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Chlamydia trachomatis infection of the male genital tract: An update

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ABSTRACT

Chlamydia trachomatis (CT) is the most prevalent cause of sexually transmitted diseases. Although the prevalence of chlamydial infection is similar in men and women, current research and screening are still focused on women, who develop the most severe complications, leaving the study of male genital tract (MGT) infection underrated. Herein, we reviewed the literature on genital CT infection with special focus on the MGT. Data indicate that CT certainly infects different parts of the MGT such as the urethra, seminal vesicles, prostate, epididymis and testis. However, whether or not CT infection has detrimental effects on male fertility is still controversial. The most important features of CT infection are its chronic nature and the presence of a mild inflammation that remains subclinical in most individuals. *Chlamydia* antigens and pathogen recognition receptors (PRR), expressed on epithelial cells and immune cells from the MGT, have been studied in the last years. Toll-like receptor (TLR) expression has been observed in the testis, epididymis, prostate and vas deferens. It has been demonstrated that recognition of chlamydial antigens is associated with TLR2, TLR4, and possibly, other PRRs. CT recognition by PRRs induces a local production of cytokines/chemokines, which, in turn, provoke chronic inflammation that might evolve in the onset of an autoimmune process in genetically susceptible individuals. Understanding local immune response along the MGT, as well as the crosstalk between resident leukocytes, epithelial, and stromal cells, would be crucial in inducing a protective immunity, thus adding to the design of new therapeutic approaches to a *Chlamydia* vaccine.

1. Chlamydial infection: an introduction

Chlamydia trachomatis (CT) is the most prevalent bacterial cause of sexually transmitted infections in humans (Brunham and Rey-Ladino, 2005). Approximately 100 million new cases of genital CT infection are diagnosed worldwide every year, although it is believed that this number is underestimated (Mylonas, 2012; Senior, 2012). Most cases occur in the developing world, where diagnostic tools and antimicrobial treatment are almost rudimentary. However, chlamydial control programs in developed countries, most of which were devised more than 20 years ago, have had little impact on the incidence of CT infections. The quantity of diagnoses has increased over the past ten years,

Abbreviations: CM, *Chlamydia muridarum*; CT, *Chlamydia trachomatis*; EB, elementary body; FGT, female genital tract; MGT, male genital tract; NOD, nucleotide oligomerization domain; PEC, primary cultures of epithelial cells; TLR, toll-like receptor; PRR, pathogen recognition receptors; RB, reticular body.

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probably because of the use of more sensitive tests and increased awareness of this pathogen. CT is transmitted almost exclusively by sexual intercourse, and like all other sexually transmitted diseases, young and sexually active people are primarily affected. Additionally, it can be transmitted vertically from mothers to newborns during labor, resulting in children with chlamydial conjunctivitis and pneumonia. Although the prevalence of chlamydial infection is similar in men and woman, current research and screening strategies are still focused mainly on women (Chen and Basil, 2003). In consequence, the importance of this pathogen in infections of the male genital tract (MGT) has been underestimated.

In this study, we aimed to review the literature on genital CT infection with a special focus on the biology of CT infection in the MGT. Reassessing the importance of studying the MGT as a reservoir of CT, we focused on chlamydial prostatitis and on the innate as well as adaptive immune response triggered after infection. These data provided an overview of the current knowledge about the pathophysiology of male genital CT infection, in order to understand the effects of chronic infections, which are of therapeutic relevance in reproduction topics. As a consequence, the data synthesized here highlight the gap in *Chlamydia* vaccine development.

2. Epidemiology

The first isolation of CT from the genital tract was performed in the 1950s. Although this finding was reported several decades ago, until the 1990s most CT genital infections in both genders were underestimated because of their asymptomatic clinical course (Paavonen, 2011). The current high incidence of CT genital infection is the result of its chronic nature and the absent or mild symptoms, which lead to an undiagnosed disease. Although the true prevalence of genital CT infection is unknown, after reviewing the epidemiological data, it can be accepted that it varies between 1 and 40%, depending on the population (Mylonas, 2012). The prevalence in our local population is 8.7% (Cuffini et al., unpublished data), comparable to the reported prevalence of a Brazilian population attending health care centers, which presented an average prevalence rate of 11.7%, ranging from 4 to 25.7% (Rodrigues et al., 2011; Ramos et al., 2011). It has been become clear that the prevalence of chlamydial infection is very similar in both men and women (Lewis et al., 2012). CT infections occur mainly in women younger than 25 years and men younger than 35 years. The mean prevalence estimated by setting is similar for men and women (Cunningham and Beagley, 2008). A typical feature and major problem of genital CT infection is to assess the extent of the infection. This occurs because the onset of the infection is generally unknown, re-exposure is quite common, and pathogen clearance is rarely followed up. Therefore, screening for chlamydial infections is not common, but remains very important in preventing bacterial spread. Since 1 January 2008, in Germany, all women younger than 25 years with statutory health insurance are eligible for yearly screening for urogenital CT infections, as well as women undergoing a planned induced abortion. The primary goal of this new preventive measure is the reduction of severe sequelae, such as tubal infertility and ectopic pregnancies (Mund et al., 2008). Primary prevention by educational programs to promote behavioral changes has not proved to be very effective. Although routine screening for CT in men and women is insufficient based on feasibility, efficacy, and cost-effectiveness, well-established screening programs administered to the sexually active young population should be considered in clinical settings with a high prevalence of *Chlamydia*, in order to reduce bacterial spread in the general population (Peipert, 2003). An accurate diagnostic screening and rescreening by physicians differentiating between acute or persistent infection might help to resolve the infection and thus avoid possible fertility-related complications. Furthermore, CT infection facilitates the transmission of HIV and might be a co-factor in human papilloma virus (HPV)-induced cervical neoplasia (Anttila et al., 2001). Understanding the natural history of infection, the host immune response, and the impact they have on subsequent pathologies is crucial for a rational vaccine design.

A vaccine against CT would be of benefit to human health and is likely to have a significant impact on health care costs. Vaccine development would be essential for controlling CT infection, especially in developing countries where a major increase in prevalence has been reported during the last decade (Götz et al., 2002; Lewis et al., 2012). Sanitary programs for diagnosis and control of CT infection carried out in developed countries cannot be used in these places, mainly because of their expensive costs, thus highlighting vaccine development as a major issue in CT infection control (Iwasaki, 2010). However, primary prevention by vaccination is problematic since the development of a CT vaccine is still a major challenge (Brunham and Rey-Ladino, 2005; Pal et al., 2010; Paavonen, 2011). CT vaccine development is difficult because of the complex antigenic structure and limited knowledge of protective antigens. Also, the nature of protective immune response and correlates of protection are not well known. Performing a protective vaccine without harmful effects is a complex task that needs further research on immune correlates of protection against CT and disease pathogenesis (Rockey et al., 2009).

3. Microbiology and infection

Chlamydia trachomatis is a small obligate intracellular Gram-negative bacterium surrounded by a rigid cell wall that needs living cells to multiply because of its inability to synthesize essential nutrients, thus strictly depending on host biosynthesis pathways (Wyrick, 2000). The chlamydial chromosome consists of approximately one million base pairs and has a capacity to encode for up to 600 proteins. Nineteen different serotypes of CT can be distinguished based on their major outer membrane protein (MOMP) characteristics. Serotypes A, B and C cause trachoma; serotypes D through K are responsible for urogenital infections, and serotype L is responsible for lymphogranuloma venereum (Brunham and Rey-Ladino, 2005; Wagenlehner et al., 2006). The cell cycle of *Chlamydia* is different to those of other bacteria. *Chlamydia*

spp. undergo two phases of reproduction: an intracellular phase of non-infectious metabolically active and replicative reticular bodies (RBs), and an extracellular phase of infectious metabolically inactive and non-replicative elementary bodies (EBs) (Schachter and Stephens, 2008; Valdivia, 2008).

The bacteria can persist in host cells in a viable, but culture-negative state (Beatty et al., 1994). Although re-infection is very likely to occur, (Witkin, 2002; Brunham and Rey-Ladino, 2005), there is another possible explanation for the sustained prevalence of genital CT infection. It has recently been revealed that CT has the capacity for rapid evolution by exchanging DNA between strains at a much higher rate than predicted, which allows this pathogen to evade the immune response and chronically persist in a host (Harris et al., 2012; Senior, 2012).

As stated above, *Chlamydia* spp. have a unique biphasic developmental cycle among obligate intracellular bacteria. It consists of the conversion of EBs to RBs, followed by RB cell division and transformation back to infective EBs. EBs infect epithelial cells entering by several potential receptors including PRRs, followed by endocytosis, which leads to the formation of membrane-bound, glycogen-enriched intracellular inclusions. Once inside the epithelial cells, CT undergoes the conversion to RBs in order to replicate, and finally converts again to infective EBs, which infect close epithelial cells. These EBs are released after host cell lysis and can survive in the extracellular environment as infectious agents (Witkin, 2002; Schachter and Stephens, 2008). After lysis of the infected host cells, mucosal epithelial cells undergo necrosis. During this process, chlamydial components, such as the endotoxin lipopolysaccharide (LPS) and bacterial proteins, trigger the host immune response. Neutrophils, monocytes, dendritic cells, lymphocytes, and plasma B cells migrate into this necrotic and ulcerous epithelium (Agrawal et al., 2009a; Al-Zeer et al., 2009). Local inflammation subsequently leads to fibrosis followed by the shriveling of connective tissue structures and scarring.

4. Pathophysiology

In women, urogenital infections caused by CT can lead to urethritis, mucopurulent cervicitis, endometritis, salpingitis, pelvic inflammatory disease, perihepatitis, periappendicitis, ectopic pregnancy, and tubal infertility (Paavonen, 2011; Kalwij et al., 2012). In men, CT is responsible for urethritis, epididymitis, epididymo-orchitis, and it is becoming increasingly accepted as a causative agent of prostatitis (Cunningham and Beagley, 2008; Ouzounova-Raykova et al., 2010; Kalwij et al., 2012). In addition, CT infection has been reported to be a major cause of reactive arthritis (Carter and Hudson, 2010). Furthermore, it has been shown that 21% of patients with unexplained arthritis have a history of urogenital chlamydial infection (Wollenhaupt et al., 1995; Senior, 2012).

Chlamydia trachomatis has been associated with reproductive dysfunction, mainly in women. Indeed, female genital CT infection has proved to have significant consequences for female fertility, tubal factor infertility and tubal ectopic pregnancy being the most frequently reported sequelae. On the other hand, the consequences of CT infection for male fertility are still under debate, with some reports arguing no effect on male fertility and others reporting decreased semen quality and impaired sperm fertilizing capacity and DNA integrity (Paavonen and Eggert-Kruse, 1999; Cunningham and Beagley, 2008; Joki-Korpela et al., 2009; Mazzoli et al., 2010).

The most important epidemiological occurrence of CT infection is as an asymptomatic infection. Although CT induces local inflammation and triggers the host immune response, infection remains subclinical in most infected individuals (Gottlieb et al., 2010). Chlamydial genital tract infections are asymptomatic in 75% of women and in up to 50% of men (Stamm, 1999; Peipert, 2003; Gonzales et al., 2004). The factors that determine whether infections develop as symptomatic or asymptomatic are unknown. However, a high prevalence of serotype E and its lack of associated clinical symptoms may suggest that this serotype might be more successful in maintaining a subclinical infection than other, less prevalent serotypes. Indeed, a successful serotype would be that one that remains undetected for a longer period of time, enhancing dissemination. Variability of the main CT antigen MOMP is presumably the result of host selection and bacterial adaptation. Thus, the MOMP sequence that elicits a milder immune response in the infected host could be an adaptive mode of evolution to escape immune pressure and might therefore confer a transmission advantage over other MOMP serotypes.

Symptomatic infected women can show signs of disease such as mucopurulent endocervical discharge, hypertrophic cervical ectopy, and friability (Marrazzo and Martin, 2007). Clinical symptoms consist of dysuria, abnormal vaginal discharge, menstrual bleeding, and lower abdominal pain. However, the lack of diagnosis and therapy favors the establishment of a chronic persistent infection that may last months to years (Gottlieb et al., 2010). As most CT infections remain asymptomatic and untreated, ascending bacteria can lead to infection of the upper genital tract and produce pelvic inflammatory disease (PID; 2–4.5% of untreated infected women) with long-term reproductive complications such as infertility or ectopic pregnancy (Gottlieb et al., 2010; Mylonas, 2012). It is noteworthy that the most important problem for subfertile infected couples is immune-mediated fallopian tube scarring, mainly associated with persistent genital CT infection (den Hartog et al., 2005, 2006, 2009; Cunningham and Beagley, 2008).

On the other hand, the best known consequences of MGT chlamydial infection are non-gonococcal urethritis, epididymitis, and epididymo-orchitis. However, they are more infrequent and less expensive to treat than complications in women. As a consequence, little attention has been paid to CT infection of the MGT, as it is underestimated and mainly considered to be a reservoir for transmission and re-infection (Chen and Basil, 2003). Although in men CT mainly affects the urethra and epididymis, it has also been reported to infect other tissues such as the prostate and seminal vesicles (Toth et al., 2000; Skerk et al., 2002; Gonzales et al., 2004; Krieger and Riley, 2004; Furuya et al., 2006; Motrich et al., 2006, 2012; Mackern-Oberti et al.,

2011a). However, the consequences of CT infection of the prostate or seminal vesicles are still to be defined.

