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Heat Shock Proteins in Immune Reactions

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ABSTRACT. The review concerns heat shock proteins and their significance in immune reactions. It focuses on problems of physiological and pathological interactions in etiology and duration of autoimmune diseases and infection processes, especially fungal infections. New trends are described in exploitation of heat shock proteins for preparation of specific protective vaccines.

CONTENTS

1 INTRODUCTION

Prokaryotic and eukaryotic cells respond to unfavorable external conditions by enhanced synthesis of a small number of specific proteins known as stress or shock proteins. These proteins represent an evolutionary highly conserved group that plays a significant role in cell adaptation to changed environmental conditions and regulates a great number of physiological cell processes (Lindquist 1986).

The best-known group of stress proteins are hsp (heat shock proteins) proteins whose synthesis is induced or enhanced by temperature change. Closely related to them are the so-called hsc (heat shock cognates). They can be identified also in non-stressed cells, but their synthesis significantly increases after heat shock. GRP (glucose regulated protein) is the term used for stress proteins which are produced in response to glucose deprivation. GRP can again be present under normal conditions and are then termed hsc (Pelham 1986). Nevertheless, the abbreviation "hsp" is used for the whole so-called stress protein superfamily. Some heat shock proteins perform so-called chaperon functions (molecular chaperons). They are bound to unfolded polypeptides during their movement in the cell, enabling the transport of these polypeptides through membranes or their integration into cell organelles (Hartl 1996).

Table I. Heat shock proteins superfamily

Family	Main members
Hsp90	Hsp90, Hsp83
Hsp70	Hsp70, BiP, Hsc70, DnaK
Hsp60	Hsp65, GroEL
Ubiquitin	Ubiquitin

The designation of these proteins includes a numeral index equal to the molar mass in kDa. The proteins are classified into families according to their molar mass. The proteins in each family have not only similar molar mass and a high degree of sequence homology, but also similar function characteristics (Plesovski-Vig 1996; Becker and Craig 1994; Table I).

2 TRANSCRIPTION OF HEAT SHOCK PROTEINS

The signal pathways activating and regulating the transcription of so-called shock genes are not yet well understood. Mechanisms regulating the expression of heat shock (hs) genes differ between prokaryotes and eukaryotes. Among prokaryotes, the regulation of hsp synthesis is best studied in *Escherichia coli*. Most genes that are expressed during heat shock are transcribed by RNA polymerase containing δ32 subunit (δH) (Yura *et al.* 1993; Kopeček and Weigl 1994). This subunit recognizes different promoter sequences, which are recognized by δ70. A gene coding the δ32 is *rpoH* (= *hptR*, *hin*) and is transcribed by the polymerase containing δ70. The level of δ32 rapidly but transiently increases following heat shock. Its maximum is reached by increasing the temperature from 30 to 42 °C within 6 min, increasing levels of DnaK and GroE acting as a negative feedback here. A higher stability of δ32 during increased temperature also contributes to

the regulation of its level under heat shock (Donati *et al.* 1990; Chaloupka 1993; Kanemori *et al.* 1994; Schmidt *et al.* 1994). In eukaryotic organisms, the transcription of shock genes is connected with the presence of specific DNA segments, so-called HSE (heat shock element) and regulation proteins hsf (heat shock factor). hsf are bound to HSE and activate the transcription of structural genes. The basic primary structure of HSE fields has the sequence 5′-nGAAn-3′ or its opposite orientation. The number of pentanucleotide repeats can vary (Burel *et al.* 1992; Chaloupka 1993; Lis and Wu 1994). hsf can react with HSE either directly under normal temperature, or after its activation through heat shock. In the former case, which known from yeasts, a non-functional HSE–hsf complex is formed, which can be activated by heat-shock induced phosphorylation of hsf. The latter way described in higher organisms consists in the formation of hsf–Hsp70 complex under normal conditions. hsf is released from the hsf–Hsp70 complex during heat shock; it forms trimers, binds to HSE and stimulates transcription. Dissociation of the hsf–Hsp70 complex occurs since the affinity of Hsp70 for denatured proteins is higher than that of hsf. This regulation is similar to the regulation of eukaryotic gene expression by enhancers. URS (upstream repressing sequence) segments which have been located in the vicinity of HSE, inhibit the synthesis of stressed proteins under normal conditions. The question of regulation of hsp transcription due to hsf phosphorylation still remains a hypothesis (Lis and Wu 1994).

