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REVIEW

Male genital tract immune response against *Chlamydia trachomatis* infection

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Abstract

Chlamydia trachomatis is the most commonly reported agent of sexually transmitted bacterial infections worldwide. This pathogen frequently leads to persistent, long-term, subclinical infections, which in turn may cause severe pathology in susceptible hosts. This is in part due to the strategies that *Chlamydia trachomatis* uses to survive within epithelial cells and to evade the host immune response, such as subverting intracellular trafficking, interfering signaling pathways and preventing apoptosis. Innate immune receptors such as toll-like receptors expressed on epithelial and immune cells in the genital tract mediate the recognition of chlamydial molecular patterns. After bacterial recognition, a subset of pro-inflammatory cytokines and chemokines are continuously released by epithelial cells. The innate immune response is followed by the initiation of the adaptive response against *Chlamydia trachomatis*, which in turn may result in T helper 1-mediated protection or in T helper 2-mediated immunopathology. Understanding the molecular mechanisms developed by *Chlamydia trachomatis* to avoid killing and host immune response would be crucial for designing new therapeutic approaches and developing protective vaccines. In this review, we focus on chlamydial survival strategies and the elicited immune responses in male genital tract infections.
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Chlamydia trachomatis and genital tract infections

Chlamydia trachomatis (CT) is the leading cause of sexually transmitted bacterial infections in both, developing and developed countries (Mylonas 2012, Gottlieb *et al*. 2013, WHO 2016). According to the World Health Organization, approximately 131 million new cases of chlamydial genital infections are diagnosed worldwide every year (WHO 2016).

CT infections mainly occur in young fertile women, who suffer from urethritis, cervicitis, endometritis, salpingitis to pelvic inflammatory disease (PID), ectopic pregnancy or tubal infertility (reviewed in (Haggerty *et al*. 2010, Kortekangas-Savolainen *et al*. 2012, Refaat *et al*. 2016). The most important feature of CT genital infections is their asymptomatic nature, leading to long-term subclinical infections responsible for permanent sequelae in the female genital apparatus (Gottlieb *et al*. 2010). In men, CT infects urethra being a major cause of male urethritis, which usually constitutes an acute episode of an underlying chronic silent infection affecting prostate, seminal vesicles, epididymis and testis (Furuya *et al*. 2005, 2009, Motrich *et al*. 2012, Mackern-Oberti *et al*. 2013).

In this review, we discuss the current knowledge regarding chlamydial recognition by epithelial cells and how these bacteria invade and survive within them. In addition, we review chlamydial strategies to evade host immune response to develop male genital tract infections.

Chlamydia trachomatis lifestyle

Attachment and invasion

CT is a gram negative bacterium that displays an obligate intracellular lifestyle involving a unique biphasic cycle with two clearly defined developmental stages (de Jesus De Haro-Cruz *et al.* 2011, Elwell *et al.* 2016). Infection starts by the bacterial-driven endocytosis of the quiescent, environmentally resistant and highly infectious elementary body (EB). Several virulence factors have been linked to EBs attachment to epithelial cells such as a new adhesin and invasin protein from CT serovar E Ctad1 (CT017) (Stallmann & Hegemann 2016), major outer membrane protein (MOMP) (Su *et al.* 1996), outer membrane complex protein B (OmcB)

(Fadel & Eley 2007) and polymorphic membrane protein D (PmpD) (Wehrl *et al.* 2004).

