Genital *Chlamydia trachomatis*: Understanding the Roles of Innate and Adaptive Immunity in Vaccine Research

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SUMMARY

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted disease worldwide, and despite significant advances in chlamydial research, a prophylactic vaccine has yet to be developed. This Gram-negative obligate intracellular bacterium, which often causes asymptomatic infection, may cause pelvic inflammatory disease (PID), ectopic pregnancies, scarring of the fallopian tubes, miscarriage, and infertility when left untreated. In the genital tract, *Chlamydia trachomatis* infects primarily epithelial cells and requires Th1 immunity for optimal clearance. This review first focuses on the immune cells important in a chlamydial infection. Second, we summarize the research and challenges associated with developing a chlamydial vaccine that elicits a protective Th1-mediated immune response without inducing adverse immunopathologies.

INTRODUCTION

*C*hlamydia trachomatis is the leading cause of bacterial sexually transmitted diseases (STDs) in humans. According to a 2008 WHO report, there are 105 million new cases of STDs due to *C. trachomatis* each year, and the infection rate has been increasing steadily ([1], [2]). When symptomatic, *C. trachomatis* infection can lead to mucopurulent endocervical discharge, hypertrophic cervix, and postcoital bleeding. In 20 to 40% of untreated women, *C. trachomatis* may reach the fallopian tubes via the endometrial epithelium and cause pelvic inflammatory disease (PID). However, because patients with *C. trachomatis* urogenital infections often do not exhibit any symptoms (75 to 90% of patients), they remain undiagnosed and untreated. This can lead to tubal factor infertility, miscarriage, or ectopic pregnancy ([3–5]), which is a life-threatening condition. Figure 1 shows pathologies caused by *C. trachomatis*. *C. trachomatis* is easily treated with antibiotics (i.e., erythromycin, azithromycin, or doxycycline), but several studies
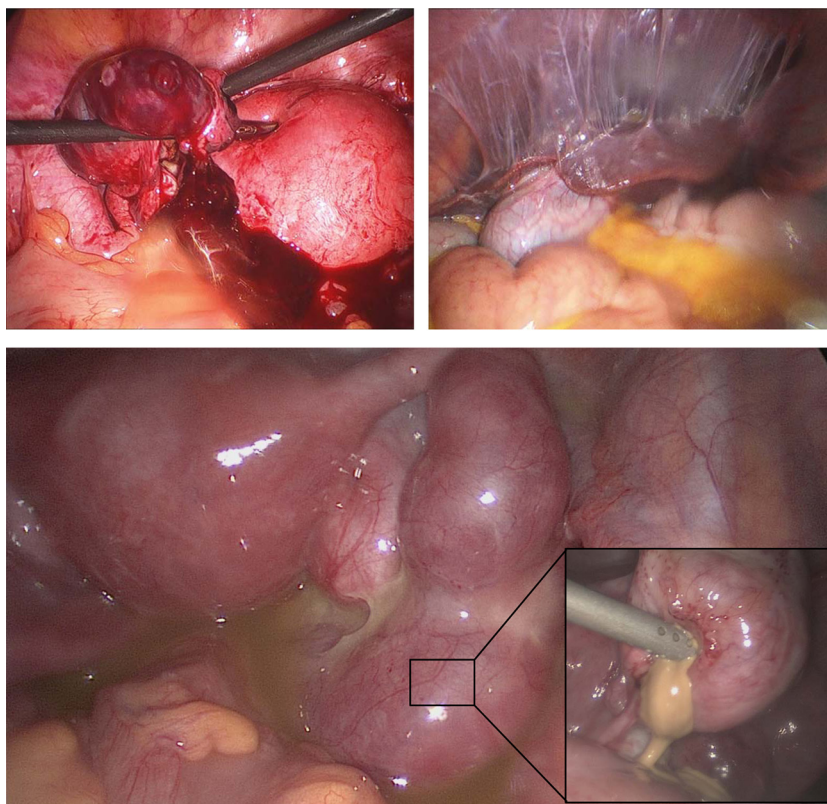
FIG 1 (Top left) Laparoscopy surgical procedure showing a ruptured left fallopian tube as a result of ectopic pregnancy. The image shows the presence of significant hemoperitoneum. The patient was positive for *Chlamydia trachomatis* by PCR. (Top right) Fitz-Hugh-Curtis syndrome caused by *Chlamydia trachomatis*. (Bottom) Pelvic inflammatory disease (PID) caused by *Chlamydia trachomatis* infection of the uterus, fallopian tubes, and ovaries. The image shows the presence of a large dilation of the right fallopian tube, representing a tubo-ovarian abscess. (Inset) Drainage of the tubo-ovarian abscess.

indicate that a year after treatment, almost one-fourth of individuals are reinfected with *C. trachomatis* (6, 7). *C. trachomatis* can also cause ocular scarring, which often leads to blindness. This disease, known as trachoma, is the leading cause of blindness worldwide (8, 9). As with *C. trachomatis* genital infection, ocular infections are often asymptomatic but can induce inflammation that leads to conjunctival scarring. Trachoma is prevalent in more than 50 countries, and the WHO estimates that 40 million people worldwide suffer from trachoma and that 1.3 million people are blind as a result of *C. trachomatis* trachoma infections (10, 11). In addition to causing urogenital and ocular disease, *C. trachomatis* can also infect the lymph nodes and the lymphatic system. This disease, termed lymphogranuloma venereum (LGV), is mostly caused by *C. trachomatis* serovars L1 to L3 (12, 13). Therefore, because of the prevalence of asymptomatic infections, recurrent infections, and the severity of genital and ocular pathologies induced by *Chlamydia*, the development of a vaccine is paramount. This review focuses largely on genital *C. trachomatis* and *C. muridarum* (a model organism that naturally infects rodents and is used largely for animal experiments) immunity and the challenges associated with generating a vaccine against these bacteria.

CHLAMYDIA BIOLOGY

C. trachomatis is a Gram-negative obligate intracellular bacterium, and chlamydial species are able to infect both humans (*C. trachomatis* and *C. pneumoniae*) and animals (*C. muridarum*, *C. suis*, *C. abortus*, *C. pecorum*, *C. psittaci*, and *C. caviae*) (14). Pres-

ently, there are 18 identified serovars of *C. trachomatis* (15). Some serovars naturally infect the eye (serovars A to C), while others infect primarily genital tissues (serovars D to K) (16). In the genital tissues, *C. trachomatis* normally infects the cervical (women) or urethral (men) epithelium layer (17). *Chlamydia* exists in two developmental forms: the elementary body (EB), which is infectious, nonreplicating, and extracellular; and the reticulate body (RB), which is noninfectious, replicating, and intracellular. The EB displays no metabolic activity and is able to survive for long periods outside the cell. Infection begins when the small (~0.2 to 0.3 μm) EB is internalized by the cell. After 8 to 10 h, the vesicle-bound EB (termed an inclusion) replicates by binary fission into the larger (~0.8 μm) RB (18). After replication, the RBs revert back to EBs, which are able to infect neighboring cells (19). *C. trachomatis* is able to avoid destruction by preventing lysosomal fusion and replicating in an inclusion outside the endocytic pathway (18). Scarring associated with *C. trachomatis* infections may be the result of increased production of inducible nitric oxide synthase (iNOS) and mediators such as activins (20, 21).

INNATE AND ADAPTIVE IMMUNITY TO *CHLAMYDIA*

Neutrophils and NK Cells

Innate immunity plays a role in controlling chlamydial infections (22). Natural killer (NK) cells and neutrophils are the first cells that are recruited to the site of a chlamydial infection. These cells are important in innate immunity and have been implicated in the

initial control of chlamydial infections. Two early studies demonstrated that human neutrophils were able to inactivate *C. trachomatis in vitro* (23, 24). Additionally, mice that were neutrophil depleted had a 10-fold greater *C. muridarum* burden in the female genital tract than neutrophil-competent mice. However, both sets of mice were able to eliminate *C. trachomatis* within the same time frame (25), suggesting that neutrophils are not critical for the resolution of infection. In fact, neutrophils are usually the first immune cells recruited to an infectious site, and compared to other leukocytes, they are short-lived (26, 27). Therefore, the most likely role for neutrophils is to reduce the initial chlamydial infection and limit it from spreading. However, recent evidence indicates that *C. trachomatis* may delay neutrophil apoptosis (28). Since neutrophils are a major source of tissue-damaging cytokines, such as matrix metalloproteinase 9 (MMP9), during acute inflammation (29), the prolonged life span of neutrophils may contribute to fibrosis and infertility associated with a chlamydial infection (30).

NK cells are known to be involved primarily in viral infections and cancer but have also been shown to be important in the early elimination of intracellular bacteria (31, 32). A study conducted by Tseng and Rank demonstrated that mice inoculated intravaginally with *C. muridarum* recruited gamma interferon (IFN-γ)-producing NK cells to the site of infection as early as 12 to 24 h after inoculation (33). Cytokine production by epithelial cells and dendritic cells (DC) has been implicated in NK cell IFN-γ production during a chlamydial challenge. Hook and colleagues demonstrated that *C. trachomatis*-stimulated human epithelial cells and DC produced interleukin-18 (IL-18) and IL-12, respectively, and that these cytokines induced NK cells to secrete IFN-γ *in vitro* (34). IFN-γ not only is important in inhibiting the growth of *Chlamydia* (35) but also is one of the main cytokines important for the induction of a Th1 immune response. Indeed, mice that were depleted of NK cells by treatment with an anti-NK-cell antibody and inoculated intravaginally with *C. muridarum* had a significant increase in the Th2-associated antibody IgG1. In contrast, Th1-associated IgG2a was the dominant antibody in mice that were not treated with an anti-NK-cell antibody and challenged with *Chlamydia* (33). A more recent study indicated that NK cells may influence Th1 immunity by modulating DC function. This investigation demonstrated that DC from intranasally *C. muridarum*-infected and NK-cell-depleted mice produced lower levels of IL-12 and a reduced capacity to stimulate CD4$^+$ T cells *in vitro*. Furthermore, DC from NK cell knockout (KO) mice that were adoptively transferred into naive mice failed to induce a Th1-mediated immune response after intranasal challenge with *C. muridarum* (36). These data suggest that early IFN-γ production by NK cells modulates DC to downregulate the Th2 response, thereby allowing expression of strong Th1-mediated immunity, which has been shown to be essential for the resolution of *Chlamydia* infection.

