Induction and Function of Type I IFNs During Chlamydial Infection

Uma M. Nagarajan

Introduction

Chlamydia trachomatis infection is the leading sexually transmitted bacterial infection (STI) in the US, as reported by CDC. The global burden of chlamydial infection is likely higher than that reported for STI, as ocular trachoma caused by chlamydiae continues to be the leading cause of preventable blindness in the world [1]. *Chlamydia* spp. also cause significant disease in livestock. In women, *C. trachomatis* is a major cause of pelvic inflammatory disease, ectopic pregnancy, and infertility [2]. Chlamydial infections can be self-limiting, providing evidence for the development of protective immune responses [3, 4]. However, infection induces mostly short-term immunity that is strain (serovar) specific, so the risk of re-infection is high, and carries an increased risk of tissue damaging effects [5]. Human epidemiologic studies also indicate increased risk of disease with repeated infection [6, 7]. Consequently, a great deal of research has focused on understanding chlamydial biology and the immune responses to chlamydial infection, with an obvious goal to develop a vaccine that will induce protective responses to *Chlamydia* while avoiding responses that lead to pathology. In this chapter, we will focus on one such innate immune response, the type I IFNs in chlamydial pathogenesis, with emphasis on their role during infection and the mechanism of induction during chlamydial infection.

U.M. Nagarajan (✉)
Division of Pediatric Infectious Diseases, University of North Carolina at Chapel Hill, 8312 MBRB bldg., Chapel Hill, NC, USA
e-mail: nagaraja@email.unc.edu

D. Parker (ed.), *Bacterial Activation of Type I Interferons*,
DOI 10.1007/978-3-319-09498-4_9

The Pathogen and Pathogenesis

Gram-negative *Chlamydia* sp. are obligate intracellular pathogens with a relatively small genome (1–1.3 Mbp) and a unique developmental cycle [8, 9]. The first step in the intracellular chlamydial infection is attachment of the infectious and metabolically inactive elementary body (EB) to the host cell surface. Once the EB enters the cell by endocytosis, it modifies the vacuole to inhibit phagolysosome fusion, and remains confined in a membrane bound vacuole, termed the "inclusion," during its entire developmental cycle [10–12]. Inside the early inclusion the EB transforms into the metabolically active reticulate body (RB) form by a process that involves DNA de-condensation [13] and reductive cleavage of the outer membrane protein complex [14, 15]. Rather than being strictly non-fusogenic with the host vesicular trafficking pathways, the chlamydial inclusion selectively fuses with sphingomyelin containing exocytic vesicles en route to the plasma membrane from the Golgi [16, 17]. Inclusion formation and acquisition of sphingomyelin are initiated very early in the cycle [17], a phenomenon driven by early chlamydial protein synthesis [18]. Inside the inclusion, the RB multiplies by binary fission [10] and the inclusion expands to occupy significant parts of the cytosol during *C. trachomatis* infection. Specific molecular triggers generated in the RBs likely due to its local environment, initiate the conversion of RBs to EBs towards the latter part of the chlamydial developmental cycle, a process that occurs asynchronously. Eventually, by multiple exit mechanisms [19], the infected cells are lysed and the released EBs go on to infect neighboring cells. The sequential conversion from the specialized EB to RB and then back to EB is a unique feature of chlamydial biology.

Unlike several facultative intracellular pathogens, chlamydiae are not equipped with toxins that damage the host cells. *C. trachomatis* strictly infects mucosal epithelial cells during a genital infection and conjunctival cells during an ocular infection. The host response to *C. trachomatis* is initiated by infected epithelial cells [20] and sustained by professional inflammatory cells and neighboring uninfected cells. Chlamydial ligands recognized by surface and intracellular pathogen recognition receptors (PRRs) initiate chemokine and cytokine production as early as 3 h post infection in vivo, suggesting that entry of viable chlamydiae into host cells is sufficient to induce a response [21]. Using the mouse model of chlamydial genital tract infection [22], it has been shown that inflammatory responses are a major determining factor in oviduct pathology. Following bacterial ascension to the oviducts, infected epithelial cells respond to bacterial signals by producing cytokines and chemokines [20] that act locally to recruit PMNs and other immune cells [21]. PMNs are partially protective in the cervix and uterus because they restrict on-going chlamydial replication, amplify cytokine signaling and reduce pathogen load by attacking infected cells [23]. However, PMN recruitment to the oviducts is excessive and prolonged, leading to distal blockage and formation of hydrosalpinx or salpangitis [21, 23–26]. The contribution of innate immune pathways, such as TLR2, IL-1R, IFNAR, TNFR in PMN recruitment and oviduct pathology has been demonstrated using gene knockout mice [27–33]. On the other

hand, $CD4^+$ Th1 cells that produce a predominant IFN-γ response are critical to the control of chlamydial genital and ocular infection, and enhanced Th1 immune responses correlate with protection from infection and disease in both animal models and humans [34–38].

