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Original Article

Bacterial Heat Shock Proteins: It's role in immunity as potent antigen and as adjuvant

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Introduction

Heat shock proteins (Hsps) are among the most highly conserved protein families in all forms of life. Although referred to as heat shock proteins, most of these proteins are expressed at significant levels in all cells under normal growths conditions and are essential for cellular growth at all physiologically relevant temperatures. Heat shock proteins perform important functions in the folding of proteins, their translocation across different compartments within a cell, as well as in the assembly of protein complexes. Heat shock proteins are also termed as molecular chaperones. Increased synthesis of heat shock proteins occurs in prokaryotic and eukaryotic cells when they are exposed to stress conditions such as hypoxia, nutrient deprivation, oxygen radicals, metabolic disruption, viral infection, phagocytosis and transformation.

As much as 15% of prokaryotic cellular protein mass is constituted by heat shock proteins under conditions of stress. The increase in the cellular content of heat shock proteins enables the cells to protect themselves from the various lethal assaults. The protection that the heat shock proteins offer to cells is primarily through their properties as molecular chaperones, whereby they interfere with the uncontrolled protein unfolding that occurs under conditions of stress. Heat shock proteins thus play a major role in cell protection following different stressful stimuli.

Another form of stress occurs upon invasion of a foreign host by a pathogen. Alterations in the cellular environment result in an increase in the cellular content of heat shock proteins within the pathogen. The host reacts to the assault by pathogens by rapidly degrading the foreign heat shock proteins. These heat shock protein derived determinants therefore form a major group of antigens inducing strong humoral

and cellular immune responses (Ziigel and Kaufmann, 1999). Heat shock proteins thus play a dual role in the cells, as antigens in a wide spectrum of infections and as molecular chaperones.

Significance of Heat Shock Proteins in Immunity

Heat shock proteins (Hsps) play a variety of important roles in immunity. For many pathogenic species, Hsps represent prominent antigens in the humoral and cellular immune response. These proteins also play an important role in presentation of antigenic peptides to the immune system. Although Hsps are widely distributed in nature, and are highly homologous among different species, the extent of their immunogenicity is different suggesting that the immunological properties of Hsps are dependent on their sequence and structure.

Role as Antigens:

The vertebrate immune system encounters an enormous variety of pathogens. Pathogen infection results in alterations in the living conditions of both the host cell and the microbe. With these changing conditions, induction of synthesis of Hsps within the pathogen has often been observed. Increased pathogen Hsp levels in cells lead to rapid degradation of the foreign proteins by the host processing machinery. Since they are so abundantly expressed, Hsps swamp the immune system with these epitopes. Pathogen-derived determinants are then efficiently presented by host cells and promote recognition of infected cells by the immune system (Kaufmann, 1991; Ziigel and Kaufmann, 1999). The abundance of Hsps, especially under conditions of stress, is one of the major factors contributing to their role as antigens. Moreover, conservation of Hsps across different life forms also appears to contribute to the antigenicity of these proteins. A high conservation results in the presence of cross-reactive epitopes on different Hsps. For the host, frequent interaction with microbes results in the generation of an immunological memory for these cross-reactive determinants. As a result, the immune system of an infected individual is already prepared to react quickly to subsequent infections. Furthermore, an immune response to the conserved epitopes of Hsps shared by different microbes may prevent colonization of the host by microbial pathogens. Thus, due to their wide distribution in nature, and high homology among different species, Hsps represent important immunogenic components of different pathogens.

Role as Antigen Presenters:

The Hsps mediate yet other powerful reactions that engage the immune system. Many Hsps have been shown to be associated *in vivo* with a large repertoire of cellular peptides, including antigenic peptides generated by degradation of the proteins within a cell. The Hsp-peptide complexes are taken up by the antigen presenting cells through the cell surface receptors. The peptides thus get presented by the

Major- Histocompatibility Complex (MHQ class I and class II molecules, which in turn stimulate the CD8+ and CD4+ T cells (Srivastava, 2002). These observations have led to the interesting role of Hsps as antigen presenters and carriers.