NK T Cells

Natural killer T cells (NK T cells) are a unique population of T lymphocytes that express typical NK cell markers (NK1.1 and NKR-P1C) and a semivariant T cell receptor (αβT cell receptor; TCR) (37). NK T cells are CD1d restricted, meaning that they are able to recognize lipids and glycolipids presented by antigen-presenting cells on CD1d receptors but not antigens from the classical major histocompatibility complex (MHC) (38). These granular cytolytic lymphocytes are able to destroy infected and cancerous cells without prior sensitization and also secrete cytokines that are important in both innate and adaptive immunity. NK T cells have demonstrated immunomodulatory roles in a wide range of diseases, such as cancer, autoimmunity, allergy, atherosclerosis, and infection (37, 38). Furthermore, these cells have been implicated in regulating both innate (macrophages [Mϕ], natural killer cells, and dendritic cells) and adaptive (B cells and conventional T cells) immune cells (39–41). Zhao et al. demonstrated that NK cells from NK T cell KO mice and from mice that had the CD1d receptors blocked by antibodies exhibited decreased IFN-γ production and proliferation in a *C. muridarum* lung infection model (37). Another study suggested that natural killer T cells may induce protective Th1 immunity by promoting proliferation, CD40 upregulation, and production of IL-12 in a DC subset (CD8α$^+$) during *C. pneumoniae* respiratory tract infections (42). However, there is conflicting evidence on whether NK T cells promote protective Th1 cell immunity or a Th2-mediated response that is characterized by bacterial pathogenesis. A study conducted by Bilenki et al. in 2005 (43) examined the role that NK T cells play in *C. muridarum* pneumonitis infection. This study demonstrated that intranasally infected CD1d-deficient mice lost less weight, exhibited less pathology, and had lower bacterial burdens, IL-14 levels, and IgE titers than wild-type (wt) mice. Additionally, wt mice that were stimulated with a known NK T cell ligand, α-galactosylceramide (α-GalCer), showed induced *C. muridarum* growth and increased IL-4 and IgE levels, suggesting that NK T cells promote a pathological Th2 response during chlamydial infection (43). However, a more recent study by Wang and colleagues demonstrated that pretreatment with α-GalCer in *C. muridarum* genital infection reduced bacterial burdens, decreased pathology, and increased the Th1-associated cytokines IFN-γ and IL-12 in both lymph nodes and genital tissues compared with those in non-α-GalCer-pretreated mice. These results suggest a role for NK T cells in protective Th1 immunity against *Chlamydia* (44).

Mϕ

Studies using both *C. trachomatis* and *C. muridarum* have shown that macrophages (Mϕ) migrate to chlamydial infection sites (45), phagocytose bacteria (46), and produce proinflammatory cytokines (47, 48). However, unlike epithelial cells, Mϕ are not a hospitable niche for chlamydial intracellular replication, as illustrated by the fact that compared to the case in epithelial cells, only a small fraction of chlamydial RBs are detected in Mϕ (49). *C. trachomatis* destruction inside Mϕ has been associated with host cell autophagy, a process by which cells degrade cytoplasmic proteins and organelles (49–51). Also, studies have demonstrated that Mϕ autophagy can enhance antigen presentation to T cells (52). Furthermore, IFN-γ has been shown to enhance both autophagy and upregulation of MHC class II molecules in Mϕ (50, 53). This is relevant because in addition to activating primed T cells, studies indicate that Mϕ can induce a humoral response in naive mice (54). Therefore, enhanced upregulation of MHC molecules containing chlamydial antigens may induce T cells to initiate both cell-mediated and antibody immune responses against *Chlamydia*. However, Jendro et al. demonstrated that *C. trachomatis*-infected human Mϕ are able to induce T cell apoptosis (55, 56). In addition to efficiently eliminating *Chlamydia* and presenting the peptides to T cells, Mϕ may also have an effect on chlamydial

infection by inducing T cell death and perpetuating a persistent infection.

DC

Dendritic cells (DC) are known to be the quintessential antigen-presenting cells (APC). Immature DC are highly phagocytic, and after internalization of pathogens, they degrade the components and present the peptides to T cells via MHC receptors. This activates the T cells to initiate a cell-mediated and/or humoral immune response. Numerous investigations have demonstrated the ability of DC to activate T cells through MHC class I/II presentation and to secrete Th1 cytokines in chlamydial infection both *in vitro* and *in vivo* (57–61). An early study conducted by Lu and Zhong showed that mice that received heat-killed (HK) *C. trachomatis*-incubated bone marrow-derived DC (BMDC) were protected against a subsequent nasal challenge with live *C. trachomatis* (62). The protective response was Th1 mediated, further demonstrating a correlation between Th1-skewed immunity and protection against chlamydial infection. In contrast, DC that were pulsed with recombinant MOMP and adoptively transferred into mice elicited primarily the Th2-associated antibody IgG1 (63). Furthermore, IL-10 (Th2-associated cytokine) knockout DC pulsed with UV-inactivated *C. trachomatis* and adoptively transferred activated a high frequency of Th1 cells (64). These data have direct relevance to vaccine development because they indicate that the types of cytokines produced and antigens processed by DC and presented to CD4$^+$ T cells mediate the Th1/Th2 balance during a chlamydial infection. There is also evidence that live *Chlamydia* is required for an optimal and protective immune response. Rey-Ladino and colleagues demonstrated that protection mediated by DC pulsed with UV-inactivated *C. trachomatis* and adoptively transferred into mice was significantly less than that in mice that were challenged with live EB-pulsed DC (65). A more recent study discovered that murine DC incubated with live *C. muridarum* presented many more peptides on their MHC class II molecules than DC that were incubated with dead EBs (66). However, *C. trachomatis* is able to limit MHC class I/II expression in APC (67). *C. trachomatis* has been shown to inhibit MHC molecules by degrading the MHC class I transcription factor RFX-5 and the MHC class II transcription factor USF-1 by secreting chlamydial protease-activating factor (CPAF) into the cytosol (68–71). DC are important for vaccine research because they are the critical links between innate and adaptive immunity. Two recent studies, using *C. trachomatis* MOMP transfected into DC (72) and DC that were incubated with recombinant CPAF (rCPAF) *in vitro* (73), illustrate the ability of DC to induce protective immunity against genital *C. trachomatis* and *C. muridarum* challenges, respectively.

T Cells

The involvement of T cells in chlamydial immunity was demonstrated almost 30 years ago, when Rank et al. observed that athymic nude mice established chronic infection with *C. muridarum* after intravaginal inoculation, but wild-type controls were able to eliminate the infection within 20 days (74). In human and mouse models, CD4$^+$ as well as CD8$^+$ T cells are able to be detected at the site of *C. trachomatis* infection (75–78). T cells are unable to recognize pathogens or antigens without the help of APC. APC such as DC and Mφ are able to phagocytose chlamydial EBs in the extracellular space or engulf infected cells harboring RBs. After phagocytosis, APC degrade chlamydial components and present

the peptides via MHC class I/II-antigen complexes. CD4$^+$ T cells recognize antigens that are presented on MHC class II, and CD8$^+$ T cells are activated by MHC class I-antigen complexes. In fact, both T cell subsets have been shown to recognize *C. trachomatis* antigens, such as outer membrane protein 2 (Omp2) (79), polymorphic outer membrane protein D (POMP-D) (80), MOMP (81–83), heat shock protein 60 (hsp60) (81, 84), chlamydial protease activating factor (CPAF) (73), PmpG, PmpF, and RpIF (77, 85). Although *Chlamydia* is able to induce a Th2-associated response by inducing IL-4 and IgG1 production, a Th1 response predominates. This response is characterized by the production of IL-12 by APC (86) and the subsequent activation of IFN-γ-producing T cells and plasma B cells that secrete Th1-associated antibodies, such as IgG2a and IgG3 (87, 88). However, a recent study demonstrated that previously *C. trachomatis*-sensitized human CD4$^+$ T cells that were restimulated *ex vivo* with inactivated (γ-irradiated) EBs secreted significantly more IL-4 than tumor necrosis factor alpha (TNF-α) and IFN-γ. This study suggests that the type of immune response (Th1 versus Th2) to *C. trachomatis* may be tissue specific (89).

While there is ample evidence that CD4$^+$ T cells play an integral part in *C. muridarum* and *C. trachomatis* infection resolution (90–93), the role for CD8$^+$ T cells has been controversial. Indeed, CD8$^+$ T cells are known to migrate to the infection site, and both human and mouse CD8$^+$ T cells have been shown to destroy cells that have been infected with *Chlamydia* (94). A recent study by Murthy and colleagues showed that wt and CD8$^+$ T knockout mice displayed similar clearances of *C. muridarum* following vaginal chlamydial challenge (95). These data support previous studies demonstrating that CD8$^+$ T cells are not critical for *C. trachomatis* clearance (45, 59, 96). Furthermore, compared to wt mice, CD8$^+$ T cell-deficient mice demonstrated less hydrosalpinx, implicating CD8$^+$ T cells in chlamydia-induced pathology (95). A study conducted by Ibana et al. showed that most of the cervical CD8$^+$ T cell populations before and after a *C. trachomatis* infection do not express the cytolytic protein perforin (97). Therefore, the lack of perforin in endocervix CD8$^+$ T cells may explain why CD8$^+$ T cells are not critical for genital chlamydial infection resolution. Although CD8$^+$ T cells are not critical for chlamydial elimination and may even contribute to chlamydial sequelae, they nonetheless may play a contributory, albeit secondary, role by regulating other cells and by their own production of IFN-γ (94).

B Cells and Antibodies

Previous studies demonstrated that in humans, *Chlamydia*-specific antibodies play a role in *C. trachomatis* protective immunity (98, 99), and numerous *C. trachomatis* proteins have been shown to induce antigen-specific antibodies (91). However, even though anti-*Chlamydia* antibodies are able to neutralize infection *in vitro* (100, 101), growing evidence shows that B cells may not be important for initial chlamydial infection but, instead, play an important role in the secondary memory response (102, 103). Several possible mechanisms by which B cells modulate immunity during reinfection include antibody-mediated neutralization and opsonization (100) and antibody-dependent cellular cytotoxicity (ADCC) (a mechanism of cell-mediated immune defense whereby cells that have antibodies attached to their surfaces are targeted for lysis) (104). Another mechanism is the formation of antigen-antibody complexes that bind Fc receptors on APC,

which then enhance phagocytosis and antigen presentation to CD4$^+$ T cells (105). A recent study suggests that in humans, antibodies may be more specific for certain chlamydial serovars. Verweij and colleagues demonstrated that in serum samples from 235 *C. trachomatis*-positive patients, anti-IgG titers specific for *C. trachomatis* serogroup B (serovars B, Ba, D, Da, E, L1, L2, and L2a) were significantly higher than titers specific for serogroup C (serovars A, C, H, I, Ia, J, K, and L3) and serogroup I (serovars F, G, and Ga) (106).

Heat shock proteins (hsp's) are proteins that influence the correct folding and unfolding of intracellular proteins. *C. trachomatis* is known to secrete hsp's during an infection, and antigenic epitopes from bacterial hsp's have proven to be strong inducers of cellular and humoral immunity. Chlamydial and human hsp60 proteins are extremely similar, with four defined epitopes having 70% homology and virtually identical amino acid sequences (107). Several studies have suggested that autoimmunity to human hsp60 is a result of cross-reactivity after a chlamydial infection (108, 109). However, a study conducted by Hjelholt and colleagues did not find a correlation between tubal infertility and antibodies specific for human hsp60 in *C. trachomatis* infections, even though the patients produced antibodies against MOMP and chlamydial hsp60 (110).

