Chlamydia trachomatis infection: host immune responses and potential vaccines

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Chlamydia trachomatis **causes genital tract infections that affect men, women, and children on a global scale. This review focuses on innate and adaptive immune responses in the female reproductive tract (FRT) to genital tract infections with *C. trachomatis.* It covers *C. trachomatis* infections and highlights our current knowledge of genital tract infections, serovar distribution, infectious load, and clinical manifestations of these infections in women. The unique features of the immune system of the FRT will be discussed and will include a review of our current knowledge of innate and adaptive immunity to chlamydial infections at this mucosal site. The use of animal models to study the pathogenesis of, and immunity to, *Chlamydia* infection of the female genital tract will also be discussed and a review of recent immunization and challenge experiments in the murine model of chlamydial FRT infection will be presented.**

CHLAMYDIAL GENITAL TRACT INFECTIONS

Epidemiology

Sexually transmitted diseases (STDs) are a major global cause of acute illness, infertility, long-term disability, and death, with severe medical and psychological consequences for millions of men, women, and children. The World Health Organization (WHO) states that "in developing countries, STDs and their complications are amongst the top five disease categories for which adults seek health care. In women of childbearing age, STDs (excluding HIV) are second only to maternal factors as causes of disease, death and healthy life lost." The presence of an untreated STD can also "increase the risk of both acquisition and transmission of HIV by a factor of up to 10."[1] Untreated infection with *C. trachomatis* is also associated with productivity losses forming a substantial portion of the economic burden of disease.[2]

As of July 2007, the most recent international estimates are that 340 million new cases of STD infections occurred worldwide in 1999 of which around 92 million were chlamydial infections affecting more women (50 million) than men. The prevalence of *Chlamydia* in adults (persons between 15 and 49 years of age) also varies across the world and is highest for females (24 million) and males (19 million) in South-East Asia followed by adults (15.8 million) in sub-Saharan Africa. The highest rate (119) of new cases of infected adults per 1,000 population has occurred in sub-Saharan Africa.[1]

Chlamydia remains the most commonly reported infectious disease in the United States. In 2005, there were 976,445 cases of genital *Chlamydia trachomatis* infections reported[3] with 496.5 cases per 100,000 population in women and 150 cases per 100,000 population in men. Even so, most *Chlamydia* cases go undiagnosed with approximately 70% of chlamydial endocervical infections in women and 50% of chlamydial urethral infections in men being asymptomatic.[4,5] It is estimated that there are approximately 2.8 million new cases of *Chlamydia* in the United States each year.[6] In addition to gender, age and race are risk factors for genital chlamydial infections. Among women, the highest age-specific rates were among the 15- to 19-year-olds (2,796.6 cases per 100,000 females) and 20- to 24-year-olds (2,691.1 cases per 100,000 females). The rate of *Chlamydia* among blacks was over eight times higher than that of whites (1,247 and 152.1 cases per 100,000, respectively).[3]

C. trachomatis infection

In vivo, the intracellular, biphasic developmental cycle of the Gram-negative bacterium *C. trachomatis* is reliant on host cell ATP and nutrients for its existence. In this unique cycle, the bacterium is normally found in two highly specialized morphologic forms—the extracellular, metabolically inactive, and infectious elementary body (EB) and the metabolically active, intracellular form known as the reticulate body (RB) that divides by binary fission within the inclusion (reviewed in ref. 7). The infectious EB enters the mucosal host cells in a process now known to be independent of host cell-surface

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heparin sulfate glycosaminoglycans[8] and following binding to a number of proposed ligands on *Chlamydia* (reviewed in ref. 9). Once inside, the epithelial cell-surface antigens of the EB appear to prevent fusion of the endosome with lysosomes,[10] allowing the EB to subsequently reorganize into the larger, replicative form of the RB. RBs successfully divide by binary fission, filling the endosome that has now become a chlamydial cytoplasmic inclusion. Multiplication then ceases after 48–72 h and nucleoid condensation occurs, enabling the RBs to transform into infectious EBs. The EBs are released from the cell and target new host cells for progression of infection.[11]

In females, *Chlamydia* targets the apical surface of polarized superficial columnar epithelial cells that are found lining the endocervix and upper reproductive tract, an attachment that has previously been shown to be enhanced in estrogen-dominant endometrial epithelial cells.[12]

Chlamydiae have the ability to cause prolonged and often subclinical infection. *In vitro*, it can be demonstrated that *Chlamydia* can enter a latent state under stressful conditions such as exposure to interferon (IFN)-γ (which depletes available tryptophan), exposure to penicillins,[13] growth in non-permissive cells,[14] or iron depletion.[15] This third or "persistent form" has been defined as a viable but non-cultivable growth stage, resulting in a long-term relationship with the infected host.[16] Persistence allows *C. trachomatis* to remain dormant in the host cell, but after the removal of stressful conditions, *C. trachomatis* can subsequently be recovered from culture. There is also accumulating evidence to support chlamydial persistence *in vivo*.[17–21] However, direct detection of Chlamydiae for the diagnosis of persistent latent infection is somewhat questionable, and thus chlamydial persistence is not accepted by all researchers in the field, as many believe that to demonstrate that chlamydial persistence occurs *in vivo*, both nucleic acid detection and viability of the organism need to be proved. It is known that serological diagnosis of chronic persistent, latent infection can be somewhat imprecise because of low detectable levels of specific antibodies, although recently it was reported that even very low levels of specific antibodies may be directly associated with a persistent infection.[22] How often persistence occurs *in vivo* is unknown, but it may be that this persistence acts as an adaptive survival mechanism for the organism. A review on chlamydial persistence has recently been published.[23]

Serovar distribution, clinical manifestations, and infectious load

The major outer membrane protein (MOMP) and its gene *ompA* of *C. trachomatis* is an antigenically diverse and abundant protein found on the surface of the organisms. Chlamydial strains causing sexually transmitted infections are differentiated into individual serovars by typing with an anti-MOMP monoclonal antibody using microimmunofluorescence, enzyme immunoassay or, more recently, by typing using PCR and reverse line blot hybridization assay.[24,25] Serovars D, Da, E, F, G, H, I, Ia, J, and K are responsible for urogenital infections. The predominant strains of *C. trachomatis* that are most prevalent worldwide are serovars E, F, D, and Ia.[26–32] Serovar E is the predominant serovar in cervical isolates[33] and urine samples[34] but was found in only 6% of rectal isolates.[33]