Role as Chaperones of Antigen Presenters:

Hsps are involved in the folding and assembly of molecules that play important roles in the immune system. These include the immunoglobulins, T-cell receptors, and the MHC complex. Members of the 70kDa heat shock protein (Hsp70) family are critically involved in the processing and presentation of antigens. Bip, a member of the Hsp70 family of stress proteins and another endoplasmic chaperone, calnexin, promote the assembly of both MHC class I and class II molecules in the Endoplasmic Reticulum (ER). Calnexin facilitates MHC class I-Transport Associated Protein (TAP) interactions and thus controls peptide binding to MHC class I molecules.

Role as Carrier Molecules:

Peptide transport from the proteasome to the ER and subsequent peptide loading of MHC class I molecules in the ER depends on a variety of Hsps including the Hsp70 and 90 kDa heat shock protein (Hsp90) families (Srivastava *et al.,* 1994). Recent studies have revealed that gp96, a member of the Hsp90 family in the ER, acts as a peptide acceptor. This protein receives peptides of cytosolic origin after their transport through the ER membrane by TAP molecules. Subsequently, gp96-peptide complexes bind to MHC and the peptides are then translocated from gp96 to MHC class I molecules in an ATP-dependent manner. The capacity of Hsps to serve as carrier molecules has also been studied extensively in murine tumor models. Transfection with mycobacterial 60 kDa heat shock protein (Hsp60) reduces the tumorogenicity of a murine macrophage tumor cell line suggesting the role of Hsp60 in delivery of immunodominant tumor antigens to the cell surface.

Role as Adjuvant:

HSPs would act as an immune adjuvant for APCs by improving antigen-specific T or B lymphocyte responses. The adjuvant function of microbial and mammalian HSPs has been investigated extensively in vitro in murine and human experimental systems. HSP70 has been described to be involved in the activation of APCs, inducing cytokine secretion and the up-regulation of molecules involved in antigen presentation (MHC class I/II and costimulatory markers) and in adhesion. In vivo, HSP70- peptide complexes or peptide-HSP70 fusion proteins have been shown to act as a chaperon of peptides and to activate the T- or B-mediated adaptive immunity. Coadministration of soluble HSP70 and gp33 peptide stimulates dendritic cell (DC) functions and converts T cell tolerance to autoimmunity in vivo. Microbial or mammalian HSP70s exert their effects on APCs, through several receptors CD91, CD40, TLR-2, and

TLR-4. HSP70 activates two of the main pathways activated by LPS and other stimulatory factors: IκB-α/NF-κB pathway and the p38 stress-activated protein kinase (p38).

Hsps have unequivocally been demonstrated to be associated with a large variety of peptides including tumor, viral, cytosolic, nuclear and secreted antigens *in vivo.* When tested for their ability to complex with peptides *in vitro,* Hsps were shown to effectively present peptides of synthetic origin to the immune system. Blachere *et al.* (1997) showed that Hsps and a few synthetic peptides, when administered alone, were non-immunogenic. However, a strong immunogenic response in a MHC class I restricted manner was elicited upon administration of the Hsp70-peptide and gp96- peptide complexes. The studies thus demonstrated that Hsps act as powerful adjuvants for generation of CD8+ responses. Use of either foreign or self-Hsps as carrier molecules for antigenic determinants thus provides a basis for applying Hsps in conjugate vaccines.

Heat Shock Proteins as Antigens

Members of the Hsps family have been described as dominant antigens in several infectious diseases. They have been typically classified according to their molecular weights, the 60kDa Hsp being denoted as Hp60, the 70kDa Hsp as Hsp70 etc.