IFN-γ

Production of IFN-γ in response to *Chlamydia* infection is critical for inhibiting chlamydial growth (17). IFN-γ can affect the survival of *Chlamydia* by several mechanisms. IFN-γ is able to enhance the phagocytic capabilities of Mφ (111) and may promote the engulfment and elimination of *Chlamydia trachomatis* (112). Iron has been shown to be important for *Chlamydia* survival (113). IFN-γ downregulation of the transferrin receptor (114), which is needed for the import of iron into the cell, may also inhibit *Chlamydia* growth by limiting the available iron to the bacterium. In fact, IFN-γ has been shown to limit iron availability in Mφ infected with *Salmonella* (115). Most *Chlamydia* species require tryptophan for survival (116). IFN-γ induces the expression of the cellular tryptophan-decyclizing enzyme indoleamine-2,3-dioxygenase (IDO), which degrades tryptophan. The lack of this essential amino acid has also been shown to cause *Chlamydia trachomatis* death through tryptophan starvation (35). However, there are chlamydial species that have adapted to tryptophan starvation by transforming into nonreplicating but viable persistent forms. After IFN-γ removal and subsequent tryptophan production, these persistent forms quickly differentiate into infectious elementary bodies. Furthermore, a recent study by Zhang and colleagues demonstrated that IFN-γ and IL-17A synergistically inhibit *Chlamydia muridarum* replication by inducing intracellular iNOS and NO production (117).

In conclusion, cell-mediated immunity that activates Mφ, NK cells, NK T cells, neutrophils, and mediators such as IL-12 and IFN-γ is required for initial clearance. However, for protective immunity, both cell-mediated immunity and humoral immunity are needed, including antigen-specific T cells and antibodies that enhance phagocytosis and subsequent degradation and presentation of bacterial components by DC for a rapid Th1-mediated immune response. Table 1 summarizes recent developments in chlamydial research, including *Chlamydia* strains and antigens used, cell types affected, and immune responses elicited.

ANIMAL MODELS

Mouse

The most commonly used animal in chlamydial research is the mouse. There are several advantages in using mice to investigate chlamydial genital infections, including availability of transgenic mouse strains, small size, low cost, and availability of mouse-specific reagents. *C. muridarum* is utilized for genital studies, because *C. muridarum* intravaginal infection closely mimics acute *C. trachomatis* infection in women. Moreover, it can cause hydrosalpinx, fibrosis, infertility, and abortion in mice (118–121). *C. muridarum* genital infection is usually resolved in 3 to 4 weeks, and the mice are partially protected against subsequent reinfections (122, 123). *C. trachomatis* is also used, but the infection in mice is less severe and is resolved more quickly than *C. muridarum* infection. Additionally, a mouse *C. trachomatis* infection requires a larger number of infectious units (118) and usually causes pathology only when injected directly into the uterus, uterine horn, or ovarian bursa (92, 124). However, *C. trachomatis* genital infection in women is mostly asymptomatic and often does not induce severe upper tract genital pathology. Thus, as Lyons et al. have argued (125), *C. trachomatis* is an appropriate model for studying chlamydial urogenital infections in mice.

Pigtailed Macaque

Although several nonhuman primate models have been used in *Chlamydia* research, including the grivet monkey (126), marmoset (127), and baboon (128), the pigtailed macaque is utilized most frequently for genital research. Indeed, it is naturally infected with *C. trachomatis* human biovars, and the female anatomy, menstrual cycle, and vaginal microflora are akin to those in humans (129). In fact, repeated *C. trachomatis* infection of macaque fallopian tubes has been shown to induce a pathology similar to that of pelvic inflammatory disease in women (130). Immune responses include Th1-skewed cytokine production after initial inoculation and systemic and local humoral responses. However, unlike the case in mice, where CD4$^+$ T cells are the dominant T cell subset, CD8$^+$ T cells predominate in macaques after chlamydial infection (118). Although the macaque model is ideal for vaccine and immunology studies, the high cost and need for adequate facilities and expertise limit its use.

Guinea Pig

Guinea pigs are naturally infected with *C. caviae*. Advantages of this model include the ability to study chlamydial sexual transmission (male guinea pigs are able to be infected with *Chlamydia*) (131), the transmission of *Chlamydia* to newborns, and the fact that guinea pig genital tract infection is similar to that by *C. trachomatis* in humans. Additionally, the guinea pig is a good model for hormonal research because humans and guinea pigs have comparable estrous cycles (118, 132, 133). Studies have indicated that CD8$^+$ T cell genital infiltrates after infection are similar to those of humans and nonhuman primates (134, 135). In contrast to the case in mice, antibodies have been implicated in the resolution of primary infection in chlamydia-infected guinea pigs (118).

Pig

In addition to the mouse, guinea pig, and nonhuman primate models, the pig has also been used for chlamydial studies. Pig and human female reproductive tracts are very similar (136), and

TABLE 1 Summary of recent developments in *Chlamydia* research

Cell type	*Chlamydia* species or antigen	Immune response	Reference(s)
Human neutrophil	*C. trachomatis*	Neutrophils incubated with *Chlamydia in vitro* were able to decrease viable bacterial count by 3 log after 60 min.	24
	C. psittaci and *C. trachomatis*	*C. psittaci* and *C. trachomatis* cultured with complement or antibody induced neutrophil chemotaxis, whereas reticulate bodies alone did not.	23
		Neutrophil phagocytosis of *C. psittaci* and *C. trachomatis* was antibody and complement independent.	
Mouse neutrophil	*C. muridarum*	Neutrophil-depleted mice had a 10-fold greater chlamydial burden in the female genital tract than neutrophil-competent mice.	25
		Both wt and neutrophil-depleted mice were able to eliminate *C. muridarum* within the same time frame.	
Human neutrophil	*C. pneumoniae*	*C. pneumoniae* delayed neutrophil apoptosis *in vitro*.	28
Guinea pig neutrophil	*C. caviae*	*Chlamydia*-infected neutrophil-depleted animals exhibited reduced ocular pathology and decreased T cells in infected conjunctivae.	232
Mouse NK cell	*C. muridarum*	*Chlamydia* induced IFN-γ-producing NK cells at the site of infection within 12–24 h after vaginal challenge.	33
		Mice depleted of NK cells and inoculated intravaginally with *C. muridarum* had a significant increase in Th2-associated IgG1 antibody, whereas Th1-associated IgG2a was the dominant antibody in nondepleted mice.	
Human NK cell	*C. trachomatis*	*C. trachomatis* induced human NK cells to secrete IFN-γ *in vitro*.	233
Human NK cell line	*C. trachomatis*	IL-18 and IL-12 from epithelial cells and DC, respectively, that were incubated with *C. trachomatis* stimulated NK cell IFN-γ production.	34
Mouse NK cell	*C. muridarum*	DC from intranasally *C. muridarum*-infected, NK cell-depleted mice produced lower levels of IL-12 and a reduced capacity to stimulate CD4$^+$ T cells *in vitro*.	36
		DC from NK cell-deficient mice that were adoptively transferred failed to induce a Th1-mediated immune response after intranasal challenge with *C. muridarum*.	
Mouse NK T cell	*C. muridarum*	NK cells from NK T cell KO and CD1d antibody-blocked mice had decreased IFN-γ production and proliferation in a *C. muridarum* lung infection.	37
	C. pneumoniae	NK T cells promote proliferation, CD40 upregulation, and production of IL-12 in a DC subset (CD8α$^+$) in *C. pneumoniae* respiratory tract infection.	42
	C. muridarum	Infected CD1d-deficient mice were less susceptible to weight loss and had less severe pathology, lower bacterial burdens, lower IL-4 levels, and lower IgE titers than wild-type mice.	43
		Wild-type mice that were stimulated with a known NK T cell ligand (α-GalCer) had induced *C. muridarum* growth and increased IL-4 and IgE levels.	
	C. muridarum	Pretreatment with α-GalCer in *C. muridarum* genital infection reduced bacterial burdens, decreased pathology, and increased the Th1-associated cytokines IFN-γ and IL-12 in both lymph nodes and genital tissues compared with the case in non-α-GalCer-pretreated mice.	44
Mouse Mφ cell line (J774) and human Mφ	*C. trachomatis*	Live and inactivated *Chlamydia* induced elevated IL-8, IL-1β, TNF-α, and IL-6.	47, 48
Mouse (RAW) and human (THP-1) Mφ cell lines	*C. trachomatis*	Live *Chlamydia* induced autophagy.	49, 50
Human Mφ	*C. trachomatis*	Live-*Chlamydia*-infected Mφ induced T cell apoptosis.	55, 56
Mouse BMDC	*C. muridarum*	DC pulsed with UV-inactivated *Chlamydia in vitro* secreted elevated levels of IL-12.	62
		DC pulsed with UV-inactivated *Chlamydia* and adoptively transferred into naive mice induced strong protection against live chlamydial lung infection.	
		IL-12$^{-/-}$ DC failed to induce Th1-dominant response and did not induce strong protection against chlamydial infection.	
	rMOMP	DC pulsed with rMOMP secreted IL-12 and induced infection-sensitized CD4$^+$ T cells to secrete IFN-γ.	63
		DC pulsed with rMOMP and adoptively transferred into naive mice generated a Th2 anti-MOMP immune response.	
	C. trachomatis	IL-10$^{-/-}$ DC pulsed with UV-inactivated *Chlamydia* caused early DC maturation and activation and an increased ability to process and present antigens and enhanced the rate of Th1 activation.	64
	C. muridarum	DC incubated with UV-inactivated *Chlamydia* expressed low levels of CD40 and CD80, secreted low levels of proinflammatory cytokines, and exhibited reduced recognition by *Chlamydia*-specific CD4$^+$ T cells.	65
		Adoptive transfer of live-EB-pulsed DC was more effective than UV-inactivated *Chlamydia* at protecting mice against a live intranasal chlamydial challenge.	