Finally, it has been accepted that persistent infection in women as well as in the MGT may have deleterious effects on fertility owing to chronic inflammation and subsequent inter-infection between the couple.

5. MGT infection

In males, CT usually first infects the single-cell columnar layer of the urethral epithelium in the form of EBs. Once inside the epithelial cells, CT undergoes the developmental cycle to RBs in order to replicate by binary fission, and finally converts again to infective EBs, which infect nearby epithelial cells leading to an ascending infection. As the primary site of infection in males is the penile urethra, CT emerges as a major cause of male urethritis (Mulcahy et al., 1987). In addition, it is well known and widely accepted that male CT infection can also cause retrograde epididymitis and epididymo-orchitis (Krishnan and Heal, 1991; Trojian et al., 2009).

On the other hand, the role of CT infection in male accessory sexual glands such as prostate and seminal vesicles has been controversial and poorly studied for decades. However, CT infection has been accepted as a cause of prostatitis/CPPS and prostato-vesiculitis. There are still some controversies regarding subsequent prostate diseases (Mulcahy et al., 1987; Zdrodowska-Stefanow et al., 2000; Krause and Bohring, 2003; Falk et al., 2004). CT infection of male accessory sex glands may have a major impact on prostate pathology since it has been shown in mouse models that CT infects and may persist in the prostate, establishing an immune-privileged niche, avoiding the host immune response. This persistent infection might lead to a chronic infection, which in turn can result in impaired male fertility, besides being a reservoir of continuous transmission of the infection (Mackern-Oberti et al., 2011a). Regarding that point, research performed in murine models focusing on the pathogenic mechanisms involved in CT infection of the MGT is still very limited (Domingue and Hellstrom, 1998; Jantos et al., 1998; Stephens, 2003; Pal et al., 2004, 2009, 2010; Skerk et al., 2004; Wagenlehner et al., 2006; Cunningham and Beagley, 2008; Motrich et al., 2012).

5.1. CT infection of the MGT, sperm quality, and infertility

Infertility is a complex and frequent problem that affects 5% of men. The cause of infertility is unknown in approximately 55% of cases and infection of the MGT has been proposed as a risk factor for male infertility (Jungwirth et al., 2012). In this regard, the role of CT infection and male infertility has not yet been proven (Paavonen and Eggert-Kruse, 1999; Cunningham and Beagley, 2008; Joki-Korpela et al., 2009). Nevertheless, several studies have evaluated the association between CT infection and poor semen quality, but whether or not this has detrimental effects on male fertility is still uncertain (Cunningham and Beagley, 2008; Joki-Korpela et al., 2009). Some reports indicate that CT infection is associated with a decrease in sperm concentration and motility and also with altered semen pH and reduced volume of the ejaculate (Idahl et al., 2004; Mazzoli et al., 2010; La Vignera et al., 2011; Pajovic et al., 2012). Conversely, other studies have revealed no association between CT infection of the MGT and altered sperm quality (Weidner et al., 1996; Habermann and Krause, 1999; Ochsendorf, 1999; Ochsendorf et al., 1999; Vigil et al., 2002; Eggert-Kruse et al., 2003; Motrich et al., 2006; de Barbeyrac et al., 2006; Gdoura et al., 2007). In summary, the available evidence is conflicting and still makes it impossible to establish a clear relationship between CT infection and semen quality.

On the other hand, some in vitro studies have demonstrated that CT is able to interact with sperm cells, affecting their function and inducing apoptosis (Hosseinzadeh et al., 2001; Eley et al., 2005; Satta et al., 2006). Apoptosis of human sperm can be induced by in vitro incubation of human sperm cells with chlamydial LPS, which has a 550-fold greater spermicidal activity than *Escherichia coli* LPS (Galdiero et al., 1994; Hosseinzadeh et al., 2003). In addition, CT serovar E can attach to human spermatozoa and influence its function leading to premature capacitation (Hosseinzadeh et al., 2000). It has been shown that chlamydial LPS interacts with CD14 on the sperm surface, thus leading to increased production of reactive oxygen species and resulting in caspase-mediated apoptosis (Eley et al., 2005). Despite having obtained all these data from in vitro studies, a clear association between CT and sperm damage has not yet been corroborated by in vivo studies. Several reports have shown that CT infection of the MGT does not produce any alteration in semen quality (Weidner et al., 1996; Habermann and Krause, 1999; Ochsendorf et al., 1999; Vigil et al., 2002; Eggert-Kruse et al., 2003; Motrich et al., 2006, 2012; de Barbeyrac et al., 2006; Gdoura et al., 2007).

5.2. CT and seminal vesiculitis

In humans, whether CT can infect seminal vesicles and lead to subclinical inflammation and pathology is still unknown because it has been much less studied mainly because the infection produces neither severe clinical symptoms nor significant sequelae. Furuya et al. pioneered the study of seminal vesiculitis caused by CT (Furuya et al., 2004). They reported the presence of inflammation in the seminal vesicles of patients with acute epididymitis and also that CT was the causative pathogen most frequently detected in seminal vesicle fluid (Furuya et al., 2004). Furuya et al. also reported the first case of seminal vesiculitis prior to acute chlamydial epididymitis in a patient whose wife had been diagnosed with chlamydial cervicitis (Furuya et al., 2005). Recent reports have demonstrated that patients with urethritis were likely to have accompanying seminal vesiculitis (Furuya et al., 2009). Also, inflammatory findings, the presence of leukocytes, as well as seminal vesicle gland dilatation on the ipsilateral side to the epididymis, were frequently found in patients with epididymitis. These findings could also be found on the contralateral side, but much less frequently (Furuya et al., 2004, 2006, 2009). CT was most frequently detected in the fluid of dilated seminal vesicles, especially in patients with epididymitis aged 40 and younger. In addition,

vesiculitis-associated symptoms disappeared simultaneously with improvement in symptoms of epididymitis after antimicrobial treatment (Furuya et al., 2004). These findings strongly suggest that seminal vesicles were involved in the urogenital inflammation process. Furthermore, some researchers have proposed that chlamydial epididymitis mainly originates from seminal vesiculitis (Krishnan and Heal, 1991).

5.3. CT and chronic prostatitis

Prostatitis is highly prevalent in men (Schaeffer, 2006). It is a state of inflammation of the prostate often described as an infection of the prostate, but it can also be an inflammation with no signs of infection. Prostatitis syndromes are classified into four categories: acute (I); and chronic (II); non-bacterial prostatitis, chronic pelvic pain syndrome (CPPS) (III); and asymptomatic inflammatory prostatitis (IV); (Krieger et al., 1999). Although acute (I) and chronic (II) bacterial prostatitis have a clear etiology and patients are good responders to antimicrobial treatment, these types of prostatitis only encompass 10% of the cases seen in clinical practice (Habermacher et al., 2006). CPPS or category III prostatitis is the most common prostatitis syndrome, constituting 90–95% of prostatitis cases. Patients with chronic non-bacterial prostatitis (CP)/CPPS have no evidence of urinary tract infection making CP/CPPS a common disease of unclear etiology (Habermacher et al., 2006). The diagnosis of this syndrome still relies mainly on reported pain in the pelvic zone, perineum, rectum, and/or prostate and is often a diagnosis of exclusion. Patients with CPPS are empirically treated because of the uncertain etiology and pathogenesis, a fact that explains the limited efficacy of the recommended treatments (Habermacher et al., 2006). The etiology of CPPS remains unknown and three main hypotheses are currently under consideration. One such hypothesis postulates a microbial etiology due to a cryptic undetected microbial pathogen, suggesting that these patients should be classified as having bacterial prostatitis. In this regard, several studies have focused on CT as an etiological agent of CPPS (Shurbaji et al., 1988; Abdelatif et al., 1991; Corradi et al., 1995; Toth et al., 2000; Weidner et al., 2002; Skerk et al., 2002; Badalyan et al., 2003; Krieger and Riley, 2004; Ostaszewska-Puchalska et al., 2004; Wagenlehner et al., 2006; Motrich et al., 2006; Ouzounova-Raykova et al., 2010). The second hypothesis postulates an autoimmune response to prostate components because of the presence of inflammation in the absence of infectious agents (Motrich et al., 2007). A third hypothesis proposed by some researchers correlates this disease with neuronal and/or psychological factors, but lacks the support of substantial experimental and clinical evidence. For diagnostic purposes, microbiological analysis, as well as evaluation of the presence of leukocytes, is performed in prostatic expression, semen and/or urine pre- and post-prostatic massage samples. After negative conventional microbiological cultures, physicians perform microbiological, cell culture or molecular tests for specific pathogen diagnosis. Assays to detect antibodies specific for microorganisms in seminal plasma and serum may be valid for sero-epidemiological studies, but not for diagnostic purposes (Taylor-Robinson, 1997; Eley, 2011). The cross reactivity of antibodies between CT and some *Chlamydophila* species and the persistence of antibody titers over time impairs the possibility of performing an accurate diagnosis and makes it difficult to distinguish between past and current infections (Gijsen et al., 2001).

Several reports have documented the prevalence of CT infection in patients with chronic prostatitis (Shurbaji et al., 1988; Abdelatif et al., 1991; Corradi et al., 1995; Toth et al., 2000; Weidner et al., 2002; Skerk et al., 2002; Badalyan et al., 2003; Krieger and Riley, 2004; Ostaszewska-Puchalska et al., 2004; Wagenlehner et al., 2006; Motrich et al., 2006; Ouzounova-Raykova et al., 2010). As has also been demonstrated in epidemiological female studies, differences in prevalence rates between studies are related to the type of samples analyzed: urethral swab, first void urine, semen or expressed prostate secretion as well as the identification technique (Krause and Bohring, 2003). The prevalence of CT infection in CP/CPPS patients has been reported to range from 8.3 to 27% (Weidner et al., 2002; Cunningham and Beagley, 2008; Ouzounova-Raykova et al., 2010). Some authors have raised concerns about the reliability of the samples used in these studies (Weidner et al., 2002). They postulated that bacterial isolation from prostate diagnostic material (expressed prostatic secretions [EPS], urethral swabs, and/or urine after prostatic massage) presents a potential risk of contamination while going through the urethra, thus limiting the interpretation of the test. However, a number of studies indicate that semen/EPS specimens are often positive for CT in patients with negative urethral swabs (Corradi et al., 1995; Skerk et al., 2002; Ostaszewska-Puchalska et al., 2004; Gdoura et al., 2007). Also, pure prostatic biopsies from CP/CPPS have demonstrated the presence of CT in the absence of urethral infection (Toth et al., 2000; Krieger and Riley, 2004). These findings undoubtedly support the role of CT as a causative or triggering agent of CP/CPPS.

Chlamydia trachomatis infection of the prostate gland could cause inflammation within the prostatic tissue, thus impairing the normal functionality of the gland, and as is well known, prostate secretions account for up to 60% of the volume of seminal plasma (Lepor and Lawson, 1994). The main function of the prostate gland is the production of large amounts of soluble proteins and components that are secreted into the ejaculate. These proteins optimize the conditions for successful fertilization, providing an adequate medium for the survival of sperm and enhancing sperm motility in the female reproductive tract (Elzanaty et al., 2002).

A key enzyme, PSA, needed for the degradation of the seminal plasma inhibitor precursor has a positive impact on sperm motility. In addition, zinc derived from prostate secretion plays an important role in stabilizing sperm chromatin (Rivero et al., 2007). It could be speculated that an inflammation of the prostate gland as a consequence of CT infection could alter the gland's functionality, which results in consequences for male fertility. As stated above, the literature concerning this issue is controversial, with some reports arguing in favor of a positive relationship between chronic prostatitis induced by CT and altered semen quality (Idahl et al., 2004; Mazzoli et al., 2010; La Vignera et al.,

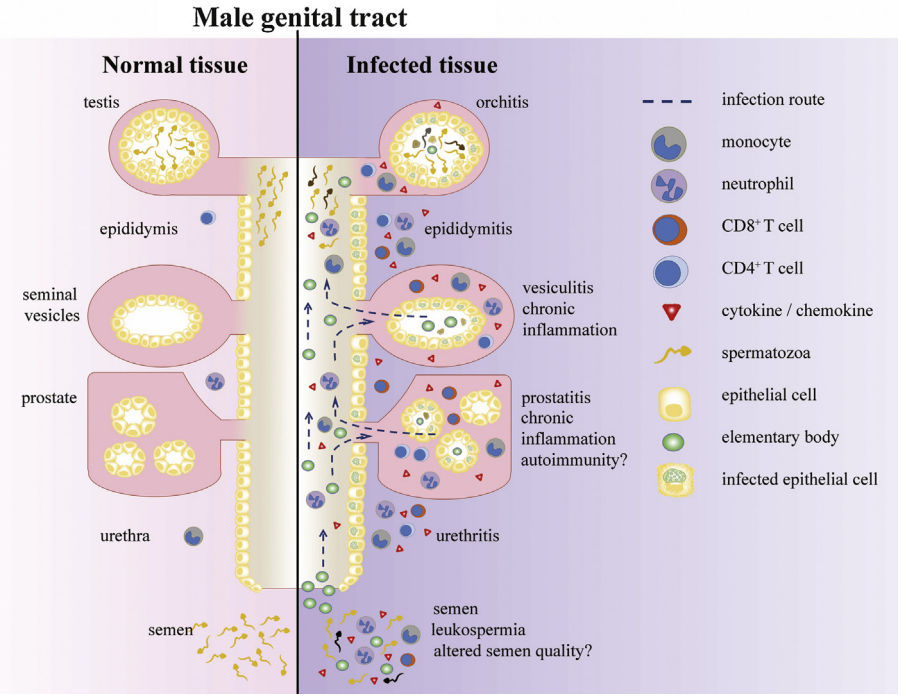

Fig. 1. Male genital tract (MGT) infection with CT. Upon exposure of the urethra to infective *Chlamydia trachomatis* (CT) elementary bodies (EBs), bacteria may produce an ascending infection in the genital tract, resulting in a diverse spectrum of clinical entities, such as acute urethritis, acute or chronic prostatitis, vesiculitis and orchitis, and related signs and symptoms: dysuria; polyuria; pelvic, perineum and testicular pain; leukospermia, etc. First, EBs interact with the urethral epithelial cells thus establishing the infection and leading to local recruitment of immune cells and inflammation. Then, the bacteria ascend to the upper genital tract and infect male accessory sex glands as prostate and seminal vesicles, where they may establish a niche and persist for extended periods of time, thus producing re-infection to other parts of the MGT. CT persistence in the male accessory sex glands recruits immune cells causing mild inflammation and epithelial cell desquamation that usually remains subclinical. CT can continue ascending to the epididymis and testis and also produce an established infection and mild inflammation. Although inflammation secondary to CT infection of the MGT causes tissue cell infiltration and the release of proinflammatory mediators to the ejaculate, that inflammation state seems to be mild and quenched by the seminal antioxidant capacity, thus not compromising male fertility. Also, the persistence of bacteria in the prostate could act as a trigger factor for autoimmune responses in susceptible individuals.

