Chlamydia trachomatis, a hidden epidemic: Effects on the female reproduction and options for treatment.

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Running head: *Chlamydia* and female reproduction

Abstract

Problem

The number of genital tract *Chlamydia trachomatis* infections is steadily increasing worldwide, with approximately 50-70% of infections asymptomatic. There is currently no uniform screening practice, current antibiotic treatment has failed to prevent the increased incidence and there is no vaccine available.

Method of Study

We examined studies on the epidemiology of *C. trachomatis* infections, the effects infections have on the female reproductive tract and subsequent reproductive health and what measures are being taken to reduce these problems.

Results

Undetected or multiple infections in females can lead to the development of severe reproductive sequelae, including pelvic inflammatory disease and tubal infertility. There are two possible paradigms of chlamydial pathogenesis, the cellular and immunological paradigms. Whilst many vaccine candidates are being extensively tested in animal models they are still years from clinical trials.

Conclusions

With no vaccine available and antibiotic treatment unable to halt the increased incidence, infection rates will continue to increase and cause a significant burden on health care systems.

Keywords

Chlamydia, female reproduction, pathogenesis, vaccine

Introduction

Chlamydia trachomatis is a Gram-negative bacterium, which has a unique biphasic developmental cycle. Genital tract *C. trachomatis* infections, caused by serovars D-K, are the most common bacterial, sexually transmitted infection, costing health care systems billions of dollars to treat not only the acute infections, but also the complications they cause.[1] A major concern with *Chlamydia* infections are that 70% of infected women and 50% of infected men are asymptomatic. In women this can lead to severe sequelae such as pelvic inflammatory disease (PID), which can then cause ectopic pregnancies and tubal infertility,[2, 3] and men can suffer from prostatitis and epididymitis.[4] Repeat or multiple infections with *C. trachomatis* increase the likelihood of these same sequelae, with a 2 – 4.5 fold increase in the risk of ectopic pregnancy, and a 4.5 – 6.4 fold increase in the chance of PID development[5]. Risk factors for contracting infection include age, with those aged 15-24 most affected, gender, with females at more risk than men, and race[6].

Recent reports from the Centre for Disease Control and Prevention (CDC) state that, in the United States, there has been an increase in infections of 7.5% from 2006 to 2007.[7] It is possible that these increases are due to the development of more specific testing procedures,[8] however a study controlling for the effects of new testing methods and demographic and sexual risk behaviours showed an independent 5% per year increase in *C. trachomatis* positivity between 1997 and 2004.[9] Even though the rates of genital tract infections continue to rise, there are no uniformly accepted screening practices, with the majority being opportunistic. Systematic reviews of the cost effectiveness of screening young asymptomatic women suggested screening is cost effective, due to the reduction in long term health costs.[10, 11]

This highlights that while infection rates continue to increase and cost health care systems billions of dollars annually, there are currently few options available to prevent the increasing incidence of infections. This emphasises the urgent need for the development of an efficacious vaccine.

Chlamydia infection and immunity

Infection

The primary site of chlamydial infections of the genital tract is the columnar epithelial cells of the endocervix of women and the urogenital epithelia of men.[12] In males, ascending infection can cause prostatitis and epididymitis,[13] this has been extensively reviewed by Cunningham and Beagley[13] and will not be addressed in this review. In females the infection can ascend the reproductive tract and cause PID and ectopic pregnancies.[11] The development of disease sequelae in women following chlamydial infection is associated with ascension of *Chlamydia* from the lower reproductive tract (LRT) into the upper reproductive tract (URT). The mechanisms that lead to this ascension are not fully understood, neither is the rate at which this happens. It is thought that *Chlamydia* can gain access to the URT of women by attachment to sperm.[14, 15] It is also possible that movement along the reproductive tract is from general flow of fluids (Fig. 1), with studies demonstrating that particles approximately the same size of sperm,[16] or radio-labelled sperm,[17] deposited into the vagina of women, could be found in the uterus within 2 min, demonstrating that rapid ascension of bacteria could occur.

