Chapter 9

Female Infertility Associated to *Chlamydia trachomatis* Infection

Agustín Luján, Silvina Fili and María Teresa Damiani

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62462

Abstract

Chlamydia trachomatis (CT) is the most common agent of bacterial sexually transmitted infections, both in developed and developing countries. It clearly constitutes a major burden on public health. Screening programs and current research are mainly focused on decreasing the high incidence of chlamydial infections as well as their associated morbidity.

The greater clinical impact of CT infections occurs in women of reproductive age. Acute CT infections are often associated to urethritis, mucopurulent cervicitis, endometritis, salpingitis, and pelvic inflammatory disease (PID). A vast proportion of CT infections are likely underestimated because of their asymptomatic clinical course. This leads to repeat and chronic infections, which have deleterious impact on the female reproductive health. Among the complications of the CT chronic infections are PID, ectopic pregnancy, preterm birth, tubal obstruction and female infertility, which are of great interest in the reproduction field.

Keywords: *Chlamydia trachomatis*, intracellular pathogens, bacterial sexually transmitted infections, tubal obstruction, female infertility

1. Introduction

Chlamydia trachomatis (CT) is the most frequent bacterial agent causing sexually transmitted infections (STIs) worldwide. According to the latest World Health Organization (WHO) report, approximately 100 million new infections occur annually [1]. According to the Centre for Disease Control (CDC), around 1.4 million new CT cases were reported in 2013, only in the United States [2].

In this chapter, we briefly present the current knowledge about the cell biology of the bacteria, reviewing the mechanisms of establishment of CT intracellular niche, the inducers of persistent

infections, and the pathogen factors that may be involved in the damage of female reproductive tract. Then, we analyze the host factors that may contribute to the development of infertility, mainly immune response and genetic predisposition, hormonal status, and sexual behavior. Undiagnosed and untreated infections, repeat and persistent infections, and coinfections are likely responsible for the detrimental sequelae on woman fertility of CT pathogenesis.

This chapter specially focuses on the consequences of chronic diseases after CT infections, mainly pelvic inflammatory disease, tubal infertility, and adverse pregnancy outcome, which are of therapeutic interest in the reproduction field.

2. The bacteria: Intracellular life cycle, *Chlamydia trachomatis* serovars and virulence factors

2.1. Chlamydial developmental cycle and intracellular niche

CT is a highly evolved pathogen that has a reduced genome, first sequenced by Stephens and collaborators in 1998. Its chromosome consists of approximately one million base pairs and encodes for up to 600 proteins [3]. Analysis of chlamydial genes reveals that this bacterium heavily depends on host cell for nutrition and replication, indicating a complex evolution for adaptation to an obligate intracellular lifestyle.

CT has tropism for genital mucosal epithelium, which promotes its own uptake into non-phagocytic cells. Chlamydial infection and propagation rely upon a unique biphasic life cycle that begins by contact of infectious, environmentally resistant, elementary bodies (EBs) with the apical surface of the epithelial cell. Several mechanisms are involved in the invasion of host cell, likely parasite-specified phagocytosis and receptor-mediated endocytosis [4]. Multiple receptors have been proposed to mediate the interaction between the EB and the host cell, among them, the mannose receptor, the mannose 6-phosphate receptor, and the estrogen receptor [5]. Other host molecules such as heparan sulfate proteoglycans [6,7], and protein disulfide isomerase also participate in EB binding to the eukaryotic cells [8]. Concomitantly, multiple bacterial adhesins and ligands such as glycosaminoglycan [9], the major outer membrane protein (MOMP) [10], OmcB [11], and PmpD [12] facilitate EB attachment to host cells. Translocated actin-recruiting phosphoprotein (TARP) is a bacterial protein that nucleates actin and promotes host cell cytoskeleton remodeling to force bacterium uptake [13–15].

The infectious EBs enter the host cell in membrane-bound vesicles that travel toward the perinucleus and fuse to form a single vacuole termed the inclusion. Once inside this modified phagosome, EBs differentiate into metabolically active but non-infectious reticulate bodies (RBs) that are the replicative bacterial forms. RBs asynchronously multiply by binary fission within the confines of the growing inclusion. After numerous rounds of replication, RBs re-differentiate back into infectious EBs to be ready for spreading to adjacent cells [16,17]. The ability of CT to cycle between resting and replicating organisms accounts for a drawback in the eradication of this intracellular pathogen. Finally, the infectious bacteria are released by

http://dx.doi.org/10.5772/62462

two independent mechanisms, the host cell lysis, or the extrusion of the inclusion [18]. A scheme of chlamydial developmental life cycle is shown in Figure 1.

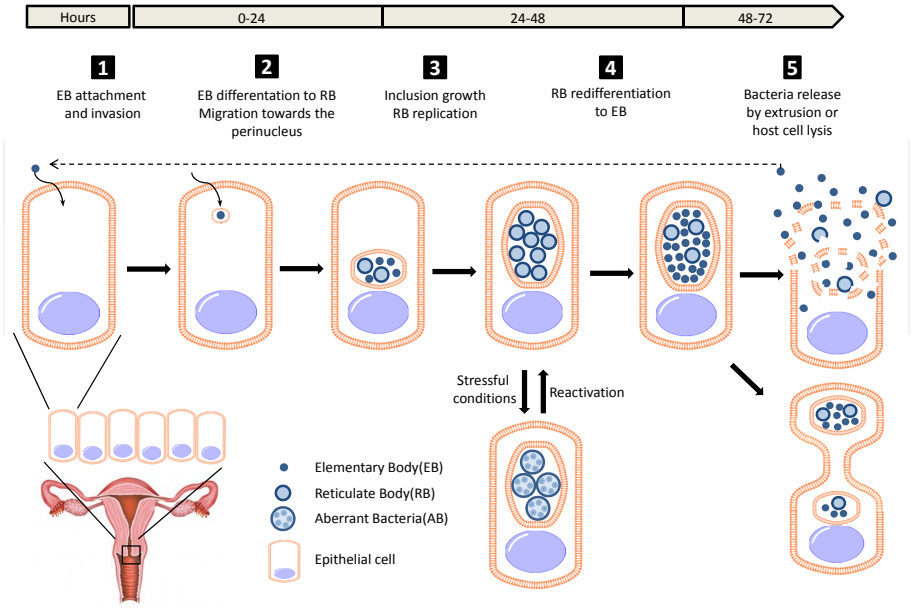

Figure 1. *Chlamydia trachomatis* developmental cycle. Chlamydial infection begins with attachment of the infectious bacterial form, the elementary body (EB) to uncharacterized host cell receptors. Signal transduction events are triggered, and EBs entry into the host cell in small vesicles. These CT-containing vesicles are actively modified by the bacteria; they travel toward the perinucleus and fuse to form a single vacuole named "the inclusion". Once internalized, EB differentiates into the replicative bacterial form, the reticulate body (RB), which multiplies by binary fission. At the end, RBs re-differentiate to EBs that are released by host cell lysis or extrusion of the inclusion. The whole cycle is completed in 40–72 hours. In a stressful environment, RBs enter a latent stage where it persists until more favorable growing conditions. These aberrant bacteria (AB) are present in persistent and chronic infections.

In response to stress, CT enters into a low replicative viable state that is termed "persistent or aberrant bacterial form", which is able to resume normal replication as soon as conditions are again favorable. Among the inducers of the persistent bacterial state stand the sphingolipid deprivation and tryptophan lack, the presence of interferon gamma (IFN-γ), or certain antibiotics such as penicillin. The evasion strategy has been linked to the capacity of these bacteria to cause latent and chronic infections. Thus, the onset of the infection is generally not detected and re-infection may occur, especially when infected couples are involved. Furthermore, the fact that CT clearance is rarely followed up, combined with the ability of this pathogen to persist, contributes to the occurrence of long-term infections [19].

