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IMMUNITY TO THE 60KD HEAT SHOCK PROTEINS

Consequences for Reproductive Outcome

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SUMMARY

A brief review on the role of the heat shock proteins (hsp), their common properties and possible consequences for early pregnancy development is described. The 60kD hsp plays an important role as immunogenic antigen of many microbial pathogens and possibly in postinfectious autoimmunity. The immune responce to hsp may cause pregnancy failure. The consequences of previous sensitization to microbial hsp and the effects of human autoantibodies to hsp, are demonstrated in a mouse embryo culture model.

Key words: 60kD heat shock proteins, embryo development, reproductive failure, preimplantation mouse embryo.

RESUMO

Foi feita neste trabalho uma rápida revisão do papel das heat shock proteínas (hsp), suas propriedades mais comuns e suas possíveis conseqüências para o desenvolvimento da gravidez em fase inicial. As hsp 60 desempenham um significativo papel como fator imunogênico de muitos microrganismos e possivelmente também nos problemas de auto-imunidade pós-infecciosas. A resposta imunológica às hsp pode estar envolvida com insucessos da gravidez. Foram descritos em modelos de culturas de embriões de camundongos as conseqüências da sensibilização prévia com hsp microbianas e os efeitos dos auto-anticorpos humanos a estas hsp.

Unitermos: *Heatshock proteínas 60kD, desenvolvimento do embrião, falha de reprodução, pré-implantação de embriões de camundongo.*

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INTRODUCTION

When cells are subjected to physiological stress they increase the production of a group of proteins called heat shock proteins (hsp). Hsp help bacterial, fungal and mammalian cells to survive adverse environmental conditions by preventing protein denaturation. The physiological and pathological significance of hsp is enormous and has been studied widely over the last decade. In this report we describe the basic principles of immune sensitization to hsp. Possible implications of immune sensitization to hsp for reproductive outcome in women are presented.

HISTORICAL ASPECTS OF HEAT SHOCK PROTEINS

Indicators of a cellular heat shock response were first discovered more than 36 years ago, when Ritossa and coworkers described in 1962 the phenomenon of puffing in salivary glands chromosomes of the fruit fly Drosophila melanogaster after exposure to heat[1]. Not many people took notice of this observation and it was not until 12 years later that the first gene products of this morphological puffing pattern were identified and the term "heat shock proteins" was created[2]. Today the genes coding for these proteins have been sequenced, their structure described, their chromosomal location defined, and their mode of interaction with nuclear heat shock transcription factors characterized[3]. Studies involving the role of hsp in basic and applied clinical medicine are numerous and involve almost every medical field including oncology, immunology and infectious diseases. Drugs modulating the total hsp expression (thus protecting integrity and homeostasis of cells and tissues) are currently in phase 2 clinical trial[4,5]. Preliminary results suggest that these novel drugs might gain new and important therapeutic applications in the future.

COMMON PROPERTIES OF HEAT SHOCK PROTEINS

In the following paragraph a number of crucial characteristics that define this group of proteins are summarized.

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1. All organisms studied ranging from prokaryotic bacteria to mammals, including man, respond to an increase in temperature by switching off the synthesis of most proteins and commencing large-scale synthesis of a few heat shock proteins. Even thermophilic organisms, whose optimal growth temperature lies between 50°C and 90°C respond to sudden temperature increase with the over-expression of hsp.
2. This type of cellular response has not very much changed during evolution. The induced hsps are very similar to one another in very different organisms (their structure has been conserved) and they share a high level of amino-acid homology.
3. The high conservation between very diverse species has important implications for autoimmune diseases.
4. Hsp serve two major functions. First, under physiological conditions, they function as molecular chaperones (intracellular housekeeping proteins) which are involved in mediating the folding of other intracellular proteins (and in some cases their assembly into oligomeric structures). In addition, they have crucial roles in the prevention of inappropriate protein associations and premature folding, intracellular transport, maintenance of proteins in an inactive form and protein degradation. Second, they are synthesized in response to a wide variety of cellular injuries which include changes in temperature but also other circumstances like the presence of free oxygen radicals, viral infections, heavy metals, ethanol, and ischemia or reperfusion injury.
5. Heat shock proteins are classified by their molecular weight (in kilo Dalton, kD) rather than by their function.

THE 60 KD HUMAN HEAT SHOCK PROTEINS (HSP60)

The 60kD heat shock proteins is one of the best-characterized molecular chaperones of both eukaryotic and prokaryotic organisms. The major properties of hsp60 are delineated in Table 1. Studies have revealed that members of the hsp60 family of heat shock proteins are dominant antigens of many pathogenic microorganisms such as Escherichia coli, Salmonella spp.[6] and Chlamydia trachomatis[7], the most common pathogen associated with tubal infertility. For more examples of hsp involvement in infections see Table 2.