Induction of Type I IFNs During Chlamydial Infection and Its Biological Implications

Induction of type I IFNs has been observed in multiple cell types including oviduct epithelial cells [39], macrophages [40, 41], fibroblasts (McCoy cells) [42], and mouse DC [43] infected in vitro with multiple chlamydial strains. Therefore, the ability to induce IFN-β in response to intracellular *C. trachomatis* infection appears relatively conserved. *C. pneumoniae*, on the other hand does not induce significant levels of IFN-β expression in epithelial cells, which could be a result of its ability to degrade TRAF3 [44].

Type I IFNs are largely inhibitory to chlamydial growth during in vitro infections. Early studies showed significant inhibition of *C. trachomatis* infectivity in HeLa cells treated with different isotypes of IFN-α [45]. IFN-β treatment of macrophages treated with LPS also resulted in significant killing of *C. psittaci*, as observed with IFN-γ-treatment and this was attributed to activation of indoleamine dioxygenase (IDO) activity. IDO decyclizes tryptophan to *N*-formyl kyneurine resulting in reduction in tryptophan pool in the cells affecting chlamydial growth [46]. Further, inhibition of chlamydial growth by TNFα was shown to be partly mediated through an autocrine function of IFN-β enhancing the activity of IDO and could be blocked by tryptophan [47]. Treatment of murine fibroblasts (L cells) with type I IFNs was also shown to significantly reduce the yield of *C. trachomatis* LGV biovar [48]. Besides its role in chlamydial killing, IFN-β was shown to contribute to IFN-γ expression and in induction of CXCL10 in mouse macrophages infected with *C. pneumoniae* [40] and *C. muridarum* [41], respectively.

The protective effect of IFN-α/β observed in vitro was not recapitulated during in vivo *C. muridarum* infection, both in the lungs and genital tract. In the lung infection model, *Ifnar*$^{-/-}$ mice showed less bacterial burden, weight loss, and less pathology in comparison to control mice, which was attributed to lower macrophage apoptosis in the absence of IFNAR signaling [49]. During genital *C. muridarum* infection, *Ifnar*$^{-/-}$ mice displayed a slightly enhanced clearance of infection and significantly reduced oviduct pathology [28]. The improved bacterial clearance in *Ifnar*$^{-/-}$ mice was associated with an increase in antigen-specific T cells in the iliac nodes, enhanced $CD4^+$ T cell recruitment to the genital tract and an increased level of the IFN-γ-inducible protein, CXCL9 in genital secretion. A similar outcome of overall enhanced infection clearance and reduced pathology was observed in genital chlamydial infection during IFN-β neutralization in wild-type mice [30]. However, in this study a slight increase in infectious burden was observed at day 4 post infection during IFN-β depletion, which was not sustained and the IFN-β depleted mice went on to clear infection at a

faster rate than the mice receiving control sera. A similar outcome of increased infection load at day 4 post infection, which was not sustained was also observed in mice deficient for the transcription factor IRF3, which is essential for IFN-β induction. These data suggest that IFN-β likely has an anti-chlamydial activity early in infection, but its negative impact on the inflammatory cells and T cells is not protective to the host. Indeed, the T cells from the iliac lymph nodes of *Irf3*$^{-/-}$ and *Ifnar*$^{-/-}$ mice displayed an enhanced antigen-specific T cell response. IRF3 KO mice also developed significant uterine pathology unlike *Ifnar*$^{-/-}$ or IFN-β depleted mice, suggesting that IRF3 could play an IFNAR/IFN-β independent role in uterine horn protection [30].

Recent discovery of a new family member of type I IFNs, IFN epsilon (IFN-ε), has generated interest due to its exclusive expression in the mouse and human genital tract [50, 51]. *Ifnε*$^{-/-}$ mice were shown to have slightly enhanced chlamydial infection, suggesting a protective role in infection. IFN-ε signals by the same receptor, IFNAR used by IFN-α/β. Possible explanations for the discordant results during infection between *Ifnε*$^{-/-}$ and *Ifnar*$^{-/-}$ could be a result of a direct role for IFN-ε in chlamydial killing, its regulation by sex hormones and/or a constitutive role in imparting resistance to genital tract epithelia in an IFNAR-independent manner.