2011; Pajovic et al., 2012), whereas other reports support the idea that no alterations are produced in semen quality and male fertility (Weidner et al., 1996; Habermann and Krause, 1999; Ochsendorf et al., 1999; Vigil et al., 2002; Eggert-Kruse et al., 2003; Motrich et al., 2006; de Barbeyrac et al., 2006; Gdoura et al., 2007).

Overall, these results indicate that CT certainly infects different parts of the MGT, such as the urethra, seminal vesicles, prostate, epididymis, and testis, thus affecting both lower and upper MGT. An important feature is the chronic nature of this infection and also the recruitment of immune cells that in turn produce a local but mild inflammation that remains subclinical in most infected individuals (Fig. 1). Whether or not urethritis, vesiculitis, prostatitis, epididymitis, and orchitis caused by CT lead to significant detrimental effects on male fertility for certain is still controversial.

6. CT and innate immune response

Identification of CT-specific immune response and subsequent immune-mediated protection is an important priority in CT research, especially for vaccine progress. The development of in vitro assays has been crucial in the identification of *Chlamydia* antigens that are involved in innate immune recognition by epithelial cells and immune cells. Moreover, cell culture infection has provided information about CT and host cell interactions, mainly regarding the innate immune response. Mucosal barriers, mainly formed by epithelial cells, usually represent the first contact between host cells and invading pathogens. Epithelial cells are crucial in immune responses because they are the first in responding to invade pathogens such as CT, through their recognition by PRRs leading to proinflammatory cytokine production. These cytokine secretions, derived from infected epithelial cells or cells encountering CT antigens, may be crucial in activating resident leukocytes as well as recruiting circulating leukocytes and thus triggering a local immune response. Because CT has a special tropism for epithelial cells that express PRRs, the study of these cells and their receptors, as well as the identification of specific mediators released after CT infection (Table 1), are important for the design of protective vaccines as well as immune therapies against persistent CT infection (Darville and Hiltke, 2010).

Toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD) proteins are PRRs that recognize different pathogen-associated molecular patterns (PAMPs) found in bacteria, viruses, yeasts, and parasites. Some examples of PAMPs are peptidoglycans, LPS, flagellin,

Table 1
Chlamydia trachomatis genital infection and host immune response.

Type of study	Study sample			Infectious agent used	Receptors and mediators involved	Response/Main findings	Reference
	Gender	Origin	Specimen				
In vitro	Female	Mouse	Oviduct epithelial cells	*Chlamydia muridarum*	TLR2/MyD88	IL-6 secretion	Derbigny et al., 2005
			Oviduct epithelial cells	*Chlamydia muridarum*	TLR3?/IRF3/TRIF	IFNβ secretion	Derbigny et al., 2007
			Oviduct epithelial cells	*Chlamydia muridarum*	TLR3	IFNβ secretion	Derbigny et al., 2010
			Oviduct epithelial cells	*Chlamydia muridarum*	TLR3	IFNβ, Il-6, CXCL10, CXCL16, CCL5 secretion	Derbigny et al., 2012
		Rat	Uterine epithelial and stromal cells	*Chlamydia muridarum*	?	IL-1α and TNF-α secretion	Kaushic et al., 2000
		Human	Cervical epithelial cells	*Chlamydia trachomatis*	TLR2/MyD88	IL-8 secretion	O'Connell et al., 2006
			Cervical epithelial cells	*Chlamydia muridarum*	P2X7R	P2X7R ligation inhibits intracellular chlamydiae	Darville et al., 2007
			Cervical epithelial cells	*Chlamydia trachomatis*	NOD1/RIP2 other PPR/ERK	IL-8 secretion	Buchholz and Stephens, 2008
	Male	Mouse	Prostate, seminal vesicle and epididymis/vas deferens primary cell culture	*Chlamydia muridarum* and *Chlamydia trachomatis*	TLR2, TLR3, TLR4, TLR9	KC secretion	Mackern-Oberti et al., 2011b
			Prostate derived CD45+ and CD45− cells from MYD88 -/-, TLR2/TLR4 -/- and wild-type mice	*Chlamydia trachomatis*	TLR2, TLR4, other TLRs/MYD88	KC and CCL2 secretion by prostate derived CD45+ cells is dependent on TLR2/TLR4. KC and CCL2 secretion by prostate derived CD45− cells is dependent on other TLRs/MYD88 signaling cascade	Mackern Oberti et al., 2011c
		Rat	Prostate epithelial cells	*Chlamydia muridarum*	TLR2, TLR4, CD14/MYD88	Prostate epithelial cells are susceptible to CM infection and respond secreting NO, TNF-α, IL-1β, IL-6, MIP-1α, MIP-1β, IL-8, MCP1, RANTES, and IP10	Mackern-Oberti et al., 2006
		Human	Urethral and Prostate epithelial cells	*Chlamydia trachomatis*	TLR2?, TLR4?	High IL-1α secretion by urethral epithelial cells. High IL-6 and IL-8 secretion by prostate epithelial cells	Al-Mously and Eley, 2007
In vivo	Female	Mouse	TLR2 -/- and TLR4 -/- mice	*Chlamydia muridarum*	TLR2, TLR4	TNF-α and MIP-2 secretion impaired in TLR2 -/- mice. Similar infection in TLR2 -/-, TLR4 -/- and *wild type* mice. Less oviduct pathology in TLR2 -/- mice	Darville et al., 2003
			TLR2 -/-, TLR4 -/-, MYD88 -/- and IFNαβR -/- mice	*Chlamydia muridarum*	IFNα and βR TLR2, TLR4/MYD88	IFNβ and subsequent IP10 secretion occur through two different pathways, one is MYD88-dependent and the another is MYD88-independent	Nagarajan et al., 2005
			CCR5 -/- mice	*Chlamydia muridarum*	CCR5	Delayed infection resolution and reduced IP10, IFNγ, RANTES and TNFα secretion. Reduced oviduct pathology in CCR5 -/- mice	Barr et al., 2005
			NOD1 -/- mice	*Chlamydia muridarum*	NOD1/RIP2	Similar control of infection in NOD1 -/-, RIP2-/- and wild-type mice	Welter-Stahl et al., 2006

Table 1 (*Continued*)

Type of study	Study sample			Infectious agent used	Receptors and mediators involved	Response/Main findings	Reference
	Gender	Origin	Specimen				
			P2X7R -/- mice	*Chlamydia muridarum*	P2X7R	Impaired IFNβ secretion/higher infectious burden in P2X7R -/- mice	Darville et al., 2007
			IFNαβR -/- mice	*Chlamydia muridarum*	IFNαR and IFNβR	Reduced infection and enhanced specific CD4 T cell response in IFNαβR−/− mice	Nagarajan et al., 2008
			IL-1β -/- mice	*Chlamydia muridarum*	TLR2, TLR3, TLR4	IL-1β is secreted by macrophages and fibroblasts. Impaired bacterial clearance and less oviduct pathology in IL-1β -/- mice	Prantner et al., 2009
			TLR9 -/- mice	*Chlamydia muridarum*	TLR9	No different infection course in TLR9 -/- and wild-type mice. TLR9 -/- mice are protected from re-infection	Ouburg et al., 2009
			TLR3 -/- mice	*Chlamydia muridarum*	TLR3	IFNβ secretion impaired in TLR3 -/- mice	Derbigny et al., 2010
			MYD88 -/- mice	*Chlamydia muridarum*	MYD88	MYD88 -/- mice develop Th2 instead Th1/Th17 responses, more severe oviduct pathology and fail to control the infection	Chen et al., 2010
			IL-17R -/- and IFNγ -/- mice	*Chlamydia muridarum*	IL-17/IFNγ	Th1 and Th17 responses help in controlling the infection	Scurlock et al., 2011
			MYD88 -/- and TLR4/MYD88 -/- mice	*Chlamydia muridarum*	TLR4/MYD88	Most severe Infection in MYD88 -/- mice. Reduced CD4 T cell recruitment and IFNγ, IL-17, IL-18 and TNF-α secretion in MYD88 -/- mice. Dispensable role of TLR4	Nagarajan et al., 2011
			TLR3 -/- mice	*Chlamydia muridarum*	TLR3	Il-6 and CCL5 secretion diminished in TLR3 -/- mice	Derbigny et al., 2012
	Male	Mouse	C3H mice	*Chlamydia muridarum*	CT-specific Th1 cellular and humoral immune response	Infected mice develop strong CT-specific Th1 immune responses and infiltration in MGT organs, closely mimicking infection in humans	Pal et al., 2004
			Severe combined Immunodeficient (SCID) and *wild-type* C3H mice	*Chlamydia trachomatis*	CT-specific Th1 immune response	Immune-deficient mice fail to elicit protective Th1 cellular and humoral immune responses and are unable to control the infection	Pal et al., 2009
			Balb/c mice	*Chlamydia muridarum*	CT-specific Th1 immune response	Immunization of mice in the urethral meatus elicits protective Th1 cellular and humoral immune responses	Pal et al., 2010
		Mouse/rat	NOD mice and Wistar rats	*Chlamydia muridarum*	Th1 immune response	CM causes an ascending infection with special tropism for the prostate and this infection triggers a prostate-autoimmune response	Mackern-Oberti et al., 2011a
		Human	Penile urethral swabs from CT-infected, CT-uninfected, and non-CT-infected, nongonococcal urethritis-infected males	*Chlamydia trachomatis*	IL-8 and CT-specific IgA and IgG	High IL-8 and CT-specific IgA and IgG levels in CT-infected patients	Pate et al., 2001

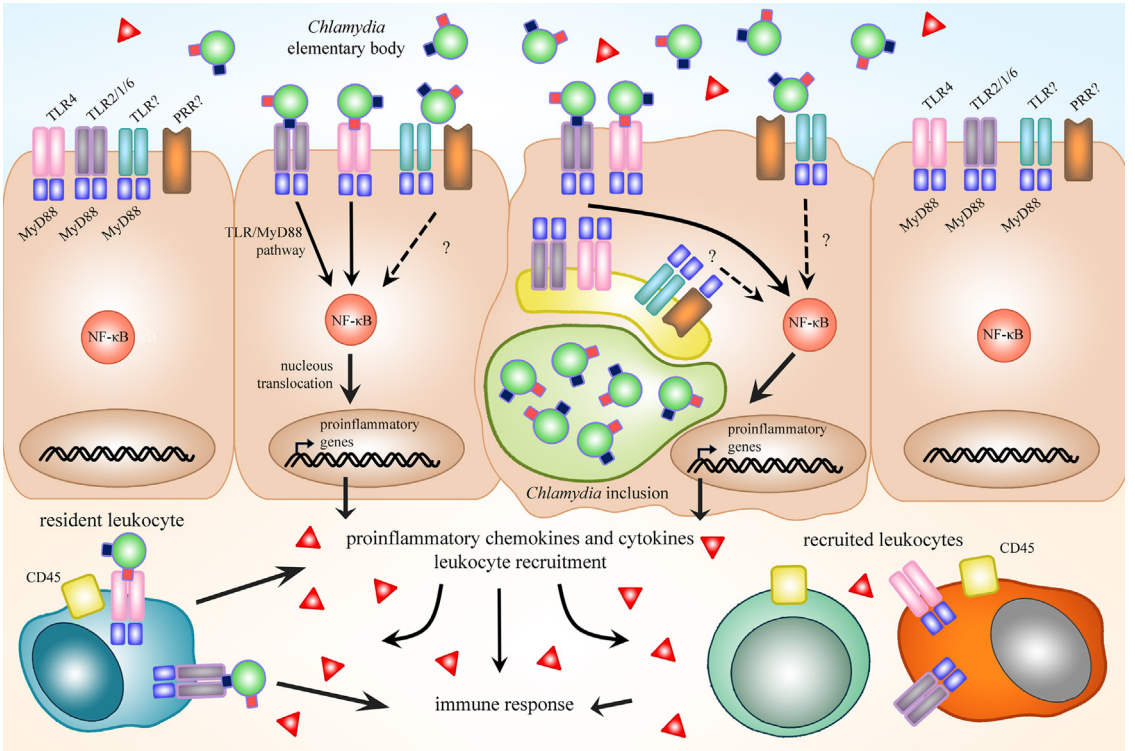

Fig. 2. Proposed mechanisms by which CT is recognized by the prostate gland cells. After ascending through the urethra, CT interacts and infects prostate epithelial cells. Extracellular CT EBs are recognized by prostate epithelial cells through toll-like receptor (TLR)1, 2, 4, and 6, thus triggering TLR signaling and NFκB nucleous translocation leading to proinflammatory genes expression and secretion. CT recognition by prostate epithelial cells is strictly dependent on the adaptor molecule MyD88, suggesting that other MyD88-dependent PRRs may be involved. It is believed that intracellular TLR2, TLR4, and TLR9 from epithelial cells may also play a role in CT recognition, thus initiating TLR signaling from this compartment. In addition, prostate resident CD45+ leukocytes may encounter and recognize CT mainly through TLR2, 1, 6, and 4 resulting in cytokine/chemokine secretion, immune cell recruitment and inflammation. Also, CT activated resident CD45+ leukocytes may interact with epithelial cells in order to augment and coordinate an effective specific immune response. Continuous activation of TLR on prostate epithelial cells and CD45+ leukocytes by a chronic CT infection may cause a state of chronic inflammation of the prostate that may impair the normal function of the gland and possibly trigger an autoimmune process in susceptible individuals.

