

Immune Pathogenesis of Asymptomatic *Chlamydia trachomatis* Infections in the Female Genital Tract

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ABSTRACT

Chlamydia trachomatis (CT) infections of the female genital tract, although frequently asymptomatic, are a major cause of fallopian-tube occlusion and infertility. Early stage pregnancy loss may also be due to an unsuspected and undetected CT infection. In vitro and in vivo studies have demonstrated that this organism can persist in the female genital tract in a form undetectable by culture. The mechanism of tubal damage as well as the rejection of an embryo may involve an initial immune sensitization to the CT 60 kD heat shock protein (HSP), followed by a reactivation of HSP-sensitized lymphocytes in response to the human HSP and the subsequent release of inflammatory cytokines. The periodic induction of human HSP expression by various microorganisms or by noninfectious mechanisms in the fallopian tubes of women sensitized to the CT HSP may eventually result in tubal scarring and occlusion. Similarly, an immune response to human HSP expression during the early stages of pregnancy may interfere with the immune regulatory mechanisms required for the maintenance of a semiallogeneic embryo. © 1995 Wiley-Liss, Inc.

KEY WORDS

Heat shock protein, spontaneous abortion, fallopian-tube occlusion, infertility

Most women who are infertile as a result of fallopian-tube occlusion have never been diagnosed as having sexually transmitted diseases. In a recent study of 283 women with tubal-factor infertility, 84% had no history consistent with an upper-genital-tract infection.¹ However, the degree of tubal damage in asymptomatic women was as severe as in women with symptomatic tubal infections. The involvement of *Chlamydia trachomatis* (CT) in tubal damage was indicated by the greatly increased prevalence of antibodies to this organism in women with asymptomatic tubal infertility.^{2,3}

This article focuses on the experimental data consistent with the hypothesis that a long-term, undetected, asymptomatic CT infection of the female genital tract both induces tubal damage and

interferes with the early stages of pregnancy through an autoimmune mechanism.

PROBLEMS IN DETECTION OF ASYMPTOMATIC CT INFECTION

In addition to being asymptomatic, CT infections are difficult to detect. Culture, the traditional "gold standard" for CT detection, is a time-consuming, technically challenging procedure because of the obligate requirement of this organism for intracellular growth within a mammalian cell. The occasional presence of inhibitors of mammalian cell growth in the tissue culture of a genital-tract sample leads to false-negative results. Antigen detection assays or DNA hybridization reactions, although more rapid than culture, suffer from a decreased sensitivity.⁴ A recently developed polymerase chain

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TABLE 1. Properties of the CT 60 kD HSP

Second most abundant CT protein in cell lysates
Loosely associated with cell surface
Present in both reticulate and elementary bodies
Only protein synthesized in nonproductive latent infections induced by IFN γ
Member of highly conserved 60 kD family of HSP
50% amino acid sequence homology to the human 60 kD HSP
Potent inducer of inflammation in animals previously infected with CT

reaction (PCR) for CT (Amplicor, Roche Molecular Systems, Branchburg, NJ), approved by the Food and Drug Administration, has now replaced the culture as the "gold standard" for detection of this organism.⁵

Recent evidence has indicated that CT may reside in the fallopian tubes in a form that cannot be cultured. In vitro, the addition of interferon-gamma (IFN- γ) to CT-infected cells led to the formation of aberrant reticulate bodies that were unable to proliferate. These forms produced minimal amounts of CT structural antigens but continued to synthesize and release the 60 kD heat shock protein (HSP). Removal of the IFN- γ , however, led to their reversion to morphologically normal reticulate bodies which then differentiated into the infectious elementary body forms of the organism.⁶ The detection of CT DNA in the inflamed fallopian tubes of women with tubal occlusions who were negative for CT by culture has also been reported.⁷ Moreover, the findings of localized inflammation and CT DNA or protein in the absence of a positive CT culture have been reported for trachoma⁸ and reactive arthritis.⁹

CT 60 kD HSP

As mentioned above, the 60 kD HSP is the major CT protein produced during a chronic, nonproductive infection. The properties of this protein are listed in Table 1. Of major interest was the demonstration of this purified protein's ability to elicit a potent inflammatory response at sites distant from the original infection, in previously infected non-human primates,¹⁰ indicating that a CT genital-tract infection could prime an individual to respond to the appearance of HSP in the absence of an active infection.