Undoubtedly, an essential issue to chlamydial growth and development is the establishment of its intracellular niche. Early chlamydial gene expression is required to inclusion generation, to avoid immune system surveillance, and to hijack host cell functions [20]. These bacteria

actively modify the inclusion membrane by exclusion or recruitment of selected host proteins, mainly Rab proteins, the master controllers of intracellular traffic [16,21]. Increasing evidence points out that the invading bacteria subvert trafficking not only to circumvent the lysosomal degradative route but also to facilitate the delivery of host nutrients to the growing inclusion [17,22]. As soon as the chlamydial inclusion is formed, it dissociates from the classical phagocytic pathway and barely interacts with endocytic vesicles [23]. Instead, chlamydial inclusion intersects Golgi-derived vesicles [24–27], multivesicular bodies [28], and lipid droplets [29,30]. By this strategy, these bacteria take over the infected cell for sphingomyelin, cholesterol, and neutral lipid acquisition like pirates [31–34]. In addition, CTs possess other mechanisms, such as transporter molecules, finely adapted to acquire amino acids, nucleotides, and energy from the host cell [22,35–38]. At present, the strategies developed by CTs to re-route intracellular trafficking and to co-opt host cell functions for their benefit are being actively studied.

2.2. Bacterial genotypes and virulence factors

Different strains of CT have been described based on genome sequencing and the antigenic properties of the major outer membrane protein (MOMP) [39]. There are more than 20 distinct serovars (serologically variant strains) of CT currently identified, on the basis of monoclonal antibody-based typing assays [40–42]. In general, CTs have been grouped into three main pathobiotypes: ocular infections (serovars A to C), sexually transmitted diseases (D to K), and lymphogranuloma venereum (L_1 to L_3). Serovars A, B, and C have tropism for the ocular epithelium, causing from acute conjunctivitis to trachoma, a serious eye disease endemic in Africa and Asia that is characterized by chronic conjunctivitis and can lead to infectious blindness. Serovars D through K have emerged as the major causing agents of sexually transmitted diseases. They preferentially infect squamocolumnar epithelial cells of female reproductive system and the male genitourinary tract. E and D serovars are isolated from genital tract infections with the most frequency worldwide. Occasionally, they cause conjunctivitis or pneumonia in newborns infected during labor. Serovars L1 to L3 are responsible for a systemic illness, the lymphogranuloma venereum that is associated with genital ulcer disease in tropical countries [43,44] (Table 1).

Chlamydia genotyping is useful to determine tissue tropism [45–47]. Several studies attempted to directly link disease severity with CT serovars; however, they often failed because of small number of samples and high variability in results [48]. Intensive research is conducted to confidently associate CT serotypes to higher pathogenic potential, clinical course, or disease outcome. Nevertheless, at present, bacterial ability to ascend and colonize female upper reproductive tract is not clearly associated to a particular CT serovar.

On the other hand, CT gene polymorphisms determine distinct antigenic challenge to the immune system [49]. Certain bacterial polymorphisms may induce an altered immune response [50]. In consequence, they are able to cause immunological disorders, especially in susceptible individuals. Chlamydial infections often precede the initiation of autoimmune diseases, and frequently, these bacteria are found within autoimmune lesions. Bacterial proteins similar to host self-proteins might be the underlying cause of diverse autoimmune diseases [51,52]. This molecular mimicry may elicit an immune response to both self and

microbial proteins. Chlamydial heat shock protein 60, DNA primase, and OmcB proteins represent the strongest cases for molecular mimicry [53]. The most frequent autoimmune diseases connected to chlamydial infections are intestinal inflammatory pathologies and rheumatic or connective-tissue diseases [54,55]. Further research is required to unravel the molecular machinery involved in the complex pathogen-host cell interaction.

Chlamydial strains and clinical diseases			
Serovars	Host	Acute	Chronic
A–C	Newborns Both sexes	Conjunctivitis	Trachoma
D–K	Newborn	Ophthalmia neonatorum	Neonatal pneumonia
	Men	Urethritis	Proctitis Epididymitis
	Women	Urethritis Cervicitis	Mucopurulent cervicitis Pelvic inflammatory disease Tubal infertility Ectopic pregnancy Premature rupture of membranes Chorioamnionitis Premature delivery Puerperal infection Cervical neoplasia
L_{1-3}	Both sexes		Lymphogranuloma venereum
Different serovars and bacterial polymorphism	Both sexes		Autoimmune diseases Reactive arthritis Collagenopathies Reiter´s syndrome Inflammatory bowel disease Crohn´s disease

Table 1. Chlamydial serovars, tissue tropism and clinical diseases. Acute and chronic pathologies occur in men, women and newborns following CT infections. Serovars A to C have tropism for ocular epithelium, causing from acute conjunctivitis to infectious blindness or trachoma. Serovars D to K infect epithelial cells of the genitourinary system, generating a broad range of acute and chronic pathologies that damage reproductive tissue and may infect newborns during labor. Serovars L_{1-3} cause lymphogranuloma venereum. Several autoimmune diseases are associated to diverse CT strains.

Several putative virulence factors have been postulated, including the polymorphic outer membrane autotransporter family of proteins (pmp), type III secretion system (TTSS) effectors, a large cytotoxin, and stress response proteins may contribute to increase the CT-associated pathogenicity. Pmp proteins are strongly immunogenic and trigger pro-inflammatory

cytokine responses [56]. Chlamydial TTSS effectors mediate the interaction with the host as they are injected to the cytoplasm and alter host cell functioning [57–59]. Important TTSS effectors are the inclusion (Inc) proteins that are bacterial proteins present at the inclusion membrane. For instance, IncA promotes the fusion of individual CT-containing vesicles to form a single inclusion [60,61]. Natural IncA bacterial mutants are associated with reduced virulence [62]. Another TTSS effector is TARP, mentioned in the previous section, as a bacterial protein that favors CT internalization via an actin recruiting mechanism [34,63]. Additionally, a chlamydial cytotoxin glycosylates the eukaryotic protein Rac1, and thereby induces actin reorganization and promotes the invasion of host cell [64,65]. Chlamydial glycolipid exoantigens [66] and the lipopolysaccharide [67] may constitute additional virulence factors. Other proteins encoded by the cryptic plasmid or related to the ability of the bacteria to survive under stressful metabolic conditions such as iron or tryptophan deprivation are thought to increase virulence and pathogenicity [68,69]. Chlamydial stress proteins, GroEL and GroES, may activate toll-like receptors and trigger a potent inflammatory response, injuring host reproductive tissues [70–73].

In addition to CT serovars and virulence molecules, other bacterial factors may be involved in the pathogenicity and chlamydial infection outcome, such as the pathogen load, route of infection, bacterial ability to enter persistent state, ascension capacity and strength to colonize genital upper tract, resistance to antibiotic treatment, and so on. Further studies are needed to determine the contribution of each bacterial factor to the development of severe damage on the female reproductive system.

3. The host: Immunological and genetic factors, age and hormonal status, and sexual behavior

3.1. Immunological and genetic factors

An important issue that contributes to CT pathogenesis is its remarkable ability to avoid the host immune system. Several strategies are displayed by these bacteria to prevent immune degradation, such as its intracellular lifestyle, its ability to escape from phagolysosomal pathway, its resistance to interferon gamma (IFN-γ), among others lesser known bacterial molecules. Actually, host immune response has opposite results on chlamydial infection outcome. A low immune response generates a suitable environment for pathogen colonization, while a strong immune response could lead to excessive inflammation and tissue damage. Pathogens own characteristics in conjunction with host genetic susceptibility are important in determining the severity of the illness.

At the site of invasion, an intense inflammation occurs, attracting different types of cells, such as macrophages, neutrophils, T and B lymphocytes, natural killers, and dendritic cells. Locally, there is an increased production of reactive oxygen species (ROS) that produces oxidative DNA damage, lipid peroxidation, energy depletion, modulation of gene expression, and proteins synthesis. Oxidative stress provokes pathologic changes that harm reproductive tissues. In

addition, a broad collection of pro- and anti-inflammatory cytokines are released including IFN-γ, tumor necrosis factor (TNF-α), interleukin (IL) IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-22, vascular endothelial growth factor (VEGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and lactoferrin. These mediators trigger cellular inflammatory responses that mediate direct damage to the tissues [74,75]. Most of them are involved in the pathophysiology of tubal infertility, birth defects, and miscarriage [76].