Hsp60:

Immune responses to Hsp60 are frequently found in a wide range of microbial infections. This Hsp family has been found to elicit humoral as well as cell mediated immune responses. For example, direct involvement of Hsp60-specific T cells has been demonstrated in a murine model of Yersiniosis. In this case, CD4+ αβT cells specific for Hsps mediate significant protection. Similarly, levels of antibodies against Hsp60 were shown to increase significantly after vaccination with a trivalent vaccine against tetanus, diphtheria, and pertussis in infants. Therefore, Hsp60 family is classified as an important immunological determinant in several pathogenic organisms. Hsp60 from various bacteria induce the release of certain pro-inflammatory cytokines and adhesion molecules. *Chlamydia pneumoniae* induces the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in human endothelial cells. *C. pneumoniae* also stimulates the production of proinflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α) and interleukin-6 (IL-6) upon infection of human monocyte derived macrophages. Moreover, human Hsp60 has been shown to elicit a potent proinflammatory response. The proinflammatory response results in the induction of TNF-OC and Nitric oxide (NO) formation in cells of the innate immune system in a Toll like receptor 4 (TLR4) dependent manners, thereby suggesting that Hsp60 also serves as a cell signaling molecule.

Hsp70:

Another member of the Hsp family that has been shown to play a major role in immunity is the 70kDa heat shock protein, Hsp70. Increased antibody levels to Hsp70 have been identified in sera of patients suffering from malaria, leishmaniasis, schistosomiasis, filiariasis, and candidiasis. Responses to pathogen derived Hsp70 are more restricted and are sometimes exclusively species specific. Moreover, recombinant Hsp70 has recently been shown to upregulate the expression of pro-inflammatory cytokines TNF-OC, interleukin-l and IL-6 in human monocytes in a CD 14 dependent pathway, transduced by Toll like receptor 2 (TLR2) and TLR4. Thus, Hsp70 also constitutes an important immunological determinant. Valentinis *et al.,* 2008 found using human monocyte-derived dendritic cells (DCs), the signal transduction pathways activated by a human recombinant HSP70 (r) HSP70 purified from eukaryotic cells. rHSP70 effectively induced a partial maturation of DCs in vitro and a significant increase in the titers of antigen-specific IgG when used as a vaccine adjuvant in vivo.

Hsp90:

Similar to Hsp60 and Hsp70 families, Hsp90 has also been shown to be involved in elicitation of the immune response. Hsp90-spedfic antibodies contribute to protection against *Candida albicans* infection. Hsp90 has also been shown to play a crucial role in LPS-mediated macrophage activation.

Vaccination with Pathogen Heat Shock Proteins:

As Hsps represent dominant antigens in numerous microbial infections, a potential use of pathogen-derived Hsps for vaccination has been suggested. Different vaccination strategies using Hsps have been successful in inducing significant protection in various infectious disease models. Immunization of mice with recombinant Hspl0 and Hsp60 from *Helicobacter pylori* protected the animals against subsequent infection and development of gastroduodenal disease. Similarly, protection against pulmonary histoplasmosis was induced upon vaccination of mice with recombinant Hsp60 from *Histoplasma capsulatum*. Protection has also been achieved by administration of naked DNA encoding the different Hsps. Mice receiving plasmid DNA encoding mycobacterial Hsp60 showed partial protection against subsequent challenge with *Mycobacterium tuberculosis.* Similar protection was achieved with plasmid DNA encoding mycobacterial Hsp70 (Colaco *et al.,* 2013).

***Mycobacterium tuberculosis* and the Immune System:**

Tuberculosis, caused by *M. tuberculosis,* is the single largest infectious cause of human mortality. Tuberculosis is responsible for about 3 million deaths and about 8 million new cases every year. Approximately one-third of the world's population harbors *M. tuberculosis* and is at a risk of developing the disease. In humans, the majority of *M. tuberculosis* infections are initially controlled and a latent

infection, without clinical signs of disease, is established. During the latent phase, low numbers of tubercle bacilli persist in dormancy inside a granulomatous containment. It is the interaction between the pathogen and the host immune system that results in protection against mycobacterial disease via the cell-mediated immunity.