To understand the mechanism behind the conflicting role of type I IFN in vitro and in vivo, the pleiotropic immune functions of this cytokine in vivo needs further understanding. Type I IFNs are a potent regulator of adaptive immunity, affecting multiple cell types, including macrophages, lymphocytes, and DCs. IFNα/β induces the expression of several interferon response genes (IRG), which are important for Th1 maturation [52]. Type I IFNs have also been implicated in the generation of cytotoxic T cells and promotion of in vivo T cell proliferation [53] and T cell survival [54]. However, type I IFNs are also known to inhibit IFNγ-induced MHC class II expression [55–57] a function that contradicts its Th1 stimulatory role. Type I IFNs have also been shown to inhibit maturation and activation of mouse Langerhans cells [58]. IFN-β has been reported to augment [59] or downregulate IL-12 and CD40 expression in DC [60]. Further, therapeutic administration of IFN-β in multiple sclerosis patients led to inhibition of IL-12, augmentation of IL-10 production [61] and inhibition of IL-1β production [62]. The paradoxical effect of IFN-β on the expression of Th1-type immune responses partly depends on the timing of DC exposure (during maturation vs. mature) to IFNβ [52]. Type I IFNs are also pro-apoptotic and induce the expression of a number of pro-apoptotic genes, which could play a major role in pathological outcomes during infection. Overall, the detrimental effect of IFN-β during chlamydial infection is a likely result of inhibition of Th1-response, a reduction in IFNγ responsiveness, and induction of an apoptotic response. These results have been largely inferred from gene knockout mice studies and antibody depletion studies. It is possible that the pathological outcome could be different if the mice were treated with recombinant IFN-β. Treatment of mice with recombinant IFN-β has been shown to downregulate IL-1β levels significantly at multiple steps [62]. Since IL-1 signaling is a major player in oviduct pathology during genital chlamydial infection, this may be protective to the oviducts during infection [29]. Therefore, the overall effects of IFN-β during chlamydial infection in vivo is likely determined by its levels in the local tissue, and assigning a beneficial or detrimental role to it would be contextual.

Mechanism of IFN-β Induction During Chlamydial Infection

Multiple host PRRs can induce IFN-β expression during viral or bacterial infection [reviewed in [63]]. Purified *E. coli* LPS is a potent stimulator for TLR4 pathway and routinely used as a positive control for TLR4 activation [64]. However, chlamydial lipopolysaccharide (LPS) has low endotoxic activity [65, 66], which is attributed to the higher hydrophobicity of its lipid A moiety with fatty acids of longer chain length and the presence of non-hydroxylated fatty acids ester-linked to the sugar backbone. Therefore, chlamydiae stimulate TLR4 poorly, although there is one report demonstrating detection of chlamydial LPS by TLR2 [67]. Besides LPS, other bacterial ligands can stimulate TLR4, as in the case of purified hsp60 from *Chlamydia* spp. [68] However, during chlamydial infection, there is limited role for TLR4 in IFN-β induction [41]. Cell invasion and intracellular growth is a prerequisite for IFN response during chlamydial infection. This prerequisite would suggest that intracellular receptors would be preferred over membrane-expressed receptors during infection.

Early studies showed that *C. muridarum*-induced IFN-β is independent of TLR2 and TLR4, and some contribution from MyD88 pathway was suggested [41]. However, no contribution of TLR7 and TLR9 in IFN-β expression was observed and TLR4-MyD88 double knockout macrophages induced similar levels of IFN-β compared to WT macrophages [69]. Further, cytosolic RNA sensing RLR and MAVS pathways were dispensable for this response [69]. This study went on to show that the adaptor for DNA sensing, STING was essential for IFN-β induction during *C. muridarum* infection in both mouse and human epithelial cells [69]. STING was found to localize in close proximity to the inclusion [69]. These data suggested that DNA sensors or chlamydial cyclic di-AMP could be contributing to this response. Indeed, recently it was shown that cyclic di-AMP is produced by *C. trachomatis* EBs [70]. The contribution of second messenger cyclic di-AMP in IFN-β expression was shown by infecting HEK293T cells overexpressing STING, and transfected with IFN-β promoter-driven luciferase reporter construct and by using fibroblasts from STING-deficient mice. Recent studies from our laboratory involved screening of multiple DNA sensors during chlamydial infection and a predominant contribution of the DNA sensor cGAS was observed in multiple cell types in response to infection using multiple *C. trachomatis* serovar [71]. The discovery of cGAS as a requirement for IFN-β induction during chlamydial infection suggests that chlamydial DNA is available for sensing on the cytosolic side of the inclusion membrane. In support of this, cGAS was found distinctly localized on the cytosolic side of the chlamydial inclusion membrane and significant co-localization of cGAS and STING was observed after infection.

Besides DNA and cyclic dinucleotide sensing by STING, other receptors have also been shown to contribute to *Chlamydia*-induced IFN-β. During *C. pneumoniae* infection in HUVEC cells, signaling through MAVS was found to be essential for IRF3 activation [72]. MAVS associates with TRAF3, leading to activation of IRF transcription factors and IFN-β expression [73]. It was reported that TLR3

contributes to IFN-β induction in mouse oviduct epithelial cells, during *C. muridarum* infection [74]. The requirement for TLR3 for IFN-β in a bacterial infection is unique to *Chlamydia*. However, it has not been shown how TLR3 interacts with the chlamydial inclusion and the nature of chlamydial ligand engaged is unclear. Since STING was shown to be essential for IFN-β expression during chlamydial infection in the same cell type [69], it is unclear if there is any interaction between the two pathways. Taken together, these data suggest the use of more than one host receptors for IFN-β induction during chlamydial infection. These differences in observation could be due to: (1) differences between *C. trachomatis* vs. *C. pneumoniae* infection, (2) use of multiple pathways in cell types tested and their ability to compensate for each other, and/or (3) infection dose. The use of multiple receptors to induce the expression of the same cytokine may not be unique to *Chlamydia* spp., as multiple receptors have been suggested to play a role in IFN-β induction during *L. monocytogenes* infection [75–78]. The signaling of type I IFN by *Chlamydia* is summarized in Fig. 1.