Murine studies using *C. muridarum* have greatly expanded the knowledge of infection kinetics, including the differential cell infiltration between the lower and upper genital tract, the rate at which this occurs,[18-24] and also the rate of infection ascension.[24-26] The infectious dose of *Chlamydia* is known to modulate the innate immune response, with greater inoculating doses causing a greater innate immune response.[26] It has been suggested that due to the greater immune responses elicited by high infectious challenge doses, the infection does not cause as great a degree of hydrosalpinx.[26] However, it has also been shown that while the infectious dose affects the degree of ascension of infection along the female reproductive tract of mice, it does not affect the pathological outcomes, such as hydrosalpinx development and cellular infiltrate.[24] This suggests that, if a similar situation occurs in humans, the development of pathological sequelae may not be affected by the sexual transmission dose. Although the number of *Chlamydia* required to establish an infection in different strains of mice,[27] and the number of *C. caviae* transmitted by an infected male guinea pig during mating is known[28] there is no data on these parameters in human infections.

Immunity

The regulation of immune responses against genital tract *C. trachomatis* infections in humans is largely unknown, due to the difficulty in obtaining samples and monitoring patients long term. Natural immunity to a single infection is known to be short lived, and serovar specific,[29] however multiple infections with different serovars induces longer term, cross serovar immunity.[30] Immune responses to infections are linked to genetic variations, with specific polymorphisms in immune response genes influencing the magnitude of immune responses to microorganisms.[31]

There have been reports indicating that women have spontaneously cleared a genital infection without medical intervention, however the exact duration of an infection cannot be determined.[32] It is also believed that antibiotic intervention increases the longer term rates of re-infection due to the inability of the person to develop protective immunity against *Chlamydia*.[33] It is widely accepted, based on animal studies, that to resolve a chlamydial genital tract infection in females, both a Th1 and Th2 response needs to be mounted. The infiltration and activation of $CD4^+$ T cells is required for the development of protective immunity and clearance of a primary infection.[34] While clearance of a primary infection is dependent on the development of cell mediated immunity, clearance of a secondary infection requires the presence and production of antibodies.[12, 35] Also an increase in infiltration of $CD8^+$ T cells,[36], B cells,[37] neutrophils[38] and dendritic cells[39] (DCs) is required. This ensures effective clearance of both the intracellular reticulate bodies and extracellular elementary bodies in the genital tracts of infected individuals. Recent studies examining cytobrush samples from the endocervix demonstrated that women infected with *C. trachomatis* had an increase in $CD3^+$, $CD4^+$ and $CD8^+$ cells and neutrophils,[40, 41] and an increase in recruitment of myeloid and plasmacytoid DCs.[41]

The first line of defense against a *Chlamydia* infection within the female reproductive tract is the mucosal barrier. Initial infection of epithelial cells causes a cascade of events leading to the increased production of pro-inflammatory cytokines and chemokines including IL-1, IL-8[42] IL-12[19], IL-6 and GM-CSF,[43] which then induces an influx of innate immune cells (Fig. 1) such as natural killer (NK) cells,[44] dendritic cells (DCs),[39] and neutrophils.[38] These cells then produce more cytokines such as IFN-γ and TNF-α, which impede further chlamydial growth. Production of various cytokines has, however, proven to be detrimental to the

mucosal barrier, with their presence linked to various tissue pathologies,[12] and this has been termed the cellular paradigm[45] (Fig. 1).

Tissue destruction leading to the development of tubal infertility and ectopic pregnancy is caused by the production of cytokines (Fig. 1), including IL-1 and IL-8, in response to infection.[42] Fallopian tube biopsy samples infected with *C. trachomatis*, with or without IL-1 receptor antagonist present, revealed that the production of IL-1 leads to destruction of the ciliated epithelium.[42] Toll-like receptor 2 (TLR-2) has also been implicated in the development of chronic pathology development in the mouse model of genital infection,[46] with TLR-2 KO mice producing lower levels of TNF-α and MIP-2, and developing significantly less oviduct pathology.[46] This supports the idea that it is the host's immune response to infection that is responsible for the damage rather than the infection itself.

It has also been suggested that the pathologies seen after an infection are linked to antigen-specific adaptive cellular responses, this is termed the immunological paradigm[47] (Fig. 1). The exact mechanism or antigen behind the immunological paradigm has yet to be determined. There are conflicting reports that pathogenesis may be linked to chlamydial heat shock protein-60 (cHSP-60) through delayed type hypersensitivity (DTH) or autoimmunity. *Chlamydia* can enter a dormant, persistent state, where, in the absence of a productive infection, there is still a low level of immune stimulation from antigen recognition. This low level stimulation is believed to cause chronic inflammatory cell infiltration.[48, 49] Originally, guinea pigs sensitized with Triton-X-100 soluble chlamydial EBs, had greater ocular delayed hypersensitivity when re-exposed to infection at other sites, including vaginal and intestinal infections.[50] Similarly, monkeys immunized against *C. trachomatis* developed a greater follicular response in the eye upon re-exposure than non-immune controls,[51] highlighting the

significance of repeated infections in terms of a delayed hypersensitivity response. T cells isolated from endometrial and salpingeal tissues, removed from PID and tubal factor infertility (TFI) patients, responded to stimulation with cHSP-60 to a greater degree than with chlamydial EBs, further supporting cHSP-60's role in DTH [52, 53] (Fig. 1). The presence of cHSP-60 antibodies has also been correlated with PID severity,[54-56] TFI[57] and more severe salpingeal pathology.[58]