HSP60 AND POSTINFECTIOUS AUTOIMMUNITY – POSSIBLE CONSEQUENCES FOR EARLY PREGNANCY

Bacterial hsp60 is highly immunogenic in man[14]. Typically, during the course of an acute infection immunity is restricted to hsp60 epitopes (antigenic regions) that are specific to the invading microorganism. However, since bacterial and human hsp are highly conserved proteins and share approximately a 50% amino acid sequence homology[15], it has been proposed that a prolonged or repeated bacterial infection can trigger immunity to conserved hsp60 epitopes that are also expressed in man[14,16]. This would result in autoimmunity to human (self) heat shock proteins. As a consequence of microbe-induced hsp60 autoimmunity the development of early human pregnancy may be inhibited.

Table 1

Properties of the 60kD family of heat shock proteins

1. Detectable in all eukaryotic and prokaryotic organisms
2. Essential chaperone proteins involved in transport, folding and assembly of protein subunits
3. Production is elevated in response to environmental stress factors in order to minimize protein denaturation
4. Highly conserved amino acid sequence throughout evolution. The human and bacterial proteins share a sequence homology of approximately 50%
5. Immune responses to conserved regions of heat shock proteins have been implicated in autoimmunity

Table 2

Examples for heat shock protein involvement during viral, bacterial, fungal and protozoan infections

Pathogen	Type of Hsp	Disease	Reference
HIV	Hsp70	Aids	8
M. tuberculosis	Hsp60 &70	Tuberculosis	9
T. pallidum	Hsp 60	Syphilis	10
N. meningitidis	Hsp60	Meningitis	11
C. albicans	Hsp90	Candidosis	12
T. cruzi	Hsp90	Chagas Disease	13

M. tuberculosis, Mycobacterium tuberculosis; T. pallidum, Treponema pallidum; N. meningitidis, Neisseria meningitidis; C. albicans, Candida albicans; T. cruzi, Trypanosoma cruzi.

Many couples with fertility problems, especially women with occluded fallopian tubes, have had a persistent and "silent" C. trachomatis genital tract infection[17,18]. Thus, conditions favorable to hsp60 autoimmunity may have been present. Heat shock proteins are also among the first proteins produced during embryogenesis and are essential for embryo development[19,20,21]. In addition, heat shock proteins are specifically expressed in the human endometrium throughout the menstrual cycle and during the postovulatory implantation phase[22]. Hsp60 expression in the human decidua at 7-11 weeks gestation has been identified[23,24], thus representing a potential target tissue for crossreacting antibodies and a source of hsp60 capable of re-activating hsp60-sensitized lymphocytes. A murine hybridoma specific for mammalian hsp60 was

Bacterial hsp60 is highly immunogenic in man.

shown to react with the surface of murine and human trophoblast, suggesting surface hsp60 expression by these cells as well[25].

A possible model for impairment of early-stage pregnancy after immune sensitization to conserved regions of the C. trachomatis hsp60 has been outlined previously[26] and is summarized in Table 3.

Table 3

Suggested mechanism of hsp60 immune mediated pregnancy failure*

1. A persistent infection (e.g., Chlamydia trachomatis) sensitizes a woman to hsp60 regions present in both microbes and man
2. Human (host) hsp60 is physiologically expressed during the pre- and peri-implantation stages of pregnancy by the embryo and the maternal decidua
3. Host hsp60 expression in early pregnancy reactivates lymphocytes previously sensitized to microbial (e.g., chlamydial) hsp60
4. The activated lymphocytes release pro-inflammatory cytokines, which induce also other lymphoid cells to release inflammatory and cytotoxic mediators
5. Cellular and humoral immune system activation disturbs immune regulatory mechanisms necessary to implantation and maintenance of the semi-allogeneic embryo
6. Alternatively a still present, but inapparent persistent microbial infection becomes reactivated during as a result of pregnancy induced immune alterations

* modified from (35).

However, the precise mechanism of hsp60-related immunopathogenesis during pregnancy remains unproven. The direct impairment of fetal development and/or fetal or maternal cell viability by anti-hsp60 antibodies or sensitized lymphocytes, or interference with immune regulatory mechanisms necessary to prevent rejection of the semi-allogenetic embryo, may induce early stage pregnancy loss.

HSP60 AND IN VITRO FERTILIZATION (IVF) OUTCOME

Women undergoing IVF with evidence of local cervical immunity to the Chlamydia trachomatis hsp60 had an increased prevalence of unsuccessful outcome as compared to antibody negative women[18]. In addition, there was a relation between cervical IgA antibodies to a conserved hsp60 epitope expressed in both the human and chlamydial proteins and the failure of successful implantation after embryo transfer in IVF patients[26]. In other infertility patients who were not undergoing IVF cervical IgA anti-human hsp60 was shown to be associated with a history of recurrent spontaneous abortion[26]. These data implicated a genital tract immune response to conserved regions of hsp60 with early stage pregnancy loss. To further elucidate the possible contribution of anti-hsp60 antibodies to reproductive failure we determined the prevalence of antibodies to the human hsp60 in maternal serum of patients undergoing infertility treatment.

The results (Table 4) indicated that serum IgG antibodies to the human 60kD heat shock protein were significantly (p=0.004) more common in patients with arrested in vitro embryo development than in IVF patients whose embryos continued to grow and were transferred to the uterus.