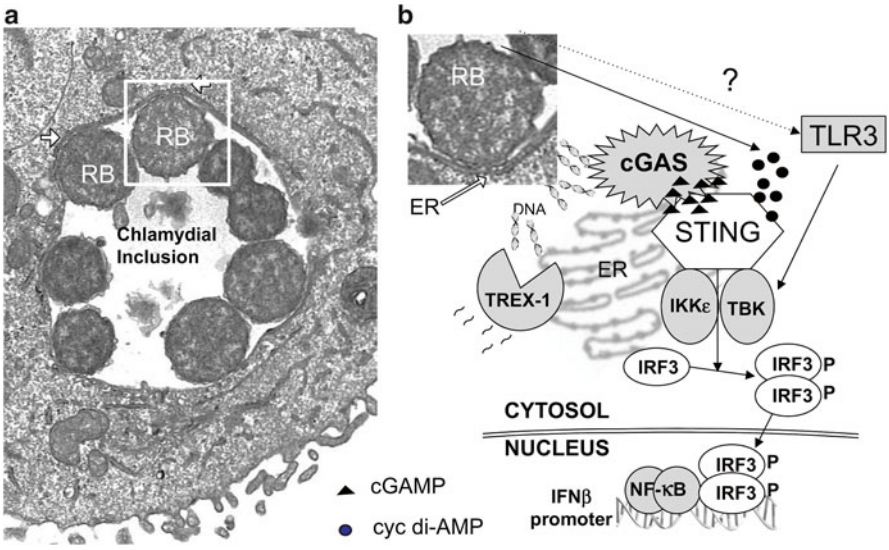

Fig. 1 Model(s) for IFN beta expression during chlamydial infection. (**a**) An electron micrograph of chlamydial inclusion containing metabolically active RBs. (**b**) An enlarged image of an RB, its interaction with the host ER outside the inclusion membrane, and the proposed model(s) for IFNβ expression during infection. At least three models have been proposed for chlamydial recognition with two demonstrating the requirement for the adaptor protein STING in IFNβ induction during infection. In the first model, chlamydial EB (not shown) produce cyc di-AMP that directly interacts with STING to result in IFN-β induction. In the second, the host DNA sensor cGAS was found to be essential for this response, with evidence for cGAMP production during infection. In support of this model, cells lacking the exonuclease TREX-1 show enhanced IFN-β expression during infection, implicating DNA as a ligand for this response. In an alternative third model, TLR3 knock down resulted in a decrease in IFN-β expression in a mouse oviduct cell line. The ligand engaged and its interaction with TLR3 is unknown

Chlamydial Ligands for IFN-β Response

The presence of enzymes essential for cyclic-di AMP synthesis and the demonstration of the presence of cyclic-di AMP in *C. trachomatis* EB [70] indicates cyclic-di AMP as a compelling ligand for IFNβ induction. Simultaneously, the significant requirement for cGAS for *Chlamydia*-induced IFN-β indicates DNA and cGAMP as a possible ligand for IFNβ induction during chlamydial infection [71]. Although this study does not show a direct interaction of DNA with cGAS, evidence for cGAMP production was provided by demonstration of its functional transfer. HeLa cells knocked down for cGAS or STING lose their ability to induce IFN-β upon infection, which was surprisingly rescued following their co-culture. These data suggest that cGAMP produced in cGAS competent cells during infection can function in trans by migrating to $STING^+$ cells to induce IFN-β. These results are based on a recent study that showed that cGAMP can cross gap junctions between epithelial cells and provide cells adjacent to an infected cell intrinsic immunity independent of IFNAR signaling [79]. cGAMP binds to the same pocket in STING as cyclic di-AMP/di-GMP, but at a much lower concentration with higher affinity [80]. Indeed, the cGAS product, 2′3′cGAMP, is a much more potent ligand of STING than all other bacterial cyclic di-nucleotides described [81]. Further, human STING is responsive only to cGAMP and unresponsive to the STING ligands CMA [82] and cyclic di-AMP/cyclic di-GMP [83], unlike mouse STING which is responsive to both cyclic dinucleotides and cGAMP [84]. These studies significantly shift the importance of cGAMP over bacterial cyclic dinucleotides during *C. trachomatis* infection in human cells. However, how chlamydial DNA is transferred to cytosol remains unclear at this point. Manzanillo et al. [85] have shown that during *Mycobacterium tuberculosis* infection, phagosomal permeabilization mediated by the bacterial ESX-1 secretion system allows cytosolic recognition pathways access to DNA [85]. Numerous studies have linked IFN-β expression to bacterial secretion systems [78, 86–88]. Small molecule inhibitors of type III secretion system (T3SS) were shown to abrogate IFN-β expression in *C. muridarum* infected cells [89], suggesting a similar role for chlamydial T3SS in permeabilization of inclusion membrane. Previous studies [90] have shown that chlamydial reticulate bodies (RB) make direct contact with the inclusion membrane, likely through T3SS. These could be potential permeabilization points where nucleic acids could leak into cytosol and made available for host recognition. It has been shown that *Chlamydia* hijacks the host ER and several ER proteins were found localized on inclusion membrane [91, 92]. The localization of the ER protein STING [69] and cytosolic cGAS in close proximity to the inclusion membrane suggest that STING could serve as a membrane scaffold for the interactions between DNA-cGAS to take place. An alternative source of DNA detected could be host mitochondrial DNA released following damage to mitochondria in *Chlamydia*-infected cells. This argument is countered by the observation that *Chlamydia* spp. inhibit host apoptosis and no mitochondrial damage has been observed in the first 24 h of infection [93]. However, in the environment of other innate receptor recognition and production of cytokines like TNFα, it