It has also been debated as to whether or not autoimmunity plays a role in the pathogenesis of chlamydial infections (Fig. 1), due to the high sequence homology between self and chlamydial HSP-60.[48] A study where mice were immunized with either cHSP-60, mouse (self) HSP-60, or a combination of the two, demonstrated that T cell proliferation in response to self-HSP-60 was only observed after immunization with both HSP-60s. A shift in cytokine secretion following *in vitro* stimulation was also observed, changing from anti-inflammatory IL-10 secretion when immunized with self-HSP-60, to pro-inflammatory IFN-γ when immunized with both.[59] This suggests that a chlamydial infection can induce autoimmunity, and this is supported by recent findings that human HSP-60 and cHSP-60-1 from *C. trachomati*s serovar D contain 4 potential T cell epitopes that display 100% identity.[59, 60] There is still some doubt over how involved cHSP-60 is in the development of autoimmunity, as, in these same experiments, immunization of mice with cHSP-60 alone did not induce cross-reactive autoimmune T cells,[59] however during an actual chlamydial infection it is highly likely that both host and chlamydial HSP-60 are produced.

This highlights that it is not necessarily the damage caused by the infection itself that leads to the development of reproductive sequelae such as PID, but rather the host's immune response to infection that may actually cause the damage.

Treatment or Prevention?

The current recommended treatments for genital tract infections caused by *C. trachomatis* are azithromycin or doxycycline.[61] Azithromycin is considered more effective due to it being a single 1g dose compared to a 7 days course of doxycycline, thereby enhancing compliance.[61] There is emerging evidence that *C. trachomatis* is developing antibiotic resistance, with clinical isolates having single and multidrug resistance when cultured *in vitro*.[62-65] Isolates have been individually resistant to tetracyclines, macrolides, fluoroquinolones,[64] or resistant to doxycycline, azithromycin and ofloxacin.[65] In addition, a study of infected women, who completed antibiotic treatment, found that 10% of the cohort was re-infected within 1 month of treatment completion, and 13% by 3 months, even though abstinence or 100% condom use was reported.[66] While the shortened duration of infections from early antibiotic treatment has reduced infection-associated reproductive sequelae, the number of case rates continues to increase.[67] This has been suggested to occur because early intervention with antibiotics interferes with the development of protective immune responses,[33] thereby increasing the risk of re-infection, and this has been termed the arrested immunity hypothesis.[67]

In light of the increasing rates of *C. trachomatis* genital infections, the asymptomatic nature of infections and the possibility of antibiotic resistance developing, there is an urgent need for the development of a vaccine that protects both from infection and from the development of pathology. Mathematical modelling has suggested that even a partially protective vaccine will dramatically decrease the rate of spread of infections and reduce economic burden.[68, 69] An efficacious vaccine will need to induce both a strong Th1 cell mediated response and a humoral response.[70] *Chlamydia* vaccine research has been ongoing for over 20 years,

exploring the efficacy of sub-unit, cellular and DNA vaccines,[70, 71] with many advances occurring, but as yet, no fully protective vaccine exists.

The greatest amount of *Chlamydia* vaccine research to date has focused on sub-unit vaccines. The use of MOMP as a vaccine candidate has been extensively studied, with varying success.[72-79] The use of this antigen has resulted from the fact that it constitutes approximately 60% of the outer membrane protein mass of the chlamydial EB.[47] However MOMP contains 4 variable domains, that are surface exposed and are antigenically variable between serovars.[80] This means any vaccine utilizing MOMP as the main antigen will elicit only serovar specific immunity, which is not appropriate considering there are 9 genital serovars (D-L) alone. This highlights that a vaccine containing only MOMP is unlikely to be successful however MOMP could be used as part of a multi-subunit vaccine. However, our recent studies of the serovar distribution of *C. trachomatis* infections in regional New South Wales suggest that a MOMP-based vaccine, containing MOMP from serovars E, F and G could potentially protect against >80% of infections in this region.[81]

