Immunity to Heat Shock Proteins and Pregnancy Outcome

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ABSTRACT

Heat shock proteins are among the first proteins produced by the zygote after fertilization. In addition, the maternal decidua also expresses heat shock proteins during the early stages of pregnancy. Autoimmunity to heat shock proteins is not typically evident in healthy women of reproductive age. However, a chronic microbial infection, such as an asymptomatic Chlamydia trachomatis upper genital tract infection, results in prolonged exposure of the immune system to the microbial 60 kDa heat shock protein (hsp60). This may result in immunity to conserved hsp60 epitopes and subsequent autoimmunity to self hsp60. Women undergoing in vitro fertilization (IVF) who never realized they had a chlamydial infection but who were positive for cervical anti-chlamydial immunoglobulin A (IgA) antibodies had a much lower pregnancy rate than did women who were negative for these antibodies. Furthermore, cervical IgA antibodies to the chlamydial hsp60, as well as to a synthetic peptide corresponding to an hsp60 epitope present in both the chlamydial and human hsp60, also correlated with IVF failure. In vitro incubation of newly fertilized human embryos in medium containing maternal serum was shown to be deleterious to embryo development if the sera was positive for antibodies reactive with human hsp60. In another study, the ability of human hsp60 to elicit a lymphocyte proliferative response (cell-mediated immunity) correlated with a history of spontaneous early stage pregnancy loss. Thus, autoimmunity to hsp60 might increase susceptibility to early stage pregnancy loss. Infect. Dis. Obstet. Gynecol. 7:35–38, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS
heat shock proteins; chlamydia; infertility

The 60 kDa heat shock protein (hsp60) is a highly conserved protein present in organisms ranging from bacteria to plants to fruit flies to man. It functions as a molecular intracellular chaperone, transporting peptides within the cell and preventing incorrect polypeptide folding and denaturation. The hsp60 molecule has not changed much during evolution; the bacterial and human proteins share almost a 50% amino acid sequence homology.

Despite this high degree of conservation, hsp60 is a dominant immunogen of most pathogenic bacteria.1 Perhaps this is due to the fact that the synthesis of bacterial hsp60 is greatly upregulated during infection, resulting in hsp60 becoming one of the most prevalent microbial proteins. Immunity following infection is typically confined to microbe-specific epitopes of the hsp60 molecule. However, prolonged and/or repeated exposure to microbial hsp60, or concomitant exposure to both the microbial and human hsp60, could result in immunity to conserved regions of the hsp60 molecule.

Heat shock proteins, including hsp60, are also expressed during the early stages of pregnancy in both the embryo2 and maternal decidua.3 Termination of the pregnancy at this early stage may

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expose complexes of hsp60 and paternal antigens to the immune system, resulting in induction of hsp60-directed autoimmunity. Women with breast cancer frequently develop autoantibodies to heat shock proteins due to exposure of heat shock protein-tumor antigen complexes to the immune system.4

We have been examining the relation between autoimmunity to hsp60 and pregnancy outcome and summarize our findings to date in this report.

ASYMPTOMATIC CHLAMYDIA TRACHOMATIS GENITAL TRACT INFECTIONS, IMMUNITY TO HSP60, AND PREGNANCY OUTCOME AFTER IN VITRO FERTILIZATION

Chlamydia trachomatis infections of the female genital tract, the major cause of infertility due to occluded fallopian tubes, are often asymptomatic and, therefore, undetected.5 Being an obligate intracellular parasite, C. trachomatis is able to evade immune defense mechanisms and remain viable within host cells for long periods of time.6 In vitro, cells chronically infected with C. trachomatis synthesize low levels of structural components but continue to produce hsp60 at high levels.7 Thus, in women with an asymptomatic and untreated chlamydial infection that leads to tubal infertility, there is long-term exposure to chlamydial hsp60. In addition, the local inflammatory response may elicit synthesis of host hsp60. It has been hypothesized that prolonged exposure to the immune system of chlamydial hsp60 and/or concomitant exposure to both the chlamydial and human hsp60 may lead to hsp60 autoantibody formation.8 Since many women with occluded fallopian tubes now seek to become pregnant via in vitro fertilization (IVF), we examined whether immunity to the chlamydial hsp60 and human hsp60 would interfere with a successful outcome.

In our initial study, endocervical specimens obtained from 216 women at the time of oocyte aspiration were tested for immunoglobulin A (IgA) antibodies to C. trachomatis and to chlamydial hsp60.11 None of these women ever had a recognized C. trachomatis infection. Among the 34.3% of women who subsequently delivered healthy infants, only five (7.3%) were positive for cervical anti-chlamydia hsp60 IgA, and one (1.5%) had IgA antibodies to chlamydial structural components. In contrast, among the 130 women whose embryo transfers did not result in a term pregnancy, 27.7% had anti-chlamydial hsp60 IgA, and 18.5% were positive for antibodies to other chlamydial antigens. Interestingly, while the majority of women with antibodies to chlamydial structural antigens were also hsp60-antibody positive, only 35% of women with IgA anti-hsp60 were also positive for antibodies to other chlamydial components. This suggested that the chlamydial hsp60 was the dominant immunogen in many of these infertile women.