is possible that mitochondrial damage occurs during in vivo infection and may also contribute to DNA sensor-mediated activation.

Future Directions and Perspectives

A fascinating feature of IFN-β inducing pathways is the resources used by the cell to detect a wide variety of pathogens to generate this important cytokine. During evolution of the immune system, viral infections likely drove this arm of innate immunity to the complex form to which it presently exists. During infection with an intracellular bacteria, the host cells responds as it would to a viral infection, detecting cytoplasmic nucleic acids and producing IFN-β. However, IFN-β is insufficient to eradicate bacterial infection and not protective to the host during in vivo infection, as in the case of chlamydial infection. In such circumstances, one can speculate that the intracellular bacteria likely exploit this antiviral pathway to its advantage. For an STI pathogen such as *C. trachomatis* that does not cause death, this would result in a prolonged infection period in the host leading to increased transmissibility. Based on studies from the mouse model, we can predict that type I IFNs likely contribute to the persistent chlamydial infection reported in humans. This could be particularly relevant during chlamydial-viral co-infection. For instance during co-infection of *C. trachomatis* with human papilloma virus, the type I IFN response resulting from the viral infection is likely to benefit *C. trachomatis* infection. Whether this results in persistent infection for either or both pathogens is not clear, although there is some evidence for *C. trachomatis* infection to be a risk factor for persistent HPV infection [94]. Over the last decade, a lot has been learnt about type I IFN induction and its role in chlamydial infection. However, the exact molecular mechanism involved in IFN-β mediating host pathology is unclear. Further, the interaction of multiple PRRs and their cell type-specific role needs further elucidation. Over the following decade, we expect discovery of antagonists that can potentially block the pathological arm of this innate response during infection, simultaneously enhancing a protective T cell response during chlamydial infection.

References

1. Tabbara KF (2001) Trachoma: a review. J Chemother 13(Suppl 1):18–22
2. Mardh PA (2004) Tubal factor infertility, with special regard to chlamydial salpingitis. Curr Opin Infect Dis 17(1):49–52
3. Parks KS et al (1997) Spontaneous clearance of *Chlamydia trachomatis* infection in untreated patients. Sex Transm Dis 24(4):229–235
4. Morre SA et al (2002) The natural course of asymptomatic *Chlamydia trachomatis* infections: 45% clearance and no development of clinical PID after one-year follow-up. Int J STD AIDS 13(Suppl 2):12–18