In addition to the inflammatory response, CT infection evokes vigorous local and systemic humoral and cell-mediated immune responses. Several CT-specific antibodies, IgG isotype rather than secretory IgA, are found in circulation and in the cervicovaginal fluid of the female genital tract. These antibodies can neutralize chlamydial antigens; nevertheless, they do not assure resolution of the infection. Antibodies toward the bacterial MOMP protein are prevalent in primary chlamydial infections, whereas antibodies against CT-hsp10 and hsp60 are present in recurrent or persistent infections and correlate with severe sequelae such as tubal infertility, ectopic pregnancy, and PID [77–79]. Regarding the cell-mediated immune response, the T-helper lymphocytes type 1 (Th1) secrete IFN-γ, IL-2, and IL-12 and play a role in the resolution of infection [80,81]. On the other hand, the T-helper lymphocytes type 2 (Th2), which support the humoral immune response, produces IL-4, IL-5, IL-6, and IL-10 and participates in the developing tubal scarring [82]. The inflammatory response occurs, at the same extent, in both initial and repeat infections, whereas T-cell responses are predominant in the latter ones [80,83].

Actually, host immune response is considered as one of the most important determinants in chlamydial infection outcome. A delicate balance between pro- and anti-inflammatory cytokines is needed to clear infection, avoiding tissue injuring. At this point, host genetic predisposition is a major player of pathology and CT-related infertility development [84].

Host genetic polymorphisms may encode aberrant or dysfunctional toll-like receptors (TLR) and nucleotide-binding oligomerization domain proteins (NOD) that do not appropriately recognize CT. These individuals have an impaired bacterial clearance and a high risk to develop an aberrant immune response, favoring CT persistence [85–88]. Identifying host genetic factors and bacterial virulence factors involved in immunoevasion and immunopathology remains a major priority in research for preventing chlamydial infection and its sequelae. Furthermore, the understanding of local innate and adaptive immune responses and their actors along the genital tract will be crucial for designing new therapeutic approaches and for developing a protective vaccine.

3.2. Age and hormonal status

CT preferentially targets young women at reproductive age. It has been reported that the highest incidence occurs in women between 16 to 24 years [89]. Therefore, the impact on female reproductive health is very important. Notwithstanding younger women are at higher risk of contracting a chlamydial infection, the rates of developing PID increase with age, being more frequent in the 30 to 40 decade [90]. After menopause, the frequency of chlamydial infections decreases substantially [91].

Accordingly, estrogen and progesterone are important for the establishment of chlamydial infection. Furthermore, estrogen receptors have been involved in the internalization of CT [92,93]. Sex hormones affect the clinical outcome of chlamydial infections; thus, follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P4), and prolactin (PRL) are involved not only in the establishment of chlamydial infection but also in the development of sequelae on reproductive tissue [94,95]. Additionally, oral contraceptive use was found to be a risk factor for CT infection. Several studies have attempted to elucidate the relation between the hormonal status at the time of infection and its contribution to tubal occlusion in genital chlamydial infection. Unfortunately, the role of sex steroids in infection outcome is far from being fully understood. Sex hormones have been shown to modulate immune responses and to have antioxidant effects in the female genital tract [96–98]. However, the mechanisms by which sex hormones may benefit or impair the colonization of genital tissues by pathogenic microorganisms should be further investigated.

3.3. Sexual behavior and other host factors

Sexual initiation at young age and a higher number of sexual partners are associated with increased risk of CT infection [99]. In a similar manner, having sex without protection favors CT contagion. This sexual behavior is also associated with higher incidence of sexually transmitted pathogens such as *Neisseria gonorrhoeae*, *Candida albicans*, *Human Immunodeficiency Virus*, among others [100–103]. Taken together, unsafe and high-risk sexual conduct is linked to impairment of women reproductive health, and particularly to tubal infertility. As it has been previously mentioned, some women have a genotypic predisposition to develop an abnormal immune response and severe inflammation following CT infection. In these women, there is a high rate of tubal obstruction and CT-related infertility, independent of sexual behavior [84].

In general, multiple sexual partners and unsafe intercourse increase the risk of CT recurrent infections and coinfection with other sexually transmitted pathogens. Thereby, the high chance to suffer repeat and chronic infections that target women reproductive tissue raises the infertility-associated pathologies.

4. Pathogen-host interplay: Establishment of latent, repeat, and persistent infections and coinfections

CT survival and replication heavily hinge on host cells. In fact, CT has evolved in relationship with human cells. However, little is known about the molecular basis of CT interaction with host epithelia and immune system. An increasing number of bacterial and host factors interplay for the establishment of chlamydial infection and determine the pathologic profile and clinical disease outcome. A simple scheme is shown in Figure 2 summarizing the main CT and host factors that jointly with environmental factors and the failure in diagnosis and treatment, lead to unsolved, latent, and long-term chlamydial infections.

http://dx.doi.org/10.5772/62462

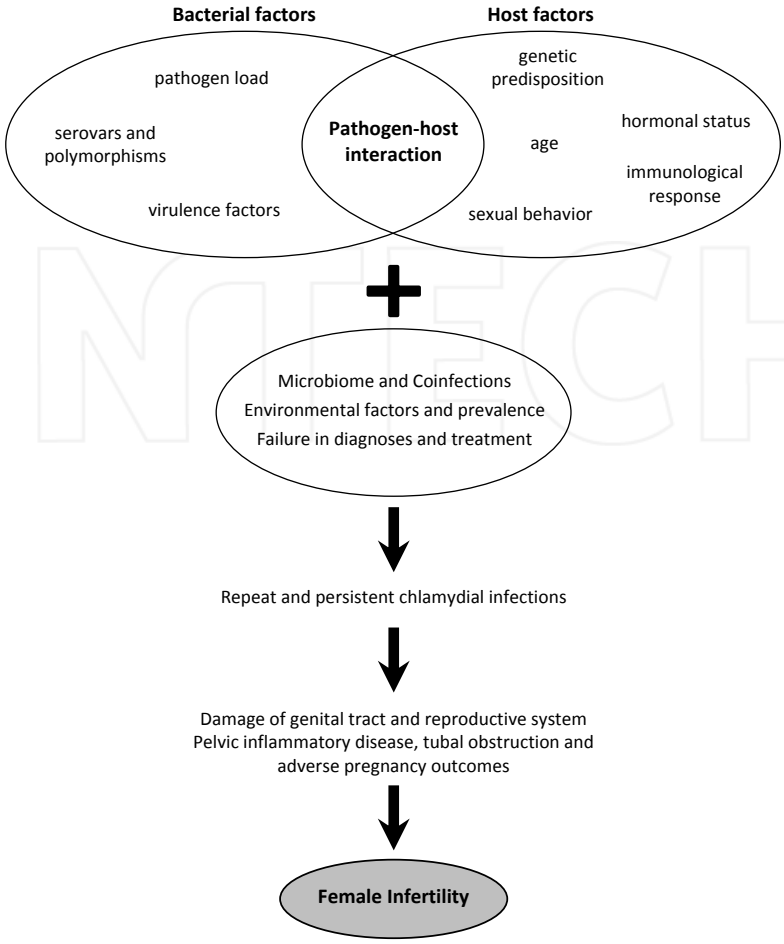

Figure 2. Bacterial and host factors involved in the development of female infertility. Bacterial factors such as CT genotypes and serovars, virulence factors, and pathogen burden, in conjunction with, host factors such as genetic predisposition and immune response, age and hormonal status, and sexual behavior participate in the establishment of the infection and pathology. Other elements that take part in the development of CT-related diseases are microbiome and coinfections with other sexually transmitted pathogens, environmental factors, and local prevalence, and most importantly, the failure in diagnosis and treatment of chlamydial infections. Taken together, these factors sustain repeat and persistent chlamydial infections that damage female reproductive health.

