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Plasmid-Deficient Chlamydia muridarum Fail to Induce Immune Pathology and Protect against Oviduct Disease

Catherine M. O’Connell, Robin R. Ingalls, Charles W. Andrews, Jr., Amy M. Scurlock, and Toni Darville

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial infection in the world. In women, genital infection can cause endometritis and pelvic inflammatory disease with the severe sequelae of ectopic pregnancy or infertility. Chlamydia sp. do not damage tissues directly, but induce an injurious host inflammatory response at the infected site. In the murine model of genital disease with Chlamydia muridarum, TLR2 plays a role in both early production of inflammatory mediators and development of chronic oviduct pathology. We report the results of studies with plasmid-cured C. muridarum mutants that retain the ability to infect the murine genital tract, but fail to cause disease in the oviduct. These mutants do not stimulate TLR2-dependent cytokine production in mice, nor in innate immune cells or epithelial cells in vitro. They induce an effective Th1 immune response, with no evidence for Th1-immune-mediated collateral tissue damage. Furthermore, mice previously infected with the plasmid-deficient strains are protected against oviduct disease upon challenge with virulent C. muridarum. If plasmid-cured derivatives of human C. trachomatis biovars exhibit similar phenotypic characteristics, they have the potential to serve as vaccines to prevent human disease.


C. trachomatis ocular infections cause trachoma, the world’s leading cause of preventable blindness (1). C. trachomatis is also the leading cause of bacterial sexually transmitted infections worldwide (2). In women, untreated infections may progress to serious reproductive tract sequelae. Observational and randomized studies yield risk estimates of 15–80% for developing pelvic inflammatory disease (3); 5–25% for ectopic pregnancy (4); and 10–20% for tubal factor infertility (5, 6) subsequent to chlamydial infection. The protective immunity that develops in response to infection is not long-lived (7), and animal models indicate that repeated infection elicits more severe disease (8–12). Although antibiotic therapy eliminates chlamydial infection, it does not ameliorate established pathology. Furthermore, the administration of antimicrobial agents may blunt the development of natural immunity to C. trachomatis in humans (13), as has been demonstrated in mouse models of infection (14).

Data from both human studies and animal models point to CD4+ Th1 cells as essential to recovery from infection and resistance to reinfection (15–22). However, the immunopathogenesis of chlamydial disease is not clear. Both cellular (23) and immunological hypotheses (24) have been proposed. Evidence that the innate immune response may be the primary mediator of pathology comes from the mouse model of genital tract infection, in which the presence of neutrophils that release and activate proteolytic molecules during acute infection correlates directly with development of fibrotic occlusion of the oviduct (25, 26).

Pattern recognition receptors (PRRs)3 found on innate immune cells enable recognition of signature structures of pathogens, called pathogen-associated molecular patterns. Engagement of PRRs, which include TLRs, by pathogen-associated molecular patterns leads to activation of innate immune cells that stimulate and direct the activity of pathogen-specific T cells (27). We have shown that TLR2-deficient mice do not develop oviduct pathology after chlamydial infection, demonstrating that TLR2 signaling is directly involved in disease development (28). Because chlamydiae are present as both extra- and intracellular/intravacuolar forms, they have the potential to stimulate multiple PRR pathways. In this study, we report novel Chlamydia muridarum mutants that retain the ability to infect the murine genital tract, but fail to cause disease. These mutants fail to stimulate TLR2-dependent signaling in cell culture and in vivo. Mice previously infected with the mutants were protected against disease when challenged with virulent C. muridarum, evidence that a protective adaptive immune response generated in the absence of TLR2 signaling is sufficient to protect from pathology caused by the innate responses induced upon infection.

Materials and Methods

Propagation of Chlamydia and isolation of C. muridarum CM3.1

All C. muridarum strains, including Nigg (29), CM972 (30), and CM3.1, were propagated in McCoy cells (American Type Culture Collection CRL-1696), as previously described (30). Bacteria were titrated by plaque assay (30) or as inclusion-forming units (IFU) (31). UV-inactivated bacteria were prepared, as described (28). The presence of the cryptic plasmid in

3 Abbreviations used in this paper: PRR, pattern recognition receptor; IFU, inclusion-forming unit; KO, knockout; MOI, multiplicity of infection; ShEC, immortalized ectocervical epithelial cell line.