A second relevant property of the CT HSP is its similarity to human HSP in having an amino acid

sequence of approximately 50% homology. It has an even stronger homology to the HSP of other pathogenic microorganisms.¹¹ Thus, an immune reaction to the CT HSP can sensitize lymphocytes to crossreacting epitopes in the HSP of man as well as other bacteria.

The human 60 kD HSP is normally sequestered within the mitochondria where it functions in protein transport and assembly and the regulation of ATPase activity.¹² However, under conditions of cellular stress due to increased temperature, infection, inflammation, or other conditions, the HSP is expressed elsewhere within the cell and on the cell's surface.¹³ The HSP binds to other proteins and prevents their denaturation until the stressful stimuli are alleviated.

IMMUNE RESPONSE TO THE CT HSP IN WOMEN WITH SALPINGITIS

Several groups of investigators have demonstrated that a humoral immune response to the CT HSP was associated with occlusion of the fallopian tubes.¹⁴⁻¹⁶ We subsequently determined that lymphocytes from most women without evidence of CT exposure as well as from women with CT infections confined to the cervix did not respond to the CT HSP or to synthetic peptide epitopes of this protein. In contrast, the lymphocytes from a high percentage of women with recurrent episodes of salpingitis exhibited cell-mediated immune responses to conserved HSP epitopes.^{17,18} This finding suggested that repeated or chronic exposure to the CT HSP was necessary to sensitize women to the epitopes also present in the human protein.

Once a woman becomes immunized to conserved epitopes of the CT HSP, any event that induces the expression of a crossreacting HSP will reactivate the sensitized lymphocytes. The observation that, in many cases of pelvic inflammatory disease, no infecting microorganism can be detected¹⁹ may be partially explained by an autoimmune mechanism of inflammation involving prior sensitization to the CT HSP and the subsequent induction of human HSP expression in the fallopian tubes as a consequence possibly of fever (elevated temperature) or a localized immune-system activation of noninfectious origin. The ascension of microorganisms not typically associated with pelvic infections into the fallopian tubes may possibly induce human HSP expression by a similar mechanism. Repeated epi-

sodes of immune activation and inflammatory cytokine release would eventually damage the epithelium and induce scar formation and occlusion.

The ability of CT to exist intracellularly in a viable but nonproliferative state⁶ may also contribute to chronic inflammatory-mediated damage. The immune response to a CT upper-genital-tract infection includes the release of IFN- γ ²⁰ and tumor necrosis factor alpha (TNF α).²¹ The 60 kD HSP is also a strong inducer of inflammatory cytokines.²² IFN- γ will inhibit the continued development of CT reticulate bodies and lead to the formation of aberrant intracellular forms.⁶ In addition, it will induce the expression of class-2 major histocompatibility complex (MHC) antigens on the surface of epithelial cells and macrophages. As suggested previously,²⁰ this reaction fosters the induction of inflammatory responses directed against epithelial membrane antigen-MHC complexes. The TNF α will interfere with fibroblast and epithelial-cell growth and further activate phagocytic cells to release products that are cytotoxic to epithelial cells. During this period of immune stimulation, the extracellular CT elementary bodies will be eradicated by the immune system. When this stage is completed, IFN- γ and TNF α production will cease and the aberrant reticulate forms will resume normal development. A recommencement of elementary-body release from the infected cells will reactivate the immune response and the reappearance of both cytokines. Repetitive cycles of active infection followed by dormancy will gradually erode the integrity of the tubal epithelia. The consequent increasing degree of scar formation will eventually compromise the patency of the fallopian tubes.

IMMUNE RESPONSE TO THE CT HSP IN EARLY STAGE PREGNANCY LOSS

Women with histories of pelvic inflammatory disease who become pregnant were shown to have a greatly increased incidence of spontaneous abortion compared with matched control women.²³ To ascertain the role, if any, of CT in early stage pregnancy loss more directly, we initiated studies of women undergoing in vitro fertilization (IVF) at the New York Hospital-Cornell Medical Center. The utilization of an IVF population allowed us to pinpoint accurately the time of conception and embryo transfer and to monitor closely the progression of the pregnancy.