Microbiome (microbial flora present at the genital tract) and the presence of other sexually transmitted pathogens may be important for the colonization of reproductive tissue and the establishment of chlamydial infection. The presence of lactobacilli in the vagina confers protection against the acquisition of chlamydial infection [104]. In contrast, indole-generating bacteria present in the microbiome or bacterial vaginosis allow CT to synthesize tryptophan, and consequently, to avoid the anti-chlamydial activity of IFN-γ [45,65]. Coinfections are more

frequent in vaginosis and women with high-risk sexual behavior [100]. Clearly, coinfections exacerbate host immune response and inflammation; therefore, they favor scarring and sequelae on female reproductive system and increase the likelihood of tubal infertility.

The worst scenarios involve silent and latent infections that underline chronic persistent chlamydial infection and frequent re-infections [1]. Chlamydial infections may persist in the female upper genital tract for months in the absence of treatment. They are often asymptomatic or alternate quiescent stages with periods of clinical manifestations [105]. Actually, chronic chlamydial infections may be because of the reactivation of asymptomatic latently infected deep-seated cells. The presence of aberrant bacterial forms responsible for chlamydial persistence constitutes a long-time challenge to the immune system. Host immunopathological response can damage the fallopian tube and generate female infertility [65,92].

Reinfections, persistent infections, and treatment failure account for repeat infections that represent a substantial proportion of the chlamydial infections detected annually [106]. Repeat infection is not the sole determinant of severe genital tract pathology and sequelae. However, repeat infection increases the risk of developing tubal obstruction and female infertility [76].

Increasing evidence indicates that long-term persistence of viable aberrant chlamydial organisms within host cells is associated with inflammatory and autoimmune diseases in extragenital tissues.

In definitive, the pathogen-host interplay and the concurrency of exogenous factors appear to be related to CT-induced immunopathology. In spite of the substantial worldwide impact of chlamydial disease, the bacterial and host factors that result in infertility are still not clear. Current efforts are focused on discovering what CT is doing to the host cells to prevent sequelae and undesired consequences of infection.

5. Mechanisms of pathogenicity and clinical course

CT infects both sexes; however, chlamydial infections constitute primarily a female health issue since the consequences are more damaging to the reproductive tissue in women than in men. In males, CT can infect urethra, epididymis, prostate, seminal vesicles, and testis. Usually, chlamydial infections are symptomatic, less frequent, and more easily eradicated in men than in women. Nevertheless, chlamydial infection in the male genitourinary tract might induce severe damage to seminiferous tubules, spermatogenesis, and sperm cells morphology that can result in impaired male fertility [107].

Here, we focus on chlamydial infections occurring in women. In first world countries, 3 to 5% of women younger than 30 years old will be infected by CT at any point in time [90,108]. And, in over 70% of the cases, CT infection of the female genitourinary tract is asymptomatic, spoiling the prevalence or incidence rates. Consequently, it is difficult to assess the incidence of sequelae, mainly PID, tubal obstruction, and female infertility, attributable to genital CT infection.

http://dx.doi.org/10.5772/62462

In women, CT is the major cause of mucopurulent cervicitis (MPC) and PID. Other manifestations of chlamydial infections are vaginitis, urethritis, salpingitis, endometritis, tubo-ovarian abscess, pelvic peritonitis, periappendicitis, and perihepatitis. The symptoms more frequently reported consist of abdominal pain, dysuria, vaginal itching, and abnormal vaginal discharge. However, the most important feature of chlamydial infection in the female genital tract is the occurrence of asymptomatic infection that remains subclinical for long periods in a high rate [109,110]. Asymptomatic ascending bacteria from the cervix may produce two groups of pathologies: (i) PID-associated complications such as chronic pelvic pain, tubal obstruction, and female infertility [111,112] and (ii) adverse pregnancy outcomes such as ectopic pregnancy, miscarriage, premature rupture of membranes, chrioamnionitis, preterm birth, stillbirths, and puerperal and neonatal infections [113–115].

General consensus in the medical community agrees that chlamydial PID is the most common preventable cause of tubal infertility and adverse pregnancy outcome [1]. However, the lack of systematic detection of the bacteria weakens these estimations, usually performed by analysis of a reduced number of cases. Approximately 20% of women with chlamydial lower genital tract infection will develop PID, 4% chronic pelvic pain, 3% tubal infertility, and 2% adverse pregnancy outcome [107]. In developing countries, the incidence and prevalence of CT infections and their harmful consequences on female reproductive tissue are not accurately known since CT infection is not routinely screened.

There is conclusive evidence that women who have suffered PID have higher risk to develop tubal infertility. A study demonstrated that 16.5% of women with abnormal laparoscopic findings likely resulting of acute PID failed to conceive, in comparison to 2.7% in control women; 10.8% developed tubal infertility, and 9.1% went through ectopic pregnancy. Therefore, PID increases the probability of permanent damage on female reproductive system. CT is the most common causing agent of PID; however, a drawback of this analysis is the lack of the identification of the infectious agent underlying the PID. Several randomized controlled trials to assess the value of CT screening found it helpful to reduce the incidence of PID among infected women [116–118]. The chance to develop tubal infertility after a single episode of PID is around 10% [112]. Furthermore, each episode of PID doubled the risk of tubal damage [119] independent of whether the infection was asymptomatic or not.

One of the most serious sequelae of PID and persistent chlamydial infections is the fibrosis and scarring obstruction of the fallopian tubes that leads to tubal infertility. However, the risk of tubal infertility due to CT infections is lower than PID incidence [76]. The proportion of tubal infertility among all causes of infertility varies from 40% in first-world countries to 85% in developing countries [1]. Undiagnosed and untreated chlamydial infections usually evolve to long-term persistent infections, in which chlamydial antigens chronically stimulate the host immune system. Abnormal humoral and cell-mediated immune responses and severe inflammation have been implicated in the development of immunopathological damage of fallopian tubes. Thus, the interaction between CT and the host becomes relevant for the illness outcome. However, the exact pathologic mechanism of CT-induced tubal damage and tubal infertility has not been elucidated yet.

In addition, acute and chronic maternal chlamydial infections constitute a significant risk factor for adverse pregnancy outcomes and newborns contagion. Untreated and persistent infections are strongly associated with ectopic pregnancy, which is the tubal development of the embryo and constitute the main cause of maternal mortality in the first trimester of pregnancy in developing countries. In addition, miscarriage, chrioamnionitis, low birth weight, stillbirth, premature rupture of membranes, and preterm birth are frequent pathological consequences of chlamydial infections [51,113,114]. Several mechanisms may contribute to the development of these pathologies, including direct fetal infection, placental damage, and severe puerperal maternal illness [120–124]. Nevertheless, the exact nature of chlamydial infection pathogenesis during pregnancy remains unexplained.

CT infections can also be vertically transmitted to newborns during labor, resulting in chlamydial conjunctivitis and/or pneumonia, which may possibly involve similar pathogenesis mechanisms to those occurring in the female genital tract.

Controversial reports point out CT as a risk factor for cervical carcinoma, independent of human papillomavirus [107]. It has been shown that CT interferes with multiple proapoptotic pathways to guarantee survival within host cells [125,126]. In addition, CT activates prosurvival signaling pathways for bacterial nutrient acquisition, expression of antiapoptotic factors, and synthesis of proinflammatory cytokines [127,128]. On the other hand, CT interferes with chromosome segregation and cytokinesis. In consequence, multinucleated cells and cells with aberrant number of chromosomes are often observed in cell cultures infected with CT [129]. Taken together, these findings suggest that persistent chlamydial infections might play a role in the development of cervical neoplasia. Further research is needed to shed light to this issue.

The high prevalence and incidence estimates worldwide, in conjunction to the diversity of clinical entities and long-term consequences of chlamydial infections, propel further research of the bacterial and host factors involved in the pathogenesis and damage to the reproductive system.

6. Important challenges: Prevention, diagnosis, and treatment of chlamydial infections

The widespread incidence and prevalence of sexually transmitted infections, especially among young population, made them a priority public-health concern worldwide. Screening, control programmes, and education in sexual behavioral aspects and contraception are fundamental for the prevention of chlamydial infections and their long-term sequelae, mainly PID and tubal infertility.