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C. muridarum was detected by PCR using the primers pMoPn F1 (5'-CG TGCAATGACTTCGAGGA-3') and pMoPn R1 (5'-TGCAAGGAGG TAAACGTTC-3').

C. muridarum CM972 was plated on confluent McCoy cell monolayers at a multiplicity of 1–2 and incubated for 2 h at 37°C without centrifugation before being overlaid with 1× DMEM, 10% FBS, 0.25% agarose, and 0.01 μg ml⁻¹ cycloheximide. The monolayers were incubated at 37°C, 5% CO₂ for 5–7 days, to allow plaques to form. Under these culture conditions, C. muridarum CM972 forms only tiny plaques at low frequency. However, large plaques resembling those formed by C. muridarum Nigg were observed occurring at a frequency of 1–2 × 10⁻⁶ relative to the primary inoculum applied to the monolayers. The plaques were harvested and individually purified by repeating the plaquing procedure without centrifugation three times before being expanded. Subsequent determination of the plaquing efficiency of the isolates was performed, as previously described (31). One isolate was selected for further study and designated C. muridarum CM3.1.

Murine infection and monitoring
Six- to 8-wk-old female C3H/HeoJ mice were obtained from The Jackson Laboratory, and mice homozygous for Tri⁻/⁻ were provided by S. Akira (Osaka, Japan). Mice were injected intravaginally, as described (31), with 30 μl of buffer containing 3 × 10⁵ IFU of C. muridarum Nigg, CM972, or CM3.1. Mice were monitored for cervicovaginal shedding, as described (31). Bacterial burden in oviduct tissues was measured by PFU determination on McCoy cells (30). Oviducts dissected free from the uterine horns of a single mouse were homogenized in 1 ml of protease inhibitor buffer, and an aliquot was removed for isolation and titration of chlamydiae. All animal experiments were preapproved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee.

Immune studies and tissue analysis
Genital tract secretions were collected and analyzed for cytokines, as described previously (28). Sera from mice were collected by retroorbital bleeds at the time of sacrifice and stored at –20°C until analyzed by ELISA, as previously described (31). Preimmune sera were used as negative controls. The titer for individual mice was determined as the highest serum dilution with an OD value greater than that of the control wells. T cell proliferation assays were performed, as described (26), with the addition of anti-CD4 (BD Biosciences) to select cell cultures to investigate CD4-T cell specificity of the response. Histopathological analysis of fixed genital tract tissues was performed, as described (31). Immunohistochemistry was performed on paraffin-embedded tissues sectioned at 5 μm, deparaffinized, and hydrated. Sections were incubated with anti-chlamydial LPS mAb (gift from Y.-X. Zhang, Boston University, Boston, MA) at a dilution of 1/1000. The immunohistochemical procedure was performed manually using the EnVision G2 System/AP visualization kit (DakoCytomation), according to the manufacturer’s instructions. The reaction was visualized by Permanent Red Chromogen, and sections were mounted in Faramount aqueous mount medium (DakoCytomation).

In vitro analysis of cellular responses
The following cell lines were examined: HEK293 cells stably expressing TLR2 and MyD88 (33) and a human papillomavirus 16/E6E7 immortalized ectocervical epithelial cell line (ShEC) (34). In addition, in vitro infection was performed in murine bone marrow-derived dendritic cells grown from bone marrow cultures following the procedure of Inaba et al. (35). The TLR2 agonist, PamCys-Ser-Lys (Axxora), or human rTNF-α (R&D Systems) were used as positive control stimulants. Cells were plated in 24-well tissue culture dishes at a density of 10⁵ cells/well. Infections were conducted by overlaying cells with a multiplicity of 0.5–5. Cells were incubated for 18–40 h at 37°C, 5% CO₂. Supernatant was harvested and assayed for IL-8 using a DuoSet ELISA kit from R&D Systems, or for IL-1α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40/p70, GM-CSF, IFN-γ, and TNF-α by multiplex bead cytokine arrays (BioSource International). All data points were assayed in triplicate and reported as the mean ± SD.