TABLE 1 (Continued)

Cell type	*Chlamydia* species or antigen	Immune response	Reference(s)
	C. muridarum	DC pulsed with live EBs presented 45 MHC class II *C. muridarum* peptides mapping to 13 proteins. In contrast, DC pulsed with heat- or UV-inactivated *Chlamydia* presented only 6 MHC class II chlamydial peptides mapping to 3 proteins.	66
		Only two epitopes were shared in common between live and inactivated *C. muridarum*.	
	Recombinant adenovirus carrying *C. trachomatis* MOMP	DC exhibited increased CD80, MHC class II, and IL-12 and were able to stimulate CD4$^+$ T cell proliferation and IFN-γ.	72
		Adoptively transferred MOMP-transfected DC generated Th1-biased cytokine production and mucosal IgA and protected mice against chlamydial genital tract infection.	
	UV-inactivated *C. muridarum* plus CpG or rCPAF plus CpG	DC pulsed with rCPAF plus CpG exhibited increased CD86, CD80, CD40, MHC class II, and IL-12 but not IL-10 and IL-4.	73
		Mice adoptively immunized with rCPAF-plus-CpG- or UV-inactivated *C. muridarum*-plus-CpG-pulsed DC produced elevated IFN-γ, IgG1, and IgG2a and exhibited reduced *Chlamydia* shedding and reduced oviduct pathology compared to infected mock-immunized mice.	
Mouse T cell	*C. muridarum*	Athymic nude mice established chronic genital tract infection, whereas wild-type mice resolved infection in 20 days.	74
	C. trachomatis T cell antigens plus AbISCO-100	A potent CD8$^+$ T cell response, polyfunctional Th1-polarized CD4$^+$ T cell responses (IFN-γ, TNF-α, and IL-2), and a high protein-specific Th1-skewed antibody response (IgG2c) were observed.	234
		Adoptive transfer of CD4$^+$ T cells and CD8$^+$ T cells to naive nonimmunized mice protected against *C. trachomatis* vaginal challenge, whereas passive transfer of immune sera did not.	
	C. muridarum MOMP plus CpG and Montanide ISA	Vaccinated mice were depleted of CD4$^+$ and CD8$^+$ T cells and challenged vaginally with live *C. muridarum*. Depletion of CD4$^+$ T cells, but not CD8$^+$ T cells, diminished vaccine-induced protection.	90
Mouse CD4$^+$ T cell	*C. trachomatis*	Genital tract *C. trachomatis* infection stimulated the activation and memory development of *C. trachomatis*-specific CD4$^+$ T cells.	92
		CD4$^+$ T cells are necessary to confer protection against *C. trachomatis* infection.	
	C. muridarum	CD4$^+$ T cell clone-induced epithelial NO production was critical for controlling replication.	93
		Most potent CD4$^+$ T cell clones were dependent on T cell degranulation for chlamydial replication control.	
Human CD4$^+$ T cell	*C. trachomatis*	CD4$^+$ T cells from women with genital tract infection that were pulsed *ex vivo* with EBs secreted significantly more IL-4 than TNF-α and IFN-γ	89
Mouse CD8$^+$ T cell	*C. muridarum*	TNF-α from CD8$^+$ T cells contributed significantly to oviduct pathological sequelae, but not bacterial clearance, following genital chlamydial challenge.	95
Human CD8$^+$ T cell	*C. trachomatis*	Endocervix effector memory CD8$^+$ T cells from *C. trachomatis*-infected women expressed low perforin levels.	97
Human B cell/antibody		A total of 21 antibody-inducing antigens were identified from *C. trachomatis*-infected patient sera.	91
Mouse B cell/antibody	Recombinant outer membrane vesicles carrying *C. muridarum* HtrA, *C. muridarum* MOMP, or a monoclonal antibody (MAb)	Mice immunized with outer membrane vesicles carrying HtrA induced specific anti-HtrA antibodies that neutralized *C. muridarum* infectivity *in vitro*.	100
		Passive immunization with sera from *C. muridarum*-infected mice conferred a marked level of protection from *C. muridarum* genital reinfection and shortened the time of infection.	102
		MOMP MAbs conferred significant level of immunity to reinfection and reduced shedding.	
	rCPAF plus CpG	Wild-type and B cell-deficient (μmT) mice vaccinated intranasally with rCPAF plus CpG and challenged vaginally with live *C. muridarum* demonstrated comparable clearances and similar reductions in pathology.	103

studies indicate that the immune systems of humans and pigs are much more related than those of mice and humans (118). Pigs are naturally infected with *C. abortus* and *C. suis*, but *C. pecorum*, *C. psittaci*, and *C. trachomatis* are also able to infect pigs (118, 137). However, although *C. suis* is highly related to *C. trachomatis* and is a natural pig pathogen, *C. suis* does not induce tubal infertility and PID. Therefore, it is difficult to use this species as a model for investigating human *C. trachomatis* urogenital pathology (118). Nevertheless, Schautteet and colleagues have used the pig model

to investigate recombinant protein-based and DNA-based vaccine candidates. These investigations demonstrated that both rPmpG and *C. trachomatis* DNA vaccines provided significant protection against *C. trachomatis* vaginal challenge. DNA mucosal immunization provided superior protection compared to that in pigs immunized intradermally (138–140), demonstrating the importance of vaccination routes.

Although animal models are extremely useful and necessary for understanding the complex nature of chlamydial infection and

TABLE 2 Advantages, disadvantages, and chlamydial protective immunity of different animal models

Species	Advantages	Disadvantages	Protective immunity	Reference(s)
Mouse	Small size, availability of reagents, low cost	*C. muridarum* does not infect humans; *C. trachomatis* infects mice only at high doses; it is difficult to extrapolate *C. muridarum* and *C. trachomatis* to human correlates.	*C. muridarum* primary genital tract infection resolves in 3–4 weeks; primary *C. muridarum* infection partially protects against reinfection; durations are shorter and bacterial loads are lower in *C. muridarum*-reinfected mice; *C. trachomatis* genital infection is milder and shorter than *C. muridarum* infection.	118
Guinea pig	Ability to study sexual transmission (female to male) and transmission to newborns; good model for hormonal research (estrous cycle similar to that in women); genital infection with *C. caviae* closely resembles *C. trachomatis* infection in women	Limited guinea pig-specific reagents	Primary genital infection with *C. caviae* is resolved in 3–4 weeks; partial immunity remains for roughly 50% of the animals' life span.	123, 132
Pig	Reproductive organs and immune system are closely related to those of humans; naturally infected with *C. suis*, which is closely related to *C. trachomatis*	Expensive, complicated to work with, lack of reagents	*C. trachomatis* shedding has been documented for up to 21 days.	132
Nonhuman primates (pigtailed macaque)	Female anatomy, menstrual cycle, and microflora similar to those in women; naturally infected with *C. trachomatis*	Expensive, need for special facilities, need for expertise	Secondary cervical challenge with *C. trachomatis* after initial resolution resulted in either no infection or a shorter and less severe infection.	141

pathology, comparing data from different animal models can be difficult. *C. muridarum*-infected mice and genitally *C. caviae*-infected guinea pigs are characterized by infections that last roughly 3 to 4 weeks (132). However, pigtailed macaque genital *C. trachomatis* infections are longer and more persistent, with bacterial shedding still occurring after 4 months (132, 141). In comparison, human studies suggest that after 1 year of untreated genital infections, half of *C. trachomatis* infections still persist (142, 143). Extrapolating data from animal studies and comparing the results to human correlates are difficult because of limited data on untreated human subjects with chlamydial infections. Nevertheless, there are data indicating that although humans are able to spontaneously clear chlamydial infection without antibiotic intervention, the time frame of such clearance can span several months to years, and the resolution appears to be more robust in older individuals (142, 144). Additionally, epidemiological data indicate that the longer an individual is infected with *Chlamydia*, the greater are the chances of clearance (142, 144). Finally, a major limitation in comparing animal models of chlamydial genital infection to human *C. trachomatis* urogenital infection is that the actual amounts of *Chlamydia* inoculated during sexual intercourse in humans are not known, so it is impossible to approximate similar doses in animal models. Table 2 summarizes the main advantages, disadvantages, and protection in the various animal models discussed above.

VACCINES

Due to increasing rates of mainly asymptomatic *C. trachomatis* infections worldwide and the adverse long-term consequences resulting from these infections (ectopic pregnancy, infertility, and preterm birth), developing an antichlamydial vaccine is of paramount importance. However, a human vaccine that elicits both T

cell and B cell immunity has been elusive. Lack of knowledge of female genital tract immunity, which is highly regulated by sex hormones during the menstrual cycle (145), a dearth of adjuvants that not only optimize the immune response to *Chlamydia* antigens but also can target the vaccine-specific immune responses at the infection site, and a limited understanding of the mechanisms by which chlamydial antigens induce protective immunity hinder human *C. trachomatis* vaccine development. A potential *C. trachomatis* vaccine ideally will induce both mucosal and systemic immune responses, but autoimmune cross-reactions with human antigens and unregulated inflammation that causes pathology must be avoided. Table 3 summarizes recent chlamydial antigens, delivery systems, routes of vaccination and infection, and the subsequent immune responses elicited.

Intact Organisms

Successful vaccines against ovine enzootic abortions have been available for many years (146). These vaccines consist of either live or inactivated *C. abortis* strains and provide proof of principle that a successful vaccine against *Chlamydia* is possible in mammals. However, these vaccines are not able to protect against infection, and the vaccines were not designed for use in humans (147, 148). Nonetheless, because of the success of these vaccines, live *C. trachomatis* bacteria were used as the first human *Chlamydia* vaccines (149). The first vaccines focused mainly on trachoma rather than genital *C. trachomatis* infection, with results ranging from limited and short-lived protection to considerable protection against infection and pathology (150, 151). However, some individuals who were challenged with *Chlamydia trachomatis* developed a pathological response that was worse than that in those who did not receive the vaccine. Notably, Grayston and colleagues vaccinated Taiwanese children at risk for trachoma with formalin-inactivated

TABLE 3 Summary of recent developments in *Chlamydia* vaccine research[a]