Individual serovars have been reported to differ among different groups of women[35] by geographic region[32] and also by specific racial groups.[36] Asymptomatic *C. trachomatis* infections are highly prevalent in young women, with genotyping results showing that serovars E, I, and D (in decreasing order) were frequent in women aged less than 30 years, whereas serovars F, E, and G (in decreasing order) were found in a group of randomly selected patients (aged 17–68 years) attending an inner-city outpatient gynecological clinic.[35] Significant differences in serotype distribution by geographic region in the United States were reported, with the rare serotypes Ba and G significantly overrepresented in San Francisco.[32] A more recent study reported that blacks were less often infected with serovars E (30 vs. 41%) and J (9 vs. 24%) but more often infected with serovar Ia (17 vs. 0%) when compared with individuals of other racial groups.[37]

The association between serovars and infectious load was also recently investigated. Similar infectious loads were reported for high-(E, F, and G) and low-(Ia, H, J, and Ja) prevalence serovars, suggesting a similar ability for *in vivo* replication despite their differing ecological successes.[38] Men had a significantly lower load than women when the genotype was F, and the probability of being infected with serovar J was 7.7-fold higher in patients with prior chlamydial infections.[38]

Current antimicrobial therapy

Current recommendations of Centers for Disease Control (CDC) for the treatment of *C. trachomatis* urethritis and cervicitis are comprehensively covered in a recent review.[39] Compliance with an effective antibiotic regimen has nevertheless reportedly been associated with an increased frequency of recurrent infection,[20,40] tubal infertility,[17] and persistent infection characterized by a non-culturable but viable state in which RBs do not mature into EBs.[29,41] An *in vitro* study of latent genital chlamydial infections using polarized human endometrial epithelial cells reported that a persistent form of *C. trachomatis* did not have the same susceptibility to antibiotics as compared with actively growing Chlamydiae, with persistent Chlamydiae phenotypically resistant to azithromycin.[42] It has recently been hypothesized that women with high chlamydial loads may be at increased risk of antibiotic treatment failure,[43] as it is known that at high multiplicities of infection (load), *in vitro* resistance can often be demonstrated to antimicrobials and genital load is probably greater in women than men.

Disease sequelae and immunopathogenesis

The initial *C. trachomatis* infection at the mucosal site may, over time, extend from the cervix via the endometrium and into fallopian tubes in some individuals who do not spontaneously clear the initial chlamydial infection. Chronic disease associated with bacterial persistence, inflammation, and tissue damage are common sequelae of infection with these organisms. The

disease sequelae caused by ascending *C. trachomatis* infections include pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility (TFI), virtually all as a result of chronic inflammation that causes fibrosis and scarring that characterize chlamydial diseases. *C. trachomatis* reportedly is responsible for approximately 25% of the greater than one million cases of PID per year in the United States. It has also been documented that one in four women infected with *C. trachomatis* has unrecognized or latent or "silent" PID that can lead to fallopian tube damage.[44] Chlamydial infections may also be linked to cervical cancer[45,46] and may also increase susceptibility to, and transmission of, HIV in women.[47]

Immunopathogenesis of *Chlamydia* disease is currently unclear. It has been proposed that persistent infections leading to chronic inflammation and tissue damage can result from interleukin-10 (IL-10) downregulation of chlamydial-specific T-cell responses.[48] Murine model studies of chlamydial genital tract infections have shown that neutrophil recruitment and activation of matrix metalloproteinase (MMP)-13, MMP-10, MMP-15, MMP-17, MMP-3, MMP-9, and MMP-12 occur during acute infection[49] and correlate directly with hydrosalpinx formation and infertility[50] and that inhibiting MMPs protects mice against chronic disease manifestations.[51]

The issue of whether chlamydial PID is a consequence of persistent infection, immunopathology, or infection linked to the initiation of autoimmunity ("molecular mimicry") remains unresolved (for a review on *Chlamydia* and antigenic mimicry, see ref. 52). The phenomenon of molecular mimicry involves products of genes conserved in the host and infecting bacteria, e.g., heat-shock protein 60 (HSP60), or protein products from dissimilar genes sharing similar structures that elicit an immune response to both self- and microbial proteins. Chlamydial proteins that mimic host self-proteins include chlamydial heat-shock protein 60 (CHSP60),[53] the DNA primase of *C. trachomatis*, and the OmcB proteins.[54] CHSP60 is considered a key antigen in the immunopathogenesis of TFI,[55–57] stimulating both humoral and cell-mediated immune (CMI) responses in women with PID or TFI. Indeed, a recent report shows that the TFI prediction model can be improved by combining tests for humoral and CMI responses to CHSP60.[58] A recent study reported that expression of CHSP60 is greatly enhanced in *C. trachomatis* serovar E propagated in an iron-deficient medium, a stress-inducing growth condition that can result in persistent forms of *Chlamydia*.[59] Infertility in women has also been associated with the presence of CHSP10.[60,61] A recent study investigated whether host genetic traits (carrying multiple single-nucleotide polymorphisms in different genes) in the bacterial sensing system played a role in *C. trachomatis*-associated TFI. The investigators reported that, following a *C. trachomatis* infection, subfertile women (i.e., those with reduced fertility with prolonged time of unwanted non-conception) carrying multiple single-nucleotide polymorphisms in four *C. trachomatis* pathogen recognition receptor genes (Toll-like receptor (TLR)9, TLR4, CD14, and CARD15/NOD2) were at increased risk of tubal pathology.[62]

FEMALE REPRODUCTIVE TRACT AND IMMUNE RESPONSES TO *CHLAMYDIA*

Unique features of the immune system

The mucosal immune system of the female reproductive tract (FRT) comprises compartments that are unique from those found at other mucosal and systemic immune sites. The FRT contains key cells of both the innate and adaptive immune systems that detect and respond to invading microbial pathogens, and these cells are found throughout the vagina, cervix, uterus, and fallopian tubes. Protective cells include polarized epithelial cells of the endocervix, uterus and fallopian tubes, macrophages and neutrophils, and natural killer (NK) cells in the uterus, although NK cells (or large granular lymphocytes) are localized throughout the FRT in large numbers but particularly within the endometrium.[63] Many distinct immunological features are found in the mucosal tissues of the FRT. Mucosal inductive sites are lacking in the FRT, although unusual menstrual cycle-dependent aggregates of CD8 cells and B cells occur in the endometrium.[64] In contrast to the dominant role of IgA-producing cells at other mucosal sites, the dominant immunoglobulin isotype in the genital tract is IgG.[65] The epithelial cells in the endometrium can constitutively express major histocompatibility complex class II and function as antigen-presenting cells, at least for reactivation of memory T cells. This latter antigen presentation is hormonally regulated.