The vaccine candidate at the forefront of current chlamydial vaccine research is chlamydial protease-like activity factor (CPAF), and is possibly the candidate closest to human clinical trials. CPAF has been extensively studied in the murine model of genital infection,[82-87] and has proven, with the use of adjuvants such as IL-12 and CpG-ODN,[82, 85] to be able to reduce the level of *Chlamydia* shed from the genital tract and prevent the development of pathology, thereby preserving reproductive health.[82, 84-86, 88] Due to CPAF being highly conserved between chlamydial species[89] and the fact that transgenic mice expressing human leukocyte antigen (HLA)-DR4 molecules, rather than mouse major histocompatibility complex class II molecules, are able to mount robust protective immune responses,[87] suggests CPAF could be

a good candidate for an efficacious vaccine, especially when used in combination with other protective antigens.

Any chlamydial vaccine candidate needs to be able to not only protect the individual from infection but also prevent the development of pathological sequelae such as infertility. This requires a vaccine to induce $CD4^+$ T cell mediated immunity, along with neutralising antibodies and importantly long lasting immunity. As yet, no single candidate is able to do this effectively and will likely require the development of a multi-subunit vaccine.

Problems with vaccine development

The advancement of molecular techniques and availability of immunologically defined animals has allowed the development of vaccine candidates and identified the likely mechanisms of protection. However, there are still many unresolved issues, including how efficacious does a vaccine have to be to prevent further spread and pathology development? As mentioned previously, mathematical modelling has indicated that even a partially protective vaccine will significantly reduce the health care burden,[68, 69] however, in light of the ability of cytokines secreted from non-immune cells to cause significant reproductive tract tissue destruction, even a low level infection has the ability to cause damage.[90] This suggests that a vaccine designed to prevent pathology development rather than infection may be more cost effective.

There are very few studies that have examined the effects of the timing of vaccination. For example it is unknown if any candidate vaccines have a therapeutic effect, or if immunization after a previously resolved infection will provide additional protection against re-exposure. Very early studies in humans indicated that individuals that had had previous ocular

infections, that were then immunized with a killed whole cell vaccine developed enhanced pathology of the eye upon re-exposure, compared to those that were not immunized.[91] This occurrence was thought to be linked to the high homology seen between cHSP-60 and self-HSP-60, contributing to autoimmunity and pathology development. Due to this, investigations using whole cell vaccines were not continued. This highlights the need to select vaccine antigens that elicit protective immunity but do not enhance pathology, particularly in individuals who might have an asymptomatic infection at the time of vaccination, or who may have had an infection prior to vaccination.

There have been no studies that have examined the effects of vaccine administration during an acute chlamydial genital infection. Therefore, it is unknown if any experimental chlamydial vaccine candidates could have a therapeutic effect, if they might reduce or enhance pathological sequelae, or if they could cause chronic, persistent infections, when administered during an infection. Knowing that *Chlamydia* can enter a persistent state when put under various pressures, including cytokine production,[92-105] it is plausible that enhancement of infection-induced immune responses by vaccination may cause the *Chlamydia* to enter this state and promote both pathology development and future reactivation of infection. It is also unknown whether any vaccine candidates administered after resolution of a previous genital infection will further boost the individual's infection-induced immune response, provide a greater level of protection and prevent further pathology development. Herpes simplex virus-2 (HSV-2) vaccine trials in women revealed that those who were HSV-1 seropositive at the time of vaccination did not mount an immune response. However, women who were we seronegative for HSV-1 and 2 mounted strong anti-HSV-2 immune responses.[106] With genital tract *Chlamydia* infection rates on the continual rise and

the asymptomatic nature of infections, these are important aspects that have yet to be examined in any way.

A vaccine designed to target the mucosal surfaces of the female reproductive tract could also be affected by reproductive cycle-associated changes in female sex hormones.[107] Studies in animal models have shown that progesterone and estradiol can affect many components of the immune response, including antigen presentation by DCs and macrophages,[108] production and transport of antibody into the FRT[109, 110] and also the induction of cell-mediated immunity.[111-113] The stage of the hormonal cycle has also been shown to influence the effectiveness of vaccination. The ability of transcutaneous and intranasal vaccination to induce immune responses in the FRT of mice is hormonally regulated,[114] with degree of antigen-specific T cell stimulation and local antibody secretion dependent on the stage of estrous when vaccination occurs.[114] A study in women, examining the effect of the route of vaccination and how the stage of the menstrual cycle affects antibody secretions in the reproductive tract, demonstrated that immunization via the nasal route induced the greatest IgA antibody secretion in vaginal secretions. However, those immunized vaginally on days 10 and 24 of their menstrual cycle had the greatest amounts of IgG and IgA in cervical secretions, compared to those immunized vaginally irrespective of cycle stage.[115] With these factors in mind, any vaccine targeted to the female reproductive tract must induce a strong CMI and humoral response that is able to overcome the effects of hormonal fluctuations.