Cervical samples were obtained at the time of oocyte retrieval from 307 IVF patients who were negative for CT by culture. These samples were reassayed by PCR for evidence of this organism.²⁴ Twenty (6.5%) of the women were positive for CT by PCR. The presence of CT was associated both with a failure to become pregnant after embryo transfer and with a spontaneous abortion after successful implantation. This study reinforced the clinical relevancy of the culture-negative, PCR-positive detection of CT and provided data that an asymptomatic CT infection may be a cause of early stage pregnancy loss.

To examine the possible role in pregnancy loss of an immune response to the CT HSP, we analyzed cervical samples from 216 IVF patients for cervical IgA antibodies to the recombinant CT HSP as well as to CT structural antigens.²⁵ Term deliveries occurred in 34.3% of the 198 patients who proceeded to embryo transfers; only 5 (7.3%) of these women were positive for anti-HSP IgA and 1 (1.5%) was positive for IgA to CT structural components. In contrast, the presence of cervical antibodies to both HSP and CT structural components strongly correlated with a poor IVF outcome. Of the women whose embryo transfers did not lead to term deliveries, anti-HSP IgA was present in 36 (27.7%) and anti-CT IgA in 24 (16.2%).

Almost all of the women sensitized to CT structural antigens also had antibodies to HSP. However, only 35% of the women with cervical IgA antibodies to HSP were sensitized to the other CT antigens. This finding suggested 3 possibilities: 1) that the HSP was the most immunogenic of the CT antigens, 2) that a latent CT infection was present in which only HSP was being produced,⁶ or 3) that the women were sensitized to the HSP from an organism other than CT and crossreacting antibodies were being detected.

The demonstration that local cervical immunity to CT was associated with a poor IVF outcome confirmed our previous studies using PCR to detect this organism.²⁵ We believe it is unlikely that the presence of either CT or anti-CT antibodies in the cervix was responsible for the observed pregnancy failures. A more likely explanation is that their detection serves as a marker for the concomitant presence of this organism in the upper genital tract where infection or immune sensitization would be responsible for early stage pregnancy termination.