Innate immunity

The innate immune system of the FRT has evolved to recognize molecular patterns (rather than antigens) on microbial invaders that are not normally found on the host. It relies on receptors that recognize conserved pathogen-associated molecular patterns found in certain microorganisms.[66] The pattern recognition receptors of the host that recognize pathogen-associated molecular patterns are expressed on cells of the innate immune system. TLRs are one group of pattern recognition receptors that are expressed on macrophages, dendritic cells (DCs), neutrophils, NK cells, and epithelial cells. Comprehensive reviews on innate and adaptive immunity in the FRT[63] and epithelial cells as sentinels in immune protection[67] have recently been published.

There are many reports on the innate immune responses to genital tract infections caused by *C. trachomatis* (for a recent review, see ref. 68). Innate immune responses to chlamydial infection of HeLa cells have been reported to differ between serovars E and L2 of *C. trachomatis*.[69] Using human epithelial cell lines, it has been reported that these cell lines fail to respond to lipopolysaccharide (LPS) (associated with absence of surface expression of CD14), indicating that non-TLR4 ligands are involved in the inflammatory response of epithelial cells to *Chlamydia*.[70] These data agree with the finding of Rasmussen *et al.*[71] that chlamydial LPS is not the component responsible for induction of the proinflammatory cytokines that upregulate the endogenous inhibitors of nuclear factor-κB. The latter component of the innate immune system is a transcription factor that is activated in response to chlamydial components.[72,73]

Experiments using TLR adaptor molecule myeloid differentiation factor 88 (MyD88)-deficient mice have demonstrated the importance of TLRs in the generation of adaptive immune responses.[74] TLR2 has been identified as the principal TLR responsible for the secretion of IL-6 and granulocyte–macrophage colony-stimulating factor in a MyD88-dependent manner in *Chlamydia muridarum*-infected cloned murine oviduct epithelial cell lines.[75] It has also been reported that the surface-exposed TLR2 plays a prominent role in the recognition process of *C. muridarum* infection but that the time course of genital infection was unaffected by the absence of TLR2, although significant reduction in oviduct pathology was noted in TLR2-knockout mice.[76] Mice deficient in MyD88 showed greater effects on clearance of *C. muridarum* genital infection *in vivo* and *in vitro*, demonstrating a prominent role for MyD88 in the induction of IFN-γ-inducible protein and IFN-β during chlamydial infection in a TLR2- and TLR4-independent manner.[77] This lack of involvement of TLR4 in the *C. muridarum* model of female urogenital chlamydial infection is largely substantiated by recent data showing a lack of involvement of CD14 (an accessory protein for TLR4 recognition) in pathogenesis in this model.[78] It has also been reported from studies with the L2 serovar of *C. trachomatis* that intracellular TLR2 and MyD88 are colocalized within the chlamydial inclusion and that expression of TLR2 was required for IL-8 secretion from infected cells.[79] In a murine model of chlamydial genital tract infection, it has also recently been reported that TLR3, or an unknown pattern recognition receptor using the Toll/IL-1R domain-containing adaptor inducing IFN-β, is responsible for IFN-β production by *C. muridarum*-infected oviduct epithelial cells.[80]

DCs, which survey epithelial surfaces, and NK cells are important components of the innate immune system. Human NK cells can lyse *C. trachomatis*-infected epithelial cell lines.[81] Epithelial cells, the primary targets of *C. trachomatis* infection, produce IL-18 in response to infection and DCs produce IL-12, and it has been reported that these factors then act together to induce IFN-γ production from NK cells.[82] The production of substantial amounts of IFN-γ favors the differentiation of T helper 1 (Th1) cells, further maximizing IFN-γ production that is crucial for resolving chlamydial infection. In addition to the type II IFN-γ, it is also known that type I IFNs have protective effects against *C. trachomatis* infections by increasing tryptophan degradation in synergism with tumor necrosis factor, IL-1, and LPS.[83] For a comprehensive review of type I IFN activity against bacterial infections, see ref. 84.

Adaptive immunity

An effective protective immune response to pathogens requires not only innate immunity, the first line of defense to prevent and control invasion of pathogens, but is also dependent upon adaptive immunity. The innate recognition of infection can lead to the induction of adaptive immune responses through both pathogen-associated molecular pattern-dependent and pathogen-associated molecular pattern-independent activation of DCs.[85]

It is well known that some elements of adaptive immunity in the genital tract are distinct and unique from other mucosal sites.[86–88] There is also evidence that the acquired immune systems in some sites (e.g., endometrium, fallopian tube, and occasionally the endocervix) and other sites (vagina, ectocervix, and occasionally the endocervix) respond differentially to chlamydial challenge with different T-cell recruitment patterns throughout the reproductive tract during infection.[89]

Humoral immunity

Humoral immunity in the FRT is reliant on the IgG- and IgA-secreting plasma cells that are found in greater abundance in the endocervix than in the vagina. In contrast to other mucosal secretions, the dominant immunoglobulin isotype is IgG rather than secretory immunoglobulin A found in the cervico-vaginal fluid of the female genital tract.[65]