Different host molecules have been associated to CT invasion such as heparan sulfate proteoglycans (HSPGs) (Kim *et al.* 2011), glycosaminoglycan (Menozzi *et al.* 2002), mannose receptor (CD206) (Kuo *et al.* 2002, Campbell *et al.* 2006), protein disulfide isomerase (PDI) (Conant & Stephens 2007), platelet-derived growth factor receptor (PDGF-R), tyrosine kinase ephrinA2 receptor (EphA2) (Moorhead *et al.* 2010) and abelson kinase (Abl) (Elwell *et al.* 2008) (Fig. 1). Interestingly, EBs have the ability to directly bind to fibroblast growth factor 2 (FGF-2), which may enable their interaction with the FGF-2 receptor, triggering its activation that leads to the uptake of CT into
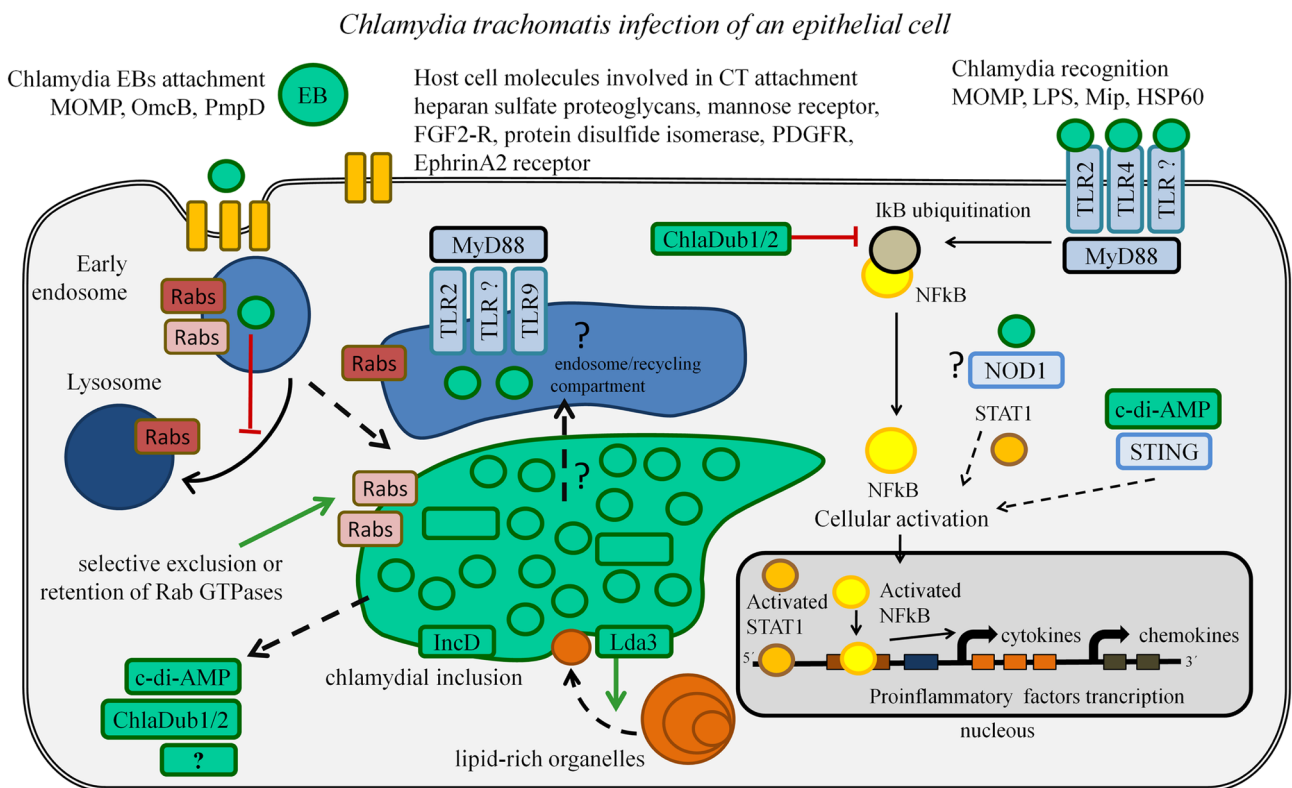

Figure 1 *Chlamydia trachomatis* infection of an epithelial cell. Several bacterial factors have been linked to EBs attachment to epithelial cells such as major outer membrane protein (MOMP), outer membrane complex protein B (OmcB) and polymorphic membrane protein subtype D (PmpD). On the other hand, several eukaryotic molecules are involved in CT binding to host cells such as heparan sulfate proteoglycans (HSPGs), mannose receptor (CD206), fibroblast growth factor 2 receptor (FGF2-R), protein disulfide isomerase (PDI), platelet derived growth factor receptor (PDGFR) and EphrinA2 receptor (EphA2). Host cells may recognize CT by different patterns recognition receptors (PRRs) such as toll-like receptor (TLRs), stimulator of interferon genes (STING) and nucleotide oligomerization domain 1 (NOD1). In turn, PRRs trigger nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation, leading to Inhibitor of NF-kB (IkB) ubiquitination and NF-kB nuclear translocation. Chlamydial components such as ChlaDub1/2 (deubiquitinating enzymes) may interfere with this signaling pathway limiting apoptosis. Once inside the host cell, CT drives modifications of the *Chlamydia*-containing vacuole or 'inclusion' by the selective exclusion or retention of Rabs on the chlamydial inclusion membrane in order to manipulate host trafficking. In this way, CT avoids degradation by inhibiting fusion with lysosomes and redirects Golgi-derived vesicles and multivesicular bodies (MVBs) to the inclusion for the acquisition of sphingolipids and cholesterol. In addition, *Chlamydia* also recruits lipid-rich organelles from the host cytosol, such as lipid droplets, through chlamydial proteins like Lda3 (Lda are proteins secreted by chlamydia that bind to cytoplasmic lipid droplets, LDs). Green boxes indicate chlamydial molecules; green circles indicate EBs; green arrows indicate host proteins associated with chlamydia inclusion; black lines indicate intracellular pathways; red lines indicate signaling pathways modulated by chlamydia. Red blunt lines indicate inhibitory signals; green lines indicate activation signals; black lines indicate intracellular localization and traffic; and dotted black lines indicate theoretical mechanism.

non-phagocytic cells (Kim *et al.* 2011). These data are summarized in Fig. 1.