dsRNA, and unmethylated bacterial DNA (O'Neill, 2002). PAMPs recognition by PRRs on epithelial cells mainly induces NFκB activation, leading to pro-inflammatory cytokines and chemokine production (Fig. 2). These immune factors mediate the recruitment of circulating leukocytes to the infected tissue and are involved in subsequent development of a specific immune response against the pathogen (O'Neill, 2002; Akira and Takeda, 2004). TLRs are commonly expressed in epithelial cells. To date, the expression of several PRRs, such as TLRs, NOD receptors, and other receptors like P2X7R, has been reported in the female reproductive tract (Fichorova et al., 2002; Pioli et al., 2004; Schaefer et al., 2004, 2005; Derbigny et al., 2005, 2007, 2010, 2012; Andersen et al., 2006; Welter-Stahl et al., 2006; Darville et al., 2007). TLRs have also been shown to be expressed in the MGT and are crucial for maintaining an appropriate microenvironment for sperm development, maturation, and storage and protection from invading pathogens (Krause and Bohring, 2003; Krause, 2008). It has been proposed that MGT exposition to PAMPs might impair male fertility, principally by the induction of a strong pro-inflammatory cytokine milieu and the production of reactive oxygen species that could induce oxidative damage to spermatozoa (Ochsendorf, 1999; Eley et al., 2005; Fraczek and Kurpisz, 2007). The expression of TLRs has been extensively studied in the testis, epididymis, prostate, and vas deferens (Nishimura and Naito, 2005; Riccioli et al., 2006; Palladino et al., 2007, 2008; Bhushan et al., 2008; Kundu et al., 2008; Mackern-Oberti et al., 2006, 2011b; Mackern Oberti et al., 2011c). TLR expression is different throughout the MGT, suggesting a tissue-specific immune surveillance. Furthermore, antimicrobial peptides were also found in the epididymis, testis, and prostate, showing the importance of epithelial cells in MGT innate immune response (Com et al., 2003). In contrast, the expression of TLRs on seminal vesicles has been poorly studied (Mackern-Oberti et al., 2011b).

Research on the innate immunity of MGT has also focused on cytokine production after bacteria or PAMPs stimulation in order to develop diagnostic tools for identifying infected tissue, as well as for a better understanding of MGT pathology, such as prostate cancer, urethritis, and prostatitis (Pate et al., 2001; Gatti et al., 2006, 2009; Al-Mously and Eley, 2007). It has been demonstrated that immortalized normal human urethral and prostate epithelial cells respond to CT producing IL1α and IL6 (Al-Mously and Eley, 2007). This cytokine response occurred in a tissue-specific pattern, suggesting a differential sensitivity

in the recognition of CT. These findings led researchers to speculate that evaluating cytokine levels could help to identify the tissues involved in MGT infections. Studies performed in our laboratory have demonstrated that primary cultures of murine MGT cells respond to PAMPs secreting proinflammatory chemokines. Prostate, seminal vesicles, and epididymis-vas deferens primary cell cultures respond to TLR2, TLR3, TLR4, and TLR9 ligands by secreting the keratinocyte-derived chemokine (KC) in a time-dependent manner (Mackern-Oberti et al., 2011b; Mackern Oberti et al., 2011c). To our knowledge, there are very few reports showing innate immune response of seminal vesicle cell cultures after TLRs ligand stimulation. These results undoubtedly show that different TLRs are expressed in MGT tissues. Furthermore, MGT primary cell cultures also respond to chlamydial LPS (Mackern-Oberti et al., 2011b). KC production after TLR ligand stimulation by primary cell cultures was different between tissues, suggesting specific sensitivity to TLR ligands or differential TLR expression among MGT tissues, thus delimiting/restricting locally immune response against pathogens, which is a common event in TLR biology.

Primary cultures from rat and mouse prostates and seminal vesicle epithelial cells (PECs) are susceptible to *Chlamydia muridarum* (CM) infection; this is a murine pathogen closely related to CT (Mackern-Oberti et al., 2006, 2011b; Mackern Oberti et al., 2011c). It has also been shown that prostate, seminal vesicle and epididymis-vas deferens primary cell cultures respond to CT infection, producing proinflammatory chemokines such as MCP1, MIP2, and KC (Mackern-Oberti et al., 2011b). In addition, we have demonstrated that rat PECs respond to CM by up-regulating pro-inflammatory cytokines and chemokines. Furthermore, TLR2 and TLR4, but not TLR5, were recruited to the chlamydial inclusion vicinity, suggesting the active role of these receptors in bacterial recognition and activation of PECs (Mackern-Oberti et al., 2006) (Fig. 2).

It has been shown that CT recognition and chemokine/cytokine response by HeLa cervical epithelial cells are also driven by NOD receptors (Buchholz and Stephens, 2008), although control of the infection seems to be similar in NOD1/RIP2-KO and wild-type mice (Welter-Stahl et al., 2006). Even though most studies on TLRs have focused on innate immune cells, the role of these receptors expressed on resident leukocytes within the MGT remains poorly understood (Bhushan et al., 2008). In this regard, our group demonstrated that CT is differentially recognized by prostate-derived CD45+ (resident leukocytes) and CD45− (epithelial and stromal) cells (Mackern Oberti et al., 2011c). Both prostate-derived cell populations expressed genes involved in TLR signaling respond to PAMPs and to CT infection. We also showed that primary cell cultures from MyD88 KO mice did not respond to CT producing KC, suggesting that CT recognition was critically affected in both prostate derived CD45+ and CD45− cells, in addition to the fact that TLRs are certainly involved in CT recognition. Furthermore, when cultures were performed with prostate-derived cells obtained from TLR2/TLR4 KO mice, we found that KC and MCP1 response by CT infected CD45− cells was partially independent of TLR2/TLR4, while CD45+ cells recognition was dependent on TLR2/TLR4, suggesting the differential role of these cells in the recognition and development of the immune response against CT (Mackern Oberti et al., 2011c). As stated above, most data about innate immune response in males and the FGT have focused on epithelial and immune cells. Nevertheless, it has been demonstrated that stromal cells from the genital tract also secrete multiple cytokines such as TNFα, IL1, IL6, IL8 after stimulation, indicating that the stromal compartment may also be an important player in the initial recognition and induction of the immune response (Kaushic et al., 2000; Hanada et al., 2003; Prantner et al., 2009).

It has been reported that dendritic cells and monocytes produce proinflammatory cytokines and chemokines in response to CT (Prebeck et al., 2003; Gervassi et al., 2004; Beagley et al., 2009). Identifying chlamydial antigens involved in MyD88/TLR dependent recognition and subsequent TLR-driven immune pathology is a major issue in *Chlamydia* research (Joyee and Yang, 2008). Several studies have shown that chlamydial LPS and HSP60 are associated with TLR2 and TLR4 recognition by monocytes or dendritic cells (Vabulas et al., 2001; Heine et al., 2003; da Costa et al., 2004). Moreover, macrophage infectivity potentiator (Mip) has been associated with TLR2 recognition (Bas et al., 2008). Although most reports state that TLR2 and TLR4 are the most important receptors in *Chlamydia* recognition, bacterial clearance is not affected in murine FGT infection of genitally infected TLR2- and/or TLR4-deficient mice (Darville et al., 2003; den Hartog et al., 2009; Nagarajan et al., 2011). However, we were unable to find any studies on MGT infection in TLR2/4 KO mice in the literature. It has been shown that FGT epithelial cells recognize *Chlamydia* mainly by TLR2 (Darville et al., 2003; Derbigny et al., 2005; O'Connell et al., 2006). In accordance, TLR2 KO female mice infected with CM showed poorer inflammatory cytokine responses and less inflammatory oviduct pathology compared with infected wild-type mice (Darville et al., 2003). Despite some discrepancies in the role of TLR2 and TLR4, the major and critical role of MyD88 in the immune response to CT is widely accepted, suggesting that, in addition to TLR2/TLR4, other PRRs are involved (Nagarajan et al., 2005; Chen et al., 2010). Regarding other PRRs, Ouburg et al. have shown that TLR9 may be involved in CT immune response. Using TLR9 KO mice, these authors showed that TLR9 KO mice were protected from re-infection compared with wild-type mice, suggesting the detrimental role of TLR9 in CT infection (Ouburg et al., 2009). On the other hand, it has recently been demonstrated that TLR3 also plays an important role in mounting an immune response to CT infection. It has been shown that infected TLR3-KO female mice demonstrated lower levels of vaginal IL-1β, IL-6, and CCL5 secretion, compromising their ability to control the infection (Derbigny et al., 2010, 2012). In addition, the role of other immune mediators like α and β interferons has been studied in female genital tract CT infection. It has been shown that infected IFNαβR-KO mice elicit a more robust specific CD4T cell response and more successfully clear the infection than their infected wild-type control counterparts (Nagarajan et al., 2008). These results demonstrated the detrimental role of type I interferons in mounting an effective immune response to control genital

CT infection. However, studies of CT infection of the MGT using either TLR3-KO or IFNαβR KO mice have not been reported to date.

All these data show that after CT infection, the local microenvironment of the host MGT may trigger different mechanisms to mount an immune response (Table 1). We postulated a scenario in which both resident leukocytes and epithelial cells would be activated in response to CT via TLR2, TLR4, and possibly other PPRs. Our results suggest that not only TLR2 and TLR4 on the surface membrane, but also these receptors recruited to the vicinity of chlamydial inclusion could participate in the activation of epithelial cells. This epithelial cell activation leads to production of inflammatory cytokines and chemokines, mediators that induce the local recruitment of immune cells, thus initiating the immune response against *Chlamydia* (Fig. 2). These observations suggest that epithelial/leukocyte crosstalk plays an important role in establishing a local immune response to CT infection in the male genital tract. This crosstalk may play a crucial role in perpetuating inflammation and leading to chronic disease. Studying the specific PRRs that mediate *Chlamydia* recognition in MGT cells would help to develop new strategies for the treatment of acute and chronic MGT pathology.

7. CT and adaptive immune response

Despite much work focusing in the immune response to CT, the reason why it takes so long to eradicate the bacterium remains unknown (Gottlieb et al., 2010). Geisler et al. analyzed the duration of untreated genital CT infection in men and showed that the probability of resolving an infection increases over time, with half of *Chlamydia* infections spontaneously resolving around 1 year after initial testing and the other half leading to persistent infections (Geisler, 2010). Persistent infection may be related to its developmental cycle and subsequent niche creation into epithelial cells, facts that would impair the immune response and favor the bacterium evading the immune system (Igietseme and Rank, 1991; Senior, 2012).

The inflammatory response is initiated and propagated by host epithelial cells, the primary target of CT infection (Stephens, 2003). The inflammatory response mounted to clear CT infection is also thought to drive reproductive tract pathogenesis and sequelae (Stephens, 2003). As stated above, epithelial cells secrete chemokines that recruit inflammatory leukocytes to the infected site, and cytokines that induce inflammation initiating an adaptive immune response (Rasmussen et al., 1997; Stephens, 2003). The continuous release of inflammatory mediators during chronic persistent infection could also lead to cell proliferation, tissue remodeling, and scarring. One paradigm states that genital tract pathogenesis subsequent to CT infection is due to the adaptive immune response to chlamydial antigens during persistent infection (Brunham and Peeling, 1994). It is believed that the CT-specific adaptive immune response induces collateral tissue damage or, if it fails to clear the infection, orchestrates inflammatory pathology during the ongoing chronic infection.