3 HEAT SHOCK PROTEINS IN INFECTION

Expression of heat shock proteins in stress conditions is also manifested during an infection process when host and pathogen interact. Cellular stress is present in both the host and the pathogen, and hsp from the Hsp65, Hsp70 and Hsp90 groups are expressed. The role of microbial hsp was studied intensively in terms of anti-infectious immunity and they were found to function as virulence factors in some microorganisms (Lamb *et al.* 1990; Lin *et al.* 1992; Ericsson *et al.* 1994; Stulík *et al.* 1999). On the other hand, these molecules also belong to immunodominant antigens and the antibody titres against bacterial hsp, especially against Hsp60, are markedly higher in infected or vaccinated individuals (Bahr *et al.* 1988, 1990; Brown and Hormeache 1989). hsp were therefore used as markers of successful vaccination in some studies (Bahr *et al.* 1988, 1990; de Graeff-Meeder *et al.* 1990). Much less attention has been paid to the significance of hsp which are expressed in the host cells.

Chemoattractans released from the infected tissue during the infection process lead to inflammatory cellulization of the infected focus. The inflammation type is determined by the physiological state of the organism and by the invading noxa. Inflammation mediators, namely derivates of arachidonic acid, reactive forms of oxygen and cytokines, are released in the damaged tissue. Except for primary alteration of the tissue by an infectious agent, these mediators take part in the development of cellular stress and induce expression of stress proteins. While induction of hsp-genes expression following enhanced formation of oxygen radicals is evident, the influence of cytokines is variable. Cytokines can affect the hsp-genes expression directly or indirectly, *e.g.*, through their pyrogenic activity. This especially concerns IL-1 and IL-2 cytokines, interferons and tumor necrosis factor (Polla and Kantegwa 1991). Numerous other relationships between cytokines and hsp have been reviewed, *e.g.*, by Polla *et al.* (1993). The significance of general functional characteristics of hsp in the development of an immune response is evident. A specific significance has been especially ascribed to Hsc78 (Hsp70), which takes part in determining the correct composition of immunoglobulin molecules. Recent data showed that Hsp70 family members play a significant role during the processing of antigens and their transport to the membrane of accessory cells. Some other possible functions of Hsp70 have been considered in this direction. It is presumed that the transport of antigens into lysosomes by hsp is involved in their further processing. It is assumed that the inducible form of Hsp72 can take an active part in the transport of antigenic peptides from the cytosol into the endoplasmatic reticulum *via* the TAP heterodimers. Hsp72 can also amplify the formation of antigenic peptides by stimulating the formation of proteasome complexes, and increases the membrane presentation of MHC I complex with endogenous antigen (Burel *et al.* 1992; Kaufmann 1994; Wells *et al.* 1998).

4 HEAT SHOCK PROTEINS AND AUTOIMMUNITY

Due to the extensive neutral homology of hsp, the specific immune response of the host against the hsp molecules produced by a number of prokaryotic organisms may induce a specific reaction against some self-antigens, especially hsp, at the same time. Moreover, the situation is complicated by the fact that anti-self hsp reactive clones of cells have also been described in healthy individuals without a manifested infection, as

well as in individuals having autoimmune disease. Both groups produced antibodies against their self hsp in high titres (Res *et al.* 1988; Burmester *et al.* 1991). This poses the question of a relationship between the immune response against hsp induced by a microorganism and that of autoimmune diseases. Thus van Eden and coworkers showed in 1988 that T lymphocyte clones, which were able to transfer adjuvant arthritis in experimental animals, were able at the same time to recognize mycobacterial Hsp60 (van Eden *et al.* 1988). Later, T lymphocytes specific for bacteria and human hsp were found in some other autoimmune diseases, *e.g.*, rheumatoid arthritis (Kaufmann 1990), multiple sclerosis (Jindal *et al.* 1989) and others (Renoir *et al.* 1986; Golubinoff *et al.* 1989). Both humoral and cellular immune responses against hsp can be considered as a simple immune response against a foreign protein, which occurs when the immune response is directed against epitopes characteristic for the hsp of the microbial pathogen, and absent in the host hsp. Alternatively, molecular mimicry occurs between the common hsp epitopes of the microorganism and those of the host, resulting in a cross-immune response. The pathophysiological consequence is autoreactive clones whose association with autoimmune disease has been interpreted in various ways. Res *et al.* (1991) described three basic concepts of immunopathological conditions based on T cell response to both self and foreign hsp:

