Pathogenesis of chlamydia induced pelvic inflammatory disease

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Introduction
Chlamydia trachomatis is the most common cause of pelvic inflammatory disease (PID) in the United States, and is an important cause of female infertility worldwide. Our understanding of the immunopathological pathways associated with genital C. trachomatis infection and reproductive sequelae such as tubal infertility and ectopic pregnancy remains incomplete. Current observations show that only a fraction of women infected with C. trachomatis develop upper genital tract infection, and only a subset of those women develop tubal infertility. Studies in macaques have shown that a single episode of chlamydia salpingitis is usually self limiting, whereas repeated infection eventually produces severe tubal scarring. These and other findings suggest that acquired immune responses to chlamydial antigens are important in pathogenesis. Other research has suggested that genetic and immunological risk factors are associated with risk of salpingitis and subsequent tubal damage. In addition, Dean et al reported that specific major outer membrane protein (MOMP) variants are associated with upper genital tract infection. Thus both bacterial and host factors appear to influence the pathogenesis of chlamydial disease.

The development of tubal scarring secondary to C. trachomatis ultimately depends upon initial infection of the female lower genital tract, usually the cervix. Once lower genital tract infection occurs, the infection must ascend via the endometrial cavity to the endosalpinx in order to cause salpingitis and finally tubal scarring. Compartmental models are useful to describe the pathogenesis of chlamydia induced infertility since each step in the causal pathway may be associated with distinct genetic, immunological, and bacterial virulence factors. In this review we will elaborate on the pathogenesis of C. trachomatis associated PID, and consider host and bacterial factors associated with chlamydial disease pathogenesis.

Epidemiological risk factors for C trachomatis pelvic inflammatory disease
Several epidemiological factors have been studied with regard to risk of chlamydia associated PID. Repeated C. trachomatis infection has been associated with both an increased risk of PID and infertility, and supports the hypothesis of immune mediated chlamydial induced pathogenesis. Oral contraceptives appear to increase the risk of acquiring cervical C. trachomatis infection, decrease the risk of PID, and have no effect on the risk of tubal factor infertility. The mechanism by which contraceptive steroids confer protection from acquiring symptomatic chlamydial PID is unknown and deserves further research. HIV-1 infection has been associated with an increased incidence of C. trachomatis infection, and increased risk of PID among CD4+ cell deficient HIV-1 infected women.

Chlamydial heat shock protein-60
A gradient in seroprevalence to chlamydial heat shock protein-60 (Chsp60) has been observed among women with different clinical manifestations of chlamydial infection. Data show that 16%–25% of fertile microimmunofluorescent (micro-IF) antibody positive women have antibody to Chsp60, in comparison with 36%–44% of women with C trachomatis cervicitis, 48%–60% of women with chlamydial PID, and 81%–90% of women with C trachomatis associated fallopian tube damage. Probably the strongest epidemiological evidence is a study of 280 female sex workers prospectively evaluated for incident C. trachomatis cervical infection and PID. Among women infected with C. trachomatis, those with antibody to Chsp60 had a twofold to threefold increased risk of PID. In a study using pigtailed macaques, purified recombinant Chsp60 was injected into subcutaneous salpingal transplants in animals previously sensitised by infection with live C. trachomatis organisms. In this experiment, delayed type hypersensitivity reaction in the salpingeal tissue was observed, characterised by mononuclear cell infiltration with a peak reaction at 48 hours; while animals not sensitised by prior C. trachomatis infection were unaffected by injection of purified Chsp60. This study provided additional evidence to support the role of Chsp60 in the immunopathogenesis of chlamydial disease.