In murine models, B cells or specific anti-chlamydial antibody responses that may enhance protective T-cell responses in the FRT have been recorded following genital chlamydial infections,[89–93] although mice incapable of producing specific antibody resolve primary chlamydial genital infection and are resistant to reinfection.[90,94] Moore *et al.*[95] report that *in vitro* anti-chlamydial antibodies increase the rate of Th1 activation by $FcR^{+/+}$ but not $FcR^{-/-}$ antigen-presenting cells, results that provide a mechanistic basis for the need for both T-cell and humoral immune responses in protective immunity to chlamydial reinfection. The results suggest that a major role for antibodies in chlamydial immunity is the enhancement of Th1 activation via FcR-mediated processes involving DCs. A recent study using the *C. muridarum* model of chlamydial genital tract infection reported that monoclonal antibodies to the chlamydial MOMP and LPS conferred significant levels of immunity to reinfection and reduced chlamydial shedding by over 100-fold.[96] These data of Morrison and Morrison[96] do not support the notion that direct neutralization is a major mechanism of antibody-mediated protection against chlamydial infection *in vivo*, but these authors suggest that antibody-dependent cellular cytotoxicity mechanisms are involved in protection. In a more recent study,[97] however, it was reported that mice genetically deficient in Fc receptors ($FcR^{-/-}$) resolved primary infection and secondary reinfection in a manner indistinguishable from ($FcR^{+/+}$) wild-type cells. These recent data imply that FcR-mediated interactions are not the only mechanism by which antibody confers protective immunity to reinfection.

Anti-chlamydial antibody responses (MOMP-specific IgA and IgG in vaginal and uterine lavage fluid and MOMP-specific IgA in serum) have been reported following various immunization routes such as transcutaneous, intranasal,[98–102] and systemic[100,103,104] immunizations, using animal models of chlamydial genital tract infection.

Antibodies are induced and present in the genital tract secretions following *C. trachomatis* infection, and the immunoprotective role of antibody-mediated immunity has been well reported (and debated) in the literature. Antibody has been shown to contribute to a protective response against genital tract reinfection with *C. trachomatis*.[105] Indeed, antibody has demonstrated

many beneficial effects against many infectious agents, including *Chlamydia*, in which CMI would be assumed to represent the key protective mechanism.[93,105,106] However, owing to the many chlamydial challenge doses that are used by different investigators, the exact role of antibody in protection against chlamydial infection is difficult to assess.

CMI

CMI is the predominant component of protective immune responses to *Chlamydia*, with the generation of specific major histocompatibility complex class II-restricted $CD4^+$ T cells playing a central role both by responding to, or by orchestrating, the activation of other protective immune components. It is the Th1 subpopulation of CD4 cells that is responsible for resolving chlamydial genital infection via an effector cytokine response, although other components such as $CD8^+$ T cells also play a role.[107–109] The protective CMI response is strongly associated with the production of IFN-γ either by $CD4^+$ or by $CD8^+$ T cells.[110–112] A recent review on T-cell responses to *C. trachomatis* infection has been published.[113]

DCs are present in the cervix and vagina of humans, mice, and macaques[114–116] and are central in T-cell priming and the induction of chlamydial-specific immunity. The form of *Chlamydia* initially encountered by DCs and the hormonal status at the time of this encounter may influence the type of adaptive immune response (protective vs. inflammatory) that is elicited by infection. Shaw *et al.*[117] showed that adoptive transfer of DCs pulsed with non-viable *Chlamydia* into naive mice afforded significant protection against subsequent challenge with viable *Chlamydia*. In a study investigating the uptake and processing of *C. trachomatis* serovar L2 by human DC, it was shown using blood-derived myeloid DCs that the entry of *C. trachomatis* could be inhibited by heparin, that activated DC produced IL-12 and tumor necrosis factor-α, but not IL-10, in DCs infected with *Chlamydia* vacuoles did not develop into inclusion bodies, and that infected DCs efficiently presented chlamydial antigens to CD4+ T cells and expanded *C. trachomatis*-specific CD8+ T cells.[118] Adoptive transfer of live-EB-pulsed bone marrow-derived DCs was more effective than that of UV-EB-pulsed DC at protecting mice against intranasal challenge with live *C. muridarum*,[119] and genetic profiling of DCs exposed to live or inactivated EBs revealed marked differences in the expression of cysteine-X-cysteine chemokines.[120]

In *C. trachomatis*-positive infertile women, the recruitment of lymphocyte subsets in the genital tract and subsequent production of Th1 and Th2 cytokines in the cervical secretions and laparoscopic specimens from the fallopian tubes were recently evaluated. Flow cytometric analysis of cervical secretions in these women revealed that the recruitment of both CD4 and CD8 lymphocytes to the genital tract was upregulated and that a variation in the production rates of different cytokines in the cervical secretions and fallopian tubes was observed.[121] These results suggest that CD8 lymphocytes may be important for local regulation of Th1/Th2 responses in the genital tract during *C. trachomatis* infection, and this would be supported by the results of Yeaman *et al.*[64] A recent comprehensive analysis was undertaken investigating *in vivo* cytokine responses to chlamydial infections in a large cohort of high-risk female adolescents before and after acquisition and before and after clearance of chlamydial infection. An increase in the endocervical production of the Th1-associated cytokine IL-12 and a decrease in IL-2 concentrations in endocervical secretions coincided closely with genital *C. trachomatis* infection.[122] Decreased local T-cell response and IFN-γ production may promote latent *C. trachomatis* infection and inflammation or immunopathology.[113] It has recently been hypothesized that IL-12 acts with other host factors to influence the recruitment of effector T lymphocytes to the infected FRT.[107]

The development of *Chlamydia*-specific T-cell receptor transgenic mice has (for the first time) enabled monitoring of *C. trachomatis*-specific T-cell responses in the genital mucosa. Using this model, it was reported that after a primary uterine infection with human *C. trachomatis* serovar L2, naïve *Chlamydia*-specific CD4+ T cells developed into Th1 effector cells in the lymph nodes, draining the genital tract in response to infection. Activated T cells migrated into the genital mucosa and secreted IFN-γ.[123]

Roan and Starnbach[124] have also generated a model using retrogenic mice that express a T-cell receptor specific for *Chlamydia*-specific T-cell antigen CrpA. This model has been used to identify and track the activity of *Chlamydia*-specific CD8+ T cells *in vivo*. They reported that *Chlamydia*-specific retrogenic T cells proliferated in draining lymph nodes and then migrated to the epithelium following genital infection with human *C. trachomatis* serovar L2.

