Chlamydia trachomatis in subfertile women undergoing uterine instrumentation
An alternative to direct microbial testing or prophylactic antibiotic treatment

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Chlamydia trachomatis is the major cause of tubal occlusion, and is also associated with IVF failure and spontaneous abortion. These infections are asymptomatic in most individuals and can persist in the genital tract for long periods of time in a form resistant to immune destruction. A significant percentage of couples seeking treatment for infertility might, therefore, harbour C. trachomatis in their genital tract. An unresolved question is what to do about this possible chlamydial persistence. Cervical, endometrial and semen samples can be tested for C. trachomatis and only positive individuals treated. Alternatively, all couples undergoing infertility treatment can receive prophylactic antibiotics. We advocate a third option, to screen and treat only individuals who are positive for systemic and/or local anti-chlamydial antibody production. Detection of species-specific C. trachomatis antibodies in peripheral blood will determine which individuals have been exposed to this organism and who, therefore, may be at risk for harbouring persistent forms. Identification of IgA antibodies in genital tract secretions may be an even better indicator of the presence of C. trachomatis in the genital tract. Circulating antibodies to the chlamydial 60kDa heat shock protein (hsp60) is a specific indicator of tubal occlusion and, furthermore, correlates with the continued presence of this micro-organism in the genital tract of non-human primates. Screening for both cervical IgA antibodies to C. trachomatis and serum IgG anti-chlamydial hsp60 appears to provide the best indication as to which women may be harbouring C. trachomatis.

Key words: antibodies/Chlamydia trachomatis/endocervix/heat shock protein/tubal infertility

Introduction

Chlamydia trachomatis genital tract infections are unique due to a lack of clinical indicators of their presence. In ~75% of infected women and 50% of infected men symptoms remain unnoticed or are minor and non-specific. Therefore, unsuspecting infected individuals do not seek medical treatment. However, C. trachomatis is a major cause of infertility (Cates and Wasserheit, 1991), ectopic pregnancy (Sziller et al., 1998) and pregnancy loss (Paavonen and Eggert-Kruse, 1999) in women, and probably contributes to infertility in men (Close et al., 1987; Munoz and Witkin, 1995; Paavonen and Eggert-Kruse, 1999).

In this article we review evidence in favour of specific C. trachomatis antibody testing as a means of identifying women at greatest risk for harbouring this organism. Although attention is focused on female partners of infertile couples, we strongly emphasize that when the female partner is being treated for this infection it is mandatory to also treat the male partner.

C. trachomatis persistence

C. trachomatis is an obligate intracellular bacterium with a unique life cycle. The extracellular form of the organism, the elementary body, attaches to and penetrates into epithelial cells. Within the cell the elementary body converts to the reticulate body, the replicating form of the organism. When a certain density of reticulate bodies is achieved the organism again converts to the elementary bodies, which are released to infect neighbouring epithelial cells. The immune system of the host is activated by chlamydial elementary bodies. Interferon (IFN)-γ and other pro-inflammatory cytokines are produced. The resulting activation of phagocytic cells limits the extent of the infection. However, exposure to IFN-γ also leads to chlamydial persistence (Beatty et al., 1994b). IFN-γ inhibits replication of the reticulate bodies. Large misshapen chlamydial forms can be seen within IFN-γ treated cells in vitro. These forms are still viable and when IFN-γ is no longer being produced, due to the apparent absence of this organism from immune recognition, replication of the reticulate
bodies resumes. In addition to IFN-γ, treatment with various antibiotics has also been shown to result in formation of chlamydial persistence, at least in vitro (Beatty et al., 1994b).

There is evidence that an asymptomatic chlamydial genital tract infection can evade immune destruction and remain within the reproductive tract for long periods of time (months or years) (McCormack et al., 1979; Ruijs et al., 1990; Golden et al., 2000). This is undoubtedly due to the induction of persistence by the immune response to this organism. The reactivation of a chlamydial infection after its apparent clearance has also been demonstrated both in experimentally infected mice (Cotter et al., 1997) and in humans (Ormsby et al., 1952; Batteiger et al., 1989). The problem for infertility practitioners, and especially for centres specializing in assisted reproduction, is how to assess patients who never knowingly had a chlamydial infection and therefore were never specifically treated with antibiotics, but who, nevertheless, have sequela consistent with possibly having had an asymptomatic chlamydial infection. Reactivation of a persistent and unexpected \textit{C. trachomatis} infection could interfere with a successful pregnancy outcome, as outlined above.