Chsp60 has important immunological characteristics. The protein is highly conserved in amino acid sequence; chlamydial and human sequences are 48% homologous. Antibody responses to the Chsp60 protein are genetically determined and in part have been mapped to the MHC locus in mice. Chsp60 immune recognition may incite an autoimmune inflammatory response through molecular mimicry. These and recent results in humans suggest that an autoimmune response to self hsp60 commonly develops following C. trachomatis infection, and have no effect on the risk of tubal factor infertility.
upper genital tract infection, and may be the consequence of an immune response cross reactive to self hsp60 and Chsp60. Autoimmune inflammation may sustain tissue damage as chlamydial growth becomes inhibited by the acquired immune response.

Genetic factors
HLA class I molecules present peptides to CD8 T lymphocytes, and thereby restrict cytotoxic CD8 T lymphocyte (CTL) responses. HLA class II molecules present peptides to CD4 T cells and restrict a range of cellular and antibody responses. Early data support the theory that genetic and immunological risk factors are associated with risk of chlamydial salpingitis, and subsequent tubal damage. HLA-A31 was associated with an increased risk of clinical PID among women with *C trachomatis* infection. Similarly, HLA class I alleles were associated with susceptibility and resistance to peritubal adhesion formation in pigtailed macaques with experimental *C trachomatis* salpingeal infection. In a Gambian population with endemic ocular *C trachomatis* infection, HLA A*6802 was associated with an increased risk of trachoma. Interestingly, while MHC class I restriction of the immune response to *C trachomatis* is supported by these investigations, HLA class II alleles were not found associated with disease outcome. In vitro studies of peripheral blood mononuclear cells from patients with and without trachoma suggest that CD4 T cells which preferentially express Th-2 cytokines may play a role in the pathogenesis of trachomatous scarring and may be associated with HLA-DQ regulation of the Th1/Th2 response. We recently studied women with tubal factor infertility in Nairobi, Kenya to investigate genetic factors associated with altered susceptibility to *C trachomatis* infection as defined by the presence of *C trachomatis* micro-IF antibody, and tubal factor infertility (CR Cohen et al, unpublished data). Ninety three women were HLA class II genotyped—47 women with tubal factor infertility and 46 women undergoing tubal ligation serving as controls. DQA*0102 was associated with resistance and DQB*0501 was associated with an increased prevalence of *C trachomatis* micro-IF antibody. HLA class II alleles were not strongly correlated with additional increased risk of tubal infertility among *C trachomatis* seropositive women (table 1). Thus these data may suggest that susceptibility to *C trachomatis* infection may be HLA class II restricted. As an alternative explanation, these HLA class II alleles may be in linkage disequilibrium with an unstudied gene which itself causes the altered risk of *C trachomatis* infection. Because of multiple comparisons, this study, as well as other epidemiological studies referenced in this section, probably overrepresents the extent of genetic associations with chlamydial infection and disease pathogenesis. Population based studies that focus on specific alleles will be required to verify these relations.

### Humoral immunity to *C trachomatis* infection

The *C trachomatis* immunodominant major outer membrane protein (MOMP) is both a target of neutralising antibodies and a serotyping antigen. Relative resistance occurs towards reinfection with the identical *C trachomatis* serological variant (serovar). Wang and Grayston recognised that *C trachomatis* infection preferentially induces serovar specific micro-IF antibody and that reinfection with a *C trachomatis* variant different from the original serovar recalled antibodies to the original serovar, a phenomenon termed original antigenic sin. Local antibody mediated neutralisation of infectivity seems probable since secretory IgA antibody to *C trachomatis* in cervical mucus has been inversely correlated with quantitative shedding of the organism in the genital tract, and antibody to MOMP can neutralise the organism in vivo. In a murine model using β cell deficient mice which do not produce antibody, resistance to *C trachomatis* infection was only modestly or not impaired in comparison with β cell intact mice. Furthermore, women with high micro-IF titres to MOMP (≥ 1:128) were not more likely to develop clinical PID. Thus, humoral immunity may not be sufficient or an absolute requirement for acquired resistance to *C trachomatis* infection or chlamydial induced PID. These findings require confirmation in humans.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Association of selected HLA class II alleles with risk of <em>C trachomatis</em> micro-IF seropositivity among a weighted sample of women in Nairobi, Kenya (CR Cohen et al, unpublished data)</th>
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<tbody>
<tr>
<td>HLA class II allele</td>
<td>Weighted sample*</td>
</tr>
<tr>
<td>DQA*0101</td>
<td>OR=1.7 (0.6–4.4)</td>
</tr>
<tr>
<td>DQA*0102</td>
<td>OR=0.4 (0.1–0.8)</td>
</tr>
<tr>
<td>DQA*0501</td>
<td>OR=2.2 (1.0–5.1)</td>
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*Weighted multivariate model adjusting for lifetime number of sex partners assuming 15% prevalence of tubal factor infertility in reproductive aged women in Nairobi, Kenya.
from animal or in vitro models. T cells accumulate at the site of infection and play a critical role in controlling the infection. During reinfection, T cells accumulate more rapidly and in larger numbers compared with primary infection. It has also been suggested that epithelial cells infected with Chlamydia may be important at initiating an inflammatory process through the secretion of proinflammatory cytokines such as IL-1 and IL-8.