Results of studies on the immune response to chlamydial infections in relevant animal models indicate that CD4 is the critical T-cell element in the protective immune response in all of these models and that variations in the degree of protective response may result from modulation of the CD4 T-cell effector functions.[125] During a primary genital infection with *C. muridarum*, CD4 cells are recruited mainly to the upper genital tract.[89]

T-cell responses in the FRT may be affected by hormonal status at the time of infection by the effects of estradiol and progesterone, either directly on T cells or indirectly on DCs or epithelial cells.

ANIMAL MODELS FOR STUDYING *CHLAMYDIAL* INFECTION OF THE FEMALE GENITAL TRACT

To study both the pathogenesis of and immunity to *Chlamydia* infection of the female genital tract, a number of animal species and chlamydial strains have been used. The most common is the murine model (using either *C. muridarum* or *C. trachomatis* human biovars), but the guinea-pig, rat, primate, and pig models are also used.[126–128]

It is known that the female mouse genital tract is susceptible both to the mouse pneumonitis biovar of *C. trachomatis* (i.e., *C. muridarum*) and to infection with human serovars of *C. trachomatis* (particularly serovars A, D, E, and L2). In both models, infection and disease outcomes are reportedly enhanced following pretreatment of animals with progesterone.[129] When

human serovars are used, the inoculum is administered directly into the upper genital tract tissues using surgical techniques[130] or it is inoculated intravaginally.[131] In a study on the course of outcome of infection in female outbred CF-1 mice infected with *C. muridarum* or human oculogenital isolates of *C. trachomatis* (serovars D, E, F, G, H, I, and K), a more rapid production and release of inclusion-forming units (IFUs) was reported for *C. muridarum* when compared to human serovar D,[126] although in C3H mice the course of infection with a strain of serovar E has been shown to cause more invasive disease.[131,132]

C. muridarum is much more virulent in mice than the human strains, and following vaginal inoculation, it results in an infection that naturally ascends from the lower genital tract (vagina and cervix) to infect the upper genital tract tissues (uterine horns and oviducts).[108,133] Human serovars cause post-infection sequelae in mice only when high doses are inoculated directly into the uterine horns, resulting in hydrosalpinx formation and infertility,[130] whereas infection by intravaginal instillation with human serovars normally resolves without these outcomes.[131] It has also been shown recently (in C3H/HeN mice) that prior intravaginal infection of mice with a human serovar E (10^6 IFU) may lead to partial protective immunity against challenge infection with serovar A or L2, as shown by reduced chlamydial shedding and a shortened infection course.[132] It was also reported that homotypic secondary challenge resulted in an increased pathological outcome, with 6 of the 10 mice challenged with serovar E in the secondary infection becoming infertile.[132] The pathology of the upper genital tract of *C. muridarum*-infected mice is comparable to that of women with post-chlamydial infection sequelae.[108,134] It was reported by Ramsey *et al*. that intraluminal occluding fibrosis of the oviduct is an outcome of genital infection with *C. muridarum* in C3H/HeN mice,[49] further supporting the use of this murine model to study the pathogenesis of chlamydial upper genital tract infection. However, it is also argued that investigating cytokine profiles in the murine genital tract during the course of infection with human biovars of *C. trachomatis* is potentially more clinically relevant than using the *C. muridarum* biovar.[135] The results of a comprehensive study using human and mouse cell lines to provide a better understanding of the innate host defense against infection by mouse-adapted (*C. muridarum*) and human-adapted (*C. trachomatis* serovar L2) chlamydial strains have been recently published and conclude that cytokine- and LPS-inducible effectors produced by human and mouse cells differ and, importantly, these responses result in chlamydial strain-specific antimicrobial activities.[136]

Improved mouse models for human chlamydial infection have been suggested based on current knowledge of host-specific IFN-γ effector activities against human serovars and *C. muridarum*. Although both *C. trachomatis* and *C. muridarum* infect and develop normally in most murine cell lines, their growth is differentially affected following the treatment of cells with IFN-γ; *C. trachomatis* growth is inhibited, whereas *C. muridarum* is resistant.[136,137] In human epithelial cells, IDO (indoleamine 2,3-dioxygenase) is induced by IFN-γ[138] and it inhibits growth of sexually transmitted infectious (STI) serovars of *C. trachomatis* by depleting intracellular tryptophan pools.[139] The ability to synthesize tryptophan from indole may be a major pathogenic mechanism enabling genital chlamydial strains to evade IDO activity. It is known that the LifA domain of *C. muridarum* cytotoxins targets mouse innate immune effectors such as p47GTPases[137] and these are mouse-specific proteins. It has recently been predicted, therefore, that a combination of GTPase-knockout, IDO transgenic mouse would be a superior model for the study of human STI using *C. trachomatis* challenge strains.[140]

To evaluate potential chlamydial vaccine candidates, it is important that the infecting dose used closely approximates the natural infecting dose in the animal model used to evaluate the protective capacity of the vaccine. As sexual transmission of *Chlamydia* has been demonstrated only in the guinea-pig model of genital tract chlamydial infection with the chlamydial agent of guinea-pig inclusion conjunctivitis (*Chlamydophila caviae*), this is a reliable and predictable model to use for vaccine studies.[141] Recently, it has been reported that female guinea-pigs received approximately 10^2 IFU by sexual transmission and that those infected by the natural route of sexual transmission shed organisms for a significantly shorter time than artificially infected animals.[142] Guinea-pigs are therefore a useful model for studying vaccine candidates for chlamydial genital tract infections.