\textit{C. trachomatis} antibody assays

Since another member of the Chlamydia genus, \textit{C. pneumoniae}, is a common respiratory tract inhabitant, many individuals have antibodies that will react with Chlamydia genus-wide antigens. In addition, antibodies to other micro-organisms may sometimes cross-react with Chlamydia (Maun et al., 1997). It is important, therefore, that \textit{C. trachomatis} antibody detection assays be species-specific. The microimmunofluorescence (MIF) assay has been long considered the ‘gold standard’ for species-specific \textit{C. trachomatis} antibody testing. The technical difficulty in performing this analysis plus the long period of learning required for correct interpretation of results has limited the MIF assay to very few microbiological laboratories. Other, less technically demanding, antibody detection assays were until recently only genus-specific and did not correlate very well with infertility or \textit{C. trachomatis} organism detection. Recently, however, species-specific \textit{C. trachomatis} antibody assays have become available. It is now possible for any clinical laboratory to perform accurate \textit{C. trachomatis} antibody determinations.

\textbf{Systemic versus local }\textit{C. trachomatis} \textbf{antibodies}

The human endocervix is part of the mucosal immune system and is distinct from systemic immunity. IgA and IgG producing plasma cells in the endocervical mucosa can be stimulated to produce antibodies that are not present in the circulation (Kuette et al., 1988). \textit{C. trachomatis} has been shown to induce a local cervical antibody response that differs from antibodies in serum. Furthermore, cervical anti-chlamydiyal antibodies correlate better with the presence of an active infection than circulating antibodies do (McComb et al., 1979).

More recent studies have demonstrated that while the presence of cervical anti-chlamydiyal IgA antibodies is correlated with IVF failure (Witkin et al., 1994), circulating anti-chlamydiyal IgG antibodies are unrelated to IVF outcome (Spandorfer et al., 1999). Although other mechanisms are possible, these observations are also consistent with a relationship between cervical IgA immunity and the continued presence of \textit{C. trachomatis} in the female reproductive tract.

\textbf{Immunity to heat shock protein}

Heat shock proteins are essential components of every living organism from bacteria to humans and their amino acid sequences have been highly conserved throughout evolution. They bind to nascent proteins and facilitate their intracellular transport and correct assembly and folding. Under conditions of stress the synthesis of several heat shock proteins is up-regulated to prevent protein denaturation and to mark degraded polypeptides for elimination from the cell (Kaufmann, 1990). When \textit{C. trachomatis} is in its persistent state, a stressful condition, synthesis of hsp60 is up-regulated while the production of other chlamydial components is down-regulated (Beatty et al., 1994a; Gerard et al., 1998). Although it is a highly conserved protein, hsp60 is also immunogenic and induces a potent pro-inflammatory immune response. The inflammatory response to chlamydial hsp60 was first demonstrated in guinea-pigs (Morrison et al., 1989). Subsequent elegant studies in rhesus monkeys established that the chlamydial hsp60 induced a potent pro-inflammatory immune reaction in the Fallopian tubes of previously infected animals and that this response was probably responsible for the development of tubal occlusion (Patton et al., 1994a).