Table 4

Relation between circulating IgG antibodies to hsp60 and IVF outcome

IVF outcome	No. subjects	No. Hsp60* (%)
No fertilization	14	1 (7.1)
Arrested embryo development	13	6 (46.2)*
Embryo transfer		
Not pregnant	75	7(9.3)
Pregnant	53	9 (17.0)

Sera from 155 women were tested. * p=0.004 vs all others.

MOUSE IN VITRO EMBRYO STUDIES

Most recently, we have investigated the direct effect of antibodies to the mammalian hsp60 on mouse embryo development in vitro. Six to eight week old mice (strain B6D2F1) were superovulated by intraperitoneal injections of pregnant mare serum gonadotropin. After mating, females exhibiting copulation plugs were sacrificed and two-cell embryos were flushed from the oviducts. A total of 249 embryos were transferred to wells of tissue culture plates containing either RPMI 1640 culture medium and 10% fetal calf sera (complete medium, or complete medium plus 100mg/ml of a monoclonal antibody to mammalian hsp60 (SPA 806, StressGen, Victoria, B.C.) or purified mouse IgG_1 (100mg/ml) as control. Embryo development was evaluated microscopically after 3,5 and 7 days in culture and the number of blastocysts, hatched blastocysts and outgrown trophoblasts was determined.

Table 5

In vitro development of mouse embryos in the presence of monoclonal antibodies to mammalian 60kD heat shock proteins

Antibody	No. tested	No. developed/Total no. examined (%) Day 3[a]	Day 5[b]	Day 7[c]
None	112	80 (72)	79 (71)	79 (71)
IgG_1	62	49 (79)	45 (73)	41 (66)
Hsp60	75	22 (29)[d]	16 (21)[d]	21 (28)[d]

[a] blastocyst stage; [b] hatched blastocyst stage; [c] outgrowth;
[d] P<0.0001 vs IgG_1

Inclusion of anti-hsp60 antibody to the culture medium inhibited embryo development at each time period

examined. At day 3, only 29% (22/75) of the embryos cultured with this antibody reached the blastocyst stage as compared with 72% (80/112) of embryos cultured in medium and 79% (49/62) cultured in medium plus mouse IgG_1 ($p<0.0001$). On day 5, hatched embryos were present in 21% (16/75) of cultures containing anti-hsp60, 71% (79/112) of cultures containing media ($p<0.0001$) and 73% (45/62) of cultures containing IgG_1. At day 7, outgrown trophoblast were observed in 28% (21/75) of cultures containing anti-hsp60, 71% (79/117) containing media and 66% (41/62) of cultures with IgG_1 ($p<0.0001$). These results are summarized in Table 5.

Hsp60 is predominately present within mitochondria.

DISCUSSION

During the preimplantation stage of mammalian embryo development many rapid changes occur. After formation of the zygote the genome of the embryo becomes activated and assumes control of subsequent cell division and differentiation. Hsp60 expression has been demonstrated in mouse embryos at this early stage[20]. Heat shock proteins gene expression occurs in 2-cell embryos concurrent with the onset of zygote gene activation[19,20]. Gene transcription for a 70kD heat shock proteins may be initiated even earlier at the 1-cell stage[27].

Hsp60 is predominately present within mitochondria. However hsp60 expression at other sites has been consistently observed. Recent immuno-electron microscopic localization studies of hsp60 revealed, that in addition to the above mentioned mitochondrial localization, 15-20% of the total hsp60 was present at discrete extra-mitochondrial sites including the cell surface[28]. Heat shock proteins are also found on the cell surface of tumor cells where they elicit an antitumor immune response[29].

In our mouse model, anti-hsp60 antibodies exhibited a detrimental effect on in vitro mouse embryo development. The mechanism(s) of anti-hsp60 inhibition of mouse embryo development in vitro is completely unknown. The zona pellucida of mouse oocytes and zygotes is permeable to macromolecules. Molecules up to 170kDa have been shown to penetrate through the zona pellucida of postovulated mouse oocytes[30]. In addition the permeability of mouse zona pellucida to IgG with the subsequent induction of embryo damage has been demonstrated[31]. The ability of IgG to enter intact cells has also been demonstrated. IgG anti-ribonucleoprotein and IgG anti-DNA were shown to penetrate into epithelial and fibroblast cells where they reacted with intranuclear antigen and induced cell death[32].

The extent of heat shock proteins gene transcription in mouse embryos varies dependent upon in vitro culture conditions[33] and by the specific inbred strain utilized[34]. Therefore, the direct relevance of the mouse embryo studies to in vitro and vivo embryo development in man remains to be definitively determined. Since in IVF the in vitro fertilized embryos are most often cultivated in medium containing maternal sera the effect of serum containing different titers of antibodies to human hsp60 and other heat shock proteins on in vitro embryo growth and in relation to as yet undefined genetic variables should be further investigated. Such studies are now in progress.

In conclusion these results suggest that a late sequelae of a persistent or chronic genital tract infection may be the development of immune sensitization to conserved epitopes of hsp60. This event might compromise the success of subsequent natural or assisted fertility attempts.

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Running title: Heat shock proteins and pregnancy outcome

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