Female pig-tailed macaque monkeys have been used by some investigators as a primate model of PID.[143–148] Male macaques have also been used to identify *C. trachomatis* serovar L2 protein antigens (p242 and TroA localized to intracellular development forms) recognized in the context of infection.[149]

Pigs have recently been used as an alternative large animal model for studying *C. trachomatis* female genital tract infections caused by oculogenital serovars. The majority of genes expressed in major porcine FRT tissues and subsequently found to be expressed ubiquitously in human genital tissues are contained in cDNA libraries that have been constructed recently.[150] specific pathogen free (SPF) outbred pigs (gilts) were evaluated for intravaginal infection (1×10^8 IFU) with *C. trachomatis* human serovar E genital isolate (strain 468). It was reported that human serovar E could ascend the genital tract of gilts, replicate in the cervical epithelium and uterine layers, and cause inflammation and pathology at the infection site.[151]

VACCINES

Immunity induced by natural chlamydial infection does not provide long-lasting protection and may contribute to pathology. A recent study involving DC pulsed with live or UV-irradiated EB reported that live EBs (but not UV-irradiated EBs) induced significant DC and neutrophil infiltration during infection, indicating that development of an anti-chlamydial response *in vitro* is greatly influenced by chlamydial viability.[152] **Table 1** summarizes recent chlamydial antigens, delivery systems, routes of immunization, and immune potentiators used for immunization against *C. trachomatis* genital tract infections. Delivery of vaccines is also summarized and includes systems (chemical, microbial-related, detergent-based, viral, cellular, DNA systems), mucosal (intranasal, intravaginal, rectal, transcutaneous), and systemic vaccination routes and also includes

Table 1 Recent immunization and challenge experiments in the murine model of chlamydial FRT infection

Mouse strain	Immunogen (adjuvant)	Route of immunization (infectious dose)	Route of challenge (challenge dose)	Immune response	Protection level	References
	MOMP/EBs					
BALB/c C57/BL6 C3H/HeN	MOMP DNA *C. muridarum*	IM (50 μg plasmids)	Ovarian bursa (10^5 IFU)	Weak DTH and antibody production	No effect	162
BALB/c	Conformational MOMP (Freund's) *C. muridarum*	IM+SC (5 μg mouse IM+5 μg mouse SC)	Ovarian bursa (10^5 IFU)	70% reduction in IFUs in vagina	Th1-like, increased IgG2a in serum	175
	MBP-MOMP (CT and CpG-containing ODNs) *C. muridarum*	TCI	IVAG (5×10^4 IFU)	MOMP-specific IgG and IgA in vaginal and uterine lavage; IFN-γ; IL-4 in CLN	IFN-γ production 50% reduction in IFUs in vagina	176
	rMOMP (VCGs) *C. trachomatis* Serovar D	IM (2×10^9 CFU)	ND	ND	Protection in naive mice following adoptive transfer of CD4+ T cells	177
	MOMP *C. muridarum* (Montanide ISA720 and CpG-1826 plus Alum)	IM+SC IM (10 μg)+SC (10μg)	Ovarian bursa (10^5 IFU)	Serum IgG (IgG2a>IgG1a) indicating Th1 response; vaginal wash IgA < controls	MOMP-plus CpG plus Montanide showed best protection at 85% clearance of infection after 6 weeks	178
	MOMP *C. muridarum* (MF59, LT-K63, LT-R72)	IM+SC+IN IM (10 μg)+SC (5 μg)+IN (5 μg)	Ovarian bursa (10^5 IFU) (note: progesterone-treated animals inducing Th1 to Th2 shift)	High serum IgG titers; balanced Th1/Th2 response; low titers if IgA and IgG in vaginal washes	No significant statistical differences to control mice Fertility studies showed no significant differences to controls	179
	Whole EBs *C. muridarum*	EBs (1×10^4 IFU)	IN (10^4 IFU) IVAG (10^6 IFU)	Serum antibody high IgG2a/IgG1 ratio indicating predominant Th1 response	Following IVAG challenge 45% reduction in chlamydial shedding	180
	RMOMP *C. muridarum* (CTA_2B cholera toxin)	IN, IVAG	IVAG (10^5 IFU)	Balanced Th1/Th2 Strong mucosal IgA and systemic antibody IgG, IgG1, IgG2a, IgG2b	Significant protection	181
BALB/c C57/BL6 C3H/HeN	Whole EBs *C. muridarum*	IN (10^4 IFU) in BALB/c and C57 mice IN (10 IFU) in C3H	Ovarian bursa (10^5 IFU)	Serum IgG and IgA (high titers), low IgM Th1 predominates Significant T cell similar in all three strains	Protective in C3H and C57 mice and 87% cleared EBs in BALB/c mice	182
BALB/c C57/BL6	Whole EBs *C. trachomatis* Serovar D grown in McCoy cells	IVAG (2×10^7 IFU) and reinfected 8 weeks later	ND	BALB/c infection (12 days) significantly shorter than C57 (43 days) Plasma IgG1 and IgG3 67% in BALB/c compared to 4% in C57BL/6 Vaginal IgA < outbred	ND	183
C57BL/6	MOMP and OmcB as VCGs *C. trachomatis* Serovar D	IM (2×10^9 CFU)	IVAG (10^5 IFU) Live serovar D	Th1, IgG2a in serum and IFN-γ produced by splenic T cells	80% of animals protected	184
	Whole EBs *C. muridarum* (treated mice with oxytetracycline following vaginal infection)	IVAG (1,500 IFU)	ND	*Chlamydia*-specific antibody and CMI in antibiotic-treated animals equal to control animals	ND	185