Studies in man have established that humoral (Brunham et al., 1985; Toye et al., 1993) and cell-mediated (Witkin et al., 1993) immunity to chlamydial hsp60 can be demonstrated in women with recurrent pelvic inflammatory disease and tubal infertility. Most importantly, the detection of anti-chlamydial hsp60 immunity in the circulation was highly correlated with the presence of tubal occlusion (Brunham et al., 1985; Toye et al., 1993; Claman et al., 1997; Ault et al., 1998). Furthermore, the chlamydial hsp60 antibodies in women with tubal infertility appeared to be unrelated to a prior \textit{C. pneumoniae} infection (Persson et al., 1999). Antibodies to recombinant human hsp60 also correlated with tubal occlusion (Spandorfer et al., 1999). Detection of immunity to specific epitopes of hsp60 that are present in both the chlamydial and human proteins (Arno et al., 1995; Domeika et al., 1998; Sziller et al., 1998) may further increase the specificity of this analysis. It has been reported that analysing for antibodies to both the chlamydial hsp60 and a second protein, polypeptide encoded by open reading frame 3 of the plasmid (pgp3), increased sensitivity and specificity (Bas et al., 2001). Cervical IgA antibodies to chlamydial hsp60 have also been detected in some women undergoing IVF and their presence was associated with adverse outcomes (Witkin et al., 1994). Importantly, studies in monkeys have also demonstrated that detection of circulating antibodies to hsp60 correlated with the continued presence of this micro-organism in the upper genital tract (Peeling et al., 1999).

The continued release of chlamydial hsp60 from persistently infected epithelial cells can eventually lead to development of
autoimmunity to the homologous human hsp60 (Withkin et al., 1996). Hsp60 is one of the first proteins expressed by mammalian zygotes after fertilization and is present on both the early stage embryo and maternal decidua (Neuer et al., 2000) (review paper). Therefore, in women with pre-existing immunity to chlamydial hsp60, exposure to human hsp60 in the early stages of pregnancy can lead to reactivation of hsp60-sensitized lymphocytes. The subsequent pro-inflammatory immune response can foster immune rejection of the developing embryo. Similarly, immunity to hsp60 has also been associated with spontaneous abortion (Kligman et al., 1998), inhibition of in-vitro development of mouse embryos (Neuer et al., 1998) and preterm birth (Ziegert et al., 1999).

One small published study with a total of only 37 pregnancies (and a low 22% overall pregnancy rate) concluded that, among women who were seropositive for antibodies to C. trachomatis, those who also had anti-chlamydial hsp60 IgG in their sera had a higher pregnancy rate after IVF than those who were chlamydial hsp60 IgG seronegative (Claman et al., 1996). Until further investigations on a greater number of subjects corroborate this observation the putative positive effect of these antibodies on IVF success must remain highly questionable.

Summary and recommendations
Prescribing antibiotics to all couples who seek treatment for infertility, besides being wasteful, is potentially dangerous in that unnecessary antibiotic administration can lead to development of antibiotic-resistant micro-organisms. Antibiotic resistance in C. trachomatis has been reported (Somani et al., 2000). In addition, short courses of prophylactic antibiotics may not be effective in eliminating C. trachomatis from the upper genital tract (Patton et al., 1994b). Directly testing infertile couples for C. trachomatis—in semen, urine, endocervix or endometrium—has a low overall sensitivity. C. trachomatis might be undetectable in the lower genital tract but remain present in the upper genital tract (Thomas et al., 2000). In women who might be harbouring C. trachomatis only in their upper genital tract, a positive antibody result will be the sole non-invasive indicator of its presence. While testing patients’ sera for anti-C. trachomatis IgG antibodies by a species-specific assay will determine previous exposure to this micro-organism, it will not provide information as to whether Chlamydia might still be present (Thomas et al., 2000). In contrast, detection of cervical IgA antibodies to C. trachomatis, combined with circulating IgG antibodies to chlamydial hsp60, will pinpoint which women are most likely to still harbour this micro-organism in their reproductive tracts.

It will initially be more expensive to test women for chlamydial antibodies and to treat only the positive patients than to non-selectively prescribe antibiotics for every infertility patient. However, in our opinion the small increased cost is more than justified. Studies on cost-effectiveness must take into account the increasing problem to society of antibiotic resistance among micro-organisms as well as potential side-effects of antibiotic usage on other body systems.

While the presence of C. trachomatis in the genital tract is a clear risk for a successful pregnancy outcome, unfortunately there are no published trials to definitively determine whether antibiotic treatment of cervical IgA-positive or serum hsp60 IgG-positive women is indeed effective in improving pregnancy outcome. Such studies are urgently needed.

References


