic processing and presentation of foreign antigens. On the basis of the morphology, rejection episodes are classified as hyperacute, acute and chronic rejections. Injury to the allograft kidney is unavoidable during transplantation by the temporary discontinuation of renal blood supply, occurring during organ retrieval and storage. This includes warm and cold ischaemia, ischaemia reperfusion injury, nephrotoxic drugs and clinical changes mentioned above. The kidney might respond to this injury phase by a temporary repair mechanism with influx of mononuclear cells and fibroblasts. On the other hand the allograft responds to the injury by expression of genes that are necessary for organ and cell survival. Among them the heat shock proteins are remarkably important. Beside their physiological functions, HSPs have been shown to be strongly involved in immunologically-mediated reactions; they can act as antigens and stimulate immune competent cells. In autoimmunity they can stimulate the immune system *via* "molecular mimicry". In murine models HSPs elicit cancer immunity and additionally, T lymphocytes specific for HSPs with cytotoxic potential were described and isolated from human osteosarcomas. It has been shown that HSP expression is modulated *in vitro* by hypoxia, cytokines, glucocorticoids and cyclosporin. Studies on the expression of HSPs in the normal kidney are so far scanty and focus on the rat and human kidney. The expression of HSP47, 60 and 70 was demonstrable in the human kidney in all compartments and in the rat. In a rat model, the constitutive expression of HSP32 and 90, but not 72 was reported.

An increased expression of HSPs was observed in acute or chronic rejected kidneys, but not in post-transplantational kidneys without rejection. T-cells reactive for HSP were isolated from rejected kidney and from rejected cardiac allografts, thereby suggesting a participation of HSPs in transplant rejection, accompanied by an increased expression of HSPs during the rejection of human kidney allografts.

Although T-lymphocytes play a major role in the development of both cellular and inflammatory immune responses leading to rejection of an allograft, the humoral part of the immune system is also of critical importance, but so far anti-HSP antibody titers during allograft rejection do not differ from normal levels. No correlation was found to either severity of rejection or an immunologically uncomplicated course.

Key Words: Heat shock proteins, kidney, allograft, transplantation, rejection, survival, expression, graft nephropathy, lymphocyte, infiltration, reperfusion, resistance.

Summary

Ad P. Koets

Mycobacterial heat shock proteins and the bovine immune system

Mycobacterial infections constitute a major threat to cattle populations worldwide. The major mycobacterial infections are tuberculosis, caused by infection with *M. bovis* (MB), and paratuberculosis, caused by infection with *M. avium ssp. paratuberculosis* (MAP). Mycobacterial heat shock proteins are potent antigens for many immunological cells, and are used widely as defined antigens to study interactions between mycobacteria and the host. This

chapter provides an overview of interactions between mycobacteria and the bovine immune system with special focus on reactivity of $\alpha\beta$ T-cells, $\gamma\delta$ T-cells and B-cells towards mycobacterial heatshock proteins (HSP) in paratuberculosis.

Key Words: Cattle, heat shock protein, immunology, *Mycobacterium paratuberculosis*, *Mycobacterium bovis*.

Summary

Johannes M. van Noort

Microbial infection generates pro-inflammatory autoimmunity against the small heat shock protein alpha B-crystallin and provides the fuel for the development of multiple sclerosis

Evidence is accumulating that heat shock proteins that are selectively expressed in only some tissues escape tolerance mechanisms and become drivers of strong pro-inflammatory responses. In this contribution, an example is described of a small HSP, viz. alpha B-crystallin, that drives inflammatory responses considered to contribute to the development of multiple sclerosis by immunological mechanisms that are unique to humans.

In humans, alpha B-crystallin is undetectable in normal lymphoid tissues and cells, allowing immune reactivity to develop against this self HSP when it becomes presented in secondary lymphoid organs under inflammatory conditions. Such presentation occurs upon infection of B-cells by the common Epstein-Barr virus. As a result, a normal adult human immune repertoire contains pro-inflammatory self-reactivity against alpha B-crystallin at the level of both helper T-cells and antibodies. While this poses little risk for autoimmunity under normal conditions, alpha B-crystallin is sometimes produced at high levels by oligodendrocytes, the myelin-forming cells in the central nervous system. When this happens, the HSP effectively becomes part of the extracellular matrix allowing its presentation *via* Class II MHC molecules. In this situation, alpha B-crystallin becomes a major driver for the local inflammatory response that causes multiple sclerosis.

Key Words: Alpha B-crystallin, autoimmunity, autoantibodies, multiple sclerosis, tolerance, Epstein Barr virus.

Summary

Kalle Söderström

HSP60-peptide interference with CD94/NKG2 receptors

HLA-E is a widely expressed non-classical MHC Class I molecule which presents a restricted set of nonameric peptides, derived mainly from the signal sequence of other MHC Class I molecules. Such HLA-E/peptide complexes can modulate both innate and adaptive immune responses as they form ligands interacting with CD94/NKG2 receptors expressed on NK cells and T-cell subsets. It is becoming increasingly apparent that HLA-E also is capable of binding non-MHC derived peptides, including peptides derived from the human heat shock protein 60 (HSP60). One such HSP60 peptide, located in the mitochondrial targeting sequence, gains access to HLA-E intracellularly, resulting in up-regulated HLA-E/HSP60 signal peptide surface levels on stressed cells. Notably, HLA-E molecules in complex with the HSP60 signal peptide are no longer recognized by CD94/NKG2 receptors. Thus, during cellular stress an increased proportion of HLA-E molecules binding HSP60 signal peptide will result in a reduced capacity to engage either inhibitory or activating forms of CD94/NKG2 receptors

expressed on major NK cell population and subsets of activated T-cells. Such stress induced peptide interference (SPI) would gradually uncouple CD94/NKG2A inhibitory recognition and provide a novel mechanism for NK cells to detect stressed cells in a peptide-dependent manner. In addition, SPI could potentially also lower the threshold for antigen-specific T-cell activation by CD94/NKG2A expressing T-cells carrying TCR specific against viral or tumor derived peptides displayed on classical MHC. Potentially, these findings may result in novel peptide-based strategies to treat infection, cancer and autoimmune diseases.

Key Words: CD94/NKG2, MHC Class I, cellular stress, peptide interference, HSP60, HLA-E, NK cells, CTL.



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