(*1*) The possibility of activation of immunopathological conditions during reaction to exogenous hsp resulting from a poorly controlled T cell response against the hsp of the invading organism. The immunopathological process can be caused by a host defense response against the exogeneous hsp, which are related to certain tissues or organs of the host. When the immune system incorrectly differentiates between self and foreign elements, it can give rise to clinical symptoms of autoimmune disease. This acute forms of reactive arthritis may be initiated by infection of gastrointestinal or urogenital tracts. The inducing infection agents can include the genera *Chlamydia*, *Shigella*, *Salmonella* or *Yersinia*. Although the organisms have never been isolated from synovial fluid in the location of reactive inflammation, the immunocompetent cells (T lymphocytes) isolated from this location responded to the extracts of these microorganisms by strong proliferation (Gaston *et al.* 1989*a*,*b*; Herman *et al.* 1989). A cross-reaction between the Hsp65 of the invading pathogen and the Hsp65 of the host is considered to be the cause of reactive inflammatory response in chlamydia infections. Such a reaction has not been confirmed for Hsp70 (Morrison *et al.* 1989; Taylor *et al.* 1990).

(*2*) Another model is based on an immunological cross-reaction between the hsp of the microbial pathogens and endogenous proteins which can differ from hsp. As shown on the example of adjuvant arthritis in Lewis' rats, reactive clones of T lymphocytes specifically activated by epitopes of microbial hsp undergo a cross-reaction with proteins in host tissues. As mentioned above, T lymphocytes capable of transferring diseases respond to the Hsp65 from *Mycobacterium tuberculosis*. The cross-reacting antigen in autologous tissue is cartilaginous proteoglycan (van Eden *et al.* 1985, 1988). The conclusions from these observations remain at the level of hypotheses because the presence of autoreactive cells need not necessarily be in a causal link with the etiology of autoimmune diseases.

(*3*) Insulin-dependent diabetes mellitus in non-obese mice represents a further experimental model of autoimmune disease. The condition occurs in these mice spontaneously and is associated with T cell reactivity against hsp65. Contrary to the previous models, this process does not involve bacterial infection (Elias *et al.* 1990).

5 HEAT SHOCK PROTEINS AND SPECIFIC IMMUNOTHERAPY

Protective vaccines against bacterial and viral pathogens are usually based on antigens that are characteristic for a given kind of microorganism and have no structural analogy to the host organism. It is therefore surprising that highly conserved proteins such as hsp, which are characterized by high sequence homology, can be effective as protective vaccines (Dixon *et al.* 1998). Hsp60 isolated from *Legionella pneumophila* belongs to the first experimentally prepared vaccines of this type. The vaccination with this protein protected guinea-pigs against lethal aerosol infection (Blander and Horowitz 1993). Similar results have also been obtained using preventive administration of Hsp60 from *Yersinia enterocolitica*. Heat shock proteins administered with adjuvants in various vaccination schemes induced a protective cellular immune response, although application of purified protein without adjuvant did not stimulate a defense reaction (Noll and Autenrieth 1996).

The protective effect of hsp has been studied intensively, especially in tuberculosis. The basic findings were that heat shock proteins having molar mass of 10, 65 and 70 kDa responded to T cell clones of tuberculosis patients, and consequently became candidates for potentially protective antigens (Barnes *et al.* 1992; Mendez-Sampiero *et al.* 1995). Moreover, Hsp65 differs from Hsp10 and Hsp70 in being produced by

mycobacteria in increased levels under conditions of intracellular parasitism. Further studies have confirmed that Hsp65 can induce immune protection comparable with BCG vaccination (bacillus Calmette-Guérin) (Silva and Lowrie 1994). A significant progress has been achieved using a new vaccination method, the so-called DNA vaccination. The basis of this vaccination technology consists in intramuscular administration of eukaryotic expression plasmid, which contains a gene sequence that codes for mycobacterial Hsp65. This vaccination method ensures a presentation of gene product (Hsp65) as an endogenous antigen through MHC I and promotes activation of the T cell immune response.

The efficiency of vaccination depends upon the presentation of the antigen, either through MHC I (genetic vaccine) or MHC II (vaccination by protein). While the vaccination by the Hsp65 ensures a short-term defense against a challenge by a virulent strain such as *M. tuberculosis*, DNA vaccination provides a long-term protection (at least 8 months in mouse model; Lowrie *et al.* 1997; Bonato *et al.* 1998). Apparently, the cause of this long-term stimulation of the immune system is the plasmid DNA encoded antigen. This plasmid persists in the episomal form in the cytoplasm of transfected cells and does not integrate into the eukaryotic chromosomes (Wolffe *et al.* 1992). Immune mechanisms apparently eliminate the cells generating endogenous antigens only slowly.