Vaccine	Advantages	Disadvantages	Antigen (Ag) and adjuvant	Ag immunization route	Model/*Chlamydia* infection route	Immune response	Reference(s)
Intact *Chlamydia*	Intact Ag, native configuration, replication, humoral/ cellular immunity	Requires refrigeration, potential to revert to virulent strains, large-scale production is difficult, possible transmission to unvaccinated individuals	Plasmid-deficient *Chlamydia* (CM972, CM3.1)		Mouse/i.v.	Elevated IgG2a (Th1), low levels of IgG1 (Th2). Mutants do not stimulate TLR2-dependent cytokine production. Infected mice with mutant *Chlamydia* and challenged with wt *Chlamydia* are protected against oviduct disease.	87
			Plasmid-deficient *Chlamydia* (L2)		Mouse/i.v.	Elevated IgG2a, low IgG1, no IgA (mucosal). No pathology in the urogenital tract induced by L2. Mice vaccinated with plasmid-deficient bacterium were not protected from infection/inflammation with secondary wt chlamydial infection.	88
Purified subunits	Do not revert to virulent strains, their use avoids undesirable antigens	Expensive to produce, purification not standardized, difficult to maintain native conformation of antigen complex	MOMP plus cholera toxin subunit B conjugated to CpG	i.m. + s.c.	Mouse/i.n.	Elevated IgG2a and IgG3 (Th1), lower IgG1 level, elevated IFN-γ (Th1).	160
			MOMP-ISCOM	i.n. or i.m.	Mouse/i.n.	i.m. route induced highest IFN-γ and IL-4 (Th2) levels.	159
			MOMP plus Freund's adjuvant	i.m. + s.c.	Mouse/i.v.	Vortexed MOMP elicited higher IgG2a than IgG1. Sonicated MOMP elicited higher IgG1 than IgG2a.	157
			MOMP plus IC31	i.m. + s.c.	Mouse/i.n.	Higher IgG1 than IgG2a.	235
			MOMP plus CpG/ Montanide	i.m. + s.c.	Rhesus macaque	Elevated IgG, IgA, IFN-γ, and TNF-α.	161
Recombinant proteins	High yields, inexpensive	Some proteins require posttranslational modification; if produced in *E. coli*, possibility of endotoxin contamination	rMOMP plus cholera toxin/ CpG or CTA1	s.l. or t.c. or i.n.	Mouse/i.n.	Elevated IFN-γ and TNF-α. i.n. immunization with MOMP plus either adjuvant protected mice from infection but not pathology. t.c. immunization with MOMP and CTA1-DD protected mice from pathology, but *Chlamydia* burden was same as that in control mice.	167
			rMOMP plus CpG/ Montanide	i.m. + s.c.	Mouse/i.n.	Vaccination protected against fibrotic scarring in lungs. Elevated IgG2a and lower levels of IgG1.	168
			rCPAF plus IL-12	i.n.	Mouse/i.v.	Increased IFN-γ and minimal IL-4. Elevated IgG2a and IgA.	170
			rCPAF plus CpG	i.n.	Mouse/i.v.	Vaccination significantly prevented infertility.	171
			rCTH1 plus CAF01	s.c.	Mouse/i.v.	T cell production of TNF-α, IL-2, and IFN-γ. Anti-CTH1 IgG2a and IgG1. Protection was solely CD4+ T cell mediated.	173
			rGlgP plus CpG	i.m.	Mouse/i.v.	Th1-dominant T cell response. Reduced hydrosalpinx severity.	172
			rMIP	i.m.	Mouse/i.v.	More IgG2a than IgG1. Elevated IFN-γ and no IL-4. Reduced hydrosalpinx severity.	174
			rCT043	i.m.	Mouse/i.n.	rCT043 reduces bacterial load in a mouse model of i.n. infection.	236
			rCT823 plus ISCOM and CT144 plus ISCOM	s.c.	Mouse/i.v.	Elevated IFN-γ, TNF-α, and IL-2. No detectable IL-4 and IL-10. Elevated IgG2c (Th1) but not IgG1.	234
			rPmpG plus GNE (adjuvant) and SctC plus GNE	s.c.	Pig/i.v.	PmpG protected better than SctC. PmpG vaccination did not elicit antibody production. SctC vaccination elicited high antibody titers.	140

Vaccine type	Advantages	Disadvantages	Construct	Route	Model/route	Findings	Reference
DNA vaccines	Cheap, easy to produce, can encode multiple epitopes, native conformation of antigenic determinants	Safety, possible genome integration, anti-DNA antibodies, not possible for nonproteins	DNA MOMP	i.m.	Mouse/i.v.	Elevated levels of IgG2a and IgG1.	177
			Priming with MOMP and secondary boost with DNA MOMP-ISCOM	i.m.	Mouse/i.n.	Elevated levels of IgG2a, IgA, and IFN-γ.	178
			DNA MOMP plus GM-CSF, enterotoxins (*E. coli*) A and B	i.n. + i.v.	Pig/i.v.	Vaccination induced significant protection against genital challenge. Protection correlated with efficient T cell priming and elevated IgA.	138
			OmpA	i.m.	Pig/i.m.	Anti-MOMP antibodies and low IL-4 production.	179
			MOMP and PorB DNA plasmid	i.m.	Mouse/i.v.	High levels of IgG2a and IgA.	182
Bacterial ghosts	Inactivation not required, and therefore relevant antigenic determinants are not denatured; easy to produce; require no refrigeration; carriage of different antigens, DNA, and drugs simultaneously; recognition and phagocytosis by APC	Presence of lipopolysaccharide (LPS)	PmpD and PorB DNA plasmid	i.m.	Mouse/i.v.	High levels of IgG2a, IgA, and IFN-γ and low levels of IL-5 (Th2).	183
Biodegradable polymers	Biodegradable, nontoxic, high encapsulation capacity, PLGAs are efficiently phagocytosed by DC and Mφ, chitosan has mucosal adhesiveness properties and enhanced penetration across mucosal barrier		rMOMP encapsulated in PLGA	s.c.	Mouse	Elevated CD4$^+$ and CD8$^+$ T cells.	189, 190
			Chitosan containing rMOMP DNA	i.m.		Elevated IFN-γ and IL-12 and reduced IL-4 and IL-10. Elevated IgG2a and reduced IgG1.	191
Vaccines from transgenic plants	Low-cost production, ease of use	Requirement for strong adjuvant	MOMP introduced into *A. thaliana* and *D. carota*				169
Gas vesicles	Able to express peptides from various genes		Gene fragments coding for MOMP, OmcB, and POMP loaded into *Halobacteria*-derived gas vesicles			Elicited Th1 cytokines in human foreskin fibroblasts.	237

a i.v., intravenous; i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; s.l., sublingual; t.c., transcutaneous.

C. trachomatis and followed their progress for 3 years. Although the children that received the inactivated chlamydial vaccine exhibited partial protection compared to nonimmunized controls, a significant proportion of the immunized individuals developed enhanced disease, ostensibly as a result of delayed-type hypersensitivity after chlamydial infection (152). A vaccine study using two different preparations of live *C. trachomatis* demonstrated short-lived and modest protection in Gambian children. However, similar to the case in the Taiwanese study, some individuals developed more severe disease after infection (153). Vaccines with live organisms are generally considered optimal because they contain virtually all of the antigenic determinants in the correct three-dimensional conformation. However, using live organisms for vaccines has drawbacks, as growing and purifying *Chlamydia* on a large scale are extremely complex. Moreover, these vaccines need cold storage, and even more importantly, there is the potential for avirulent strains to revert back to infectious wild-type strains (154).

Because of the safety issues of live vaccines, research switched to organisms that were heat or chemically inactivated. The major disadvantages of these types of vaccines are the absence of replication and a suboptimal immune response, necessitating the need for revaccination and adjuvants. Heat or chemical bacterial inactivation may also release unwanted and detrimental components, which can have deleterious effects or degrade protein antigenic determinants, thereby reducing the degree of protection. Recently, plasmid-deficient *Chlamydia* strains have been used in vaccine research, with conflicting results. O'Connell et al. demonstrated that a strain of *C. muridarum* (Nigg) which lacks a plasmid and is defective in the ability to accumulate glycogen did not cause inflammatory pathology in mice. Furthermore, the plasmid-deficient bacterium protected mice against a secondary infection with plasmid-competent virulent *C. muridarum* (87). However, a different group demonstrated that mice vaccinated with an attenuated plasmidless *C. trachomatis* strain (L2R) were not protected from colonization and inflammatory pathology after a secondary challenge with wild-type *C. trachomatis*, although there were reductions in infectious burdens at early time points (88).

Subunit Antigenic Determinants

Another vaccine strategy utilized is the administration of purified antigenic determinants known to elicit an immune response. Subunit vaccines are safer than attenuated or heat- or chemically inactivated organisms because they are unable to cause infection and because virulent components that may cause pathology can be avoided. One of the most well-studied vaccine candidates for *C. trachomatis* is MOMP. This membrane protein contains several conserved CD4$^+$ T, CD8$^+$ T, and B cell epitopes (155). An early study conducted by Pal and colleagues demonstrated that *C. muridarum* COMP (chlamydial outer membrane complex), a chlamydial outer membrane with a cysteine-cross-linked protein shell, significantly protected mice against genital challenge, whereas MOMP did not (156). Several years later, the same group administered a different preparation of *C. muridarum* MOMP along with Freund's adjuvant. This new, purified-and-refolded MOMP–Freund's adjuvant preparation significantly reduced bacterial burdens after a chlamydial genital challenge, demonstrating the importance of adjuvants and a correct MOMP configuration in eliciting a protective immune response (157). Tifrea et al. discovered that a polymer that keeps membrane proteins soluble

(Amphipol) in aqueous solution was able to stabilize MOMP (158). Another group immunized mice with a *C. trachomatis* MOMP-ISCOM vaccine. ISCOM (immune-stimulating complex), which is composed mainly of cholesterol, phospholipids, and saponin, is known to induce both cell-mediated and antibody responses when used as an adjuvant. Inoculation with MOMP-ISCOM was able to elicit a Th1 antigen-specific response, and vaginal infection was cleared within 1 week (159). A *C. muridarum* native MOMP preparation combined with an adjuvant consisting of the subunit B cholera toxin conjugated to CpG (CTB-CpG) induced significant cell-mediated and antigen-specific antibody responses against intranasal infection with *C. muridarum* (160). A nonhuman primate model was used to demonstrate the efficacy of a vaccine formulated with native MOMP. Rhesus macaques that were immunized intramuscularly and subcutaneously along with the adjuvants CpG-2395 and Montanide ISA 720 produced high levels of Th1 cytokines (IFN-γ and TNF-α) and *C. trachomatis*-specific IgG and IgA (161). Drawbacks of subunit vaccines include the facts that extracting, refolding, and purifying protein complexes such as MOMP are very expensive and that purifications are not standardized, so differences in extraction methods may influence the conformation of the protein epitopes and the vaccine efficacy. The advent of protein arrays has aided in the identification of potential immunodominant antigen vaccine candidates. Cruz-Fisher et al. designed a protein chip array that was incubated with sera from mice that were infected with *C. muridarum* (162). From a total of 909 proteins, 71 were recognized by the array. Another array using sera from *C. trachomatis*-infected women recognized over 700 chlamydial proteins (163).

Recombinant Proteins

The advent of recombinant DNA technology has made it possible to produce large quantities of bacterial proteins. Thus, different attempts have been made to use rMOMP in *C. trachomatis* vaccines. Unfortunately, producing rMOMP with its native conformational epitopes intact on a large scale is challenging, and full-length rMOMP is toxic in some expression systems (164, 165). Evidence suggests that differences in MOMP conformation may affect its ability to act as a vaccine. In 2009, a comparison of vaccines using native or recombinant MOMP demonstrated that natural MOMP was superior than rMOMP in its ability to protect against chlamydial challenge (166). However, other studies using rMOMP with and without adjuvants demonstrated protection against *Chlamydia* (167, 168). In 2011, Kalbina and colleagues designed a chimeric construct containing genes that correlate with two different MOMP regions and introduced the construct into a bacterium (*Escherichia coli*) and two plants (*Arabidopsis thaliana* and *Daucus carota*). The stable integration of the transgene was demonstrated in *A. thaliana* and *D. carota* plants over several generations. The rMOMP purified from *E. coli* was used to produce antibodies in rabbits, and these antibodies recognized the proteins in *E. coli*, *A. thaliana*, *D. carota*, and *C. trachomatis*. The stability of the construct in the offspring plants suggests that this system may be useful for large-scale production of rMOMP (169).