Routine screening allows the identification of asymptomatic carriers of CT and contributes to the early detection of chlamydial infections. Appropriated diagnostic services are required to obtain reliable results. The most confident tests for the detection of CT involve nucleid acid amplification techniques that are not available in all laboratories, especially in third-world countries. Nucleic acid amplification tests are more sensitive than culture or antigen tests.

Improvement in molecular diagnostics will lead to improvement in treatment and prevention of damage to reproductive tissues. It has been shown that screening is cost-effective even in low prevalence populations, due to the high costs of treatment of complications resulting from undiagnosed and untreated chlamydial infections [130]. The implement of additional targeted screening of women at risk will contribute to reduce the CT-associated infertility.

Additionally, clinicians should be aware about the latest therapeutic management of chlamydial cervicitis and PID. Different antibiotics such as azithromycin, doxycycline, ofloxacin, erythromycin, and amoxicillin may be useful for treating genital chlamydial infections [131]. The antibiotic selection depends on the characteristics of the drug itself like pharmacokinetics, half-life, and bioavailability, concentration in mucous membranes and genital tissues, development of gastrointestinal tract side effects, safe use in pregnancy, as well as, the characteristics of the host and the clinical course of chlamydial infection. The effectiveness of therapy relies on timely treating sex partners and to abstain from sexual intercourse until completing the whole antibiotic scheme.

Unceasing efforts are conducted for the development of a chlamydial vaccine. Nevertheless, until now, no effective chlamydial vaccines are available. The knowledge of bacterial factors involved in pathogenicity will help in addressing optimal vaccine design that prevents not only chlamydial infection but also progression to infertility.

Acknowledgements

The authors acknowledge CONICET (PIP), Foncyt (PICT 2116) and UNCuyo grants to M.T.D. for their support in research. They apologize to those investigators whose work could not be cited because of space constraints.

Author details

Agustín Luján, Silvina Fili and María Teresa Damiani*

*Address all correspondence to: meteresadamiani@gmail.com

Laboratory of Phagocytosis and Intracellular Transport, IHEM-CONICET, School of Medicine, University of Cuyo, Mendoza, Argentina

References

[1] World Health Organization. Prevalence and incidence of selected sexually transmitted infections, Chlamydia trachomatis, Neisseria gonorrhoeae, syphilis and Tricho-

monas vaginalis: Methods and results used by WHO to generate 2005 estimate. WHO. 2011;1–38.

[2] CDC. Incidence, Prevalence, and Cost of Sexually Transmitted Infections in the United States. CDC Fact Sheet. 2013;40(February):1–4.

[3] Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, et al. Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. Science (Internet). 1998;282(5389):754–759.

[4] Hybiske K, Stephens RS. Mechanisms of Chlamydia trachomatis entry into nonphagocytic cells. Infect Immun. 2007;75(8):3925–3934.

[5] Cocchiaro JL, Valdivia RH. New insights into Chlamydia intracellular survival mechanisms. Cell Microbiol. 2009;11(11):1571–1878.

[6] Dautry-Varsat A, Subtil A, Hackstadt T. Recent insights into the mechanisms of Chlamydia entry. Cell Microbiol. 2005;7(12):1714–1722.

[7] Chen JC, Stephens RS. Chlamydia trachomatis glycosaminoglycan-dependent and independent attachment to eukaryotic cells. Microb Pathog. 1997;22(1):23–30.

[8] Abromaitis S, Stephens RS. Attachment and entry of chlamydia have distinct requirements for host protein disulfide isomerase. PLoS Pathog. 2009;5(4):1–12.

[9] Menozzi FD, Pethe K, Bifani P, Soncin F, Brennan MJ, Locht C. Enhanced bacterial virulence through exploitation of host glycosaminoglycans. Mol Microbiol. 2002;43(6):1379–1386.

[10] Su B, Karint M. Mitogen-activated gene expression protein kinase cascades and regulation of gene expression. Curr Opin Immunol. 1996;8(3):402–411.

[11] Fadel S, Eley A. Chlamydia trachomatis OmcB protein is a surface-exposed glycosaminoglycan-dependent adhesion. J Med Microbiol. 2007;56(1):15–22.

[12] Mölleken K, Schmidt E, Hegemann JH. Members of the Pmp protein family of Chlamydia pneumoniae mediate adhesion to human cells via short repetitive peptide motifs. Mol Microbiol. 2010;78(4):1004–1017.

[13] Engel J. Tarp and Arp: How Chlamydia induces its own entry. Proc Natl Acad Sci U S A. 2004;101(27):9947–9948.

[14] Lane BJ, Mutchler C, Al Khodor S, Grieshaber SS, Carabeo RA. Chlamydial entry involves TARP binding of guanine nucleotide exchange factors. PLoS Pathog. 2008;4(3):1–11.

[15] Jewett TJ, Miller NJ, Dooley CA, Hackstadt T. The conserved tarp actin binding domain is important for chlamydial invasion. PLoS Pathog. 2010;6(7):1–11.

[16] Damiani MT, Gambarte Tudela J, Capmany A. Targeting eukaryotic Rab proteins: A smart strategy for chlamydial survival and replication. Cell Microbiol. 2014;16(9): 1329–1338.

[17] Gambarte Tudela J, Capmany A, Romao M, Quintero C, Miserey-Lenkei S, Raposo G, Goud B, Damiani MT. The late endocytic Rab39a GTPase regulates the interaction between multivesicular bodies and chlamydial inclusions. J Cell Sci. 2015;128(16):3068–3081.

[18] Hybiske K, Stephens RS. Mechanisms of host cell exit by the intracellular bacterium Chlamydia. Proc Natl Acad Sci U S A. 2007;104(27):11430–11435.

[19] Beatty WL, Byrne GI, Morrison RP. Repeated and persistent infection with Chlamydia and the development of chronic inflammation and disease. Trends Microbiol. 1994;2(3):94–98.

[20] Bastidas RJ, Elwell CA, Engel JN, Valdivia RH. Chlamydial intracellular survival strategies. Cold Spring Harb Perspect Med. 2013;3(5): 1–20.

[21] Rzomp KA, Scholtes LD, Briggs BJ, Whittaker GR, Scidmore MA. Rab GTPases are recruited to chlamydial inclusions in both a species-dependent and species-independent manner. Infect Immun. 2003;71(10):5855–5870.

[22] Hackstadt T, Fischer ER, Scidmore MA, Rockey DD, Heinzen RA. Origins and functions of the chlamydial inclusion. Trends Microbiol. 1997;5(7):288–293.

[23] Fields KA, Hackstadt T. The chlamydial inclusion: Escape from the endocytic pathway. Annu Rev Cell Biol. 2002;18:221–245.

[24] Moore ER, Fischer ER, Mead DJ, Hackstadt T. The chlamydial inclusion preferentially intercepts basolaterally directed sphingomyelin-containing exocytic vacuoles. Traffic. 2008;9(12):2130–2140.

[25] Rejman Lipinski A, Heymann J, Meissner C, Karlas A, Brinkmann V, Meyer TF, et al. Rab6 and Rab11 regulate Chlamydia trachomatis development and golgin-84-dependent Golgi fragmentation. PLoS Pathog. 2009;5(10): 1–12.

[26] Capmany A, Damiani MT. Chlamydia trachomatis intercepts golgi-derived sphingolipids through a rab14-mediated transport required for bacterial development and replication. PLoS One. 2010;5(11): 1–17.

[27] Leiva N, Capmany A, Damiani MT. Rab11-Family of Interacting Protein 2 associates with chlamydial inclusions through its Rab-binding domain and promotes bacterial multiplication. Cell Microbiol. 2013;15(1):114–129.

[28] Beatty WL. Trafficking from CD63-positive late endocytic multivesicular bodies is essential for intracellular development of Chlamydia trachomatis. J Cell Sci. 2006;119(Pt 2):350–359.

[29] Cocchiaro J, Kumar Y, Fischer ER, Hackstadt T, Valdivia RH. Cytoplasmic lipid droplets are translocated into the lumen of the Chlamydia trachomatis parasitophorous vacuole. Proc Natl Acad Sci U S A. 2008;105(27):9379–9384.

[30] Saka HA, Valdivia R. Emerging roles for lipid droplets in immunity and host-pathogen interactions. Annu Rev Cell Dev Biol. 2012;28(1):411–437.