5. Woolridge RL et al (1967) Long-term follow-up of the initial (1959–1960) trachoma vaccine field trial on Taiwan. Am J Ophthalmol 63(5)Suppl:1650–1655
6. Hillis SD et al (1997) Recurrent chlamydial infections increase the risks of hospitalization for ectopic pregnancy and pelvic inflammatory disease. Am J Obstet Gynecol 176(1 Pt 1): 103–107
7. Kimani J et al (1996) Risk factors for *Chlamydia trachomatis* pelvic inflammatory disease among sex workers in Nairobi, Kenya. J Infect Dis 173(6):1437–1444
8. Wyrick PB (2000) Intracellular survival by *Chlamydia*. Cell Microbiol 2(4):275–282
9. Bavoil PM, Hsia R, Ojcius DM (2000) Closing in on *Chlamydia* and its intracellular bag of tricks. Microbiology 146(Pt 11):2723–2731
10. Eissenberg LG, Wyrick PB (1981) Inhibition of phagolysosome fusion is localized to *Chlamydia psittaci*-laden vacuoles. Infect Immun 32(2):889–896
11. Heinzen RA et al (1996) Differential interaction with endocytic and exocytic pathways distinguish parasitophorous vacuoles of *Coxiella burnetii* and *Chlamydia trachomatis*. Infect Immun 64(3):796–809
12. Scidmore M, Fischer E, Hackstadt T (1996) Sphingolipids and glycoproteins are differentially trafficked to the *Chlamydia trachomatis* inclusion. J Cell Biol 134(2):363–374
13. Grieshaber NA et al (2004) Chlamydial histone-DNA interactions are disrupted by a metabolite in the methylerythritol phosphate pathway of isoprenoid biosynthesis. Proc Natl Acad Sci U S A 101(19):7451–7456
14. Hatch TP, Allan I, Pearce JH (1984) Structural and polypeptide differences between envelopes of infective and reproductive life cycle forms of *Chlamydia* spp. J Bacteriol 157(1):13–20
15. Hatch TP, Miceli M, Sublett JE (1986) Synthesis of disulfide-bonded outer membrane proteins during the developmental cycle of *Chlamydia psittaci* and *Chlamydia trachomatis*. J Bacteriol 165(2):379–385
16. Hackstadt T, Scidmore MA, Rockey DD (1995) Lipid metabolism in *Chlamydia trachomatis*-infected cells: directed trafficking of Golgi-derived sphingolipids to the chlamydial inclusion. Proc Natl Acad Sci U S A 92(11):4877–4881
17. Hackstadt T et al (1996) *Chlamydia trachomatis* interrupts an exocytic pathway to acquire endogenously synthesized sphingomyelin in transit from the Golgi apparatus to the plasma membrane. EMBO J 15(5):964–977
18. Scidmore MA et al (1996) Vesicular interactions of the *Chlamydia trachomatis* inclusion are determined by chlamydial early protein synthesis rather than route of entry. Infect Immun 64(12):5366–5372
19. Hybiske K, Stephens RS (2007) Mechanisms of host cell exit by the intracellular bacterium *Chlamydia*. Proc Natl Acad Sci U S A 104(27):11430–11435
20. Rasmussen SJ et al (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(1):77–87
21. Rank RG et al (2010) Host chemokine and cytokine response in the endocervix within the first developmental cycle of *Chlamydia muridarum*. Infect Immun 78(1):536–544
22. Barron AL et al (1981) A new animal model for the study of *Chlamydia trachomatis* genital infections: infection of mice with the agent of mouse pneumonitis. J Infect Dis 143(1):63–66
23. Rank RG et al (2008) Chlamydiae and polymorphonuclear leukocytes: unlikely allies in the spread of chlamydial infection. FEMS Immunol Med Microbiol 54(1):104–113
24. Imtiaz MT et al (2007) A role for matrix metalloproteinase-9 in pathogenesis of urogenital *Chlamydia muridarum* infection in mice. Microbes Infect 9(14–15):1561–1566
25. Lee HY et al (2010) A role for CXC chemokine receptor-2 in the pathogenesis of urogenital *Chlamydia muridarum* infection in mice. FEMS Immunol Med Microbiol 60(1):49–56
26. Frazer LC et al (2011) Enhanced neutrophil longevity and recruitment contribute to the severity of oviduct pathology during *Chlamydia muridarum* infection. Infect Immun 79(10):4029–4041
27. Darville T et al (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(11): 6187–6197