Recombinant proteins other than MOMP have also been shown to be potential vaccine candidates. In 2007, Murphy and colleagues investigated the potential of rCPAF to induce an immune response that would resolve chlamydial infection. Mice immunized intranasally with rCPAF and IL-12 (a Th1 cytokine) demonstrated increased IFN-γ production and minimal IL-4 (a Th2

cytokine) production and elevated IgG2a (Th1) and IgA (mucosal) antibody levels, displayed markedly reduced bacterial burdens upon *C. muridarum* genital inoculation, and were protected against pathological consequences of *Chlamydia* infection compared with mock-immunized mice (170). The same group demonstrated that rCPAF intranasal vaccination may prevent infertility from repeated genital *C. muridarum* infections in mice (171). Mice immunized with recombinant chlamydial glycogen phosphorylase (GlgP) and intravaginally challenged with live *C. muridarum* elicited Th1 immunity that included antichlamydial antibodies and reduced hydrosalpinx severity. Additionally, mice that were immunized with GlgP demonstrated less shedding on day 14 post-vaginal challenge (172). Olsen et al. utilized two recombinant proteins in a subunit chlamydial vaccine. The fusion protein CTH1 consisted of CT443 (OmcB), which has been shown to elicit cell-mediated and antibody responses, and CT521 (rl 16), a protein known to be a target during chlamydial infection in humans. Immunization with CTH1 along with the strong Th1-inducing adjuvant CAF01 elicited TNF-α, IL-2, and IFN-γ production from T cells, as well as large amounts of both Th1 (IgG2a) and Th2 (IgG1) CTH1-specific antibodies. The vaccine significantly reduced bacterial burdens after vaginal infections with live *C. trachomatis* and *C. muridarum* (173). Lu and colleagues screened 5 recombinant chlamydial antigens that were previously found to react with sera from intravaginally *C. muridarum*-infected mice as chlamydial vaccine candidates. Only Mip (macrophage inflammatory protein) induced pronounced protection, which was characterized by a Th1-dominant T cell response and anti-Mip antibodies (174).

Plasmid DNA

DNA vaccines work by injecting a plasmid that carries a specific gene of interest within the host. The product of the gene can then be expressed by inducing an immune response. DNA vaccines have several advantages compared with other vaccination strategies. DNA is easy to purify, and plasmids can be constructed relatively quickly (175). Additionally, DNA vaccines can encode multiple epitopes that are in the native three-dimensional configuration and avoid the problem associated with attenuated organisms which are able to revert back to virulent forms. However, as with other vaccine strategies, DNA vaccines have some disadvantages. In autoimmune diseases such as lupus, anti-DNA antibodies are produced, and introduction of a DNA plasmid into the host may result in autoimmunity. Also, because DNA encodes proteins, DNA vaccines are generally used for protein-based antigens (176). In 1999, Pal and colleagues immunized mice with a *C. trachomatis* MOMP DNA vaccine. When the mice were vaginally challenged with *C. trachomatis*, the immune response was modest, and immunized mice were not protected against infection (177). The following year, Dong-Ji et al. demonstrated that immunization with DNA-MOMP and boosting with MOMP-ISCOM conferred more protection against *C. trachomatis* than that in mice that were immunized only with MOMP-ISCOM (178). More recently, two studies using a pig model assessed the efficacy of DNA chlamydial vaccines. Schautteet et al. combined aerosol-vaginal delivery of a DNA vaccine encoding MOMP coadministered with DNAs encoding three different adjuvants (granulocyte-macrophage colony-stimulating factor [GM-CSF] and *E. coli* enterotoxin subunits A and B). Mice immunized with the DNA vaccine were significantly protected against genital *C. trachomatis* challenge (138). Ou and colleagues, using a pig model, demonstrated that an OmpA-based DNA vaccine elicited more antigen-specific IgG antibodies and a larger T cell proliferative response than those in controls after a vaginal infection with *C. abortus* (179). A plasmid encoding MOMP epitopes inserted into a human papillomavirus (HPV) was used to assess the ability of a MOMP DNA vaccine to protect against vaginal *C. trachomatis* infection. Immunization elicited a Th1 response characterized by low IL-4 production and antibodies against MOMP (180). All of these recent studies demonstrate the feasibility of DNA-based vaccines, and this approach thus deserves further study.

OTHER CHLAMYDIAL VACCINES AND DELIVERY SYSTEMS

BGs

Bacterial ghosts (BGs) are bacterium-based empty shells that do not contain internal components but retain their outer morphological structure and can be loaded with peptides, drugs, or DNA (181). In 2007, a vaccine system in which a DNA plasmid that encoded *C. trachomatis* MOMP and the porin protein (PorB) inserted into a BG was used. Animals that were immunized intramuscularly with the DNA-bacterial ghost vaccine completely resolved a *C. trachomatis* genital infection by 2 weeks postinfection. The inflammatory response was Th1 mediated, characterized by high levels of IgA and IgG2a (182). More recently, Eko and colleagues used a BG that contained PorB and chlamydial polymorphic membrane protein D (PmpD) proteins to evaluate its ability to induce chlamydial immunity. Intramuscular immunization elicited high levels of Th1-associated IgG2a antibody, mucosa-associated IgA antibody, and IFN-γ (Th1) and low levels of IL-5 (Th2) in response to an intravaginal *C. muridarum* infection (183).

Biodegradable Polymers

PLGA (poly-D,L-lactide-coglycolide) is an FDA-approved polysaccharide that can encapsulate peptides, proteins, or DNA. PLGAs are efficiently phagocytosed by DC and Mφ (184, 185), and PLGA antigens are able to be presented on MHC class I/II molecules, thus activating CD4$^+$ and CD8$^+$ T cells (186, 187). Chitosan is a chitin-derived polysaccharide and has several properties that make it a useful vaccine delivery system, including its mucoadhesiveness and enhanced penetration capacity across mucosal barriers (188). Two recent studies using recombinant MOMP encapsulated in PLGA demonstrated enhanced induction of Th1 cytokines and cellular and antibody immune responses (189, 190). Cambridge et al. demonstrated that MOMP was expressed in the tissues and organs of mice that were intramuscularly injected with chitosan nanoparticles containing recombinant MOMP DNA (191).

Gas Vesicles

Gas vesicles are gas-containing structures that provide buoyancy and are found in some bacteria and archaea. These protein structures are hollow, rigid, and lipid-free and allow diffusion of gases across the membrane. In fact, gas vesicles from *Halobacterium* spp. have been used in vaccine research (192, 193). Gas vesicles are desirable for use as a delivery system for human vaccines because they are nontoxic to humans and are able to be phagocytosed efficiently by APC (194). Furthermore, exogenous bacterial DNAs that encode particular proteins are able to be inserted into the

structure, resulting in expression of these proteins on the gas vesicle surface (192–194). Studies have shown that in the absence of adjuvants, *Halobacteria* gas vesicles that displayed viral peptides elicited a long-lasting immune response characterized by immunological memory in mice (193). *Halobacteria*-derived gas vesicles that were loaded with gene fragments coding for MOMP, OmcB (outer membrane complex B), and POMP-B (polymorphic outer membrane B) and expressed on the surface were able to elicit a Th1 cytokine profile in human foreskin fibroblasts *in vitro*. Furthermore, antibodies specific for the recombinant proteins were confirmed using sera from *Chlamydia*-positive patients, suggesting that this could be an effective antigen delivery system for a *Chlamydia* vaccine (192).

ADJUVANTS

Live attenuated or intact inactivated whole-organism vaccines usually do not require additional components to induce a robust immune response. However, vaccines that comprise subunits of the original organism often induce a suboptimal immune response and therefore require substances, termed adjuvants, that are intended to enhance the immunogenicity of these vaccines. Natural adjuvants can come from the organism itself, such as Toll-like receptor (TLR) ligands, or can be endogenous cytokines/chemokines produced in response to a challenge. The main goal of artificial or naturally derived adjuvants is to induce immunity that closely resembles a natural immune response to the intended pathogen. Therefore, identifying adjuvants that elicit a protective immune response *in vivo* is going to be one of the main challenges for developing an effective chlamydial vaccine. There are several components that are required for a successful vaccine, including activation of innate immunity, costimulation of immune cells, cytokine production, antigen presentation, and immune modulation, and adjuvants can contribute to all of these signals. Even though various natural and synthetic adjuvants have been utilized in basic research for over 70 years, only a few adjuvants are currently licensed for use in human vaccines. These include alum (aluminum hydroxide), AS04 (monophosphoryl lipid A [MPL]-alum), AS03 and MF59 (squalene-based adjuvants), and liposomes (195). Some adjuvants bind with the antigen and are used as delivery systems. Delivery system adjuvants stabilize the antigen and allow the antigen to be released slowly, thereby contributing to costimulation of immune cells and possible uptake by antigen-presenting cells, such as DC. Examples of antigen delivery system adjuvants include calcium phosphate, tyrosine, liposomes, virosomes, emulsions, nanoparticles, ISCOMs, virus-like particles, and alum (196). However, even though, until recently, alum has been the only FDA-approved adjuvant, it does not induce IL-12 production, weakly activates DC, and induces a Th2-mediated antibody response (197, 198). Therefore, it is a poor adjuvant if the intended outcome is Th1-mediated immunity. Another class of adjuvants influence the immune response by directly activating immune cells. These components are recognized as "danger signals" via receptors, such as TLRs, of innate immune cells. The subsequent cytokine secretion, internalization, and presentation of the antigen to CD4$^+$ T lymphocytes activate the T cells, which can then initiate an adaptive immune response. These adjuvants, termed potentiators, are usually purified bacterial or viral components or synthetic molecules that are structurally similar to the intended natural organism component. Examples of immune potentiators are MPL, MDP (*N*-acetyl-muramyl-L-alanyl-D-isoglu-

tamine), CpG, bacterial or viral components, lipopeptides, and double-stranded RNA (dsRNA) (196).

Numerous adjuvants, such as those mentioned in this review (e.g., Freund's adjuvant, ISCOMs, CTB-CpG, CpG, and bacterial ghosts), have been used in chlamydial vaccine research, with various results. Recent research has added other new antigen/adjuvant candidates, with encouraging results. A study by Yu and colleagues investigated the ability of liposomes, CpG, alum, and the squalene water-in-oil emulsion adjuvant Montanide coadministered with the chlamydial protein PmpG to mediate protective immunity against *C. muridarum*. The results demonstrated that two liposomal adjuvants, DDA-MPL and DDa-TDB, were superior compared to the other adjuvants. Additionally, protection against chlamydial infection was better when the liposomal adjuvant DDA-MPL was administered with 7 different T cell antigens compared to immunization with just MOMP (199). This highlights the various opportunities to further improve vaccine candidates by identifying the optimal epitope-adjuvant combination.

VACCINATION ROUTES

Vaccine efficacy is defined not only by the type of antigen and adjuvant used but also by the administration route, since lymphocytes primed by antigens *in vivo* are endowed with specialized homing programs guiding their migration to specific mucosal sites (200). Once naive T cells are primed in a lymph node, a global switch of their homing program occurs, which enables them, while trafficking through the blood circulation, to detect chemokines and adhesion molecules which direct them to their tissue destination. Furthermore, T cell homing to the genital mucosa involves either α1β1, α4β1 (201), or α4β7/E selectin (202) in *Chlamydia*-infected mice. Both systemic and mucosal immunization routes have been shown to be able to induce both antibody- and cell-mediated immune responses in the genital tract, with intranasal immunization often being more effective (203, 204). Overall, mucosal immunization routes were more effective at preventing genital challenges with a variety of pathogens (205–209).