Conflicting evidence in mice supports the role of CD4 and/or CD8 T cells in protective immunity to C. trachomatis. Using a gene knockout model, one study demonstrated that MHC class II restricted T cell responses were absolutely required for the development of protective immunity, whereas the MHC class I restricted T cell pathway was not required. Yet, other researchers found that C. trachomatis elicited a CD8 CTL line from C. trachomatis infected animals which, when transferred to uninfected animals, conferred immunity. This study suggested that CTL or cytokine (IFN-γ) secreting CD8 T lymphocytes may limit the establishment of chlamydia infection. Recent tissue based studies in non-human primates have demonstrated induction of mRNA for IFN-γ, IL-2, IL-6, and IL-10, but not IL-4 by single and repeated chlamydial infection of salpingal tissue. These findings suggest that Th-1 cytokines (IFN-γ and IL-2) may play a role in enhanced intracellular killing of C. trachomatis. In parallel murine experiments, anti-IFN-γ antibody treatment resulted in significantly prolonged infection while passive administration of IFN-γ to chronically infected nude/nude mice caused resolution of infection in some animals. CD8 cells predominate in the human endometrium, and are the dominant infiltrating lymphocyte phenotype during acute and repeated C. trachomatis infection in the macaque salpingal infection model. Although the evidence is stronger for CD4 T cells, both CD4 and CD8 T cell restricted mechanisms are likely to be important in C. trachomatis associated immunity and disease, affecting different stages of the pathogenic pathway. In the future, it will be important to ascertain the T cell mechanisms that regulate immunity versus immunopathology.

Bacterial virulence factors

Although most of this review has focused on host factors in chlamydial disease pathogenesis, we need to be cognizant that immunopathogenesis is regulated through multiple interactions between the bacteria and the host. Since serovars F and E have been associated with an increased and decreased risk of severe upper genital tract infections, respectively, it is logical to propose that certain chlamydial genotypes elicit different immune responses. To understand HLA restriction of C. trachomatis infection and pathogenesis it will be important to determine which antigens bind HLA haplotypes that may confer resistance or increased susceptibility to chlamydial infection and that influence risk of upper genital tract infection and subsequent tubal scarring.

Summary

Further research is necessary to elucidate the pathogenesis of chlamydial PID. It is hoped that these endeavors will eventually lead to a vaccine to prevent not only chlamydia infection, but also chlamydia associated infertility, ectopic pregnancy, and chronic pelvic pain. In the meantime we need to develop strategies to prevent primary and secondary chlamydia infection and its sequelae. Recently, Scholes et al demonstrated that a population based approach to identify and test women at high risk for cervical C. trachomatis infection effectively reduced risk of PID. Hopefully, through the use of public health measures, we can see similar decreases of chlamydia associated genital tract disease worldwide.