Internalized EBs grow into a membrane-bound compartment known as the inclusion, in which they differentiate into larger, metabolically active and highly replicative reticulate bodies (RBs). After several rounds of replication, RBs are asynchronically turned back into EBs. At the end of the developmental cycle, which lasts 42–76 h, the inclusion occupies most of the cell cytoplasm and contains these new EBs, which are released to the extracellular environment either by cellular lysis or extrusion. Consequently, infection is spread into neighboring cells (Valdivia 2008). Along the developmental cycle, chlamydial lipopolysaccharide (cLPS) and other chlamydial molecules including c-di-AMP are sensed by host receptors such as toll-like receptor 4 (TLR4) and a cytosolic cyclic-nucleic acid-sensing protein named stimulator of interferon genes (STING) leading to NFκB and NLPR3-inflammasome activation (Barker *et al.* 2013, Finethy *et al.* 2015, Sixt *et al.* 2017, Webster *et al.* 2017). By these mechanisms, CT triggers host innate immune response and impairs mucosal homeostasis (Mackern-Oberti *et al.* 2011a, Barker *et al.* 2013). On the other hand, chlamydial plasmid is crucial for inducing immunopathology, which may be mediated by a toll-like receptor 2 (TLR2)-dependent mechanism. In agreement, plasmid-free CT or *Chlamydia muridarum* (CM) displayed an attenuated infection (O'Connell *et al.* 2007, 2011, Lei *et al.* 2014). Some Inc proteins such as incC, CT229, CT288, CT383 and CT449 are involved in bacterial replication contributing to inclusion membrane stability (Weber *et al.* 2017). Furthermore, these chlamydial proteins create a replicative niche that avoid innate immune recognition (Weber *et al.* 2017). A general view of bacterial and eukaryotic molecules involved in CT attachment to and invasion of host cells is shown in Fig. 1.

Chlamydia trachomatis and host signaling

CT has developed several molecular mechanisms to subvert or dampen the host immune response. Among them, CT prevents nuclear translocation of nuclear factor κ B (NFκB) by the release of the chlamydial proteins ChlaDub1 and ChlaDub2 (deubiquitinating enzymes) that in turn interfere with inhibitor of NF-kB (IkB) ubiquitination (Misaghi *et al.* 2006) (Fig. 1). Additionally, CT exerts anti-apoptotic activity by triggering a pathway driven by mitogen-activated protein kinases, originally called extracellular signal-regulated kinases (MAPK/ERK signaling) (Kun *et al.* 2013). Hence, CT ensures the survival of the infected cell. CT also modulates PLCγ1 (phospholipase Cγ1) and Akt (protein kinase B) signaling pathways to promote chlamydial attachment, internalization and inclusion formation (Subbarayal *et al.* 2015). Indeed, upon chlamydial infection, there is a strong and sustained infection-mediated ERK activation, which in turn leads to upregulation of EphrinA2 receptor (Subbarayal *et al.* 2015). Although the mechanism is still unclear and controversial, the chlamydial secreted protease (CPAF) may also promote cell survival (Yang *et al.* 2016). In Fig. 1, we describe the main signaling pathways that are affected by chlamydial infection. Since most of them are also involved in the regulation of host immune response, it is likely that through common molecules, CT manipulates not only the intracellular environment of the infected cell but also overall host immunity.

Chlamydia trachomatis controls intracellular transport

CT has evolved highly specialized mechanisms to survive within human epithelial cells being able to scavenge nutrients from the host cell while being restricted into the inclusion (Saka & Valdivia 2010).

CT drives modifications in host vesicular transport by selective exclusion or retention of Rab GTPases (family of small Ras-like GTPases) on the chlamydial inclusion membrane (Hackstadt 2012, Damiani *et al.* 2014). Rab5 and Rab7, which control transport in the phagocytic pathway, phagosome maturation and finally, the fusion with lysosomes, are conveniently excluded from the inclusion membrane (Rzomp *et al.* 2003, Hackstadt 2012). On the other hand, Rabs belonging to the endocytic recycling pathway like Rab4 and Rab11 are selectively retained on the chlamydial inclusion membrane. Furthermore, Rab6 and Rab14, GTPases that participate in intra-Golgi and post-Golgi transport, are recruited by CT to redirect endogenously synthesized host lipids (Rzomp *et al.* 2006, Rejman Lipinski *et al.* 2009, Capmany & Damiani 2010). In addition, chlamydial inclusions interact with multivesicular bodies (MVBs, host organelles rich in cholesterol and sphingomyelin) through a Rab39a-mediated mechanism (Gambarte Tudela *et al.* 2015). Some Rab-interacting proteins have been found associated to chlamydial inclusions such as Rab6 effector Bicaudal D1, a dual Rab11- and Rab14-binding protein known as family of interacting protein 2 (FIP2), and the oculocerebrorenal syndrome of Lowe protein 1 (OCRL) that binds to multiple Rabs (some isoforms of Rab1, Rab3, Rab5, Rab6, Rab8, Rab13, Rab22 and Rab35) (Rzomp *et al.* 2006, Moorhead *et al.* 2007, 2010, Leiva *et al.* 2013) (Fig. 1).

