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ASYMPTOMATIC CHLAMYDIA TRACHOMATIS FEMALE GENITAL TRACT INFECTIONS

Immune Mechanism of Infertility and Improved Means of Detection

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Chlamydia trachomatis, and obligate intracellular microorganism, is the leading cause of tubal occlusion-related infertilily and ectopic pregnancy in industrialized countries. In addition, since the majority of infections in both women and men are asymptomatic, infected individuals often do not seek medical treatment and continue to infect new partners. Currently, there is an epidemic of asymptomatic *C. trachomatis* genital tract infections. There is an urgent need to increase the availability of *C. trachomatis* testing for women as well as utilization of the most sensitive and specific detection assays.

In an in vitro system, *C. trachomatis* does not visibly damage the fallopian tubes[1]. Current opinion holds that the immune response to infection, and not the infection per se, is the cause of the tubal damage. In women, where fallopian tube blockage does not occur, a *C. trachomatis* upper genital tract infection can cause ectopic pregnancy or early stage pregnancy loss.

In this article, the mechanism leading to persistent *C. trachomatis* infection will be outlined, the subsequent induction of induced immune-mediated fallopian tube damage and pregnancy loss delineated, and new developments in specimen collection and *C. trachomatis* detection in women will be discussed.

DEVELOPMENT OF INAPPARENT, CULTURE-NEGATIVE C. TRACHOMATIS FALLOPIAN TUBE INFECTION

Several studies of the sequela of *C. trachomatis* ocular or genital tract infections have demonstrated that this organism can persist within cells for long periods of time[2,3], in a form that will not replicate in culture[4-6]. Re-creation of proliferation-negative chlamydial persistence has recently been accomplished in vitro. This allowed determination of the probable mecha-

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nism for persistence that occurs in vivo. It must be acknowledged, however, that the mechanism of in vivo chlamydial persistence has not been definitively established.

Chlamydial persistence in epithelial cells appears to occur by an immune-mediated mechanism. In vitro, the addition of pro-inflammatory cytokine products of activated T lymphocytes and macrophages, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), disrupts the *Chlamydia* life cycle. Replication of the chlamydial reticulate bodies is arrested and large aberrant reticulate body-like forms accumulate within the cell [7,8]. Although the aberrant forms cannot replicate they remain viable, however, and when the cytokines are removed normal reticulate body proliferation recommences with their subsequent differentiation into infectious elementary bodies and cell lysis.

Applying the above results to the analogous in vivo situation, the following scenario has been envisioned[9,10]. *C. trachomatis* is sexually transmitted to a woman and asymptomatically infects the endocervix. In the absence of treatment, the organism gradually ascends up the genital tract and colonizes the fallopian tube epithelium. Chlamydial replication and release of elementary bodies into the lumen activates the woman's immune system and IFN-γ, TNF-α and other pro-inflammatory mediators are released. This results in clearance of the extracellular infection. However, the cytokines also induce arrest of the organisms and their persistence in a viable form. Once the extracellular organisms are eliminated, immune system activation abates and IFN-γ and TNF-α are no longer released. In the absence of these mediators, the aberrant reticulate bodies convert to their typical morphology and resume replication. The conversion of reticulate bodies to elementary bodies and subsequent lysis of the cell leads to the infection of neighboring epithelial cells. By the development of persistence *C. trachomatis* is able to evade immune destruction. With each succeeding cycle of active replication and persistence, more and more epithelial cells are damaged. In addition, the pro-inflammatory cytokines also induce release of cytotoxic molecules that further damage the integrity of the tubal epithelium. Eventually tissue destruction and the resulting scar formation becomes sufficient to interfere with the patency of the fallopian tubes. This results in increased susceptibility to ectopic pregnancy as well as to tubal factor infertility.

Chlamydia trachomatis, and obligate intracellular microorganism.

INVOLVEMENT OF IMMUNITY TO THE 60KD HEAT SHOCK PROTEIN (HSP60) IN C. TRACHOMATIS PATHOGENESIS

One of the proteins of *C. trachomatis*, hsp60, has been implicated in immune pathogenesis. The properties of this protein are summarized in Table. Hsp60 is present in every known organism and is one of the most highly conserved proteins. Under conditions where chlamydial growth is arrested by IFN-γ and unculturable persistent forms of the organism predominate hsp60 synthesis continues at a high level while little or no production of the other chlamydial antigens takes place[11]. The chlamydial hsp60 has been shown to induce a delayed hypersensitivity response in guinea pigs[12] and monkeys[13] that were previously sensitized to thisorganism. In addition, there is evidence from a number of different laboratories that immunity to the chlamydial hsp60 is associated with salpingitis[14], ectopic pregnancy[15] and tubal occlusion[16] in women. In fact, the presence of circulating antibodies to the chlamydial hsp60 has been shown to be a risk factor for subsequent development of fallopian tube inflammation[17]. Thus, under conditions of chlamydial persistence, hsp60 is preferentially released and an immune response to this protein is generated. *C. trachomatis*-induced cervicitis or an initial episode of acute salpingitis do not usually induce immunity to hsp60[14]. A prolonged exposure of the immune

Tabela

Characterization of the C. Trachomatis hsp60

1. The second most abundant protein in lysates of *C. trachomatis*-infected cells
2. Associated with the surface of both reticulate bodies and elementary bodies
3. The major protein synthesized by IFN-γ treated infected cells
4. Inducer of a delayed hypersensitivity response in animals previously infected with *C. trachomatis*
5. A member of the 60kD heat shock protein family
6. Almost 50% amino acid sequence homology with the human hsp60

system to hsp60 is apparently required for development of immunity to this highly conserved protein.