Conclusions

Chlamydia trachomatis genital infections are continually increasing, with females at the greatest risk of infection. Due to the asymptomatic nature of infections possibility of the development of severe reproductive impairment if multiple infections occur and antibiotic

treatment failing, there is an urgent need for the development of a vaccine that prevents further spread of infection and pathological damage to the female reproductive tract. However, any vaccine candidate's efficacy needs to not be affected by the infection status of the individual, and must be able to effectively target the reproductive mucosa, irrespective of the hormonal status.

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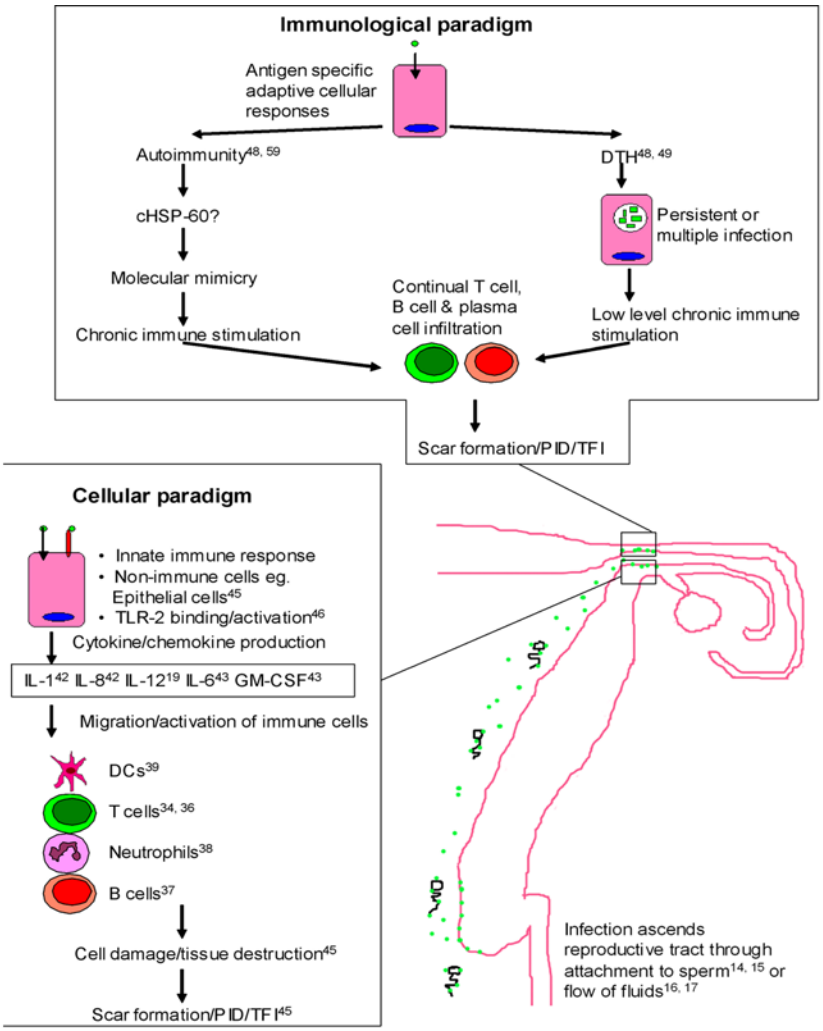

Figure 1: The paradigms of chlamydial pathogenesis in the female reproductive tract. *Chlamydia* ascends the reproductive tract either by attachment to sperm or from the general flow of fluids, infecting the upper female reproductive tract, leading to the development of pathology. Pathogenesis is thought to occur through either an innate, non-immune cellular response (Cellular paradigm), or an antigen specific adaptive cellular response (Immunological paradigm). Green circles: *Chlamydia* particles; DTH: Delayed-type hypersensitivity; cHSP-60: chlamydial heat-shock protein-60; PID: Pelvic inflammatory disease; TFI: Tubal factor infertility; TLR: Toll-like receptor; IL: Interleukin; GM-CSF: Granulocyte-macrophage colony-stimulating factor.