A wealth of data supports the central role of IFNγ-producing CD4+ T cells, mainly known as T helper 1 (Th1) cells, in the clearance of the infection (Cain and Rank, 1995; Morrison et al., 1995; Barr et al., 2005; Miyairi et al., 2010; Scurlock et al., 2011; Nagarajan et al., 2011). Most of these data come from studies on FGT, while reports studying the role of CD4+ T cells on CT infection of the MGT are scarce (Pal et al., 2004, 2009, 2010). The limited data available provided by studies about CT infection of human genital tract support the idea that some degree of protective immunity develops after infection; however, protection appears to be partial, as can be noticed after CT re-exposure. In animal models, the evidence strongly supports the development of protective immunity, but immunity against re-infection is protective only in the short term (Rank and Whittum-Hudson, 2010). Data obtained from animal models suggest that the key elements involved in the resolution of the infection by the immune response include: trafficking of *Chlamydia*-specific CD4+ T cells to the genital site; production of Th1-type cytokines including IFN-γ, which inhibits intracellular chlamydial replication; and the presence of IgG antibody at the genital site, which can neutralize extracellular EBs (Miyairi et al., 2010; Rank and Whittum-Hudson, 2010; Pal et al., 2004, 2009, 2010; Scurlock et al., 2011). Although CD8+ T cells and dendritic cells are also recruited to the infected site, they do not seem to play a central role in the clearance of the infection (Mittal et al., 2004; Agrawal et al., 2009a,b). Serum and genital IgA and IgG specific to CT antigens are usually detected in infected females (Pate et al., 2001; Ghaem-Maghami et al., 2003; Agrawal et al., 2007; Geisler, 2010), but their precise role in the resolution of the infection remains unclear.

Although published studies on human cellular immune response to CT infection are limited, they mainly show that human mucosal lymphocytes and peripheral blood mononuclear cells skewed toward Th1 rather than Th2 predominance (producing high levels of IFNγ and low levels of IL-10 after stimulation with chlamydial antigens) (Cohen et al., 2000; Debattista et al., 2002; Agrawal et al., 2009c). It is believed that skewing toward Th1 rather than Th2 protects from sequelae and is effective in the clearance of the infection. However, the specific profile of the immune response that leads to resolution rather than tissue damage promotion remains undefined. The Th17 subset was discovered years ago and has been implicated in several autoimmune diseases (Medzhitov, 2007). This new T helper cell subset might explain how ongoing inflammation causes damage, leading to sequelae without clearing the infection. Th17 cells induce proinflammatory responses and recruit large numbers of neutrophils to the infected site, but do not secrete IFN-γ. Cytokines that influence Th1 and Th2 lineage commitment, specifically IFNγ and IL-4, inhibit the development of Th17 cells (Kolls and Lindén, 2004; Cruz et al., 2006). Furthermore, IL-1, which is released from infected epithelial cells, has been shown to promote Th17 differentiation (Benwell and Lee, 2010). However, little is known about the precise role of Th17 cells in the immune response to CT infection. Experiments performed using IL-17 KO mice have shown that Th17 cells help to control CT genital infection, but there are other ongoing immune mechanisms that successfully deal with the infection in their absence (Scurlock et al., 2011).

All these data indicate that further studies focusing on the specific Th profile, as well immune-mediated pathogenesis, are promptly required to improve the whole understanding of CT infections.

8. Animal models of infection

Animal models of CT infection have started to shed light on the mechanisms involved in the protective immune response. In recent years, this field has experienced significant progress in the development of CT infection models focusing on MGT, mainly for evaluating new treatments and vaccine development (Pal et al., 2004, 2009, 2010) (Table 1). These experimental models allow the knowledge of the immunopathology of CT infection to be deepened; in addition, they aid the interpretation of clinical findings from studies on human infection (Brunham and Rey-Ladino, 2005). There are numerous studies of FGT infection with CT. Most of these focus on the effects of chronic inflammation associated with declining fertility rates, findings comparable to what is observed in female infections (Miyairi et al., 2010; Shao et al., 2012). Research performed in women as well as FGT infection models have allowed a significant improvement in understanding the physiopathology of underlying *Chlamydia*-associated female infertility. It is surprising that although similar prevalence and an extensively accepted role for *Chlamydia* in the development of male urethritis, epididymitis, orchitis, and prostatitis, research focusing on the pathogenic mechanisms involved in MGT infections is still very limited (Domingue and Hellstrom, 1998; Pal et al., 2004; Motrich et al., 2006, 2012; Cunningham and Beagley, 2008; Mackern-Oberti et al., 2011a,b; Mackern Oberti et al., 2011c). Until recent years, male animal models of CT infection were lacking, thus hindering the study of the infection course after natural exposition as well the adaptive immune response. Therefore, the importance of this pathogen in MGT pathology has been neglected for decades. One of the main drawbacks of the experimental models of MGT infection is the exposure route of the pathogen applied in the different studies. In experimental models of MGT infection, it is very important to induce infection through the most natural route of infection in order to obtain results comparable to what occurs in humans. Often, the infection route used is by direct inoculation into the target tissue. Jantos et al. performed one of the first studies focusing on the MGT, inoculating *Chlamydia psittaci* into the vas deferens and then looking for the bacteria in the prostate, epididymis, and testis (Jantos et al., 1998). Because of this artificial exposition, the results are only partially comparable to findings observed in humans. Pal et al. developed a model of MGT infection with CM in which infection develops after the inoculation of bacteria into the urinary meatus. In these studies, CM was isolated from the urethra, bladder, epididymis, and testis after inoculation (Pal et al., 2004, 2009, 2010), thus inducing an ascending infection that may have similar characteristics to human exposure and infection. The authors further evaluated the role of both adaptive and innate immunity against *Chlamydia* using C3H/SCID immunodeficient mice. Deficient animals did not control the infection, suggesting that the adaptive immune response is crucial for developing protective immunity against CT (Pal et al., 2010). Published reports studying the role of CD4+ T cells in CT infection of the MGT are scarce. Nonetheless, IFNγ-producing CD4+ T helper type 1 (Th1) responses have been suggested to be critical for bacterial clearance in FGT infection (Ito and Lyons, 1999; Rottenberg et al., 2002; McClarty et al., 2007). Studies about infection susceptibility and tropism of this bacterium to male accessory glands are hard to follow in detail. Our group developed a model of MGT infection with *Chlamydia* by the inoculation of CM into the urinary meatus of rats and mice. We showed that inoculation of CM into the meatus was capable of inducing an ascending infection of the urogenital tract, with the prostate and seminal vesicles highly susceptible to being affected, establishing a long-term infection, as the bacteria could be detected up to 80 days post-infection. Although CT has been extensively associated with human prostatitis; to our knowledge, this is the first report of a mice model of CT chronic infection affecting the prostate and seminal vesicles (Mackern-Oberti et al., 2011a; Motrich et al., 2012). As was also observed by Wang et al., histological lesions were observed in the urethra, bladder, seminal vesicles, and prostate after the infection. The lesions were more commonly observed in prostate tissue, indicating that this organ is highly susceptible in becoming injured by the infection (Wang et al., 2010; Mackern-Oberti et al., 2011a). They consisted of pro-inflammatory cell infiltrates, mostly composed of lymphocytes, with high numbers of both CD8+ and CD4+ cells. These findings are similar to the clinical observations from CT-infected women with PID (Shao et al., 2012). Furthermore, the special tropism of CT for the prostate and seminal vesicles in experimental models are also in agreement with clinical findings in CT-infected men, which have shown that CT infects male accessory glands and persists over a long period of time, constituting a pathogen reservoir (Mulcahy et al., 1987; Zdrodowska-Stefanow et al., 2000; Krause and Bohring, 2003). Moreover, the persistence of the bacterium in the prostate may favor its antibiotic treatment owing to the limited permeability of the prostate capsule to drugs (Wang et al., 2005). The generation of an in vivo infection model has also allowed us to analyze semen samples. We were able to evaluate sperm quality in semen and epididymal sperm samples; these data had not been previously reported in animal models. When we evaluated whether CM infection of the MGT was able to induce alterations in semen quality, we observed no differences between control and infected mice (Motrich et al., 2012). These findings support the hypothesis that the presence of CT in the MGT does not result in detectable alterations in semen quality parameters such as motility, sperm concentration, and viability. As ROS produced by the CT infection process would be the most important factor in damaging sperm cells, it is very likely that low-molecular weight and enzymatic antioxidants present in secretions of MGT play an active role in suppressing the deleterious effects of ROS, leading to a preservation of fertility (Ochsendorf et al., 1999; Abdul-Sater et al., 2010). However, it has been proposed (Cunningham and Beagley, 2008) that the lack of significant changes in semen quality does not ensure that CT infection of MGT does not lead

to alterations in male fertility. To go further and use our animal model of infection, we performed fertility studies by mating infected or control males with sexually mature fertile females. In agreement, we observed that the fertility potential of infected males was similar to that observed in control males, suggesting once again that MGT CT infection does not compromise male fertility (Motrich et al., 2012). These findings could occur because, despite the presence of inflammatory factors potentially harmful to sperm cells, the protective mechanisms of the MGT are able to neutralize the injurious factors produced by CT infection (Ochsendorf et al., 1999). All this information leads us to believe that despite having a CT chronic infection, no major alterations in semen quality and subfertility conditions are produced.

It is well known that infections can trigger or promote autoimmune processes. Some reports suggest that persistent chlamydial infection induces self immune responses against HSP60 by molecular mimicry of chlamydial HSP60, promoting the development of autoimmune arthritis, multiple sclerosis, atherosclerosis, vasculitis, diabetes, and thyroiditis (Bachmaier and Penninger, 2005; Swanborg et al., 2006; Cappello et al., 2009). Our group also demonstrated that genital CM infection of male Wistar rats favored the rupture of immune tolerance to prostate antigens in a significant number of animals (Mackern-Oberti et al., 2011a). When autoimmune-prone animals such as non-obese diabetic (NOD) mice were studied, we found that CM infection favored the development of autoimmune response to prostate antigens. The development of self-immune responses to prostate antigens in infected animals supports the hypothesis that infection with this bacterium in autoimmune-susceptible hosts may favor the development of organ-specific autoimmune disease (Mackern-Oberti et al., 2011a). To our knowledge, this is the only report that has associated chlamydial infection of the MGT with an autoimmune response to prostate antigens. These findings may help to improve the current understanding of human CP/CPPS pathology.

9. Conclusions

The special tropism of CT for the prostate during the course of an MGT infection favors its continuous presence, leading to the production of cytokines/chemokines that may induce a state of chronic inflammation. In genetically susceptible individuals, the chronic inflammation caused by CT may evolve at the onset of an autoimmune process. Understanding the local immune response to the infection in each tissue of the MGT, as well the crosstalk between resident leukocytes, epithelial and stromal cells would be crucial in inducing protective immunity, thus helping in the design of new therapeutic approaches to a *Chlamydia* vaccine. New animal models of infections, as well as the full characterization of MGT immunobiology, may help in disease prevention and intervention. It is important to note that even though chronic chlamydial infection presents with substantial inflammatory events, it does not produce alterations in semen quality and subfertility problems. Immune intervention targeting future identified pathology mediators remains a principal priority in chlamydial research for males, as well as female disease prevention and treatment. This review provides the evidence necessary to examine the interactions between CT and MGT tissues, mainly in the male accessory sex glands, which are of therapeutic relevance in microbiology and inflammation research.

References

Abdelatif, O.M., Chandler, F.W., McGuire, B.S., 1991. *Chlamydia trachomatis* in chronic abacterial prostatitis: demonstration by colorimetric in situ hybridization. Hum. Pathol. 22, 41–44.

Abdul-Sater, A.A., Saïd-Sadier, N., Lam, V.M., Singh, B., Pettengill, M.A., Soares, F., Tattoli, I., Lipinski, S., Girardin, S.E., Rosenstiel, P., Ojcius, D.M., 2010. Enhancement of reactive oxygen species production and chlamydial infection by the mitochondrial nod-like family member NLRX1. J. Biol. Chem. 285, 41637–41645.

Agrawal, T., Vats, V., Salhan, S., Mittal, A., 2007. Mucosal and peripheral immune responses to chlamydial heat shock proteins in women infected with *Chlamydia trachomatis*. Clin. Exp. Immunol. 148, 461–468.

Agrawal, T., Vats, V., Salhan, S., Mittal, A., 2009a. The mucosal immune response to *Chlamydia trachomatis* infection of the reproductive tract in women. J. Reprod. Immunol. 83, 173–178.

Agrawal, T., Vats, V., Wallace, P.K., Singh, A., Salhan, S., Mittal, A., 2009b. Recruitment of myeloid and plasmacytoid dendritic cells in cervical mucosa during *Chlamydia trachomatis* infection. Clin. Microbiol. Infect. 15, 50–59.

Agrawal, T., Gupta, R., Dutta, R., Srivastava, P., Bhengraj, A.R., Salhan, S., Mittal, A., 2009c. Protective or pathogenic immune response to genital chlamydial infection in women—a possible role of cytokine secretion profile of cervical mucosal cells. Clin. Immunol. 130, 347–354.

Akira, S., Takeda, K., 2004. Toll-like receptor signalling. Nat. Rev. Immunol. 4, 499–511.

Al-Mously, N., Eley, A., 2007. Interaction of *Chlamydia trachomatis* serovar E with male genital tract epithelium results in secretion of proinflammatory cytokines. J. Med. Microbiol. 56, 1025–1032.

Al-Zeer, M.A., Al-Younes, H.M., Braun, P.R., Zerrahn, J., Meyer, T.F., 2009. IFN-γ-inducible irga6 mediates host resistance against *Chlamydia trachomatis* via autophagy. PLoS ONE 4, e4588.