Numerous immunization routes have been used for chlamydial vaccinations, including oral (210), intranasal (211), intravaginal (139), subcutaneous (212), intramuscular (213), perivaginal (212), perisacral (212), sublingual (214), and colonic (124) routes. A study using purified MOMP with a *Borrelia* surface protein as an adjuvant demonstrated that in two different mouse strains, intramuscular-plus-subcutaneous and perivaginal-plus-perisacral immunization elicited high systemic and mucosal serum antibody titers. In contrast, the mice that received the MOMP-adjuvant vaccine intranasally were characterized by low serum titers (212). However, Cunningham et al. showed that intranasal vaccination with rMOMP resulted in antibodies (IgG and IgA) specific for MOMP in the genital tract, demonstrating that intranasal administration may target immunity to the reproductive tract (215). Several studies comparing the protective abilities of various vaccination routes demonstrated that combined mucosal and systemic inoculation may be optimal. Ralli-Jain and colleagues demonstrated that a MOMP-adjuvant combined sublingual (mucosal), intramuscular (systemic), and subcutaneous (systemic) vaccination regimen showed the best protection following intranasal *C. trachomatis* challenge (214). Another group demonstrated that mice immunized by combined mucosal and systemic routes with *C. muridarum* recombinant MOMP plus CpG/Montanide not only showed the strongest antibody and cell-mediated

responses after vaginal challenge with *C. muridarum* but also were protected against infertility (124).

POSTVACCINATION PROTECTION

Postvaccination protection can vary depending on the antigen, immunization route, adjuvant, and infection model. Yu and colleagues investigated the ability of live versus inactivated *Chlamydia* to protect against a subsequent chlamydial vaginal infection. In their studies, the mice were immunized with either live or UV- or heat-inactivated *C. muridarum* and challenged (at 6 weeks postvaccination for live EBs or 2 weeks postvaccination for inactivated EBs) with live *C. muridarum* intravaginally. Mice that were vaccinated with inactivated *Chlamydia* exhibited little to no protection, whereas live-EB-immunized mice had virtually no bacterial titers in cervicovaginal washes at 6 days postchallenge (66). UV-inactivated bacteria are often alive and have their components intact but are unable to replicate (216), whereas heat inactivation kills bacteria and often denatures protein epitopes (217). Therefore, these results indicate a requirement for replicating bacteria that contain nondenatured epitopes in their original conformation to induce protective immunity that significantly reduces or eliminates bacterial shedding at the site of infection. A guinea pig-*C. psittaci* genital model demonstrated the effectiveness of live chlamydial vaccination and the importance of vaccination routes. Animals were vaccinated by four different routes (intravenous, subcutaneous, oral, and ocular) with either live or UV-inactivated *C. psittaci* and were challenged intravaginally with live *C. psittaci*. All immunized animals exhibited a reduction in genital infection, except for guinea pigs that received UV-inactivated *Chlamydia* orally. Live *C. psittaci* immunization induced greater resistance to challenge than that with UV-inactivated *C. psittaci* immunization, and all routes of immunization (intravenous versus subcutaneous versus ocular versus oral) induced similar protective responses (218). Two studies investigating the use of plasmid-deficient *C. muridarum* and *C. trachomatis* as attenuated live vaccines demonstrated different results in terms of bacterial burdens and pathology in a genital infection model. Mice vaccinated with mutant *C. muridarum* strains were protected against oviduct disease but exhibited bacterial burdens similar to those in wild-type *C. muridarum*-vaccinated controls (87). Plasmid-deficient *C. trachomatis* (L2)-vaccinated mice were not protected against infection or inflammatory disease but exhibited a reduction in infectious burden 1 to 2 weeks after challenge with wild-type *C. trachomatis* (88). These results demonstrate the challenges associated with using different chlamydial strains in mouse models to understand protective immunity and pathology during *Chlamydia* infection. MOMP is one of the most investigated components of *Chlamydia* in vaccine research, and depending on the source (DNA, purified protein, or recombinant protein), preparation, and serovar, it can have varied results in its efficacy in protection against chlamydial burden and pathology. Shaw et al. demonstrated that mice intravenously receiving rMOMP-pulsed BMDC were not protected against live genital *C. muridarum* challenge and had vaginal shedding similar to that of unimmunized control mice (63). In contrast, mice immunized intravenously with Ad-MOMP (a recombinant adenovirus carrying the *C. trachomatis* serovar E MOMP gene)-transfected BMDC exhibited smaller bacterial genital burdens, less pathology, and minimal loss of body weight compared to controls (72). However, mice vaccinated with MOMP DNA and challenged intravaginally with *C. muridarum* demonstrated

vaginal shedding and fertility rates similar to those for mice vaccinated with control plasmids (177). Collectively, these results indicate that the origin of antigen (recombinant MOMP versus a virus carrying the MOMP gene) and the type of chlamydial strain (*C. muridarum* versus *C. trachomatis*) may significantly affect how DC present proteins to T cells and modulate protective immunity and pathology in a chlamydial infection. Pal et al. showed that mice immunized intramuscularly and subcutaneously with a vortexed preparation of native MOMP plus Freund's adjuvant were significantly protected against *C. muridarum* genital challenge in terms of the number of inclusion-forming units (IFUs) and the length of time the mice shed viable organisms compared to sonicated-MOMP-vaccinated mice (157), indicating that the preparation of native MOMP is important for its ability to act as a vaccine antigen. Table 4 summarizes postvaccination protection, including animal models, vaccination antigens, and times of postvaccination chlamydial challenge.

Antibiotic intervention studies in Canada, Vietnam, and Africa have yielded important insights into the development of protective immunity to *Chlamydia* in humans. Collectively, the data suggest that early antibiotic treatment for chlamydial infection may increase the number of individuals who are susceptible to reinfection by disrupting the development of adaptive immunity. Therefore, early antibiotic intervention may paradoxically increase the prevalence of *Chlamydia* in the population over the long term. For example, an epidemiological study analyzing *C. trachomatis* urogenital cases discovered that after antibiotic intervention, the incidence of *Chlamydia* reinfection cases rose by 4.6% (219). These results have also been observed for trachoma (220, 221).

CHALLENGES FOR A CHLAMYDIAL VACCINE

There are many critical questions that still need to be addressed in order to develop a chlamydial vaccine in the future. How does this intracellular bacterium induce pathogenesis in the host? How does *Chlamydia* mediate the immune response, and by what mechanism does *Chlamydia* induce sequelae during infection? Why do most patients remain asymptomatic and not develop pathology, whereas others develop severe PID? What type of human genetic polymorphisms may predispose a given individual to a chlamydial infection and pathology?

Indeed, regarding genetic susceptibility to chlamydial infection, two good reviews are available, by Morrè et al. and Lal et al. (222, 223). In cases of persistent infection, what are the characteristics of immunity that allow the infection to persist? We still do not completely understand the role that antibodies play in chlamydial infection and how Th1- versus Th2-mediated immunity is regulated at different infection sites (especially in the female genital tract). Furthermore, a better understanding of mucosal immunity may allow the development of a more specific vaccine. What type of chlamydial antigens should be used in a potential vaccine, and how will the vaccine be prepared and delivered? One might need to produce the desired vaccine antigen in a heterologous host, thus bypassing the difficulties of growing an intracellular bacterium or purifying a specific protein. Because APC differentially modulate immunity to *Chlamydia* depending on the type of antigen (i.e., recombinant, native, from viral transfection, or whole, intact bacteria), antigen origin is important for developing a vaccine to *Chlamydia*. The fact that most infected patients remain asymptomatic suggests that different strategies of vaccina-

TABLE 4 Summary of postvaccination protection[a]

Infection model/Chlamydia species	Animal model	Ag/vaccination route	Time (days) of postvaccination challenge	Assessment after postvaccination challenge	Vaccination protection	Reference
Genital (i.v.)/C. muridarum	Mouse	Plasmid-deficient C. muridarum (CM972, CM3.1)/i.v.	98	Bacterial burden (4–45 days), pathology (day 42)	Mice vaccinated with mutant C. muridarum strains were protected against oviduct disease but exhibited bacterial burdens similar to those of wt C. muridarum-vaccinated controls.	87
Genital (i.v.)/C. trachomatis serovar D	Mouse	Plasmid-deficient C. trachomatis (L2)/i.v.	35	Bacterial burden (3–28 days), pathology (day 14)	Vaccinated mice were not protected against infection or inflammatory disease but exhibited reductions in infectious burden 3 to 7 days after challenge with wt C. trachomatis. On days 14 to 28, there was no difference between vaccinated and control groups.	88
Genital (i.v.)/C. muridarum	Mouse	Purified MOMP (vortexed or sonicated) plus Freund's adjuvant/i.m. and s.c.	14	Bacterial burden (day 42)	Mice immunized with vortexed MOMP plus Freund's adjuvant were significantly protected against Chlamydia challenge in terms of the number of IFUs and the length of time the mice shed viable organisms compared to sonicated-MOMP-vaccinated mice.	157
	Mouse	rCPAF plus IL-12/i.n.	30	Bacterial burden (4–30 days), pathology (12–80 days)	Vaccinated mice exhibited significant reductions in bacterial shedding as early as 8 days postchallenge compared with controls. 80% of vaccinated mice successfully resolved infection by day 15. In contrast, control mice (rCPAF or IL-12) were still heavily infected by day 15. By day 18, 100% of vaccinated mice had resolved the infection. Vaccination reduced hydrosalpinx, oviduct dilation, and fibrosis at 80 days postchallenge.	170
	Mouse	rCPAF plus CpG/i.n.	30	Fertility assessment, breeding (day 80)	Mice vaccinated with rCPAF plus CpG and challenged with Chlamydia exhibited fertility similar to that of nonimmunized controls. After a secondary challenge, nonimmunized controls displayed significant reductions in fertility compared to immunized mice.	171
	Mouse	CTH1 plus CAF01 (adjuvant)/s.c.	42	Bacterial burden (3–35 days)	Compared to control mice, CTH1-plus-adjuvant-immunized mice exhibited significantly less vaginal bacterial shedding 7, 14, and 21 days after chlamydial challenge. Days 14 to 21 showed the most efficient protection.	173
	Mouse	rGlgP plus CpG/i.m.	30	Bacterial burden (7–28 days), pathology (day 60)	Immunized mice were characterized by a significant reduction of live organisms in the vagina by day 14 and a reduced severity of hydrosalpinx at 60 days postchallenge.	172
	Mouse	rMIP/i.m.	30	Bacterial burden (4–30 days), pathology (day 60)	Mice immunized with MIP and C. muridarum EBs (positive control) had significantly reduced vaginal bacterial shedding as early as 12 days after intravaginal chlamydial challenge. On day 21, most of the MIP-immunized mice exhibited dramatically reduced bacterial burdens compared with negative-control mice vaccinated with glutathione S-transferase (GST). Hydrosalpinx severity was less severe in MIP-vaccinated mice than in GST-immunized controls.	174
Genital (i.v.)/C. trachomatis serovar D	Mouse	rCT823 plus AblSCO (adjuvant) or CT144 plus AblSCO/s.c.	7	Bacterial burden (3–21 days)	Vaccination with CT823 plus adjuvant reduced cervicovaginal bacterial burdens by 10 log postchallenge compared to controls (UV-inactivated EBs). Vaccination with CT144 plus adjuvant reduced bacterial burdens by 1/2 log on day 7 and then 2- to 3-fold by days 10 and 14 compared to controls.	234
Genital (i.v.)/C. muridarum	Mouse	MOMP DNA plasmids/i.m.	60	Bacterial burden (7–42 days), fertility assessment, breeding (day 42)	Mice vaccinated with MOMP DNA and challenged with C. muridarum exhibited vaginal shedding and fertility rates similar to those of mice vaccinated with control plasmids.	177
Genital (i.v.)/C. trachomatis serovar D	Mouse	MOMP plus PorB DNA plasmid plus Vibrio cholerae ghost (VCG)/i.m.	21	Bacterial burden (3–24 days)	Compared to controls, VCG-MOMP/PorB-immunized mice exhibited significant differences in vaginal C. trachomatis shedding in terms of duration and intensity, and the infection was completely resolved by 2 weeks postchallenge.	182