Table 1 Continued

Mouse strain	Immunogen (adjuvant)	Route of immunization (infectious dose)	Route of challenge (challenge dose)	Immune response	Protection level	References
	MOMP, i.e., rVCG-gD2 rVCG expressing chlamydial MOMP and HSV-2 glycoprotein D *C. trachomatis* Serovar D	IM (2×10^9 IFU)	IVAG (10^7 IFU live serovar D) and 4×10^4 PFU	Chlamydial and HSV-2 Serum and vaginal secretory IgA and IgG2a Mucosal and systemic Th1 responses (IFN-γ)	Prophylactically protected from genital challenge with high doses of live *Chlamydia* and HSV-2	186
	MOMP (immunodominant T-cell epitopes) As recombinant influenza (rIV-CT) *C. trachomatis* Serovar D	IN (10^5 PFU rIV-CT)	IVAG (10^5 IFU *C. trachomatis* serovar D)	Strong Th1 response against intact chlamydial EBs High frequency of *Chlamydia*-specific Th1 in genital draining lymphoid tissues	Significant protective immunity as seen by reduced shedding of Chlamydiae and rapid clearance of infection	187
C3H/HeN	Whole EBs *C. muridarum* and *C. trachomatis* Serovar E (1°) or serovars A, E, or L2 (2°) infection	IVAG (10^4 IFU) *C. muridarum* (10^6 IFU) *C. trachomatis*	IVAG	Mice infected with *C. muridarum* became infertile with hydrosalpinx formation whether or not infected previously with *C.trachomatis* Mice infected with *C. trachomatis* remained fertile unless chalenged with homologous *Ctr* serovar	Homotypic challenge leads to exacerbated disease pathology	132
C3H/HeN and BALB/c	MOMP *C. muridarum* (OspA of *Borrelia burgdorfori*)	IM and SC or IN (10 IFU) in C3H IN (10^4 IFU) BALB/c or PV+PSac	Ovarian bursa (10^5 IFU)	Predominantly Th2 response in MOMP-immunized animals; MOMP (in) no detectable vaginal antibody	IM+SC gave 50% reduction in IFUs in vagina and significant protection against infertility	188
	MOMP *C. muridarum* (MF59, LT-K63, LT-R72)	IM+SC+IN IM (10 μg)+ SC (5 μg)+ IN (5 μg)	Ovarian bursa (10^5 IFU) Controls: 10 IFU per mouse	Mixed Th1/Th2	Mice immunized with MF59 and LTR72 as adjuvants had more embryos than controls	179
Outbred CF-1	Whole EBs *C. trachomatis* Serovar D or H	IVAG (1–3×10^7 IFU)	ND	Plasma and vaginal secretions from serovar D-infected mice contained antibodies to more antigens that serovar H	Serovar D more virulent (longer duration of infection) and immunogenic (higher serum IgG and vaginal IgA) Serovar D showed homotypic and heterotypic protection	189
	Non-MOMP antigens					
BALB/c	*C. muridarum* TC0512 (putative OMP), TC0757 (conserved hypothetical protein), TCO439 (adherence factor), TCO767/768 hypothetical protein)	GG (intra-abdominally)	IVAG (5×10^4 IFU)	Predominantly Th2 (IL-4, IL-10); IgG1>IgG2a	Partial protection with enhanced clearance of infection	169

Table 1 Continued

Mouse strain	Immunogen (adjuvant)	Route of immunization (infectious dose)	Route of challenge (challenge dose)	Immune response	Protection level	References
	rCPAF *C. muridarum* (IL-12) (CpG)	IN (15 μg CPAF) (0.5 μg recombinant murine IL-12)	IVAG (5×10^4 IFU)	Significant anti-CPAF IgG2a, IgG1, and IgA in serum and vaginal fluids	Enhanced resolution of infection; 80% animals resolved infection by day 15	190–192
C57BL/6	CrpA *C. trachomatis* Serovar L2 Recombinant vaccinia virus (Vac1505) expressing CrpA	IP (10^7 PFU)	IVAG (10^7 IFU)	Significant expansion of CD8+ T cells The response to this epitope correlates with clearance of the organism from infected mice	Partial protective immunity	124,193
	Cta1 *C. trachomatis* Serovar L2	NA (adoptive transfer of a CD4+ T-cell epitope)	IVAG (10^7 IFU)	Activation and proliferation of T cells in lymph nodes draining the genital tract	ND	194
BALB/c/cByJ	Cap1 *C. trachomatis* L2 Recombinant vaccinia virus expressing Cap1 CD8+ T-cell epitope	IP (10^6 PFU)	IVAG (10^6 IFU)	Stimulation of MHC class I-restricted CD8+ T cells	Modest but significant protection	195
C3H/HeJ	GLXA Chlamydia glycolipid exoantigen C Anti-idiotypic antibodies (poly lactic microspheres)	SC, PO, or IN	IVAG (2,000 $TCID_{50}$)	Serum antibody	Reduced shedding (serovar K)	196
C3H/HeN	*pgp3* *C. trachomatis* Serovar D DNA vector	ID (100 μg DNA)	IVAG (10^7 IFU)	Humoral and mucosal anti-pgp3 antibodies	Resistance to reinfection	197
Transgenic HLA-DR4	rCPAF *C. muridarum* (IL-12)	IN (15 μg rCPAF)	IVAG 1,500 IFU	*Chlamydia*-specific IFN-γ and antibody production	Infection resolved within 30 days	190

CLN, caudal and lumbar lymph nodes; Cap1, class I accessible protein; CFU, colony-forming unit; CMI, cell-mediated immune response; CPAF, chlamydial protease-like activity factor; CpG-1826, unmethylated bacterial CpG DNA (ODN no. 1826); CrpA, cysteine-rich protein A; Cta1, Chlamydia-specific T-cell antigen 1; DTH, delayed-type hypersensitivity; EBs, elementary bodies; FRT, female reproductive tract; GG, GeneGun; HSV-2, herpes simplex virus type 2; ID, intradermal; IFN, interferon; IFU, inclusion-forming unit; IL, interleukin; IM, intramuscular; IN, intranasal; IP, intraperitoneal; IVAG, intravaginal; LTK63, heat-labile Escherichia coli enterotoxin; LT-R72, a mutant of the heat-labile enterotoxin of Escherichia coli; MBP-MOMP, maltose binding protein fused to major outer membrane protein; MF59, an oil-in-water adjuvant emulsion containing squalene; MHC, major histocompatibility complex; Montanide ISA720, water-in-oil adjuvant Montanide ISA720 (ICC-1132/ISA 720); NA, not available; ND, not done; ODN, oligodeoxy nucleotide; OMP, outer membrane protein; PFU, plaque-forming unit; PO, per oral; PSac, peri-sacral; PV, peri-vaginal; rCPAF, recombinant CPAF; rMOMP, recombinant MOMP; rVCG, recombinant VCG; SC, subcutaneous; TCI, transcutaneous; $TCID_{50}$, tissue culture infectious $dose_{50}$; Th, T helper cell; VCG, Vibrio cholerae ghosts.

immune potentiators and mucosal adjuvants currently being trialled (**Table 1**).