Andersen, J.M., Al-Khairy, D., Ingalls, R.R., 2006. Innate immunity at the mucosal surface: role of toll-like receptor 3 and toll-like receptor 9 in cervical epithelial cell responses to microbial pathogens. Biol. Reprod. 74, 824–831.

Anttila, T., Saikku, P., Koskela, P., Bloigu, A., Dillner, J., Ikäheimo, I., Jellum, E., Lehtinen, M., Lenner, P., Hakulinen, T., Närvänen, A., Pukkala, E., Thoresen, S., Youngman, L., Paavonen, J., 2001. Serotypes of chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA 285, 47–51.

Bachmaier, K., Penninger, J.M., 2005. Chlamydia and antigenic mimicry. Curr. Top. Microbiol. Immunol. 296, 153–163.

Badalyan, R.R., Fanarjyan, S.V., Aghajanyan, I.G., 2003. Chlamydial and ureaplasmal infections in patients with nonbacterial chronic prostatitis. Andrologia 35, 263–265.

Barr, E.L., Ouburg, S., Igietseme, J.U., Morré, S.A., Okwandu, E., Eko, F.O., et al., 2005. Host inflammatory response and development of complications of *Chlamydia trachomatis* genital infection in CCR5-deficient mice and subfertile women with the CCR5delta32 gene deletion. J. Microbiol. Immunol. Infect. 38, 244–254.

Bas, S., Neff, L., Vuillet, M., Spenato, U., Seya, T., Matsumoto, M., Gabay, C., 2008. The proinflammatory cytokine response to *Chlamydia trachomatis* elementary bodies in human macrophages is partly mediated by a lipoprotein, the macrophage infectivity potentiator, through TLR2/TLR1/TLR6 and CD14. J. Immunol. 180, 1158–1168.

Beagley, K.W., Huston, W.M., Hansbro, P.M., Timms, P., 2009. Chlamydial infection of immune cells: altered function and implications for disease. Crit. Rev. Immunol. 29, 275–305.

Beatty, W.L., Byrne, G.I., Morrison, R.P., 1994. Repeated and persistent infection with Chlamydia and the development of chronic inflammation and disease. Trends Microbiol. 2, 94–98.

Benwell, R.K., Lee, D.R., 2010. Essential and synergistic roles of IL1 and IL6 in human Th17 differentiation directed by TLR ligand-activated dendritic cells. Clin. Immunol. 134, 178–187.

Bhushan, S., Tchatalbachev, S., Klug, J., Fijak, M., Pineau, C., Chakraborty, T., Meinhardt, A., 2008. Uropathogenic *Escherichia coli* block MyD88-dependent and activate MyD88-independent signaling pathways in rat testicular cells. J. Immunol. 180, 5537–5547.

Brunham, R.C., Peeling, R.W., 1994. *Chlamydia trachomatis* antigens: role in immunity and pathogenesis. Infect. Agents Dis. 3, 218–233.
Brunham, R.C., Rey-Ladino, J., 2005. Immunology of Chlamydia infection: implications for a *Chlamydia trachomatis* vaccine. Nat. Rev. Immunol. 5, 149–161.
Buchholz, K.R., Stephens, R.S., 2008. The cytosolic pattern recognition receptor NOD1 induces inflammatory interleukin-8 during *Chlamydia trachomatis* infection. Infect. Immun. 76, 3150–3155.
Cain, T.K., Rank, R.G., 1995. Local Th1-like responses are induced by intravaginal infection of mice with the mouse pneumonitis biovar of *Chlamydia trachomatis*. Infect. Immun. 63, 1784–1789.
Cappello, F., Conway de Macario, E., Di Felice, V., Zummo, G., Macario, A.J.L., 2009. *Chlamydia trachomatis* infection and anti-Hsp60 immunity: the two sides of the coin. PLoS Pathog. 5, e1000552.
Carter, J.D., Hudson, A.P., 2010. The evolving story of Chlamydia-induced reactive arthritis. Curr. Opin. Rheumatol. 22, 424–430.
Chen, L., Lei, L., Chang, X., Li, Z., Lu, C., Zhang, X., Wu, Y., Yeh, I.-T., Zhong, G., 2010. Mice deficient in MyD88 develop a Th2-dominant response and severe pathology in the upper genital tract following *Chlamydia muridarum* infection. J. Immunol. 184, 2602–2610.
Chen, M.Y., Basil, D., 2003. Screening for genital *Chlamydia trachomatis* infection: are men the forgotten reservoir? Med. J. Aust. 179, 124–125.
Cohen, C.R., Nguti, R., Bukusi, E.A., Lu, H., Shen, C., Luo, M., Sinei, S., Plummer, F., Bwayo, J., Brunham, R.C., 2000. Human immunodeficiency virus type 1—infected women exhibit reduced interferon-γ secretion after *Chlamydia trachomatis* stimulation of peripheral blood lymphocytes. J. Infect. Dis. 182, 1672–1677.
Com, E., Bourgeon, F., Evrard, B., Ganz, T., Colleu, D., Jégou, B., Pineau, C., 2003. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. Biol. Reprod. 68, 95–104.
Corradi, G., Konkoly Thege, M., Pánovics, J., Molnár, G., Bodó, A., Frang, D., 1995. Is seminal fluid a suitable specimen for detecting chlamydial infection in men? Acta Microbiol. Immunol. Hung. 42, 389–394.
Cruz, A., Khader, S.A., Torrado, E., Fraga, A., Pearl, J.E., Pedrosa, J., Cooper, A.M., Castro, A.G., 2006. Cutting edge: IFN-γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. J. Immunol. 177, 1416–1420.
Cunningham, K.A., Beagley, K.W., 2008. Male genital tract chlamydial infection: implications for pathology and infertility. Biol. Reprod. 79, 180–189.
da Costa, C.U.P., Wantia, N., Kirschning, C.J., Busch, D.H., Rodriguez, N., Wagner, H., Miethke, T., 2004. Heat shock protein 60 from Chlamydia pneumoniae elicits an unusual set of inflammatory responses via toll-like receptor 2 and 4 in vivo. Eur. J. Immunol. 34, 2874–2884.
Darville, T., Hiltke, T.J., 2010. Pathogenesis of genital tract disease due to *Chlamydia trachomatis*. J. Infect. Dis. 201, 114–125.
Darville, T., O'Neill, J.M., Andrews, C.W., Nagarajan, U.M., Stahl, L., Ojcius, D.M., 2003. Toll-like receptor-2, but not toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. J. Immunol. 171, 6187–6197.
Darville, T., Welter-Stahl, L., Cruz, C., Sater, A.A., Andrews Jr., C.W., Ojcius, D.M., 2007. Effect of the purinergic receptor P2X7 on Chlamydia infection in cervical epithelial cells and vaginally infected mice. J. Immunol. 179, 3707–3714.
Debattista, J., Timms, P., Allan, J., Allan, J., 2002. Reduced levels of gamma-interferon secretion in response to chlamydial 60 kDa heat shock protein amongst women with pelvic inflammatory disease and a history of repeated *Chlamydia trachomatis* infections. Immunol. Lett. 81, 205–210.
de Barbeyrac, B., Papaxanthos-Roche, A., Mathieu, C., Germain, C., Brun, J.L., Gachet, M., Mayer, G., Bébéar, C., Chene, G., Hocké, C., 2006. *Chlamydia trachomatis* in subfertile couples undergoing an in vitro fertilization program: a prospective study. Eur. J. Obstet. Gynecol. Reprod. Biol. 129, 46–53.
den Hartog, J.E., Land, J.A., Stassen, F.R.M., Kessels, A.G.H., Bruggeman, C.A., 2005. Serological markers of persistent *C. trachomatis* infections in women with tubal factor subfertility. Hum. Reprod. 20, 986–990.
den Hartog, J.E., Morré, S.A., Land, J.A., 2006. *Chlamydia trachomatis*-associated tubal factor subfertility: immunogenetic aspects and serological screening. Hum. Reprod. Update 12, 719–730.
den Hartog, J.E., Lyons, J.M., Ouburg, S., Fennema, J.S., de Vries, H.J., Bruggeman, C.A., Ito, J.I., Peña, A.S., Land, J.A., Morré, S.A., 2009. TLR4 in *Chlamydia trachomatis* infections: knockout mice, STD patients and women with tubal factor subfertility. Drugs Today 45, 75–82.
Derbigny, W.A., Kerr, M.S., Johnson, R.M., 2005. Pattern recognition molecules activated by *Chlamydia muridarum* infection of cloned murine oviduct epithelial cell lines. J. Immunol. 175, 6065–6075.
Derbigny, W.A., Hong, S.-C., Kerr, M.S., Temkit, M., Johnson, R.M., 2007. *Chlamydia muridarum* infection elicits a beta interferon response in murine oviduct epithelial cells dependent on interferon regulatory factor 3 and TRIF. Infect. Immun. 75, 1280–1290.
Derbigny, W.A., Johnson, R.M., Toomey, K.S., Ofner, S., Jayarapu, K., 2010. The *Chlamydia muridarum*-induced IFN-β response is TLR3-dependent in murine oviduct epithelial cells. J. Immunol. 185, 6689–6697.
Derbigny, W.A., Shobe, L.R., Kamran, J.C., Toomey, K.S., Ofner, S., 2012. Identifying a role for toll-like receptor 3 in the innate immune response to *Chlamydia muridarum* infection in murine oviduct epithelial cells. Infect. Immun. 80, 254–265.
Domingue, G.J., Hellstrom, W.J.G., 1998. Prostatitis. Clin. Microbiol. Rev. 11, 604–613.
Eggert-Kruse, W., Rohr, G., Kunt, B., Meyer, A., Wondra, J., Strowitzki, T., Petzoldt, D., 2003. Prevalence of *Chlamydia trachomatis* in subfertile couples. Fertil. Steril. 80, 660–663.
Eley, A., 2011. How to detect *Chlamydia trachomatis* in males? J. Androl. 32, 15–22.
Eley, A., Pacey, A.A., Galdiero, M., Galdiero, M., Galdiero, F., 2005. Can *Chlamydia trachomatis* directly damage your sperm? Lancet Infect. Dis. 5, 53–57.
Elzanaty, S., Richthoff, J., Malm, J., Giwercman, A., 2002. The impact of epididymal and accessory sex gland function on sperm motility. Hum. Reprod. 17, 2904–2911.
Falk, L., Fredlund, H., Jensen, J.S., 2004. Symptomatic urethritis is more prevalent in men infected with *Mycoplasma genitalium* than with *Chlamydia trachomatis*. Sex. Transm. Infect. 80, 289–293.
Fichorova, R.N., Cronin, A.O., Lien, E., Anderson, D.J., Ingalls, R.R., 2002. Response to *Neisseria gonorrhoeae* by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling. J. Immunol. 168, 2424–2432.
Fraczek, M., Kurpisz, M., 2007. Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. J. Androl. 28, 325–333.
Furuya, R., Takahashi, S., Furuya, S., Kunishima, Y., Takeyama, K.O.H., Tsukamoto, T., 2004. Is seminal vesiculitis a discrete disease entity? Clinical and microbiological study of seminal vesiculitis in patients with acute epididymitis. J. Urol. 171, 1550–1553.
Furuya, R., Takahashi, S., Furuya, S., Takeyama, K., Tsukamoto, T., 2005. A patient with seminal vesiculitis prior to acute chlamydial epididymitis. J. Infect. Chemother. 11, 250–252.
Furuya, R., Takahashi, S., Furuya, S., Takeyama, K., Masumori, N., Tsukamoto, T., 2006. Chlamydial seminal vesiculitis without symptomatic urethritis and epididymitis. Int. J. Urol. 13, 466–467.
Furuya, R., Takahashi, S., Furuya, S., Saitoh, N., Ogura, H., Kurimura, Y., Tsukamoto, T., 2009. Is urethritis accompanied by seminal vesiculitis? Int. J. Urol. 16, 628–631.
Galdiero, F., Sommese, L., Gorga, F., Galdiero, E., Rizzo, A., Ajello, M., 1994. Toxic effect on human spermatozoa by *Chlamydia trachomatis* purified lipopolysaccharide. FEMS Microbiol. Lett. 115, 197–200.
Gatti, G., Rivero, V., Motrich, R.D., Maccioni, M., 2006. Prostate epithelial cells can act as early sensors of infection by up-regulating TLR4 expression and proinflammatory mediators upon LPS stimulation. J. Leukoc. Biol. 79, 989–998.
Gatti, G., Quintar, A.A., Andreani, V., Nicola, J.P., Maldonado, C.A., Masini-Repiso, A.M., Rivero, V.E., Maccioni, M., 2009. Expression of toll-like receptor 4 in the prostate gland and its association with the severity of prostate cancer. Prostate 69, 1387–1397.
Gdoura, R., Kchaou, W., Chaari, C., Znazen, A., Keskes, L., Rebai, T., Hammami, A., 2007. *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. BMC Infect. Dis. 7, 129.
Geisler, W.M., 2010. Duration of untreated, uncomplicated *Chlamydia trachomatis* genital infection and factors associated with chlamydia resolution: a review of human studies. J. Infect. Dis. 201, 104–113.
Gervassi, A., Alderson, M.R., Suchland, R., Maisonneuve, J.F., Grabstein, K.H., Probst, P., 2004. Differential regulation of inflammatory cytokine secretion by human dendritic cells upon *Chlamydia trachomatis* infection. Infect. Immun. 72, 7231–7239.
Ghaem-Maghami, S., Ratti, G., Ghaem-Maghami, M., Comanducci, M., Hay, P.E., Bailey, R.L., Mabey, D.C.W., Whittle, H.C., Ward, M.E., Lewis, D.J.M., 2003. Mucosal and systemic immune responses to plasmid protein pgp3 in patients with genital and ocular *Chlamydia trachomatis* infection. Clin. Exp. Immunol. 132, 436–442.
Gijsen, A.P., Land, J.A., Goossens, V.J., Leffers, P., Bruggeman, C.A., Evers, J.L.H., 2001. Chlamydia pneumoniae and screening for tubal factor subfertility. Hum. Reprod. 16, 487–491.
Gonzales, G.F., Muñoz, G., Sánchez, R., Henkel, R., Gallegos-Avila, G., Díaz-Gutierrez, O., Vigil, P., Vásquez, F., Kortebani, G., Mazzolli, A.,