Current evidence indicates that CT acquires lipids not only by hijacking Golgi-derived exocytic vesicles or multivesicular bodies (MVBs). Actually, CT has the ability to recruit lipid droplets (LDs, neutral lipid rich organelles) by the interaction with chlamydial protein Lda3 (Kumar *et al.* 2006, Cocchiaro *et al.* 2008). In agreement, in the absence of LDs, CT replication is significantly reduced (Saka *et al.* 2015). In fact, lipid droplet proteome in epithelial cells is modified in response to CT infection (Saka *et al.* 2015), (Fig. 1).

Interestingly, CT also takes advantage of non-vesicular-mediated mechanisms for nutrient acquisition. Inclusion membrane protein IncD interacts with the endoplasmic reticulum (ER)-to-Golgi ceramide transfer protein CERT and the ER resident protein VAPB at ER-*Chlamydia trachomatis* inclusion membrane contact sites to acquire host sphingomyelin (Derre *et al.* 2011, Elwell *et al.* 2011). The main vesicular transport pathways and host lipid sources co-opted by CT are schematized in Fig. 1.

Chlamydia trachomatis and persistent infections

Interferon-γ (IFN-γ) has an important role in the immune response to CT (Johansson *et al.* 1997, Perry *et al.* 1999, Gondek *et al.* 2009, Sherchand *et al.* 2016). The induction of indoleamine-2,3-dioxygenase 1 (IDO1) by this cytokine results in depletion of intracellular tryptophan, which in turn imposes nutritional stress to CT, given that these bacteria are tryptophan auxotrophs (Byrne *et al.* 1986). In response to the nutritional stress, also caused by sphingolipids deprivation, CT enters into a low replicative viable state identified by the presence of persistent or aberrant bacterial forms, which are able to resume normal replication as soon as conditions are favorable again. Persistent bacteria are linked to infection chronification (Beatty *et al.* 1994*a*,*b*, Wyrick 2010).

In summary, current knowledge supports the concept that CT ensures its survival, development and replication by hijacking multiple vesicle pathways to seize host cells for its own benefit. These findings have been achieved by *in vitro* studies in epithelial cells. However, whether similar mechanisms occur inside infected immune cells such as dendritic cells (DCs) and macrophages affecting T cell priming remain elusive.

Innate immune response against *Chlamydia trachomatis*

Epithelial cells as the first immune barrier

Urethra or vagina/endocervix epithelia are physical barriers in which takes place the first contact between the host and CT. Male (MGT) and female genital tract (FGT) epithelial cells can recognize CT through pattern
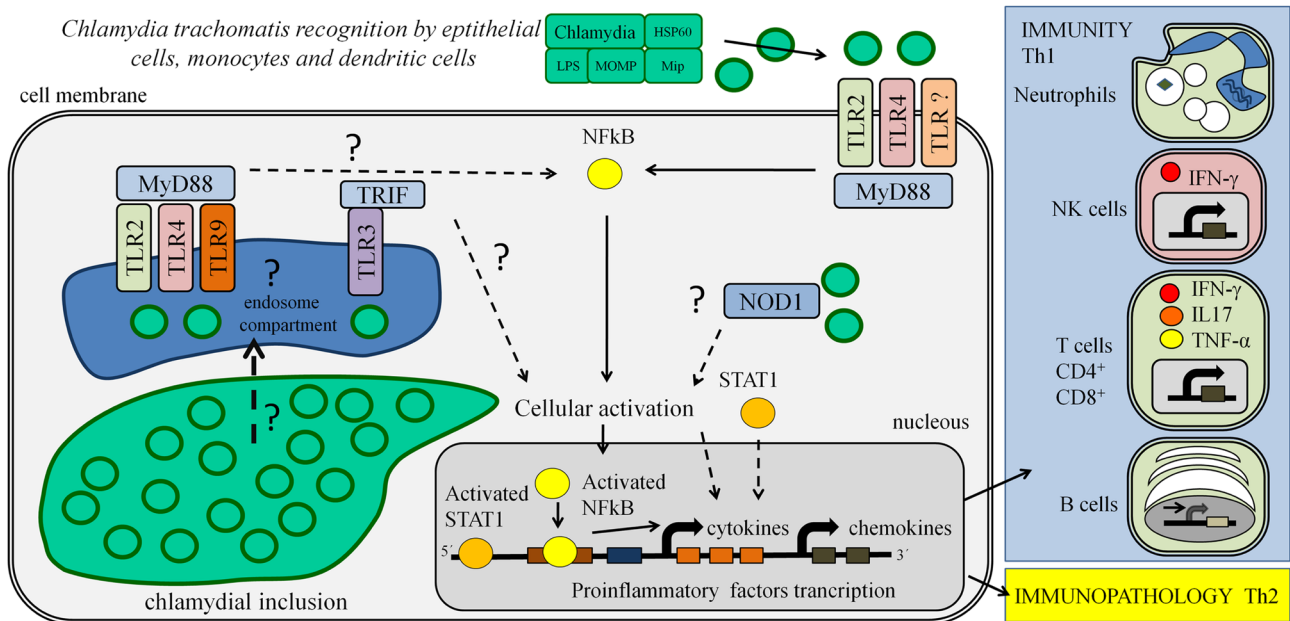