[31] Derré I, Swiss R, Agaisse H. The lipid transfer protein CERT interacts with the Chlamydia inclusion protein IncD and participates to ER-Chlamydia inclusion membrane contact sites. PLoS Pathog. 2011;7(6): 1–13.

[32] Elwell CA, Jiang S, Kim JH, Lee A, Wittmann T, Hanada K, et al. Chlamydia trachomatis co-opts gbf1 and cert to acquire host sphingomyelin for distinct roles during intracellular development. PLoS Pathog. 2011;7(9): 1–20.

[33] Robertson KD, Gu L, Rowe RK, Beatty WL. Inclusion biogenesis and reactivation of persistent Chlamydia trachomatis requires host cell sphingolipid biosynthesis. PLoS Pathog. 2009;5(11): 1–9.

[34] Clifton DR, Fields KA, Grieshaber SS, Dooley CA, Fischer ER, Mead DJ, et al. A chlamydial type III translocated protein is tyrosine-phosphorylated at the site of entry and associated with recruitment of actin. Proc Natl Acad Sci U S A. 2004;101(27): 10166–10171.

[35] Grieshaber S, Swanson JA, Hackstadt T. Determination of the physical environment within the Chlamydia trachomatis inclusion using ion-selective ratiometric probes. Cell Microbiol. 2002;4(5):273–283.

[36] Vandahl BBS, Stensballe A, Roepstorff P, Christiansen G, Birkelund S. Secretion of Cpn0796 from Chlamydia pneumoniae into the host cell cytoplasm by an autotransporter mechanism. Cell Microbiol. 2005;7(6):825–836.

[37] Trentmann O, Horn M, Van Scheltinga ACT, Neuhaus HE, Haferkamp I. Enlightening energy parasitism by analysis of an ATP/ADP transporter from chlamydiae. PLoS Biol. 2007;5(9):1938–1951.

[38] Derré I, Pypaert M, Dautry-Varsat A, Agaisse H. RNAi screen in Drosophila cells reveals the involvement of the tom complex in Chlamydia infection. PLoS Pathog. 2007;3(10):1446–1458.

[39] Mylonas I. Female genital Chlamydia trachomatis infection: Where are we heading? Arch Gynecol Obstet. 2012;285(5):1271–1285.

[40] Stephens RS, Sanchez-Pescador R, Wagar EA, Inouye C, Urdea MS. Diversity of Chlamydia trachomatis major outer membrane protein genes. J Bacteriol. 1987;169(9): 3879–3885.

[41] Baehr W, Zhang YX, Joseph T, Su H, Nano FE, Everett KD, et al. Mapping antigenic domains expressed by Chlamydia trachomatis major outer membrane protein genes. Proc Natl Acad Sci U S A. 1988;85(11):4000–4004.

[42] Millman K, Black CM, Johnson RE, Stamm E, Jones RB, Hook EW, et al. Population-based genetic and evolutionary analysis of Chlamydia trachomatis urogenital strain variation in the United States. J Bacteriol. 2004;186(8):2457–2465.

[43] Stothard DR, Boguslawski G, Jones RB. Phylogenetic analysis of the Chlamydia trachomatis major outer membrane protein and examination of potential pathogenic determinants. Infect Immun. 1998;66(8):3618–3625.

[44] Brown WJ, Rockey DD. Identification of an antigen localized to an apparent septum within dividing chlamydiae? Infect Immun. 2000;68(2):708–715.

[45] Nelson DE, Virok DP, Wood H, Roshick C, Johnson RM, Whitmire WM, et al. Chlamydial IFN-gamma immune evasion is linked to host infection tropism. Proc Natl Acad Sci U S A. 2005;102(30):10658–10663.

[46] Borges V, Nunes A, Ferreira R, Borrego MJ, Gomes JP. Directional evolution of chlamydia trachomatis towards niche-specific adaptation. J Bacteriol. 2012;194(22):6143–6153.

[47] Jeffrey BM, Suchland RJ, Quinn KL, Davidson JR, Stamm WE, Rockey DD. Genome sequencing of recent clinical Chlamydia trachomatis strains identifies loci associated with tissue tropism and regions of apparent recombination. Infect Immun. 2010;78(6):2544–2553.

[48] Dean D, Oudens E, Bolan G, Padian N, Schachter J. Major outer membrane protein variants of *Chlamydia trachomatis* are associated with severe upper genital tract infections and histopathology in San Francisco. J Infect Dis 1995;172(4):1013–1022.

[49] Brunham RC, Plummer FA, Stephens RS. Bacterial antigenic variation, host immune response, and pathogen-host coevolution. Infect Immun. 1993;61(6):2273–2276.

[50] Mascellino MT, Ciardi MR, Oliva A, Cecinato F, Hassemer MP, Borgese L. Chlamydia trachomatis detection in a population of asymptomatic and symptomatic women: Correlation with the presence of serological markers for this infection. New Microbiol. 2008;31(2):249–256.

[51] Domeika M, Domeika K, Paavonen J, Mårdh PA, Witkin SS. Humoral immune response to conserved epitopes of Chlamydia trachomatis and human 60-kDa heat-shock protein in women with pelvic inflammatory disease. J Infect Dis. 1998;177(3): 714–719.

[52] Swanborg RH, Boros DL, Whittum-Hudson JA, Hudson AP. Molecular mimicry and horror autotoxicus: Do chlamydial infections elicit autoimmunity? Expert Rev Mol Med. 2006;8(29):1–23.

[53] Linhares IM, Witkin SS. Immunopathogenic consequences of Chlamydia trachomatis 60 kDa heat shock protein expression in the female reproductive tract. Cell Stress Chaperones. 2010;15(5):467–473.

[54] Peña AS, Karimi O, Crusius JBA. A new avenue to investigate: The autophagic process. From Crohn's disease to Chlamydia. Drugs Today. 2009;45(Suppl. B):113–117.

[55] Carter JD, Inman RD, Whittum-Hudson J, Hudson AP. Chlamydia and chronic arthritis. Ann Med. 2011;44(8):1–9.

[56] Henderson IR, Nataro JP. Virulence functions of autotransporter proteins. Infect Immun. 2001;69(3):1231–1243.

[57] Subtil A, Blocker A, Dautry-Varsat A. Type III secretion system in Chlamydia species: Identified members and candidates. Microbes Infect. 2000;2(4):367–369.

[58] Betts HJ, Wolf K, Fields KA. Effector protein modulation of host cells: Examples in the Chlamydia spp. arsenal. Curr Opin Microbiol. 2009;12(1):81–87.

[59] Beeckman DSA, Vanrompay DCG. Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. Clin Microbiol Infect. 2009;15(1):11–17.

[60] Hackstadt T, Scidmore MA, Shaw EI, Fischer ER. The Chlamydia trachomatis IncA protein is required for homotypic vesicle fusion. Cell Microbiol. 1999;1(2):119–130.

[61] Delevoye C, Nilges M, Dehoux P, Paumet F, Perrinet S, Dautry-Varsat A, et al. SNARE protein mimicry by an intracellular bacterium. PLoS Pathog. 2008;4(3): 1–14.

[62] Geisler WM, Suchland RJ, Whittington WL, Stamm WE. Quantitative culture of Chlamydia trachomatis: relationship of inclusion-forming units produced in culture to clinical manifestations and acute inflammation in urogenital disease. J Infect Dis. 2001;184(10):1350–1354.

[63] Jewett TJ, Fischer ER, Mead DJ, Hackstadt T. Chlamydial TARP is a bacterial nucleator of actin. Proc Natl Acad Sci U S A. 2006;103(42):15599–15604.

[64] Belland RJ, Scidmore MA, Dean D, Hogan D, Whitmire WM, McClarty G, et al. Chlamydia trachomatis cytotoxicity associated with complete and partial cytotoxin genes. Proc Natl Acad Sci U S A. 2001;98(24):13984–13989.

[65] Carlson JH, Hughes S, Hogan D, Cieplak G, Sturdevant DE, McClarty G, et al. Polymorphisms in the Chlamydia trachomatis cytotoxin locus associated with ocular and genital isolates. Infect Immun. 2004;72(12):7063–7072.