Bustos-Obregón, E., 2004. Update on the impact of *Chlamydia trachomatis* infection on male fertility. Andrologia 36, 1–23.
Gottlieb, S.L., Berman, S.M., Low, N., 2010. Screening and treatment to prevent sequelae in women with *Chlamydia trachomatis* genital infection: how much do we know? J. Infect. Dis. 201, 156–167.
Götz, H., Lindbäck, J., Ripa, T., Ramstedt, K., Ekdahl, K., Arneborn, M., 2002. Is the increase in notifications of *Chlamydia trachomatis* infections in Sweden the result of changes in prevalence, sampling frequency or diagnostic methods? Scand. J. Infect. Dis. 34, 28–34.
Habermacher, G.M., Chason, J.T., Schaeffer, A.J., 2006. Prostatitis/chronic pelvic pain syndrome. Annu. Rev. Med. 57, 195–206.
Habermann, B., Krause, W., 1999. Altered sperm function or sperm antibodies are not associated with chlamydial antibodies in infertile men with leucocytospermia. J. Eur. Acad. Dermatol. Venereol. 12, 25–29.
Hanada, H., Ikeda-Dantsuji, Y., Naito, M., Nagayama, A., 2003. Infection of human fibroblast-like synovial cells with *Chlamydia trachomatis* results in persistent infection and interleukin-6 production. Microb. Pathog. 34, 57–63.
Harris, S.R., Clarke, I.N., Seth-Smith, H.M.B., Solomon, A.W., Cutcliffe, L.T., Marsh, P., et al., 2012. Whole-genome analysis of diverse *Chlamydia trachomatis* strains identifies phylogenetic relationships masked by current clinical typing. Nat. Genet. 44, 413–419.
Heine, H., Müller-Loennies, S., Brade, L., Lindner, B., Brade, H., 2003. Endotoxic activity and chemical structure of lipopolysaccharides from *Chlamydia trachomatis* serotypes E and L2 and *Chlamydophila psittaci* 6BC. Eur. J. Biochem. 270, 440–450.
Hosseinzadeh, S., Brewis, I.A., Pacey, A.A., Moore, H.D.M., Eley, A., 2000. Coincubation of human spermatozoa with *Chlamydia trachomatis* in vitro causes increased tyrosine phosphorylation of sperm proteins. Infect. Immun. 68, 4872–4876.
Hosseinzadeh, S., Brewis, I.A., Eley, A., Pacey, A.A., 2001. Co-incubation of human spermatozoa with *Chlamydia trachomatis* serovar E causes premature sperm death. Hum. Reprod. 16, 293–299.
Hosseinzadeh, S., Pacey, A.A., Eley, A., 2003. *Chlamydia trachomatis*-induced death of human spermatozoa is caused primarily by lipopolysaccharide. J. Med. Microbiol. 52, 193–200.
Idahl, A., Boman, J., Kumlin, U., Olofsson, J.I., 2004. Demonstration of *Chlamydia trachomatis* IgG antibodies in the male partner of the infertile couple is correlated with a reduced likelihood of achieving pregnancy. Hum. Reprod. 19, 1121–1126.
Igietseme, J.U., Rank, R.G., 1991. Susceptibility to reinfection after a primary chlamydial genital infection is associated with a decrease of antigen-specific T cells in the genital tract. Infect. Immun. 59, 1346–1351.
Ito, J.I., Lyons, J.M., 1999. Role of gamma interferon in controlling murine chlamydial genital tract infection. Infect. Immun. 67, 5518–5521.
Iwasaki, A., 2010. Antiviral immune responses in the genital tract: clues for vaccines. Nat. Rev. Immunol. 10, 699–711.
Jantos, C.A., Augustin, J., Durchfeld-Meyer, B., Baumgärtner, W., Schiefer, H.G., 1998. Experimental genital tract infection with *Chlamydia psittaci* (GPIC agent) in male rats. Infection 26, 126–130.
Joki-Korpela, P., Sahrakorpi, N., Halttunen, M., Surcel, H.-M., Paavonen, J., Tiitinen, A., 2009. The role of *Chlamydia trachomatis* infection in male infertility. Fertil. Steril. 91, 1448–1450.
Joyee, A.G., Yang, X., 2008. Role of toll-like receptors in immune responses to chlamydial infections. Curr. Pharm. Des. 14, 593–600.
Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G., Krausz, C., 2012. European Association of Urology Guidelines on Male Infertility: the 2012 update. Eur. Urol. 62, 324–332.
Kalwij, S., French, S., Mugezi, R., Baraitser, P., 2012. Using educational outreach and a financial incentive to increase general practices' contribution to chlamydia screening in South-East London 2003–2011. BMC Public Health 12, 802.
Kaushic, C., Grant, K., Crane, M., Wira, C.R., 2000. Infection of polarized primary epithelial cells from rat uterus with *Chlamydia trachomatis*: cell–cell interaction and cytokine secretion. Am. J. Reprod. Immunol. 44, 73–79.
Kolls, J.K., Lindén, A., 2004. Interleukin-17 family members and inflammation. Immunity 21, 467–476.
Krause, W., 2008. Male accessory gland infection. Andrologia 40, 113–116.
Krause, W., Bohring, C., 2003. Male infertility and genital chlamydial infection: victim or perpetrator? Andrologia 35, 209–216.
Krieger, J.N., Riley, D.E., 2004. Chronic prostatitis: Charlottesville to Seattle. J. Urol. 172, 2557–2560.
Krieger, J.N., Nyberg, L., Nickel, J.C., 1999. NIH consensus definition and classification of prostatitis. JAMA 282, 236–237.
Krishnan, R., Heal, M., 1991. Study of the seminal vesicles in acute epididymitis. Br. J. Urol. 67, 632–637.
Kundu, S.D., Lee, C., Billips, B.K., Habermacher, G.M., Zhang, Q., Liu, V., Wong, L.Y., Klumpp, D.J., Thumbikat, P., 2008. The toll-like receptor pathway: a novel mechanism of infection-induced carcinogenesis of prostate epithelial cells. Prostate 68, 223–229.
La Vignera, S., Vicari, E., Condorelli, R.A., D'Agata, R., Calogero, A.E., 2011. Male accessory gland infection and sperm parameters (review). Int. J. Androl. 34, e330–e347.
Lepor, H., Lawson, R.K., 1994. Enfermedades de la próstata. Editorial Panamericana. Bs As, Argentina.
Lewis, D., Newton, D., Guy, R., Ali, H., Chen, M., Fairley, C., Hocking, J., 2012. The prevalence of *Chlamydia trachomatis* infection in Australia: a systematic review and meta-analysis. BMC Infect. Dis. 12, 113.
Mackern-Oberti, J.P., Maccioni, M., Cuffini, C., Gatti, G., Rivero, V.E., 2006. Susceptibility of prostate epithelial cells to *Chlamydia muridarum* infection and their role in innate immunity by recruitment of intracellular toll-like receptors 4 and 2 and MyD88 to the inclusion. Infect. Immun. 74, 6973–6981.
Mackern-Oberti, J.P., Motrich, R.D., Breser, M.L., Cejas, H., Cuffini, C., Maccioni, M., Rivero, V.E., 2011a. Male rodent genital tract infection with *Chlamydia muridarum*: persistence in the prostate gland that triggers self-immune reactions in genetically susceptible hosts. J. Urol. 186, 1100–1106.
Mackern-Oberti, J.P., Maccioni, M., Breser, M.L., Eley, A., Miethke, T., Rivero, V.E., 2011b. Innate immunity in the male genital tract: *Chlamydia trachomatis* induces keratinocyte-derived chemokine production in prostate, seminal vesicle and epididymis/vas deferens primary cultures. J. Med. Microbiol. 60, 307–316.
Mackern Oberti, J.P., Breser, M.L., Nuñez, N., Maccioni, M., Rodríguez, N., Wantia, N., Ertl, T., Miethke, T., Rivero, V.E., 2011c. Chemokine response induced by *Chlamydia trachomatis* in prostate derived CD45+ and CD45− cells. Reproduction 142, 427–437.
Marrazzo, J.M., Martin, D.H., 2007. Management of women with cervicitis. Clin. Infect. Dis. 44, 102–110.
Mazzoli, S., Cai, T., Addonisio, P., Bechi, A., Mondaini, N., Bartoletti, R., 2010. *Chlamydia trachomatis* infection is related to poor semen quality in young prostatitis patients. Eur. Urol. 57, 708–714.
McClarty, G., Caldwell, H.D., Nelson, D.E., 2007. Chlamydial interferon gamma immune evasion influences infection tropism. Curr. Opin. Microbiol. 10, 47–51.
Medzhitov, R., 2007. Recognition of microorganisms and activation of the immune response. Nature 449, 819–826.
Mittal, A., Rastogi, S., Reddy, B.S., Verma, S., Salhan, S., Gupta, E., 2004. Enhanced immunocompetent cells in chlamydial cervicitis. J. Reprod. Med. 49, 671–677.
Miyairi, I., Ramsey, K.H., Patton, D.L., 2010. Duration of untreated chlamydial genital infection and factors associated with clearance: review of animal studies. J. Infect. Dis. 201, 96–103.
Morrison, R.P., Feilzer, K., Tumas, D.B., 1995. Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in *Chlamydia trachomatis* genital tract infection. Infect. Immun. 63, 4661–4668.
Motrich, R.D., Cuffini, C., Mackern Oberti, J.P., Maccioni, M., Rivero, V.E., 2006. *Chlamydia trachomatis* occurrence and its impact on sperm quality in chronic prostatitis patients. J. Infect. 53, 175–183.
Motrich, R.D., Maccioni, M., Riera, C.M., Rivero, V.E., 2007. Autoimmune prostatitis: state of the art. Scand. J. Immunol. 66, 217–227.
Motrich, R.D., Sanchez, L., Maccioni, M., Mackern Oberti, J.P., Rivero, V.E., 2012. Male rat genital tract infection with *Chlamydia muridarum* has no significant consequence on male fertility. J. Urol. 183, 1213–1220.
Mulcahy, F.M., Bignell, C.J., Rajakumar, R., Waugh, M.A., Hetherington, J.W., Ewing, R., Whelan, P., 1987. Prevalence of chlamydial infection in acute epididymo-orchitis. Genitourin. Med. 63, 16–18.
Mund, M., Sander, G., Potthoff, P., Schicht, H., Matthias, K., 2008. Introduction of *Chlamydia trachomatis* screening for young women in Germany. J. Dtsch. Dermatol. Ges. 6, 1032–1037.
Mylonas, I., 2012. Female genital *Chlamydia trachomatis* infection: where are we heading? Arch. Gynecol. Obstet. 285, 1271–1285.
Nagarajan, U.M., Ojcius, D.M., Stahl, L., Rank, R.G., Darville, T., 2005. *Chlamydia trachomatis* induces expression of IFN-γ-inducible protein 10 and IFN-β independent of TLR2 and TLR4, but largely dependent on MyD88. J. Immunol. 175, 450–460.
Nagarajan, U.M., Prantner, D., Sikes, J.D., Andrews Jr., C.W., Goodwin, A.M., Nagarajan, S., Darville, T., 2008. Type I interferon signaling exacerbates *Chlamydia muridarum* genital infection in a murine model. Infect. Immun. 10, 4642-4628.
Nagarajan, U.M., Sikes, J., Prantner, D., Andrews Jr., C.W., Frazer, L., Goodwin, A., Snowden, J.N., Darville, T., 2011. MyD88 deficiency leads to decreased NK cell gamma interferon production and T cell recruitment during *Chlamydia muridarum* genital tract infection, but