The human and *C. trachomatis* hsp60 have almost a 50% amino acid sequence homology [18]. Thus, another consequence of a persistent chlamydial infection is the possibility of development of autoimmunity to regions of the chlamydial hsp60 that are conserved in the human hsp60. The presence of cell-mediated[19] and humoral[20,21] autoimmunity to conserved regions of hsp60 in individuals sensitized to *C. trachomatis* has been demonstrated using synthetic peptides.

The human hsp60 is expressed during the early stages of pregnancy by both the embryo [22,23] and maternal decidua[24]. Thus, in women previously sensitized to conserved regions of the chlamydial hsp60 expression of the human hsp60, during pregnancy, could result in reactivation of hsp60-sensitized lymphocytes. The resulting pro-inflammatory immune response may interfere with pregnancy-induced regulatory mechanisms and lead to immune rejection of the embryo. In fact, women with cervical IgA antibodies to the chlamydial hsp60[25] or to a conserved hsp60 epitope[21] have a poorer outcome following in vitro fertilization and embryo transfer than do women lacking these antibodies.

INTROITAL TESTING FOR C. TRACHOMATIS

According to the Centers for Disease Control, reported *C. trachomatis* infections in women have increased 145% in the United States and 253% in New York State between 1991 and 1995. This apparent increase is mainly due to an appreciation that 70% of chlamydial infections, in women, are asymptomatic and so screening programs of asymptomatic women have been markedly expanded. In addition, improved *Chlamydia* detection assays, notably gene amplification techniques, have also become available. It has become apparent, that the more one looks for *C. trachomatis* infections in women, and the more sensitive the detection assay, the more *C. trachomatis* one will find. It is naive and harmful to women's fertility to underestimate, based on assays that are less than state of the art, the need for *C. trachomatis* screening in a given population.

In an in vitro system, C. trachomatis *does not visibly damage the fallopian tubes.*

In addition to infertility and early stage pregnancy loss *C. trachomatis* is a major cause of pelvic inflammatory disease (PID), preterm birth, ectopic pregnancy, chronic pelvic pain and perinatal infections[26]. *C. trachomatis* infections also facilitate HIV transmission. Although difficult to accurately document, it has been estimated that 20-40% of women with a *C. trachomatis* lower genital tract infection that is not promptly and adequately treated will develop PID. Among the PID patients 20% will become infertile and 9% will suffer an ectopic pregnancy[27]. Teenagers and young adults are at highest risk for contracting a *C. trachomatis* infection. They are most likely to have multiple sexual partners, engage in intercourse without the use of a condom and have a higher physiological and immune susceptibility to this organism. Increased access to screening for this organism, coupled with sensitive methods of detection, provides the easiest and most cost effective means of reducing the incidence of these sequela. Barriers to effective screening of some female populations, especially high risk adolescents, are the lack of ready access to a health care facility, embarrassment associated with sexual activity and the need for a speculum-based examination to obtain an endocervical sample for analysis.

Recent studies by several different investigators have demonstrated that combined with gene amplification technologies such as the polymerase chain reaction or ligase chain reaction, *C. trachomatis*[28,29], *Trichomonas vaginalis*[28,30] and *Neisseria gonorrhoeae*[31] could be detected in specimens obtained from the entrance to the vagina (introitus). The sensitivity and specificity of these findings were comparable to those obtained using endocervical swabs. This strongly suggests that women should be able to obtain their own specimens for STD testing in privacy, without the need for a clinic or trained personnel. Furthermore, since viable organisms are not required for detection, the samples should be stable at ambient temperature for several days. This would allow specimens to be collected and transported to a central location, or even sent by mail, for subsequent analysis. In-

troital specimen collection is preferable to urine sampling based on the ease of handling and improved stability. Anonymous testing would also be available by the woman sending in a sample with a code number instead of her name. All of these steps should greatly increase the numbers of women for whom STD testing in general, and *C. trachomatis* testing in particular, would be available and acceptable. Detection and subsequent effective treatment of recently acquired chlamydial infections should greatly decrease the incidence of late sequela of this infection, reduce the need for expensive hospitalizations as well as curb the spread of asymptomatic *C. trachomatis* infections.

Chlamydial persistence in epithelial cells appears to occur by an immune-mediated mechanism.

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