[66] Vora GJ, Stuart ES. A role for the glycolipid exoantigen (GLXA) in chlamydial infectivity. Curr Microbiol. 2003;46(3):217–223.

[67] Brade L, Nurminen M, Makela PH, Brade H. Antigenic properties of *Chlamydia trachomatis* lipopolysaccharide. Infect Immun. 1985;48(2):569–572.

[68] Omsland A, Sixt BS, Horn M, Hackstadt T. Chlamydial metabolism revisited: Interspecies metabolic variability and developmental stage-specific physiologic activities. FEMS Microbiol Rev. 2014;38(4):779–801.

[69] Wood H, Fehlner-Gardner C, Berry J, Fischer E, Graham B, Hackstadt T, et al. Regulation of tryptophan synthase gene expression in Chlamydia trachomatis. Mol Microbiol. 2003;49(5):1347–1359.

[70] Witkin SS, Jeremias J, Toth M, Ledger WJ. Cell-mediated immune response to the recombinant 57-kDa heat-shock protein of Chlamydia trachomatis in women with salpingitis. J Infect Dis. 1993;167(6):1379–1383.

[71] Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Cutting edge: Heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. J Immunol. 2000;164(1):13–17.

[72] LaVerda D, Albanese LN, Ruther PE, Morrison SG, Morrison RP, Ault KA, et al. Seroreactivity to Chlamydia trachomatis Hsp10 correlates with severity of human genital tract disease. Infect Immun. 2000;68(1):303–309.

[73] Kinnunen A, Molander P, Morrison R, Lehtinen M, Karttunen R, Tiitinen A, et al. Chlamydial heat shock protein 60-specific T cells in inflamed salpingeal tissue. Fertil Steril. 2002;77(1):162–166.

[74] Jha R, Srivastava P, Salhan S, Finckh A, Gabay C, Mittal A, et al. Spontaneous secretion of interleukin-17 and -22 by human cervical cells in Chlamydia trachomatis infection. Microbes Infect. Elsevier Masson SAS; 2011;13(2):167–178.

[75] Spear GT, Kendrick SR, Chen HY, Thomas TT, Bahk M, Balderas R, et al. Multiplex immunoassay of lower genital tract mucosal fluid from women attending an urban STD clinic shows broadly increased IL1ß and lactoferrin. PLoS One. 2011;6(5):1–7.

[76] Low N, Egger M, Sterne JAC, Harbord RM, Ibrahim F, Lindblom B, et al. Incidence of severe reproductive tract complications associated with diagnosed genital chlamydial infection: The Uppsala Women's Cohort Study. Sex Transm Infect. 2006;82(3): 212–218.

[77] Agrawal T, Vats V, Salhan S, Mittal A. Mucosal and peripheral immune responses to chlamydial heat shock proteins in women infected with Chlamydia trachomatis. Clin Exp Immunol. 2007;148(3):461–468.

[78] Srivastava P, Jha R, Bas S, Salhan S, Mittal A. In infertile women, cells from Chlamydia trachomatis infected sites release higher levels of interferon-gamma, interleukin-10 and tumor necrosis factor-alpha upon heat-shock-protein stimulation than fertile women. Reprod Biol Endocrinol. 2008;6:20.

[79] Mascellino MT, Margani M, Oliva A. Helicobacter pylori: Determinant and markers of virulence. Dis Markers. 2009;27(3-4):137–156.

[80] Darville T, Hiltke TJ. Pathogenesis of genital tract disease due to Chlamydia trachomatis. J Infect Dis. 2010;201(S2):114–125.

[81] Morrison SG, Su H, Caldwell HD, Morrison RP. Immunity to murine Chlamydia trachomatis genital tract reinfection involves B cells and CD4+ T cells but not CD8+ T cells. Infect Immun. 2000;68(12):6979–6987.

[82] Holland MJ, Bailey RL, Conway DJ, Culley F, Miranpuri G, Byrne GI, et al. T helper type-1 (Th1)/Th2 profiles of peripheral blood mononuclear cells (PBMC); responses to antigens of Chlamydia trachomatis in subjects with severe trachomatous scarring. Clin Exp Immunol. 1996;105(3):429–435.

[83] Horne AW, Stock SJ, King AE. Innate immunity and disorders of the female reproductive tract. Reproduction. 2008;135(6):739–749.

[84] Lal JA, Malogajski J, Verweij SP, De Boer P, Ambrosino E, Brand A, et al. Chlamydia trachomatis infections and subfertility: Opportunities to translate host pathogen genomic data into public health. Public Health Genomics. 2013;16(1-2):50–61.

[85] Ingalls RR, Rice PA, Qureshi N, Takayama K, Juey Shin Lin, Golenbock DT. The inflammatory cytokine response to Chlamydia trachomatis infection is endotoxin mediated. Infect Immun. 1995;63(8):3125–3130.

[86] Prebeck S, Kirschning C, Dürr S, da Costa C, Donath B, Brand K, et al. Predominant role of toll-like receptor 2 versus 4 in Chlamydia pneumoniae-induced activation of dendritic cells. J Immunol. 2001;167(6):3316–3323.

[87] Heine H, Müller-Loennies S, Brade L, Lindner B, Brade H. Endotoxic activity and chemical structure of lipopolysaccharides from Chlamydia trachomatis serotypes E and L2 and Chlamydophila psittaci 6BC. Eur J Biochem. 2003;270(3):440–450.

[88] Welter-Stahl L, Ojcius DM, Viala J, Girardin S, Liu W, Delarbre C, et al. Stimulation of the cytosolic receptor for peptidoglycan, Nod1, by infection with Chlamydia trachomatis or Chlamydia muridarum. Cell Microbiol. 2006;8(6):1047–1057.

[89] Walker J, Tabrizi SN, Fairley CK, Chen MY, Bradshaw CS, Twin J, et al. Chlamydia trachomatis incidence and re-infection among young women – Behavioural and microbiological characteristics. PLoS One. 2012;7(5):1–9.

[90] Price MJ, Ades AE, de Angelis D, Welton NJ, Macleod J, Turner K, et al. Incidence of Chlamydia trachomatis infection in women in England: Two methods of estimation. Epidemiol Infect. 2014;142(3):562–576.

[91] Bender N, Herrmann B, Andersen B, Hocking JS, van Bergen J, Morgan J, et al. Chlamydia infection, pelvic inflammatory disease, ectopic pregnancy and infertility: Cross-national study. Sex Transm Infect. 2011;87(7):601–608.

[92] Hall JV, Schell M, Dessus-Babus S, Moore CG, Whittimore JD, Sal M, et al. The multifaceted role of oestrogen in enhancing Chlamydia trachomatis infection in polarized human endometrial epithelial cells. Cell Microbiol. 2011;13(8):1183–1199.

[93] Borth N, Massier J, Franke C, Sachse K, Saluz HP, Hänel F. Chlamydial protease CT441 interacts with SRAP1 co-activator of estrogen receptor and partially alleviates its co-activation activity. J Steroid Biochem Mol Biol. 2010;119(1–2):89–95.

[94] Sonnex C. Influence of ovarian hormones on urogenital infection. Sex Transm Infect. 1998;74(1):11–19.

[95] Agrawal T, Vats V, Wallace PK, Salhan S, Mittal A. Role of cervical dendritic cell subsets, co-stimulatory molecules, cytokine secretion profile and beta-estradiol in development of sequalae to Chlamydia trachomatis infection. Reprod Biol Endocrinol. 2008;6:46.

[96] Wan C, Latter JL, Amirshahi A, Symonds I, Finnie J, Bowden N, et al. Progesterone Activates Multiple Innate Immune Pathways in Chlamydia trachomatis-Infected Endocervical Cells. Am J Reprod Immunol. 2014;71(2):165–177.

[97] Beagley KW, Gockel CM. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunol Med Microbiol. 2003;38(1):13–22.

[98] Kaushic C, Zhou F, Murdin AD, Charles R, Zhou FAN. Effects of estradiol and progesterone on susceptibility and early immune responses to Chlamydia trachomatis infection in the female reproductive tract. Infect Immun.2000 Jul;68(7):4207–4216.