Figure 2 Proposed mechanisms by which CT is recognized by epithelial cells and leukocytes. After ascending through the urethra, CT interacts and infects epithelial cells. Extracellular CT EBs are recognized by epithelial cells by toll-like receptors (TLR)1, 2, 4 and 6, thus triggering TLR signalling and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) nucleous translocation leading to proinflammatory gene expression and secretion. CT recognition by epithelial cells is strictly dependent on the adaptor molecule myeloid differentiation primary response 88 (MyD88), suggesting that other MyD88-dependent PRRs may be involved. It is believed that intracellular TLR2, TLR4 and TLR9 from epithelial cells may also have a role in CT recognition and so initiating TLR signaling from this compartment. In addition, CD45+ leukocytes may encounter and recognize CT mainly by TLR2, 1, 6 and 4 resulting in cytokine/chemokine secretion, immune cell recruitment and inflammation. Also, CT activated tissue resident CD45+ leukocytes may interact with epithelial cells in order to augment and coordinate an effective specific immune response driven by Neutrophils, NK cells, Th1 T cells and B cells. In contrast, a Th2 driven immune response may result in non-protective leading to pathology. Also, continuous activation of TLR on epithelial cells and CD45+ leukocytes by a chronic CT infection may cause a state of chronic inflammation of the male and female genital tract, which may impair tissue normal function and possibly trigger immunepathology process in susceptible individuals. Black lines indicate intracellular signaling and dotted black lines indicate theoretical mechanisms.

recognition receptors (PRRs) leading to nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), p38 MAP kinase pathway (P38) and c-Jun N-terminal kinases (JNK) activation and the production of pro-inflammatory cytokines and chemokines (Al-Mously & Eley 2007, Mackern-Oberti *et al.* 2013). These immune mediators may activate resident leukocytes and recruit others from the periphery, which in turn modulate the adaptive immune response (O'Neill 2002, Sellami *et al.* 2014). These interactions between CT and the host are summarized in Figs 1 and 2.

Chlamydia trachomatis recognition by PRRs in the FGT has been extensively explored, whereas the knowledge about PRR expression and CT recognition in the MGT is limited. It has been proposed that PRRs signaling may converge in MyD88 and thus immunopathology would occur by this molecular signaling (Nagarajan *et al.* 2005, Chen *et al.* 2010). MyD88-deficient mice failed to develop a protective immune response during *Chlamydia muridarum* (CM) genital tract infection, while mounting a specific T helper type 2 (Th2)-like immunity. As expected, female infected MyD88 KO mice showed increased bacterial burden in the upper genital tract with severe pathology, associated with a reduction in IFN-γ production by natural killer (NK) cells and decreased levels of IL-17, IL-18 and TNF-α (Chen *et al.* 2010, Nagarajan *et al.* 2011). In addition, *in vitro* studies indicated that nucleotide-binding oligomerization domain-containing protein 1(NOD1) participates in CT recognition leading to IL-8 production by FGT epithelial cells, which in turn may collaborate in the recruitment of leukocytes initiating the immune response (Buchholz & Stephens 2008).

Different MGT tissues such as urethra, testis, epididymis, prostate and vas deferens express TLRs showing a specific tissue distribution (Al-Mously & Eley 2007, Palladino *et al.* 2007, 2008, Mackern-Oberti *et al.* 2011*a*,*b*, Winnall *et al.* 2011). Indeed, primary cultures of cells from different MGT tissues actively produce chemokines when stimulated with different TLR ligands, including polyinosinic:polycytidylic acid (Poly I:C), CpG oligodeoxynucleotides (CpG), *E. coli* LPS and chlamydial LPS (Mackern-Oberti *et al.* 2011*a*). Furthermore, prostate cell primary cultures from TLR2/4 double KO mice displayed an altered chemokine production in response to CM infection (Mackern Oberti *et al.* 2011*b*). Interestingly, prostate cell primary cultures from MyD88 KO mice showed a more pronounced decrease in chemokine production than TLR2/4 double KO mice suggesting that an additional PRR is involved in CT recognition (Mackern Oberti *et al.* 2011*b*) (Fig. 2). Moreover, TLR2 and TLR4, but not TLR5, were recruited to the chlamydial inclusion vicinity, suggesting an active role of these receptors in bacterial recognition and activation of MGT epithelial cells (Mackern-Oberti *et al.* 2006). Urethral and prostate epithelial cells respond to CT-producing IL-1α and IL-6 in a tissue-specific pattern, suggesting a differential sensitivity to CT recognition that may be due to a particular TLRs expression (Al-Mously & Eley 2007). These molecular mechanisms are summarized in Fig. 2 and Table 1.

Besides CT recognition by epithelial cells, it is important to consider bacteria recognition by resident leukocytes (Aflatoonian & Fazeli 2008, Mackern Oberti *et al.* 2011*b*). In the FGT, it has been reported that resident macrophages were the main source of IL-1β in response to CT infection, which may collaborate with epithelial cells in mounting an immune response (Prantner *et al.* 2009). Both prostate resident leukocytes (CD45+ sorted cells) and epithelial/stromal cells (CD45− sorted cells) express genes involved in TLR signalling but exhibit different responses to CT infection (Mackern Oberti *et al.* 2011*b*). In fact, CD45+ and CD45− cells from MyD88 KO mice do not respond to CT infection (Mackern Oberti *et al.* 2011*b*). However, while CT recognition by CD45+ cells is dependent on TLR2/TLR4, CD45− cells keep responding to CT, to a certain extent, independently of TLR2/TLR4, suggesting a crucial role of these cells in initiating an immune response against CT (Mackern Oberti *et al.* 2011*b*) (Fig. 2 and Table 1).