Statistics
The ANOVA plus post hoc test was used to analyze differences in cytokine production among various in vitro groups. Statistical comparisons between the murine strains for level of infection and cytokine production over the course of infection were made by a two-factor (days and murine strain) ANOVA with post hoc Holm-Sidak test as a multiple comparison procedure. The Wilcoxon rank sum test was used to compare the duration of infection in the respective strains over time. The Kruskal-Wallis one-way ANOVA on ranks was used to determine significant differences in the pathological data between groups. The χ² test for determination of significant differences in sample proportions was used to compare frequencies of pathological findings between specific groups. SigmaStat software was used (SPSS).

Results
Plasmid-deficient derivatives of C. muridarum strain Nigg have different phenotypes in cell culture
We recently reported curing C. muridarum Nigg of its resident 7.5-kb plasmid using novobiocin (30). This strain, CM972, displays altered plaque morphology (plaque diameter reduced >0.5-fold) (Fig. 1A) and reduced infectivity in cell culture when compared with its parent. The mechanism underlying the attachment/uptake defect in this strain is not known, but the deficiency is overcome when the bacteria are inoculated into cells via centrifugation. Subsequently, we noted that inoculation of confluent McCoy cell monolayers in a plaque assay with high titer preparations of CM972 without centrifugation resulted in the recovery of plaques with wild-type morphology (Fig. 1A) at a frequency of
Subculturing of these derivatives of CM972 revealed that they were able to infect McCoy cells at efficiencies comparable to that of Nigg. In all other respects, these derivatives of CM972 resembled the parental strain. Plasmid DNA was undetectable by PCR (Fig. 1B) or Southern hybridization (data not shown); the isolates were unable to accumulate glycogen within intracytoplasmic inclusions, but could be stained with a C. muridarum-specific mAb directed against the major outer membrane protein MOMP (30). A single isolate, designated CM3.1, was selected for further study.

A comparison of the infectious yield of CM972 with its parent, Nigg, when grown in synchronous culture at a multiplicity of infection (MOI) of 0.5 (30) revealed no difference, suggesting that once inside the cell, both strains replicated and formed infectious progeny equally. To evaluate the growth properties of CM3.1 and to compare chlamydial growth of all three strains throughout the developmental cycle, we infected confluent monolayers of McCoy cells growing in medium supplemented with cycloheximide with either C. muridarum Nigg, CM972, or CM3.1 at a MOI of 0.5 via centrifugation. The infected cells were harvested at 4-h intervals over the course of a 36-h infection and titrated for chlamydial. No difference was observed between the strains over the course of the chlamydial developmental cycle (Fig. 1C). Each strain displayed eclipse phases (the period of time during infection when only non-infectious reticulate bodies are present in the infected cells) of equal duration, and a similar yield of infectious progeny from ~24 h postinfection. This indicated that the phenotypic changes noted for both CM972 and CM3.1 did not affect the strains’ abilities to replicate in cell culture.

The course of murine lower genital tract infection with plasmid-deficient strains resembles that produced by the parental Nigg strain

Three independent experiments were conducted in which groups of five progesterone-treated C3H mice were each inoculated intravaginally with 3 \times 10^5 IFU of C. muridarum strain Nigg, CM3.1, or CM972. The intensity and duration of infection were determined by quantitative culture of cervical swabs taken at intervals through day 42 (Fig. 2A). No difference in the rate of resolution was seen among the groups. The intensity of infection with CM972 was 10-fold lower than that of CM3.1 and Nigg between days 2 and 10, but indistinguishable thereafter (Fig. 2A). Thus, mice infected with CM972, which manifests an attachment/uptake defect in vitro, experience an infection that is slightly decreased in intensity early on, but later parallels that of CM3.1 and Nigg.

Mice infected with CM972 or CM3.1 display minimal oviduct pathology

Mice were sacrificed 42 days postinfection, and genital tract tissues were harvested and examined grossly and histologically for pathology (31). Hydrosalpinx was only observed in oviducts of mice infected with Nigg, whereas the oviducts from mice infected with the plasmid-deficient strains appeared normal. Histologically, inflammatory parameters scored in the ectocervix, endocervix, and uterine horns did not differ among the groups (data not shown). In contrast, significant differences were observed in the oviducts from mice infected with Nigg when compared with CM3.1 and CM972 (Fig. 2B). Acute (neutrophils), chronic (lymphocytes), and plasma cell infiltrates were absent, and oviduct dilatation was significantly reduced in mice infected with plasmid-deficient strains (Fig. 2B) and did not differ from a control group of mice that were mock infected (31). Oviducts from Nigg-infected mice were dilated and distended, and exhibited a loss of plicae and a flattened or denuded epithelium with copious inflammatory infiltrates (Fig. 3A). In contrast, the oviducts from mice infected with CM972 (Fig. 3B) and CM3.1 (Fig. 3C) appeared normal, with plicated epithelium and open, unobstructed lumens. These observations were striking because C3H mice are particularly prone to develop pathology as a consequence of chlamydial infection (31, 36).
The absence of oviduct pathology in mice infected with plasmid-deficient C. muridarum is caused by a failure to activate signal transduction via TLR2.