28. Nagarajan UM et al (2008) Type I interferon signaling exacerbates *Chlamydia muridarum* genital infection in a murine model. Infect Immun 76(10):4642–4648
29. Nagarajan UM et al (2012) Significant role of IL-1 signaling, but limited role of inflammasome activation, in oviduct pathology during *Chlamydia muridarum* genital infection. J Immunol 188(6):2866–2875
30. Prantner D et al (2011) Interferon regulatory transcription factor 3 protects mice from uterine horn pathology during *Chlamydia muridarum* genital infection. Infect Immun 79(10): 3922–3933
31. Prantner D et al (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(12):5334–5346
32. Cheng W et al (2008) Caspase-1 contributes to *Chlamydia trachomatis*-induced upper urogenital tract inflammatory pathologies without affecting the course of infection. Infect Immun 76(2):515–522
33. Murthy AK et al (2011) Tumor necrosis factor alpha production from $CD8^+$ T cells mediates oviduct pathological sequelae following primary genital *Chlamydia muridarum* infection. Infect Immun 79(7):2928–2935
34. Rank RG, Soderberg LS, Barron AL (1985) Chronic chlamydial genital infection in congenitally athymic nude mice. Infect Immun 48(3):847–849
35. Rank RG, Barron AL (1983) Effect of antithymocyte serum on the course of chlamydial genital infection in female guinea pigs. Infect Immun 41(2):876–879
36. Rank RG et al (1992) Effect of gamma interferon on resolution of murine chlamydial genital infection. Infect Immun 60(10):4427–4429
37. Cotter TW et al (1997) Dissemination of *Chlamydia trachomatis* chronic genital tract infection in gamma interferon gene knockout mice. Infect Immun 65(6):2145–2152
38. Johansson M et al (1997) Studies in knockout mice reveal that anti-chlamydial protection requires TH1 cells producing IFN-gamma: is this true for humans? Scand J Immunol 46(6): 546–552
39. Johnson RM (2004) Murine oviduct epithelial cell cytokine responses to *Chlamydia muridarum* infection include interleukin-12-p70 secretion. Infect Immun 72(7):3951–3960
40. Rothfuchs AG et al (2001) IFN-alpha beta-dependent, IFN-gamma secretion by bone marrow-derived macrophages controls an intracellular bacterial infection. J Immunol 167(11): 6453–6461
41. Nagarajan UM et al (2005) Chlamydia trachomatis induces expression of IFN-gamma-inducible protein 10 and IFN-beta independent of TLR2 and TLR4, but largely dependent on MyD88. J Immunol 175(1):450–460
42. Devitt A et al (1996) Induction of alpha/beta interferon and dependent nitric oxide synthesis during *Chlamydia trachomatis* infection of McCoy cells in the absence of exogenous cytokine. Infect Immun 64(10):3951–3956
43. Shaw JH et al (2001) Expression of genes encoding Th1 cell-activating cytokines and lymphoid homing chemokines by chlamydia-pulsed dendritic cells correlates with protective immunizing efficacy. Infect Immun 69(7):4667–4672
44. Wolf K, Fields KA (2013) *Chlamydia pneumoniae* impairs the innate immune response in infected epithelial cells by targeting TRAF3. J Immunol 190(4):1695–1701
45. de la Maza LM et al (1985) Interferon-induced inhibition of *Chlamydia trachomatis*: dissociation from antiviral and antiproliferative effects. Infect Immun 47(3):719–722
46. Carlin JM, Weller JB (1995) Potentiation of interferon-mediated inhibition of *Chlamydia* infection by interleukin-1 in human macrophage cultures. Infect Immun 63(5):1870–1875
47. Shemer-Avni Y, Wallach D, Sarov I (1988) Inhibition of *Chlamydia trachomatis* growth by recombinant tumor necrosis factor. Infect Immun 56(9):2503–2506
48. Rothermel CD, Byrne GI, Havell EA (1983) Effect of interferon on the growth of *Chlamydia trachomatis* in mouse fibroblasts (L cells). Infect Immun 39(1):362–370
49. Qiu H et al (2008) Type I IFNs enhance susceptibility to *Chlamydia muridarum* lung infection by enhancing apoptosis of local macrophages. J Immunol 181(3):2092–2102

50. Fung KY et al (2013) Interferon-epsilon protects the female reproductive tract from viral and bacterial infection. Science 339(6123):1088–1092
51. Hermant P et al (2013) IFN-epsilon is constitutively expressed by cells of the reproductive tract and is inefficiently secreted by fibroblasts and cell lines. PLoS One 8(8):e71320
52. Nagai T et al (2003) Timing of IFN-beta exposure during human dendritic cell maturation and naive Th cell stimulation has contrasting effects on Th1 subset generation: a role for IFN-beta-mediated regulation of IL-12 family cytokines and IL-18 in naive Th cell differentiation. J Immunol 171(10):5233–5243
53. Tough DF, Borrow P, Sprent J (1996) Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science 272(5270):1947–1950
54. Belardelli F et al (2002) Interferon-alpha in tumor immunity and immunotherapy. Cytokine Growth Factor Rev 13(2):119–134
55. Heise MT et al (1998) Murine cytomegalovirus infection inhibits IFN gamma-induced MHC class II expression on macrophages: the role of type I interferon. Virology 241(2):331–344
56. Lu HT et al (1995) Interferon (IFN) beta acts downstream of IFN-gamma-induced class II transactivator messenger RNA accumulation to block major histocompatibility complex class II gene expression and requires the 48-kD DNA-binding protein, ISGF3-gamma. J Exp Med 182(5):1517–1525
57. Devajyothi C et al (1993) Inhibition of interferon-gamma-induced major histocompatibility complex class II gene transcription by interferon-beta and type beta 1 transforming growth factor in human astrocytoma cells. Definition of cis-element. J Biol Chem 268(25): 18794–18800
58. Fujita H et al (2005) Type I interferons inhibit maturation and activation of mouse Langerhans cells. J Invest Dermatol 125(1):126–133
59. Gautier G et al (2005) A type I interferon autocrine-paracrine loop is involved in Toll-like receptor-induced interleukin-12p70 secretion by dendritic cells. J Exp Med 201(9): 1435–1446
60. McRae BL, Beilfuss BA, van Seventer GA (2000) IFN-{beta} differentially regulates CD40-induced cytokine secretion by human dendritic cells. J Immunol 164(1):23–28
61. Byrnes AA, McArthur JC, Karp CL (2002) Interferon-beta therapy for multiple sclerosis induces reciprocal changes in interleukin-12 and interleukin-10 production. Ann Neurol 51(2): 165–174
62. Guarda G et al (2011) Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 34(2):213–223
63. Stetson DB, Medzhitov R (2006) Type I interferons in host defense. Immunity 25(3): 373–381
64. Tapping RI et al (2000) Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for *Escherichia* and *Salmonella* lipopolysaccharides. J Immunol 165(10):5780–5787
65. Heine H et al (2003) Endotoxic activity and chemical structure of lipopolysaccharides from *Chlamydia trachomatis* serotypes E and L2 and *Chlamydophila psittaci* 6BC. Eur J Biochem 270(3):440–450
66. Rund S et al (1999) Structural analysis of the lipopolysaccharide from *Chlamydia trachomatis* serotype L2. J Biol Chem 274(24):16819–16824
67. Erridge C et al (2004) Lipopolysaccharides of *Bacteroides fragilis*, *Chlamydia trachomatis* and *Pseudomonas aeruginosa* signal via toll-like receptor 2. J Med Microbiol 53(Pt 8):735–740
68. Bulut Y et al (2002) Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. J Immunol 168(3):1435–1440
69. Prantner D, Darville T, Nagarajan UM (2010) Stimulator of IFN gene is critical for induction of IFN-beta during *Chlamydia muridarum* infection. J Immunol 184(5):2551–2560
70. Barker JR et al (2013) STING-dependent recognition of cyclic di-AMP mediates type I interferon responses during *Chlamydia trachomatis* infection. mBio 4(3)