Vaccine development against chlamydial infections has encompassed the use of inactivated, live whole organisms, subunit vaccines based on MOMP preparations, novel highly immunogenic components of chlamydial organisms that are other potential vaccine targets, recombinant protein and peptide vaccines, DNA vaccines to the more recent use of intranasal delivery of subunit vaccine using live attenuated influenza viruses as delivery vectors for chlamydial vaccines.[153]

In the 1950s and 1960s, early human vaccine studies focused on the use of inactivated or live whole organisms (reviewed in ref.[129]). In humans, whole organism vaccination against trachoma reduced disease and produced short-term protection in some individuals;[154–156] however, reinfection exacerbated disease in others resulting from stimulation of enhanced delayed-type hypersensitivity reactions. The use of whole-cell organisms was essentially abandoned following these results; however, once a stable genetic system for transformation of *Chlamydia* is developed, it may be possible to produce a live

attenuated vaccine provided any immunopathological damage is overcome.

Technology enabling the identification, isolation, and purification of antigenic determinants of *Chlamydia* has shifted the focus from a whole-cell vaccine approach to epitope, peptide, oligosaccharide, oligoglycopeptide, or subunit vaccines. The most promising vaccine candidate to date has been the 40 kDa Omp-1 antigen, also known as the MOMP. The results of MOMP-based vaccine studies have reported diverse degrees of immunogenicity and various levels of protective immunity[106,109,157–168] (**Table 1**). The results with MOMP-based vaccines might imply either that MOMP alone is an inadequate vaccine candidate or that potentially better delivery systems and adjuvants are required for optimal protective effects using MOMP as candidate vaccine antigen.

Chlamydial genomics and proteomics are currently proving to be useful tools for the identification of novel immunogenic antigens[169–173] other than MOMP. Some of these antigens have also recently been trialled in vaccine studies with varying degrees of protection reported (see **Table 1**) and these may indeed be combined with chlamydial MOMP in future multicomponent vaccines.

Definitive infection control of chlamydial infections is highly likely to be achievable through a safe and efficacious vaccine in the near future. This will require identifying protective chlamydial antigens in animal models as well as identifying effective adjuvants and delivery systems that target subunit vaccines to immune inductive sites or secondary lymphoid tissues and that will be safe for use in humans.

Many studies have been focused on identifying protective chlamydial antigens as vaccine components and these include individual research groups and pharmaceutical companies. The strategies that have been used include identifying antigens that elicit antibodies that bind to the chlamydial cell surface, mucosal immunization of animals with a chlamydial protease-like activity factor, a protein secreted into the cytosol of *Chlamydia*-infected host cell (recombinant protein was administered to mice intranasally or intraperitoneally with CpG oligodeoxynucleotides), and our own novel approach using expression library immunization.

The antigens that have been identified include a pan-genus antigen, outer membrane protein epitopes, proteins found to be infection-specific, and a conserved surface-exposed polymorphic protein D. Using expression library immunization, our study identified six novel vaccine antigens that conferred protection against a genital tract challenge infection in mice and these included genes for hypothetical proteins and housekeeping genes, including a DNA gyrase subunit, TC0462, and the ATP-dependent Clp protease, ATP-binding subunit ClpC, TC0559.

Vaccine strategies that are promising include mucosal delivery, which is also utilized for several vaccines, such as the cholera, typhoid, and original polio vaccines that are administered orally, and the live attenuated influenza A and B vaccine that is delivered intranasally. Mucosal delivery of a chlamydial vaccine has been shown to induce high levels of *Chlamydia*-specific IFN-γ following immunization intranasally with live *C. muridarum* and resistance to reinfection with *C. muridarum*.

How close are we to a realistic vaccine? From our own investigations, we believe that we are only a couple of years away from clinical trials. We have already accumulated significant data using primarily our mouse genital tract model but using both *C. muridarum* and *C. trachomatis* strains. We have also discovered several novel protective antigens and aim to use a combination of these in our final vaccine product rather than simply using a single antigen as in most previous studies. We have also identified antigens that elicit strong antibody as well as Th1 type immune response characterized by the production of tumor necrosis factor-α and IFN-γ, both of which are crucial for chlamydial clearance in primary and secondary infections in humans.

CONCLUSION AND FUTURE PROSPECTS

Today, there is no vaccine for genital tract infections in humans caused by *C. trachomatis*.

Owing to the different life-cycle forms (EB, RB, persistent form) and serovar differences in surface proteins such as MOMP, a vaccine based on a single antigen is unlikely to generate protective immunity. Progress has, however, been made in our knowledge of humoral and cellular immunity to chlamydial infections (both protective vs. proinflammatory), as well as in the identification of potential vaccine candidate antigens other than MOMP that could be incorporated into a subunit vaccine while avoiding components that mediate immunopathological damage. Globally, the severity and economic impact of genital tract diseases in humans caused by *C. trachomatis* necessitates the development of an affordable efficacious prophylactic vaccine to control infections. The challenges in developing such a vaccine include difficulty in preparing a 100% safe and effective product, the increasingly high costs of testing (identified as $802 million in 2000 (ref. 174)), and also the consideration that end users of the vaccine have to be motivated by the risk of an infection and disease that is only a potential future threat to their health and well-being. An ideal vaccine must include heterotypic cross-protection against the many serotypes of *C. trachomatis* as well as one that can achieve sterilizing immunity in the FRT. However, a vaccine that prevented ascending infection and reduced shedding would have a major economic benefit and there are many papers modeling this scenario. The natural reproductive cycle and its effects on the physiology and immunity in the FRT may make this latter goal difficult to achieve; however, mucosally administered vaccines are capable of inducing long-lasting immune responses in the genital tract. A more feasible goal of a prophylactic chlamydial vaccine might be to protect women from disease rather than infection, and this might well be achieved by developing vaccines both for men and women. This is a future prospect that increases our current optimism for the development of an efficacious vaccine against sexually transmitted diseases.

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DISCLOSURE

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