In subsequent evaluations, 122 of the above IVF subjects were evaluated for cervical IgA antibodies to synthetic peptides corresponding to unique or conserved epitopes of the chlamydial hsp60.8 Antibodies to a single epitope, corresponding to amino acids 260–271 in the chlamydial hsp60, were found to be immunodominant in our subjects. This epitope is present in both the chlamydial and human hsp60.12 Furthermore, antibodies to this epitope correlated with the occurrence of a transient “biochemical” pregnancy followed by embryo loss. Biochemical pregnancies were detected in 22.2% of women with anti-hsp60 IgA, as opposed to only 7.4% of women without anti-hsp60 IgA. Thus, genital tract antibodies to a conserved region of hsp60 in women with previously undetected exposure to C. trachomatis was associated with early stage pregnancy failure after embryo transfer in women undergoing IVF.

In a third study, the detection of IgA antibodies to C. trachomatis in follicular fluids of women undergoing IVF were correlated with the presence of human hsp60 antigen in these fluids.13 All women positive for human hsp60 had tubal infertility and failed to become pregnant in their IVF cycle. This documented that human hsp60 was expressed in some women with C. trachomatis-associated tubal infertility.

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ated with high-titer antibody to chlamydial surface antigens and to salpingitis. Furthermore, antibodies to the chlamydial hsp60 epitope were associated with antibodies to human hsp60. In contrast, detection of antibodies to the hsp60 of Escherichia coli was not related to the presence of anti-human hsp60 immunoglobulin G (IgG).\textsuperscript{16} These studies further support the suggestion that C. trachomatis upper genital tract infections can result in sensitization to self-hsp60 epitopes in some women and, furthermore, that this immunity is related to the development of infertility.

**ANTIBODIES TO HUMAN HSP60 AND INFERTILITY**

Cervical samples from 91 women with primary or secondary infertility and from women not trying to conceive or who were fertile were analyzed for IgA antibodies reactive with the recombinant human hsp60.\textsuperscript{8} Detection of these antibodies was highly associated with primary infertility ($P = 0.003$). Furthermore, cervical IgA anti-human hsp60 was related to detection of the pro-inflammatory cytokines interferon-γ and tumor necrosis factor-α in the cervix. Conversely, there was no relation between antibodies to hsp60 and detection of the anti-inflammatory cytokine interleukin-10 in the cervix. This suggested that genital tract autoimmunity to hsp60 was associated with a pro-inflammatory immune response. This may interfere with immune regulatory mechanisms essential to prevent rejection of the developing embryo.

A more direct effect of hsp60 autoantibodies on embryo development was also identified. Prior to embryo transfer to the uterus, in-vitro-fertilized embryos are grown in vitro in medium that contains the maternal serum. It was observed that there was an association between arrested embryo development in vitro and the presence in maternal serum of autoantibodies reactive with human hsp60.\textsuperscript{17} Further evaluations using mouse embryos and monoclonal antibodies to mammalian hsp60 demonstrated that antibodies to hsp60 were associated with impaired embryo development.\textsuperscript{18} This strongly suggested that hsp60 was expressed on the surface of the early embryo and might be a target for autoantibodies.

In two studies from the United States\textsuperscript{8} and France,\textsuperscript{16} circulating IgG antibodies to the human hsp60 were shown to be associated with a history of recurrent abortion. In contrast, circulating antibodies to C. trachomatis surface components were not associated with first-trimester pregnancy failure.

**CELL-MEDIATED IMMUNITY TO HUMAN HSP60 AND SPONTANEOUS ABORTIONS**

Inflammation leading to immune rejection is typically cell-mediated. It was therefore of considerable interest to examine whether cell-mediated immunity to hsp60 was also related to pregnancy failure. Our study involved 110 female partners of infertile couples who were undergoing IVF and 41 fertile control women. Peripheral blood mononuclear cells (PBMC) were collected and assayed for proliferative responses to the human hsp60 and the E. coli hsp60.\textsuperscript{19} A lymphocyte proliferative response to the human hsp60 was more prevalent in the IVF patients (21.8%) than in the fertile controls (7.3%, $P < 0.05$). In contrast, equivalent percentages of fertile (31.7%) and infertile (29.1%) women responded to the E. coli hsp60. Among the IVF patients, cell-mediated immunity was associated with a history of spontaneous abortion. Only 12% of women with no history of spontaneous abortions—as opposed to 40.7% of women with at least one spontaneous abortion—had a positive PBMC proliferative response to human hsp60 ($P = 0.003$). The relation between therapeutic abortion and immunity to hsp60 was also examined. In contrast to the above results, there was no discernible relation between a history of therapeutic abortion and a cell-mediated immune response to human hsp60. This lack of an association with induced abortion strongly suggested that an active and prolonged rejection process was necessary for development of cell-mediated immunity to self hsp60. Hsp60 readily associates with other proteins, leading to immunity to both molecules.\textsuperscript{20} Therefore, prolonged exposure of the maternal immune system to paternal antigen–hsp60 autoantigen complexes from disrupted cells may be one mechanism leading to hsp60 autoimmunity. Alternatively, an infectious event may be responsible for both spontaneous pregnancy termination and induction of hsp60 autoimmunity.

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