Mycobacterial Heat Shock Proteins as Antigens:

In mycobacterial infections, reactivity to Hsps has been shown to predominate. Most of the antigens identified upon mycobacterial infection or vaccination in mice or humans are involved in the T-cell response.

Hsp10:

Although the *Escherichia coli* and human 10 kDa Hsps (Hsp10) do not elicit strong immune response (Richardson *et al,* 2001), the mycobacterial homologue has been found to be strongly immunogenic. The *Mycobacterium leprae* and *M. tuberculosis* Hsp10 have previously been shown to be important T-cell antigens. Approximately one third of the *M. leprae* reactive T cells cross react with Hsp10. Specific immune responses to Hsp10 include production of antibodies, T-cell proliferation and delayed-type hypersensitivity. Moreover, immunodominant T-cell epitopes have recently been mapped to regions of the *M. leprae and M. tuberculosis* Hsp10.

Hsp60:

Hsp60 has been shown to be an immunodominant target of the humoral and T-cell response in mice and humans. Hsp60-specific antibodies have been detected in patients with tuberculosis and leprosy, and also in mice after infection with *M. tuberculosis*. Moreover, CD4+ αβT cells specific for the mycobacterial Hsp60 have been found in patients with leprosy or those vaccinated with *M. bovis* BCG. Surprisingly, about 20% of all mycobacterium-reactive CD4+ αβT cells in mice immunized with killed *M. tuberculosis* are specific for Hsp60. These studies suggest a protective role for Hsp60-specific T cells in mycobacterial infection.

Hsp70:

The mycobacterial Hsp70 is a dominant antigen during the human T-cell response to mycobacterial infection despite the conserved sequence with the human homologue. T-cell recognition of the *M. leprae* Hsp70 antigen occurs in the context of multiple HLA-DR phenotypes and has been shown to be species specific. Mycobacterial Hsp70 has been proposed to be utilized in subunit vaccine design since it contains a variety of T-cell epitopes presented with multiple HLA-DR molecules.

Mycobacterial Heat Shock Proteins as Potential Vaccine Candidates

Currently, the only available vaccine against tuberculosis is *Mycobacterium bovis* Bacille Calmette-Guerin, the BCG vaccine. The extent of protection offered by BCG against tuberculosis is currently intensely debated, thus soliciting search for a better vaccine. Hsps of mycobacteria have been shown to be potential candidates in the development of subunit vaccines. Recently, promiscuous epitopes from the mycobacterial Hsp60 have been identified. These epitopes were responsible for recognition by the CD4+ T cells in association with the HLA-DR molecules. These have thus been implicated in the design and development of vaccine against mycobacterial diseases. Immunization with the mycobacterial Hsp65 antigen induces protection against *M. leprae* and *M. tuberculosis* in mouse models of infections. In addition, DNA vaccination in mice with *M. tuberculosis* Hsp65 antigen has been shown to provide protection against challenge with *M. tuberculosis*. Mycobacterial Hsp70 has also been shown to act as a vaccine vehicle capable of eliciting both humoral and cell-mediated immune response.

Importantly, Hsp70 fusion proteins induced these immune responses without adjuvants thus suggesting that Hsp70 functions as an exceptionally powerful carrier, capable of eliciting both T cell and B cell responses. Hsps are abundant intracellular molecules possessing a range of housekeeping and cytoprotective functions. However, under certain circumstances, these proteins are released from cells into the extracellular environment. As secreted proteins the Hsps possess a range of immunoregulatory activities. Bacterial Hsps induce expression of pro-inflammatory cytokines and intercellular cell adhesion molecules on host endothelial cells. In addition, these proteins promote antigen presentation to the immune system by chaperoning peptides to the antigen presenting cells. Their role as antigen presenters is a result of a very different role that the Hsps play in the intracellular milieu, that of molecular chaperones. As chaperones, the Hsps promote the correct folding and assembly of other cellular proteins under normal and stressed conditions. This they perform by interacting with the unfolded or misfolded polypeptides and preventing their aggregation thus providing the nascent polypeptides a chance to fold into the correct conformation.

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