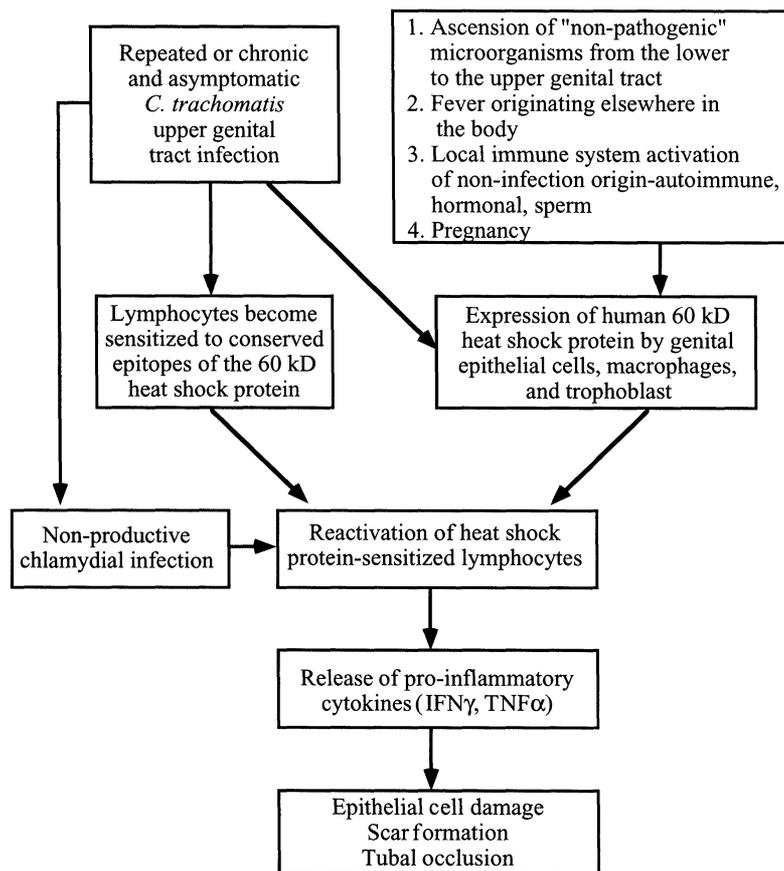


Fig. 1. Proposed mechanism of CT-induced autoimmune-mediated fallopian-tube occlusion or early stage pregnancy loss.

There is evidence that the human 60 kD HSP is expressed on the surface of decidual epithelial cells during early pregnancy.²⁶ Similarly, murine hybridomas specifically responsive to the 60 kD HSP have been shown to react with human trophoblasts.²⁷ Therefore, there appears to be ample human HSP expression during the early stages of pregnancy to activate HSP-sensitized lymphocytes and induce a localized, autoimmune inflammatory response.

The proposed sequence of events leading from a CT infection to immune sensitization to conserved HSP epitopes to tubal scarring or pregnancy failure is illustrated in Figure 1.

CT INFECTION AND ANTISPERM ANTIBODIES

Women with circulating antibodies against their partners' motile sperm have a reduced probability

for conception.²⁸ Fortunately, because of the immunosuppressive properties of sperm and seminal fluid,²⁹ most sexually active women do not produce antisperm antibodies. However, the wives of men with asymptomatic CT infections detected by PCR have been shown to have an increased prevalence of IgG and IgA antisperm antibodies in their sera.³⁰ Most probably, the CT, which can nonspecifically adhere to sperm,³¹ acts as an adjuvant in inducing an immune response to both CT and sperm-specific antigens.³² Therefore, another consequence of an asymptomatic genital-tract CT infection may be an increased susceptibility to the development of antisperm antibodies and immunological infertility.

When a woman who is sensitized to sperm, thus having antisperm antibodies, engages in coitus, the production of IFN- γ is stimulated³³ by the interaction between antibody-coated sperm and T lymphocytes.³⁴ Therefore, when sperm reach the fallopian tubes of such a woman, they can interact with resi-

TABLE 2. Screening for CT in asymptomatic sexually active women

Whenever there is a new sexual partner
When there is unexplained infertility or spontaneous abortion
When either partner of an infertile couple has antisperm antibodies
Prior to an IVF cycle
When a pregnant woman has a history of sexually transmitted disease or infertility

dent lymphocytes,³⁵ producing a localized increase in the IFN- γ concentration and, as mentioned above, the induction of inflammatory responses to epithelial-cell membrane components. The subsequent expression of HSP at the sites of inflammation would result in a further exacerbation of localized tissue damage in women sensitized to HSP.

SCREENING FOR CT GENITAL-TRACT INFECTIONS

In light of the asymptomatic nature of most CT genital-tract infections as well as the ability of this organism to evade immunological defenses, persist for long periods,³⁶ and gradually ascend from the lower to the upper genital tract, the most prudent means to reduce the incidence of CT-related infertility and pregnancy loss is the periodic screening of asymptomatic women (and men) who are at risk for acquiring this infection. Through such screening, newly acquired infections of the cervix (and the male genital tract) could be identified and antibiotic treatment promptly initiated prior to the infection of other regions of the genital tract and resultant immune sequelae.

Various studies have been published predicting which groups of women would most benefit from selective CT screening or advocating universal screening.³⁷⁻⁴¹ Further verification among different subpopulations of women will help to pinpoint future testing priorities. In addition, a more concerted effort is needed to educate women about the prevalence of CT infection, the lack of symptoms following an infection, the serious consequences of harboring an undetected infection, and the indications for screening. Our suggestions for CT testing in asymptomatic women are listed in Table 2.

Only through the education of patients and health-care providers coupled with the identification and prompt, effective treatment of *asymptomatic* female and male carriers of CT genital-tract

infections can we hope to diminish the incidence of CT-associated infertility and pregnancy complications.

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