Our prior studies have established an essential role for TLR2 in the induction of oviduct pathology associated with C. muridarum infection (28). Although TLR2 knockout (KO) mice experienced an unaltered infection course, they exhibited a marked reduction in chronic oviduct pathology compared with wild-type mice. The reduced oviduct pathology was associated with significantly lower levels of TNF-α and MIP-2 in genital secretions during the first week of infection. The striking reduction in immune pathology observed in mice infected with CM972 or CM3.1 led us to hypothesize that the plasmid-deficient strains failed to stimulate TLR2-dependent signaling. We compared TNF-α and MIP-2 levels in genital tract secretions of mice infected with the plasmid-deficient strains with those from mice infected with Nigg during the first 10 days of infection. Significantly reduced levels of TNF-α (Fig. 4A) and MIP-2 (data not shown) were observed, resembling the decreased responses described in Nigg-infected TLR2 KO mice (28).

Purified and rested bone marrow-derived dendritic cells were incubated with chlamydiae at a MOI of 1 or 2, as described previously for macrophages (28). After 24 h, an aliquot of supernatant was removed and assayed for cytokines. Intracellular staining of the infected DCs with anti-chlamydial LPS Ab 3 and 24 h postinfection revealed that all three strains of C. muridarum had been taken up and eliminated by 24 h. No inclusions were observed, consistent with the report of Zhang et al. (37) that C. muridarum does not replicate in DCs. Dendritic cell supernatants were analyzed for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40/p70, GM-CSF, IFN-γ, and TNF-α. Of the cytokines assayed, incubation of the DCs with Nigg resulted in significant increases above medium controls for IL-6, IL-12p40/p70, TNF-α, and GM-CSF. Dendritic cells incubated with CM3.1 (Fig. 4B) or CM972 (data not shown) secreted these cytokines at significantly reduced levels. Furthermore, the cytokine responses elicited by the plasmid-deficient strains were similar to those induced by Nigg in TLR2 KO DCs (Fig. 4B).

Cervical epithelial cells play a critical role in early immune signaling in response to infection by sexually transmitted pathogens. We previously reported that primary and immortalized human cervical epithelial cells express a variety of TLRs, with the exception of TLR4 and the associated protein MD-2 (38). In subsequent studies, we showed that infection of ShEC cells with C. trachomatis resulted in a dose-dependent induction of IL-8 secretion that was entirely dependent on MyD88 expression (32). Using HEK293 cells stably transfected with TLR2 or TLR4/MD-2, we determined that chlamydial-induced IL-8 secretion was predominately TLR2 dependent, with minimal TLR4-dependent activity (32). To investigate the effect of infection by C. muridarum Nigg and its plasmid-deficient derivatives on the secretion of IL-8 by relevant epithelial cells, ShEC and HEK293/TLR2 cells were infected in triplicate. After 24-h incubation, the culture supernatants were analyzed for the presence of IL-8 by ELISA. In both the ShEC cells (Fig. 4C) and HEK293/TLR2 cells (Fig. 4D), a dose-dependent increase in IL-8 secretion occurred in response to infection with Nigg. However, infection with CM972 or CM3.1 did not result in any increase in IL-8 secretion over that seen with cells cultured in medium alone (Fig. 4C, 4D, and 4F). The infectious progeny from these cell cultures were enumerated on McCoy cells, and no differences were observed between the strains (data not shown). Thus, although the plasmid-deficient strains productively infect human epithelial cells at a level equivalent to Nigg, active replication of the attenuated strains failed to elicit TLR2-dependent cell activation.

Infection of mice with either CM972 or CM3.1 results in Th1-predominant adaptive immune responses as seen in mice infected with the parental Nigg strain.