71. Zhang Y, Yeruva L, Marinov A, Prantner D, Wyrick PB, Lupashin V, Nagarajan UM (2014) The DNA Sensor, Cyclic GMP-AMP Synthase, Is Essential for Induction of IFN-β during Chlamydia trachomatis Infection. J Immunol Sep 1;193(5):2394–404
72. Buss C et al (2010) Essential role of mitochondrial antiviral signaling, IFN regulatory factor (IRF)3, and IRF7 in *Chlamydophila pneumoniae*-mediated IFN-beta response and control of bacterial replication in human endothelial cells. J Immunol 184(6):3072–3078
73. Saha SK et al (2006) Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif. EMBO J 25(14):3257–3263
74. Derbigny WA et al (2011) The *Chlamydia muridarum*-induced IFN-beta response is TLR3-dependent in murine oviduct epithelial cells. J Immunol 185(11):6689–6697
75. Yang P et al (2010) The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. Nat Immunol 11(6):487–494
76. Soulat D et al (2006) Cytoplasmic *Listeria monocytogenes* stimulates IFN-beta synthesis without requiring the adapter protein MAVS. FEBS Lett 580(9):2341–2346
77. Ishikawa H, Ma Z, Barber GN (2009) STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 461(7265):788–792
78. Crimmins GT et al (2008) *Listeria monocytogenes* multidrug resistance transporters activate a cytosolic surveillance pathway of innate immunity. Proc Natl Acad Sci U S A 105(29): 10191–10196
79. Ablasser A et al (2013) Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. Nature 503(7477):530–534
80. Wu J et al (2013) Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339(6121):826–830
81. Zhang X et al (2013) Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. Mol Cell 51(2):226–235
82. Cavlar T et al (2013) Species-specific detection of the antiviral small-molecule compound CMA by STING. EMBO J 32(10):1440–1450
83. Conlon J et al (2013) Mouse, but not human STING, binds and signals in response to the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid. J Immunol 190(10):5216–5225
84. Gao P et al (2013) Structure-function analysis of STING activation by c[G(2′,5′)pA(3′,5′)p] and targeting by antiviral DMXAA. Cell 154(4):748–762
85. Manzanillo PS et al (2012) *Mycobacterium Tuberculosis* activates the DNA-dependent cytosolic surveillance pathway within macrophages. Cell Host Microbe 11(5):469–480
86. Stetson DB, Medzhitov R (2006) Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. Immunity 24(1):93–103
87. Roux CM et al (2007) Brucella requires a functional type IV secretion system to elicit innate immune responses in mice. Cell Microbiol 9(7):1851–1869
88. Stanley SA et al (2007) The type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. J Immunol 178(5): 3143–3152
89. Prantner D, Nagarajan UM (2009) Role for the chlamydial type III secretion apparatus in host cytokine expression. Infect Immun 77(1):76–84
90. Wilson DP et al (2006) Type III secretion, contact-dependent model for the intracellular development of chlamydia. Bull Math Biol 68(1):161–178
91. Dumoux M et al (2012) Chlamydiae assemble a pathogen synapse to hijack the host endoplasmic reticulum. Traffic 13(12):1612–1627
92. Giles DK, Wyrick PB (2008) Trafficking of chlamydial antigens to the endoplasmic reticulum of infected epithelial cells. Microbes Infect 10:1494–1503
93. Fan T et al (1998) Inhibition of apoptosis in chlamydia-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. J Exp Med 187(4):487–496
94. Silins I et al (2005) *Chlamydia trachomatis* infection and persistence of human papillomavirus. Int J Cancer 116(1):110–115