Heat shock proteins as immunodominant antigens represent a significant factor in both pathogenesis and immunoprotection in mycotic infections. The majority of knowledge has been obtained from studies of candidoses and histoplasmosis. The key role in candidosis is played by the breakdown product of Hsp90, which is the antigen with a molar mass of 47 kDa (Mathews and Burnie 1989). The host organism is able to control the disease process or eliminate the fungal infection by humoral antibodies specific for this antigen. Newborn mice treated by antibodies against mouse IgM were more susceptible to candidosis than the control group and also more susceptible than mice having a congenital or an induced defect of cell immunity (Cutler 1976; Rogers *et al.* 1976). The significance of antibody response to antigen 47 kDa has been verified by a serum analysis of patients with systemic candidosis. A rising titre of antibodies against this antigen correlates regularly with improvement or clinical state, while specific antibodies against 47 kDa antigen are not detected in lethal candida infections (Matthews *et al.* 1984, 1987). Sera of patients having recovered from systemic candidoses and having a high titre of anti-47 kDa antibodies were prophylactically administered to mice. The death rate of these mice was reduced by 50 % or more after their exposure to a lethal dose of *Candida albicans* (Mathews *et al.* 1991). Epitope mapping of Hsp90 has shown that protective antibodies are specific against evolutionarilly conserved epitopes common for Hsp90 of both man and the fungus, such as the LKVIRK field (Matthews and Burnie 1992). According to Matthews and Burnie (1992) it is also possible to predict a possible mechanism of the protective function of specific antibodies: the LKVIRK field represent a multi-binding center of many proteins and its blockade by a specific antibody inhibits the chaperon functions of Hsp90 and can be fatal for the microorganism in stress infection conditions.

While Hsp90 is considered to be a protective antigen in candida infections, the views of a similar importance of hHsp70 are not uniform (Gomez *et al.* 1992; Hiroshi *et al.* 1997; Maresca and Kobayashi 1994; Matthews and Burnie 1996). A completely opposite effect of Hsp70 on the immune response has been found by, *e.g.*, Bromuro *et al.* (1998) who studied a protective effect of induced immune response against Hsp70 and two of its fragments on the model of mouse candidosis. The fragments were: 21-kDa- from the C-terminal end, and 28-kDa fragment from the N-terminal end of Hsp70. All three antigens were strongly immunogenic both for induction of humoral antibodies and for cellular immune response. Surprisingly, immunization by these antigens enhanced the manifestations of systemic mouse candidosis. Allendoerfer *et al.* (1996) studied a protective effect of a recombinant Hsp70 from *Histoplasma capsulatum* and confirmed the induction of humoral as well as cellular immune reaction. However, in this case the vaccination by recombinant protein also had no significance for defense against the challenge by a virulent strain.

On the other hand, vaccination using a recombinant Hsp60 from *H. capsulatum*, prepared by transfection of *E. coli*, induced a cellular immune response protecting experimental mice against intranasally administrated sublethal doses of fungal cells (Gomez *et al.* 1995).

6 CONCLUSIONS

Heat shock proteins have a number of significant functions in the immune response of an organism. The Hsp65, Hsp70 and Hsp90 from microorganisms were identified as the main goals for antibodies and T-cell immune response in patients infected by pathogenic bacteria, fungi or parasites. T-cell epitopes for specific bacterial Hsp65 and epitopes conserved between bacterial and host Hsp65 were determined. Epitopes that are selectively specific for the self hsp have never been identified. These data show a two-fold role of immune response against hsp. The first role is the elimination of pathogenic microorganisms that derives

from autoreactive clones and acts quickly before humoral and cellular responses to specific microbial antigens start. The second role consists in the elimination of stressed self cells with a sufficient hsp concentration on their surfaces; while normal cells do not present these hsp and are thus not affected. Autoreactive clones of immunocompetent cells are apparently an integral part of the immune system acting as a regulation element influencing homeostasis within the immune system. Expression of the self hsp serves as an universal marker of defective cells and is a factor which supports immune surveillance of senescent, malignant and infected cells (Kaufmann 1994).

The significance of heat shock proteins in the etiology of autoimmune diseases is not yet quite understood. It is apparently determined by the manner of presentation of these antigens as well as by the mechanisms of central and peripheral tolerances. The hsp itself, either bacterial or human, is not apparently the true cause of autoimmune diseases; for instance, autoimmune arthritis has not yet been successfully induced by repeated immunization using Hsp60 from *M. tuberculosis* (Gaston 1997). The role of hsp in pathogenesis of autoaggressive immune processes is apparently of no direct etiological importance but seems to depend on the genetical or pathophysiological background. The fact that some heat shock proteins can be utilized as immunodominant antigens for vaccination and induction of protective immune response against relevant microbial pathogens, underlines the importance of their further detailed study.

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