Chlamydia trachomatis infection of dendritic cells

CT can efficiently infect and replicate into human dendritic cells (DCs) leading to the production of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, IL-18 and TNF-α (Gervassi *et al.* 2004, Agrawal *et al.* 2013, Datta *et al.* 2014). Macrophages could also be infected by CT but displaying a different bacterial development such as non-conventional inclusion formation (Herweg & Rudel 2016, Zuck *et al.* 2016). Recently, it has been reported CT could also complete its replication cycle in induced pluripotent stem cell-derived macrophages (Yeung *et al.* 2017).

Chlamydial LPS and heat shock protein 60 (HSP60) were the first antigens described to be recognized by TLR2 and TLR4 expressed on monocytes and DCs (Vabulas *et al.* 2001, Prebeck *et al.* 2003). Similarly, macrophage infectivity potentiator has also been reported to be recognized by TLR2 on macrophages leading to the production of IL-1β, TNF-α, IL-6 and IL-8 (Bas *et al.* 2008). Interestingly, chlamydial MOMP, a surface-exposed antigen, has the ability to signal via TLR2 in TLR-expressing transfected cells and in TLR2-competent endocervical End/E6E7 cells leading to IL-8 and IL-6 production (Massari *et al.* 2013). Although the molecular mechanisms underlying cellular recognition remain to be determined, several polymorphic membrane proteins induce innate immune responses (Vasilevsky *et al.* 2016). Chlamydial lipoproteins D381, D541 and D775 induce the production of pro-inflammatory cytokines mediated by TLR1/2/MyD88 and TLR2/CD14/MyD88 signaling

Table 1   Innate immune mediators involved in CT infection of the genital tract.

Innate immunity	Tissue	Cell type	Type of study	Response	References
TLR2	MGT; FGT; leukoctes	Epithelial cells; prostate; seminal vesicles; vas deferens; oviduct cells; cervical cells; leukocytes	in vitro; in vivo; mice; rat; human	Cytokine and chemokine production (TNF-α, IL1-β, IL-6, IL-8, KC, MCP1, IL-12); involved in immunity and immunopathology; binding of chlamydial LPS, HSP60, Mip and MOMP	Mackern-Oberti et al. (2006, 2011a,b, 2013); Al-Mously and Eley (2007); Massari et al. (2013); Bas et al. (2008); Sellami et al. (2014); Palladino et al. (2007, 2008); O'Neill (2002)
TLR4	MGT; FGT; leukoctes	Epithelial cells; prostate; seminal vesicles; vas deferens; oviduct cells; cervical cells; leukocytes	in vitro; in vivo; mice; human	Cytokine and chemokine production; no major impact in vivo; binding to LPS and HSP60	Mackern-Oberti et al. (2006, 2011a,b); Al-Mously & Eley (2007); Sellami et al. (2014); O'Neill (2002)
TLR3	FGT	Cervical cells	in vitro; mouse	Cytokine production; TLR3 deficient leads to more suscptibility to replication	Aflatoonian & Fazeli (2008)
TLR5	MGT	Prostate	in vitro; rat	No major impact in vitro	Mackern-Oberti et al. (2006); Palladino et al. (2007, 2008)
MyD88	MGT; FGT	Prostate; leukocytes; cervical cells, oviduct	in vitro; in vivo; mouse	Involved in innate and adaptive immune response, oviduct pathology; MyD88 KO develop a Th2 immunity; IFN-g reduction	Mackern-Oberti et al. (2011a,b, 2013); Nagarajan et al. (2005, 2011); Chen et al. (2010); O'Neill (2002)
STAT1	FGT	Cervical cells, oviduct	in vitro; mouse	Contribute to IL-1β response in CT infection	Prantner et al. (2009)
NOD1	FGT	Cervical cells	in vitro; mouse; human genetic studies	Contribute to IL-8 response in CT infection; certain polymorphism may confer lower risk of infection	Buchholz & Stephens (2008)

but not by TLR4 pathway (Wang et al. 2017) (Table 1). Interestingly, vaccination with chlamydial membrane proteins such as highly conserved type III secretion system (T3SS) proteins CopB, CopD and CT584 and MOMP induce neutralizing antibodies, enhance T cell responses and reduce bacterial load from the vagina (Cheng et al. 2014, Pal et al. 2015, Bulir et al. 2016, de la Maza et al. 2017).

When TLR4 engagement is abrogated by a blocking monoclonal antibody, DCs fail to produce IL-1, IL-6 and TNF-α in response to CT (Gervassi et al. 2004). Surprisingly, adoptive transfer of mice with long-term infected DCs caused an in vivo infection showing that infective CT also develops within DCs and may be a potential reservoir with still unknown evasion mechanisms (Rey-Ladino et al. 2007, Rescigno 2015).