[99] Macleod J, Salisbury C, Low N, McCarthy A, Sterne JAC, Holloway A, et al. Coverage and uptake of systematic postal screening for genital Chlamydia trachomatis and prevalence of infection in the United Kingdom general population: Cross sectional study. BMJ. 2005;330(7497):940.

[100] Wiesenfeld HC, Hillier SL, Krohn MA, Landers DV, Sweet RL. Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection. Clin Infect Dis. 2003;36(5):663–668.

[101] Korn AP, Hessol NA, Padian NS, Bolan GA, Donegan E, Landers DV, et al. Risk factors for plasma cell endometritis among women with cervical Neisseria gonorrhoeae, cervical Chlamydia trachomatis, or bacterial vaginosis. Am J Obstet Gynecol. 1998;178(5):987–990.

[102] Da Silva CS, Adad SJ, De Souza MAH, Barcelos ACM, Terra APS, Murta EFC. Increased frequency of bacterial vaginosis and Chlamydia trachomatis in pregnant women with human papillomavirus infection. Gynecol Obstet Invest. 2004;58(4):189–193.

[103] Quintero CA, Tudela JG, Damiani MT. Rho GTPases as pathogen targets: Focus on curable sexually transmitted infections. Small GTPases. 2015;(May):1–11.

[104] Gong Z, Luna Y, Yu P, Fan H. Lactobacilli inactivate Chlamydia trachomatis through lactic acid but not H2O2. PLoS One. 2014;9(9):e107758.

[105] Richmond SJ, Sparling PF. Genital chlamydial infections. Am J Epidemiol. 1976;103(5):428–435.

[106] Batteiger BE, Tu W, Ofner S, Pol B Van Der, Diane R, Orr DP, et al. Repeated Chlamydia trachomatis genital infections in adolescent women. J Infect Dis. 2011;201(1): 42–51.

[107] Paavonen J, Eggert-Kruse W. Chlamydia trachomatis: Impact on human reproduction. Hum Reprod Update. 1999;5(5):433–447.

[108] Lewis D, Newton DC, Guy RJ, Ali H, Chen MY, Fairley CK, et al. The prevalence of Chlamydia trachomatis infection in Australia: A systematic review and meta-analysis. BMC Infect Dis. 2012;12(1):113.

[109] Bé Bé Ar C, De Barbeyrac B. Genital Chlamydia trachomatis infections. Clin Microbiol Infect. 2009;15:4–10.

[110] Haggerty CL, Gottlieb SL, Taylor BD, Low N, Xu F, Ness RB. Risk of sequelae after Chlamydia trachomatis genital infection in women. J Infect Dis. 2010;(2):S134–S155.

[111] Chernesky MA, Lee H, Schachter J, Burczak JD, Stamm WE, McCormack WM, et al. Diagnosis of Chlamydia trachomatis urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. J Infect Dis. 1994;170(5):1308–1311.

[112] Hillis SD, Joesoef R, Marchbanks PA, Wasserheit JN, Cates W, Westrom L. Delayed care of pelvic inflammatory disease as a risk factor for impaired fertility. Am J Obstet Gynecol. Mosby; 1993;168(5):1503–1509.

[113] Martin DH, Koutsky L, Eschenbach DA, Daling JR, Alexander ER, Benedetti JK, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal Chlamydia trachomatis infections. JAMA. 1982;247(11):1585–1588.

[114] Gravett MG. Independent associations of bacterial vaginosis and Chlamydia trachomatis infection with adverse pregnancy outcome. JAMA. 1986;256(14):1899.

[115] Gencay M, Koskiniemi M, Saikku P, Puolakkainen M, Raivio K, Koskela P, et al. Chlamydia trachomatis seropositivity during pregnancy is associated with perinatal complications. Clin Infect Dis. 1995;21:424–426.

[116] Oakeshott P, Kerry S, Aghaizu A, Atherton H, Hay S, Taylor-Robinson D, et al. Randomised controlled trial of screening for Chlamydia trachomatis to prevent pelvic inflammatory disease: The POPI (prevention of pelvic infection) trial. BMJ. 2010;340:c1642.

[117] Herzog SA, Althaus CL, Heijne JC, Oakeshott P, Kerry S, Hay P, et al. Timing of progression from Chlamydia trachomatis infection to pelvic inflammatory disease: A mathematical modelling study. BMC Infect Dis. 2012;12(1):187.

[118] Price MJ, Ades AE, De Angelis D, Welton NJ, MacLeod J, Soldan K, et al. Risk of pelvic inflammatory disease following Chlamydia trachomatis infection: Analysis of prospective studies with a multistate model. Am J Epidemiol. 2013;178(3):484–492.

[119] Paavonen J, Lehtinen M. Immunopathogenesis of chlamydial pelvic inflammatory disease: The role of heat-shock proteins. Infect Dis Obs Gynecol. 1994;2(3):105–110.

[120] Khader SA, Gopal R. IL-17 in protective immunity to intracellular pathogens. Virulence. 2010;1(5):423–427.

[121] Agrawal T, Bhengraj AR, Vats V, Salhan S, Mittal A. Expression of TLR 2, TLR 4 and iNOS in cervical monocytes of Chlamydia trachomatis-infected women and their role in host immune response. Am J Reprod Immunol. 2011;66(6):534–543.

[122] Gupta R, Vardhan H, Srivastava P, Salhan S, Mittal A. Modulation of cytokines and transcription factors (T-Bet and GATA3) in CD4 enriched cervical cells of Chlamydia trachomatis infected fertile and infertile women upon stimulation with chlamydial inclusion membrane proteins B and C. Reprod Biol Endocrinol. 2009;7:84.

[123] Balasubramaniam ES, Van Noorden S, El-Bahrawy M. The expression of interleukin (IL)-6, IL-8, and their receptors in fallopian tubes with ectopic tubal gestation. Fertil Steril. 2012;98(4):898–904.

[124] Lewis ME, Belland RJ, Abdel Rahman YM, Beatty WL, Aiyar AA, Zea AH, et al. Morphologic and molecular evaluation of Chlamydia trachomatis growth in human endocervix reveals distinct growth patterns. Front Cell Infect Microbiol. 2014;4:71.

[125] Fan T, Lu H, Hu H, Shi L, McClarty GA, Nance DM, et al. Inhibition of apoptosis in chlamydia-infected cells: Blockade of mitochondrial cytochrome c release and caspase activation. J Exp Med. 1998;187(4):487–496.

[126] Rajalingam K, Al-younes H, Müller A, Meyer TF, Szczepek AJ, Rudel T, et al. Epithelial cells infected with Chlamydophila pneumoniae are resistant to apoptosis. Infect Immun. 2001 Dec;69(12):7880–7888.

[127] Buchholz KR, Stephens RS. The extracellular signal-regulated kinase/mitogen-activated protein kinase pathway induces the inflammatory factor interleukin-8 following Chlamydia trachomatis infection. Infect Immun. 2007;75(12):5924–5929.

[128] Rajalingam K, Sharma M, Lohmann C, Oswald M, Thieck O, Froelich CJ, et al. Mcl-1 is a key regulator of apoptosis resistance in Chlamydia trachomatis-infected cells. PLoS One. 2008;3(9) :1–11.

[129] Grieshaber SS, Grieshaber NA, Miller N, Hackstadt T. Chlamydia trachomatis causes centrosomal defects resulting in chromosomal segregation abnormalities. Traffic. 2006;7(8):940–949.

[130] Puolakkainen M, Hiltunen-Back E, Reunala T, Suhonen S, Lähteenmäki P, Lehtinen M, et al. Comparison of performances of two commercially available tests, a PCR assay and a ligase chain reaction test, in detection of urogenital Chlamydia trachomatis infection. J Clin Microbiol. 1998;36(6):1489–1493.

[131] Horner PJ. Azithromycin antimicrobial resistance and genital Chlamydia trachomatis infection: Duration of therapy may be the key to improving efficacy. Sex Transm Infect. 2012;88(3):154–156.