Multiple studies of murine chlamydial infection report a preponderance of IgG2a vs IgG1, reflective of a Th1-dominant response. Using ELISA (31), we detected C. muridarum elementary body-specific IgG2a in serum taken from mice infected with Nigg, CM972, or CM3.1 on day 28 postinfection. Titers of IgG2a were not different among the groups (mean ± SD log10 IgG2a = 3.3 ±
Prior infection with plasmid-deficient C. muridarum protects against the development of chlamydial disease after challenge with strain Nigg

A single experiment was conducted in which groups containing five progesterone-treated C3H mice were each inoculated intravaginally with $3 \times 10^5$ IFU of C. muridarum strain Nigg, CM3.1, or CM972. The intensity and duration of infection were monitored until resolution (Fig. 4A). The courses of infection were similar between the groups, as previously determined (Fig. 5A). Ninety-eight days postprimary infection, each group was challenged with $3 \times 10^5$ IFU of C. muridarum strain Nigg. Mice in all three groups became infected upon challenge with the wild-type strain (Fig. 5B). The intensity and duration of challenge infection with Nigg were reduced compared with primary infection in all three groups, but this decrease was statistically significant ($p < 0.001$) only for mice primarily infected with Nigg; $p = 0.05$ for mice primarily infected with CM972 and $p = 0.18$ for mice primarily infected with CM3.1. Clearance of challenge infection with Nigg was delayed in mice primarily infected with the plasmid-deficient strains ($p = 0.02$ for Nigg/Nigg vs CM3.1/Nigg; $p = 0.05$ for Nigg/Nigg vs CM972/Nigg; $p = 0.03$ for CM3.1/Nigg vs CM972/Nigg by two-way repeated measures ANOVA).

The mice were sacrificed 42 days after challenge, and genital tract tissues were harvested and examined for histopathology (31). Eight of 10 oviducts from mice primarily infected with Nigg exhibited hydrosalpinx, compared with 2 of 10 oviducts from mice primarily infected with CM3.1. In mice primarily infected with CM972, hydrosalpinx was not seen. Inflammatory parameters were statistically significantly higher in mice primarily infected with Nigg compared with mice primarily infected with either CM972 or CM3.1 (Fig. 5C). Furthermore, 6 of 10 oviducts from Nigg/Nigg-infected mice showed evidence of scarring fibrosis, a pathological finding not observed for the mice primarily infected with the plasmid-deficient strains and challenged with Nigg (Fig. 5, D and E).

| FIGURE 4. | Infection with plasmid-deficient strains of C. muridarum leads to reduced levels of cytokines largely dependent on TLR2. A, Means ± SD TNF-α in genital secretions of five C3H/HeN mice infected with Nigg (▲), CM972 (○), or CM3.1 (■); $p = 0.025$ for Nigg vs CM3.1; $p = 0.017$ for Nigg vs CM972; $p = 0.06$ for CM3.1 vs CM972 (two-way repeated measures ANOVA). B, Plasmid-deficient strains induce TNF-α responses in wild-type DCs similar to those induced by Nigg in TLR2 KO DCs. Supernatants from wild-type DCs (▲) or TLR2 KO DCs (■) cultured 24 h with MOI = 2 of Nigg or CM3.1, or the positive control TLR2-ligand, Pam3Cys, were assayed for TNF-α by bead array. Bars = means ± SD calculated from triplicate wells in a single representative experiment. Plasmid-deficient strains of C. muridarum fail to induce TLR2-dependent production of IL-8 in human cervical epithelial cells (C) or HEK293/TLR2 cells (D). Supernatants 24 h postinfection with Nigg (▲), CM972 (■), or CM3.1 (■) at MOIs of 0.5, 1, and 5 were assayed by ELISA for IL-8. Bars = means ± SD IL-8 concentration calculated from triplicate wells. |
**FIGURE 5.** Prior infection with plasmid-deficient *C. muridarum* does not protect against infection, but protects against disease after challenge with strain Nigg. Quantitative IFU from cervical swabs obtained after A, primary infection with Nigg (▲), CM972 (○), or CM3.1 (●), and B, challenge infection with Nigg after resolution of a primary infection with either strain Nigg (▲), CM972 (○), or CM3.1 (●). Numbering in B reflects days following primary infection. Data points are means ± SD of mice positive for infection on each day with *n* = 5 per group. C, The oviducts of mice primarily infected with plasmid-deficient *C. muridarum* and challenged with Nigg exhibit minimal pathology. Acute, chronic, plasma cell infiltrates and oviduct dilatation were significantly decreased in mice primarily infected with CM3.1 or CM972 and challenged with Nigg vs mice primarily infected with Nigg and challenged with Nigg. Each bar = median pathology score calculated from 10 oviducts. Nigg = ■. CM972 = □. CM3.1 = △. *, *p* < 0.001 for CM3.1/Nigg vs Nigg/Nigg; @, *p* < 0.001 for CM3.1/Nigg vs Nigg/Nigg; CM972/Nigg vs Nigg/Nigg, *D* and *E*, Fibrotic oviduct occlusion occurs as a consequence of Nigg/Nigg infection. Significant tubal dilatation and fibrosing obliteration of the oviduct lumen (arrowhead) were observed in mice primarily infected with Nigg and challenged with Nigg (D, ×10; E, ×50).