Disease (route of challenge)/species	Species	Vaccine/route	No.	Measurement	Outcome	Reference
Genital (i.v.)/*C. muridarum*	Mouse	PmpD and PorB DNA plasmids plus VCG/i.m.	14	Bacterial burden (3–24 days)	Mice vaccinated with VCG containing a PmpD or PorB DNA plasmid or both shed 2.5-log fewer vaginal chlamydial IFUs than controls (VCG containing an irrelevant chlamydial antigen [gD2]) at 3 days postchallenge.	183
	Mouse	Adoptively transferred BMDC that were pulsed with rMOMP *in vitro*/intravenous	14	Bacterial burden (day 7)	Vaccinated mice that received BMDC pulsed with rMOMP were not protected against live chlamydial challenge and had vaginal shedding similar to that of unimmunized control mice at 7 days postchallenge.	63
	Mouse	Live or inactivated (UV or heat) *C. muridarum*, with or without CpG/i.n.	42 (live EBs), 14 (UV- or heat-inactivated EBs)	Bacterial burden (day 6)	Vaccination with live EBs significantly reduced bacterial shedding compared to that with both UV- and heat-inactivated *C. muridarum* at 6 days postchallenge.	66
	Mouse	Adoptively transferred BMDC that were transfected with a recombinant adenovirus carrying the *C. trachomatis* serovar E MOMP gene (Ad-MOMP)/intravenous	21	Body weight (2–10 days), bacterial burden (2–10 days), pathology (day 11)	Vaccinated mice that received Ad-MOMP-transfected BMDC exhibited lower bacterial genital burdens, less pathology, and minimal loss of body weight compared to controls.	72
	Mouse	Adoptively transferred BMDC that were pulsed with UV-inactivated *C. muridarum* plus CpG or rCPAF plus CpG *in vitro*/s.c.	30	Bacterial burden (3–30 days), pathology (day 80)	Mice immunized with adoptively transferred BMDC that were pulsed with either UV-inactivated EBs plus CpG or rCPAF plus CpG demonstrated significantly reduced vaginal shedding at 15 days postchallenge and reduced oviduct pathology at 80 days postinfection compared to mock-immunized mice.	73
	Mouse	Sera from (convalescent) previously vaginally *C. muridarum*-infected wt mice; MAb to MOMP			CD4+ T cell- and antibody-deficient mice that had previously resolved a chlamydial infection were immunized with convalescent-phase sera. These mice exhibited decreased vaginal bacterial shedding (from >100,000 IFUs to <10 IFUs) and a shortened duration of infection compared to control mice. Immunization with a MAb specific for MOMP reduced bacterial shedding >100-fold.	102
Genital (i.v.)/*C. trachomatis* serovar E	Pig	MOMP DNA plus GM-CSF, enterotoxins (*E. coli*) A and B/i.n. plus i.v., i.d.	21	Bacterial burden (2–25 days), pathology (day 25)	Vaginal shedding was less in i.n. plus i.v. vaccinated pigs than in pigs that were i.d. vaccinated. However, neither group of vaccinated pigs (i.n. plus i.v. or i.d.) completely resolved the infection. Vaccinated pigs exhibited significantly fewer lesions in urogenital tissues.	139
	Pig	PmpG plus GNE (adjuvant) and SctC plus GNE/s.c.	41	Bacterial burden (2–23 days), pathology (day 23)	PmpG gave better protection than SctC after vaginal challenge with *C. trachomatis*, characterized by low antibody titers and minor pathology. SctC-vaccinated pigs exhibited significant pathology but more antibody production after *Chlamydia* challenge.	140
Genital (i.v.)/*C. psittaci*	Guinea pig	Live or UV-inactivated *C. psittaci*/intravenous, s.c., oral, or ocular	14	Bacterial burden (3–24 days)	All immunized animals exhibited reductions in genital infection, except for guinea pigs that received UV-inactivated *Chlamydia* orally and nonimmunized controls. Live compared to UV-inactivated *C. psittaci* immunization induced more resistance to challenge, and all routes of immunization (i.v. vs s.c. vs ocular) induced similar protective responses.	218

a Abbreviations: i.v., intravaginal; i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; i.d., intradermal.

tion will be needed. Indeed, both preventing the primary acute infection and curing persistent/chronic infection might be investigated. What type of immunization route(s) and how many vaccinations are optimal? Should mucosal or systemic vaccinations be utilized separately or in combination? How many vaccinations should be administered, and what would be the optimal intervals between vaccinations? In order to enhance or select a specific immune response, novel adjuvants might have to be developed. Although animal experiments cannot replace clinical trials, they are nonetheless necessary, and deciding which animal model to utilize is an important factor in understanding and analyzing protective immunity, pathology, and immunological mechanisms during chlamydial infection. Thus, there is still much research to be done on the biology of *C. trachomatis* and on the pathogenesis of genital chlamydial infection. Developing a chlamydial vaccine will entail further research on the antigenicity of chlamydial proteins and on novel and more effective vaccine delivery systems.

When should individuals be vaccinated? A prophylactic chlamydial vaccine should be administered before the infection is normally acquired, which usually means the early teens, and preferably before sexual activity. However, the vaccine should be effective enough to provide protection throughout sexual life and may need to be readministered throughout the individual's lifetime to be optimally effective. Who should be vaccinated? Should only men or women be vaccinated, or both sexes? A similar debate occurred when the HPV vaccine Gardasil became available. In Australia, Gardasil was directly approved for both men and women in 2006, whereas the U.S. FDA initially approved it only for women. The reason for approving only women was that the efficacy studies (phase III) were performed in women, and thus no efficacy was assessed at that time for men (protection against genital warts). However, because immunity bridging data were available for both boys and girls, Australia recommended vaccination for both sexes. Computer simulations have demonstrated that more than 80% female vaccination would achieve sufficient coverage and would be more cost-effective than vaccinating both males and females (224, 225). Also, it was calculated that with such high vaccine coverage, herd immunity would be enough to reduce HPV circulation (226). Currently, the U.S. Centers for Disease Control and Prevention (CDC) recommends HPV vaccination for females aged 11 to 12 years of age, with catch-up vaccination at 13 to 16 years of age, and the HPV vaccine was recently approved for use in boys and men aged 9 to 26 years to prevent genital warts (227). If *Chlamydia* vaccination programs were to be directed mainly at females, the possible rationale would be because *Chlamydia*-related morbidity and mortality are higher among women (infertility and ectopic pregnancy). A reason for male inclusion would be to further decrease *Chlamydia trachomatis* prevalence in the population and indirectly improve the protection of women. Since men and women are equally susceptible to genital chlamydial infection (228), a *Chlamydia trachomatis* vaccine for both sexes should at least be discussed. However, although recent studies have demonstrated that vaccinating both sexes has a beneficial impact on *Chlamydia*-related morbidity, similar to the case with the HPV vaccine, targeting women is more effective than targeting men (229). *Chlamydia* vaccination in groups with the highest risks of infection should be prioritized (multiple partners, sex workers, and immunocompromised individuals), independent of gender. Since a large proportion of HIV-positive homosexual men test positive for rectal LGV and non-LGV *Chlamydia trachomatis*

(230), and since *Chlamydia trachomatis* is associated with an increased risk of HIV transmission (231), this group would also benefit from a *Chlamydia* vaccine. However, a chlamydial vaccine for these groups would be more for its therapeutic potential than as a prophylactic measure. Finally, who will pay for the cost of vaccination to prevent what is mostly a chronic silent infection? How do we convince decision-makers that the current epidemic of subfertility due to the silent *C. trachomatis* outbreak will have such a huge negative financial impact at the societal level that vaccine development should now be considered a public health priority?

CONCLUSIONS

Chlamydial infection is a public health concern worldwide, and a vaccine that stimulates multiple arms of the adaptive immune system and avoids immunopathological consequences would be the best solution for controlling this sexually transmitted disease. Unfortunately, a partial or fully protective vaccine has yet to be developed, highlighting the complex nature of the immunobiology mounted against this intracellular parasitic bacterium. The immune response to chlamydial infection is dynamic and involves cells and mediators from both arms of the host's immune system. Clearance of a chlamydial infection requires a coordinated immune response between innate immune cells, such as Mφ, neutrophils, NK cells, NK T cells, and DC, and cells important in both cell-mediated and humoral adaptive responses, such as $CD4^+$ T cells, $CD8^+$ T cells, and B cells. Activation and clonal expansion of T cells occur through cognate interactions with DC that present chlamydial antigens on their MHC molecules, and B cells produce antichlamydial antibodies through interaction with these clonal T cells. However, persistent infection seems to induce chronic inflammation and tissue damage. A shift from Th1 to Th2 immunity also appears to induce scarring and immunopathology. It is therefore essential to understand these immunological dynamics in order to develop a vaccine that is both effective and long-lasting and does not have the deleterious effects associated with unregulated inflammation. Further research is needed to identify novel adjuvants that enhance the immune response and antigens that induce a protective T cell response and antichlamydial antibodies.

A mathematical model developed by Gray and colleagues demonstrated that a fully protective vaccine administered to adolescents before they are sexually active would be able to significantly decrease *Chlamydia trachomatis* infection in 20 years. In addition, the model predicted that vaccinating 100% of women would have a greater epidemiological impact than vaccinating both sexes (229). Unfortunately, there are risks and ethical questions associated with vaccination programs, as demonstrated by the first *Chlamydia* vaccine using a live attenuated bacterium (149). Thus, research is needed to develop an efficient and safe chlamydial vaccine.

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