Interestingly, only viable EBs induce DC maturation with classical upregulation of major histocompatibility complex 2 (MHC-II), CD40, CD80, CD83, CD86 and intercellular adhesion molecule 1 (ICAM-1) expression, production of high levels of IL-12, TNF-α, keratinocyte chemoattractant (KC), macrophage inflammatory protein 2 (MIP-2) and enhancing T cell priming (Rey-Ladino et al. 2005, Zaharik et al. 2007, Agrawal et al. 2013). Although much work has been done in mice and human monocytes, macrophages and DCs, it is important to note that resident myeloid cells from genital tract may display different immune response (Da Silva & Barton 2016).

Chlamydia trachomatis and the adaptive immune response

Although CT-infected hosts develop adaptive immune responses, the bacterium manages to evade host immune effectors by building a niche inside epithelial cells (Igietseme & Rank 1991, Bragina et al. 2001, Kokab et al. 2010). As previously mentioned, epithelial cells and resident leukocytes could secrete a wide spectrum of pro-inflammatory chemokines after CT recognition leading to the local recruitment of leukocytes (Deruaz & Luster 2015). Both humoral and cell-mediated immune responses have been implicated in inducing protection against CT infection (Morrison et al. 2000, Loomis & Starnbach 2002). It has been extensively demonstrated that the T cell–mediated immune response is crucial for protection, as T cell or MHC-II-deficient mice develop more severe and long-lasting CT infections (Rank et al. 1985, Morrison et al. 1995) (Table 2).

Most studies indicate that the Th1/IFN-γ response is crucial for controlling CT infection (Cocchiaro et al. 2008, Li et al. 2008, Gondek et al. 2009). IFN-γR-deficient mice preferentially induce prominent Th2 responses and are susceptible to develop severe and long-lasting FGT infections (Gondek et al. 2009). In fact, macrophages from IFN-γR-deficient mice show increased numbers of intracellular CT inclusions and produce lower levels of nitric oxide (Johansson et al. 1997). Interestingly, in vitro studies suggest that IFN-γ is crucial in mounting effector

Table 2   Adaptive immune mediators involved in CT infection of the genital tract.

Adaptive immunity	Tissue	Cell type	Type of study	Response	References
T cells	FGT	T cells; cervical, oviduct	*in vivo; mouse*	T cells deficiency leads to CT chronic infection	Loomis & Starnbach (2002); Morrison *et al.* (1995); Rank *et al.* (1985)
Th1; CD4+ T cells; cytokines; IFN-g	FGT; MGT	CD4+ T cells; cervical, oviduct	*in vivo; mouse; human; human genetic studies*	IFN- is crucial for immune protection to CT infection by different mechanisms includding epithelial cell activation; certain IL-12 polymorphisms confer more risk to infection; Th1 cells may reside indiffernt sites of FGT and also be modulated by Estrogen	Kokab *et al.* (2010); Deruaz & Luster (2015); Morrison *et al.* (2000); Johansson *et al.* (1997); Al-Zeer *et al.* (2009); Barral *et al.* (2014); Cohen *et al.* (2005); Cocchiaro *et al.* (2008); Gondek *et al.* (2009); Li *et al.* (2008); Pal *et al.* (2010); Davila *et al.* (2014); Su *et al.* (2004); Mackern-Oberti *et al.* (2011c); Shao *et al.* (2012)
Th2; IL-4	FGT	CD4+ T cells; cervical, oviduct	*in vivo; mouse; human*	Th2 leads to developing unresolve infections; more susceptibility to tubal factor infertility	Johansson *et al.* (1997); Agrawal *et al.* (2009); Vicetti Miguel *et al.* (2013); Hawkins *et al.* (2002)
Th17; IL-17; IL23	FGT	CD4+ T cells; cervical, oviduct	*in vivo; mouse; human*	IL-17 deficiency leads to a decreased Th1 immunity; TNF-α as a compensatory cytokine; partially involved in oviduct immunopathology; infection course unaffected	Scurlock *et al.* (2011); Nagarajan *et al.* (2011); Frazer *et al.* (2013); O'Meara *et al.* (2014); Yu *et al.* (2010); Andrew *et al.* (2013); Vicetti Miguel *et al.* (2013); Russell *et al.* (2016)
CD8+ T cells	FGT	CD8+ T cells; cervical, oviduct	*in vivo; mouse; human*	Controversial role of CD8+ T cells in CT immunity; involved in immunopathogenesis	Deruaz & Luster (2015); Agrawal *et al.* (2009); Mittal *et al.* (2004); Vlcek *et al.* (2015); Manam *et al.* (2013, 2015); Nogueira *et al.* (2015)