**Discussion**

Despite the availability of suitable animal models for the study of genital tract infection by *C. trachomatis*, understanding of the mechanisms by which the bacterium causes disease has been limited by the lack of defined genetic mutants. In this work, we describe a pair of *C. muridarum* mutants that are attenuated in their ability to cause disease, but retain the ability to infect their murine host. The plasmid-deficient strain CM972 was previously noted to express a marked attachment/uptake defect in cell culture (30). In the mouse model of genital tract infection with CM972, a 10-fold reduced infectious burden was seen during the first 2 wk of infection. This observation may explain why plasmid-deficient strains are so rarely observed among clinical isolates of *C. trachomatis*. Spontaneous loss of the plasmid during infection may lead to a competitive disadvantage and ultimately reduced transmissibility. However, the attachment and uptake defect associated with CM972 were not responsible for the failure of this strain to induce oviduct pathology because strain CM3.1, a spontaneous mutant derived from CM972, does not induce oviduct pathology, whereas it infects normally in cell culture and in vivo. Thus, loss of the plasmid from *C. trachomatis* is pleiotropic, impacting two virulence-associated phenotypes in addition to the ability of the chlamydiae to accumulate glycogen within their inclusion (30, 39).

An explanation for the attenuation of CM972 and CM3.1 was derived from our discovery that neither strain is capable of stimulating a TLR2-dependent response. The signaling defect was consistently observed in sentinel cells such as DCs and cervical epithelium, and was confirmed in reconstituted TLR2-expressing epithelial cells. The possibility that the plasmid-deficient strains were limited in their ability to infect oviduct epithelium was ruled out as a contributor to reduced pathology because inclusions could be observed via immunohistochemical staining and infectious bacteria were recovered from dissected oviducts of infected mice.

Several explanations can be proposed for how the plasmid regulates TLR2-dependent signaling, as follows: directly, by failing to express a plasmid-encoded TLR2 ligand, or indirectly, by regulation of an enzyme or pathway responsible for expression or modification of the ligand. Using confocal microscopy, we have determined that TLR2 colocalizes to inclusions produced by in vitro infection of HEK293/TLR2 cells with CM972 and CM3.1 (data not shown), as observed previously for Nigg (32). Thus, trafficking of TLR2 to the inclusion is not affected by plasmid loss. However, we have not ruled out the potential for plasmid control of chlamydial products that influence trafficking of coadaptor molecules required for TLR2 signaling. Additional work is needed to identify the TLR2 ligand, but the DC cytokine cellular response that we have described will serve as a bioassay for the evaluation of candidate molecules.

Multiple studies have revealed that genetic polymorphisms of PRRs (40, 41) and their adaptor molecules (42) are associated with susceptibility to disease. However, the effects of TLR polymorphisms are not necessarily detrimental, considering that specific polymorphisms in TLR4 have been linked to resistance to Legionnaires’ disease and decreased atherosclerosis (43, 44). Our data...
suggest the possibility that a genetic predisposition or other endogenous events (45) that lead to enhanced TLR2 signaling could predispose women to more severe pathology subsequent to chlamydial genital infection.

In this model of genital infection with C. muridarum, TLR2 is dispensable with respect to resolution of infection, and is solely dispensable with respect to immune damage in trachoma and genital tract infections. In Effects of Microbes on the Immune System. M. W. Cunningham and R. S. Fujinami, eds. Lippincott Williams & Wilkins, Philadelphia, pp. 41–55.


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