a predominant Th1 response and enhanced monocytic inflammation are associated with infection resolution. Infect. Immun. 79, 486–498.
Nishimura, M., Naito, S., 2005. Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. Biol. Pharm. Bull. 28, 886–892.
Ochsendorf, F.R., 1999. Infections in the male genital tract and reactive oxygen species. Hum. Reprod. Update 5, 399–420.
Ochsendorf, F.R., Özdemir, K., Rabenau, H., Fenner, T., Oremek, R., Milbradt, R., Doerr, H.W., 1999. *Chlamydia trachomatis* and male infertility: chlamydia-IgA antibodies in seminal plasma are *C. trachomatis* specific and associated with an inflammatory response. J. Eur. Acad. Dermatol. Venereol. 12, 143–152.
Ostaszewska-Puchalska, I., Zdrodowska-Stefanow, B., Badyda, J., Bułhak-Kozioł, V., Puciło, K., Darewicz, B., 2004. Antichlamydial antibodies in the serum and expressed prostatic secretion in prostatitis. Arch. Immunol. Ther. Exp. 52, 277–283.
Ouburg, S., Lyons, J.M., Land, J.A., den Hartog, J.E., Fennema, J.S., de Vries, H.J., Bruggeman, C.A., Ito, J.I., Peña, A.S., Lundberg, P.S., Morré, S.A., 2009. TLR9 KO mice, haplotypes and CPG indices in *Chlamydia trachomatis* infection. Drugs Today 2, 83–93.
Ouzounova-Raykova, V., Ouzounova, I., Mitov, I.G., 2010. *Chlamydia trachomatis* be an aetiological agent of chronic prostatic infection? Andrologia 42 (May), 176–181.
O'Connell, C.M., Ionova, I.A., Quayle, A.J., Visintin, A., Ingalls, R.R., 2006. Localization of TLR2 and MyD88 to *Chlamydia trachomatis* inclusions: evidence for signalling by intracellular TLR2 during infection with an obligate intracellular pathogen. J. Biol. Chem. 281, 1652–1659.
O'Neill, L.A.J., 2002. Toll-like receptor signal transduction and the tailoring of innate immunity: a role for Mal? Trends Immunol. 23, 296–300.
Paavonen, J., 2011. *Chlamydia trachomatis* infections of the female genital tract: state of the art. Ann. Med. 44, 18–28.
Paavonen, J., Eggert-Kruse, W., 1999. *Chlamydia trachomatis*: impact on human reproduction. Hum. Reprod. Update 5, 433–447.
Pajovic, B., Radojevic, N., Vukovic, M., Stjepcevic, A., 2012. Semen analysis before and after antibiotic treatment of asymptomatic chlamydia- and ureaplasma-related pyospermia. Andrologia 44, 18–28.
Pal, S., Peterson, E.M., de la Maza, L.M., 2004. New murine model for the study of *Chlamydia trachomatis* genitourinary tract infections in males. Infect. Immun. 72, 4210–4216.
Pal, S., Sarcon, A.K., de la Maza, L.M., 2009. C3H male mice with severe combined immunodeficiency cannot clear a urethral infection with a human serovar of *Chlamydia trachomatis*. Infect. Immun. 77, 5602–5607.
Pal, S., Sarcon, A.K., de la Maza, L.M., 2010. A new murine model for testing vaccines against genital *Chlamydia trachomatis* infections in males. Vaccine 28, 7606–7612.
Palladino, M.A., Johnson, T.A., Gupta, R., Chapman, J.L., Ojha, P., 2007. Members of the toll-like receptor family of innate immunity pattern-recognition receptors are abundant in the male rat reproductive tract. Biol. Reprod. 76, 958–964.
Palladino, M.A., Savarese, M.A., Chapman, J.L., Dughi, M.-K., Plaska, D., 2008. ORIGINAL ARTICLE: localization of toll-like receptors on epididymal epithelial cells and spermatozoa. Am. J. Reprod. Immunol. 60, 541–555.
Pate, M.S., Hedges, S.R., Sibley, D.A., Russell, M.W., Hook, E.W., Mestecky, J., 2001. Urethral cytokine and immune responses in *Chlamydia trachomatis*-infected males. Infect. Immun. 69, 7178–7181.
Peipert, J.F., 2003. Genital chlamydial infections. N. Engl. J. Med. 349, 2424–2430.
Pioli, P.A., Amiel, E., Schaefer, T.M., Connolly, J.E., Wira, C.R., Guyre, P.M., 2004. Differential expression of toll-like receptors 2 and 4 in Tissues of the human female reproductive tract. Infect. Immun. 72, 5799–5806.
Prantner, D., Darville, T., Sikes, J.D., Andrews Jr., C.W., Brade, H., Rank, R.G., Nagarajan, U.M., 2009. Critical role for interleukin-1beta (IL-1beta) during *Chlamydia muridarum* genital infection and bacterial replication-independent secretion of IL-1beta in mouse macrophages. Infect. Immun. 77, 5334–5346.
Prebeck, S., Brade, H., Kirschning, C.J., da Costa, C.P., Dürr, S., Wagner, H., Miethke, T., 2003. The Gram-negative bacterium *Chlamydia trachomatis* L2 stimulates tumor necrosis factor secretion by innate immune cells independently of its endotoxin. Microbes Infect. 5, 463–470.
Ramos, B., Polettini, J., Marcolino, L., Vieira, E., Marques, M., Tristão, A., Nunes, H., Rudge, M., Silva, M., 2011. Prevalence and risk factors of *Chlamydia trachomatis* cervicitis in pregnant women at the genital tract infection in obstetrics unit care at Botucatu Medical School. J. Low Genit. Tract Dis. 15, 20–24.
Rank, R.G., Whittum-Hudson, J.A., 2010. Protective immunity to chlamydial genital infection: evidence from animal studies. J. Infect. Dis. 201, 168–177.
Rasmussen, S.J., Eckmann, L., Quayle, A.J., Shen, L., Zhang, Y.X., Anderson, D.J., Fierer, J., Stephens, R.S., Kagnoff, M.F., 1997. Secretion of proinflammatory cytokines by epithelial cells in response to Chlamydia infection suggests a central role for epithelial cells in chlamydial pathogenesis. J. Clin. Invest. 99, 77–87.
Riccioli, A., Starace, D., Galli, R., Fuso, A., Scarpa, S., Palombi, F., De Cesaris, P., Ziparo, E., Filippini, A., 2006. Sertoli cells initiate testicular innate immune responses through TLR activation. J. Immunol. 177, 7122–7130.
Rivero, V.E., Motrich, R.D., Maccioni, M., Riera, C.M., 2007. Autoimmune etiology in chronic prostatitis syndrome: an advance in the understanding of this pathology. Crit. Rev. Immunol. 27, 33–46.
Rockey, D.D., Wang, J., Lei, L., Zhong, G., 2009. Chlamydia vaccine candidates and tools for chlamydial antigen discovery. Expert Rev. Vaccines 8, 1365–1377.
Rodrigues, M., Fernandes, P., Haddad, J., Paiva, M., Souza, Mdo., Andrade, T., Fernandes, A., 2011. Frequency of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma* species in cervical samples. J. Obstet. Gynaecol. 31, 237–241.
Rottenberg, M.E., Gigliotti-Rothfuchs, A., Wigzell, H., 2002. The role of IFN-γ in the outcome of chlamydial infection. Curr. Opin. Immunol. 14, 444–451.
Satta, A., Stivala, A., Garozzo, A., Morello, A., Perdichizzi, A., Vicari, E., Salmeri, M., Calogero, A.E., 2006. Experimental *Chlamydia trachomatis* infection causes apoptosis in human sperm. Hum. Reprod. 21, 134–137.
Schachter, J., Stephens, R.S., 2008. Biology of *Chlamydia trachomatis*. In: Holmes, K.K., Sparling, P.F., Stamm, W.E. (Eds.), Sexually Transmitted Diseases. McGraw-Hill Companies, Inc., New York, pp. 555–574.
Schaefer, T.M., Desouza, K., Fahey, J.V., Beagley, K.W., Wira, C.R., 2004. Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. Immunology 112, 428–436.
Schaefer, T.M., Fahey, J.V., Wright, J.A., Wira, C.R., 2005. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). J. Immunol. 174, 992–1002.
Schaeffer, A.J., 2006. Chronic prostatitis and the chronic pelvic pain syndrome. N. Engl. J. Med. 355, 1690–1698.
Scurlock, A.M., Frazer, L.C., Andrews Jr., C.W., O'Connell, C.M., Foote, I.P., Bailey, S.L., Chandra-Kuntal, K., Kolls, J.K., Darville, T., 2011. Interleukin-17 contributes to generation of Th1 immunity and neutrophil recruitment during *Chlamydia muridarum* genital tract infection but is not required for macrophage influx or normal resolution of infection. Infect. Immun. 79, 1349–1362.
Senior, K., 2012. Chlamydia: a much underestimated STI. Lancet Infect. Dis. 12, 517–518.
Shao, R., Wang, X., Wang, W., Stener-Victorin, E., Mallard, C., Brännström, M., Billig, H., 2012. From mice to women and back again: causalities and clues for Chlamydia-induced tubal ectopic pregnancy. Fertil. Steril. 98, 1175–1185.
Shurbaji, M.S., Gupta, P.K., Myers, J., 1988. Immunohistochemical demonstration of chlamydial antigens in association with prostatitis. Mod. Pathol. 1, 348–351.
Skerk, V., Schönwald, S., Krhen, I., Markovinović, L., Beus, A., Kuzmanović, N.-Š., Kružić, V., Vince, A., 2002. Aetiology of chronic prostatitis. Int. J. Antimicrob. Agents 19, 471–474.
Skerk, V., Krhen, I., Schonwald, S., Cajic, V., Markovinovic, L., Roglic, S., Zekan, S., Andracevic, A.T., Kruzic, V., 2004. The role of unusual pathogens in prostatitis syndrome. Int. J. Antimicrob. Agents 24, 53–56.
Stamm, W.E., 1999. *Chlamydia trachomatis* infections: progress and problems. J. Infect. Dis. 179, 380–383.
Stephens, R.S., 2003. The cellular paradigm of chlamydial pathogenesis. Trends Microbiol. 179, 380–383.
Swanborg, R.H., Boros, D.L., Whittum-Hudson, J.A., Hudson, A.P., 2006. Molecular mimicry and horror autotoxicus: do chlamydial infections elicit autoimmunity? Expert Rev. Mol. Med. 8, 1–23.
Taylor-Robinson, D., 1997. Evaluation and comparison of tests to diagnose *Chlamydia trachomatis* genital infections. Hum. Reprod. 12, 113–120.
Toth, M., Patton, D.L., Campbell, L.A., Carretta, E.I., Mouradian, J., Toth, A., Shevchuk, M., Baergen, R., Ledger, W., 2000. Detection of chlamydial antigenic material in ovarian, prostatic, ectopic pregnancy and semen samples of culture-negative subjects. Am. J. Reprod. Immunol. 43, 218–222.

Trojian, T.H., Lishmak, Ti., Heiman, S.D., 2009. Epididymitis and orchitis: an overview. Am. Fam. Physician 79, 583–587.

Vabulas, R.M., Ahmad-Nejad, P., da Costa, C., Miethke, T., Kirschning, C.J., Häcker, H., Wagner, H., 2001. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to Activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J. Biol. Chem. 276, 31332–31339.

Valdivia, R.H., 2008. Chlamydia effector proteins and new insights into chlamydial cellular microbiology. Curr. Opin. Microbiol. 11, 53–59.

Vigil, P., Morales, P., Tapia, A., Riquelme, R., Salgado, A.M., 2002. *Chlamydia trachomatis* infection in male partners of infertile couples: incidence and sperm function. Andrologia 34, 155–161.

Wagenlehner, F.M.E., Naber, K.G., Weidner, W., 2006. Chlamydial infections and prostatitis in men. BJU Int. 97, 687–690.

Wang, S.A., Papp, J.R., Stamm, W.E., Peeling, R.W., Martin, D.H., Holmes, K.K., 2005. Evaluation of antimicrobial resistance and treatment failures for *Chlamydia trachomatis*: a meeting report. J. Infect. Dis. 191, 917–923.

Wang, Y., Nagarajan, U., Hennings, L., Bowlin, A.K., Rank, R.G., 2010. Local host response to chlamydial urethral infection in male guinea pigs. Infect. Immun. 78, 1670–1681.

Weidner, W., Ludwig, M., Thiele, D., Floren, E., Zimmermann, O., 1996. Chlamydial antibodies in semen: Search for "silent" chlamydial infections in asymptomatic andrological patients. Infection 24, 309–313.

Weidner, W., Diemer, T., Huwe, P., Rainer, H., Ludwig, M., 2002. The role of *Chlamydia trachomatis* in prostatitis. Int. J. Antimicrob. Agents 19, 466–470.

Welter-Stahl, L., Ojcius, D.M., Viala, J., Girardin, S., Liu, W., Delarbre, C., Philpott, D., Kelly, K.A., Darville, T., 2006. Stimulation of the cytosolic receptor for peptidoglycan, nod1, by infection with *Chlamydia trachomatis* or *Chlamydia muridarum*. Cell. Microbiol. 8, 1047–1057.

Witkin, S.S., 2002. Immunological aspects of genital chlamydia infections. Best Pract. Res. Clin. Obstet. Gynaecol. 16, 865–874.

Wollenhaupt, J., Kolbus, F., Weissbrodt, H., Schneider, C., Krech, T., Zeidler, H., 1995. Manifestations of Chlamydia induced arthritis in patients with silent versus symptomatic urogenital chlamydial infection. Clin. Exp. Rheumatol. 13, 453–458.

Wyrick, P.B., 2000. Intracellular survival by Chlamydia. Cell. Microbiol. 2, 275–282.

Zdrodowska-Stefanow, B., Ostaszewska, I., Darewicz, B., Darewicz, J., Badyda, J., Puciło, K., Bułhak, V., Szczurzewski, M., 2000. Role of *Chlamydia trachomatis* in epididymitis. Part I: direct and serologic diagnosis. Med. Sci. Monit. 6, 1113–1118.