mechanisms by promoting the accumulation of different interferon-γ-inducible regulatory immunity-related GTPase (Irg) molecules such as Irga6, Irgd, Irgm2 and Irgm3 at the chlamydial inclusion, targeting this vacuole to lysosomes for degradation (Al-Zeer *et al.* 2009) (Table 2). In mice, furthermore, Irga6-deficient cells are more susceptible to CT infection and fail to respond to IFN-γ indicating that Irga6 is crucial for mounting an IFN-γ response (Al-Zeer *et al.* 2009). Although studies evaluating cell-mediated immune responses to CT infection in humans are limited, a protective response associated to a reduced risk of pathology and reinfection is related to the Th1/CD4+ T cells/IFN-γ axis (Barral *et al.* 2014). It has been reported that IFN-γ production by CD4+ T cells in response to chlamydial HSP60 is associated to protection against CT (Cohen *et al.* 2005) (Table 2). In MGT, it remains to be determined whether Th1/IFN-γ axis is involved in CT resistance (Pal *et al.* 2010). Nevertheless, researchers must be cautious when interpreting data obtained from the mouse model, especially when using its natural pathogen CM instead of CT, because of differences in bacterial replication and host recognition (O'Connell *et al.* 2011).

Gradual loss of protective immunity against CT generally occurs and is consistent with decreased CD4+ T cell numbers in the genital tract (Ramsey *et al.* 1989, Kelly & Rank 1997). CD4+ T cells that are recruited to the female upper genital tract highly express a heterodimeric integrin receptor a4β7 and the blockage of this homing receptor leads to severe infection. The latter highlights the need of a vaccine that promotes a long-term expression of this adhesion molecule on

CT-specific Th1 CD4+ cells (Davila *et al.* 2014). Th2-specific T cells fail to induce protective immunity against CT displaying lower expression levels of this homing receptor (Hawkins *et al.* 2002). However, in an experimental model of CT infection of the MGT using C57BL/6 mice, it was reported that Th2-like cells transfer controls CT infection and prevents tissue damage (Sobinoff *et al.* 2015).

During the last years, the role for the Th17/IL-17 axis in the resolution of CT infection has also been investigated (Scurlock *et al.* 2011, Andrew *et al.* 2013, Frazer *et al.* 2013, Vicetti Miguel *et al.* 2013). Interestingly, IL-17-R-deficient mice showed a reduced Th1/IFN-γ response against CT although they efficiently cleared the infection and displayed a more robust TNF-α-induced immune response with similar severity of oviductal pathology (Scurlock *et al.* 2011). By contrast, CT-infected IFN-γ-deficient mice showed a more severe immunopathology with higher levels of IL-17 that may contribute to misbalance the Th profiles (Scurlock *et al.* 2011). Immunization protocols indicate that Th17 responses collaborate with both, the induction of immune protection and development of FGT pathology (Yu *et al.* 2010, O'Meara *et al.* 2014).

Regarding cytotoxic T cells, it has been reported that CD8+ T cells are also recruited to the infected mucosa but their role in the infection resolution is still under debate (Mittal *et al.* 2004, Agrawal *et al.* 2009). After CT infection, CD8+ T cells contribute to oviductal pathology secreting TNF-α (Manam *et al.* 2015). In addition, adoptive CD8+ T cell transfer experiments showed that CT-specific CD8+ T cells mediate immunopathogenesis

in the FGT during CT infection (Manam *et al.* 2013, Vlcek *et al.* 2015). However, immunization protocols indicate that CD8+ T cell response may mediate immune protection against CT (Nogueira *et al.* 2015). Additionally, women with lower FGT infection exhibit higher numbers of *Chlamydia*-specific CD8+ T cells than those with upper FGT infection (Russell *et al.* 2016) More compelling data are currently needed to ascertain the precise role of CD8+ T cells in CT infection of the genital tract (Table 2).

Pal and coworkers by inoculating male wild-type and severe combined immunodeficient mice showed the induction of a Th1 immune response and the requirement of the adaptive immune response to clear the bacterium from MGT (Pal *et al.* 2004, Su *et al.* 2004). CT infection of the MGT actively induces CD4+ and CD8+ T cell recruitment to the prostate gland, which is similar to that observed in infected women with PID (Mackern-Oberti *et al.* 2011*c*, Shao *et al.* 2012). We have also reported for the first time that chronic MGT infection with CM may promote the loss of immune tolerance to prostate antigens (Mackern-Oberti *et al.* 2011*c*). These findings could help in understanding the underlying mechanisms of chronic pelvic pain syndrome and chronic prostatitis (Table 2).

Conclusions

CT modifies intracellular trafficking by modulating several host molecules in order to avoid degradation by phagocytosis, a major mechanism of the innate immune system. By using the same strategy, CT not only evades lysosome fusion but also redirects nutrient-rich vesicles to the chlamydial inclusion. CT also avoids degradative host cell response by inhibiting transcription factors and preventing apoptotic cell death. CT infection of the FGT and MGT induces the production of cytokines/chemokines by epithelial cells and leukocytes that may initiate the specific adaptive immune response as well as drive immunopathology in susceptible hosts. Understanding Th1-driven immune response and identifying key CT targets at the genital tract are crucial for designing an efficient vaccine to control chlamydial transmission and disease, and thereby remain as major priority in CT research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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