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National Institute of Allergy and Infectious Diseases workshop report: "Chlamydia vaccines: The way forward"

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ABSTRACT

Chlamydia trachomatis (Ct), an intracellular pathogen, is the most common bacterial sexually transmitted infection. In addition to acute cervicitis and urethritis, Ct can lead to serious sequelae of significant public health burden including pelvic inflammatory disease (PID) and infertility. Ct control efforts have not resulted in desired outcomes such as reduced incidence and reinfection, and this highlights the need for the development of an effective Ct vaccine. To this end, NIAID organized a workshop to consider the current status of Ct vaccine research and address critical questions in Ct vaccine design and clinical testing. Topics included the goal(s) of a vaccine and the feasibility of achieving these goals, animal models of infection including mouse and nonhuman primate (NHP) models, and correlates of protection to guide vaccine design. Decades of research have provided both whole cell-based and subunit vaccine candidates for development. At least one is currently in clinical development and efforts now need to be directed toward further development of the most attractive candidates. Overall, the discussions and presentations from the workshop highlighted optimism about the current status of Ct vaccine research and detailed the remaining gaps and questions needed to move vaccines forward.

1. Introduction and objectives

The World Health Organization (WHO) estimates that 131 million cases of sexually transmitted *Chlamydia trachomatis* (Ct) infections occurred in 2012, with estimated incidence highest among women in the WHO Region of the Americas (72 per 1000) and Western Pacific Region (56 per 1000) [1]. Ct is a public health priority because infection in women can cause pelvic inflammatory disease (PID) and other long-term sequelae including infertility and ectopic pregnancy. An estimated 10–15% of cervical infections spread to the fallopian tubes and approximately 10–15% of these result in infertility [2]. Given that an estimated 68 million cases of Ct occur among women each year, and most are likely left untreated, up to 1 million new cases of infertility could result annually on a global scale.

As the vast majority of infections are asymptomatic, current Ct control programs are based on screening and treatment, and are not available in most of the world because they are costly, complex to roll out, and difficult to bring to scale. For instance, in the US alone, only about half of women eligible for screening are actually tested [3]. Furthermore, existing programs have not clearly resulted in reduced infection incidence, and reinfection remains all too common. The complexities of current Ct control efforts highlight the need for continued work toward an effective vaccine.

A recent cost-effectiveness model of a hypothetical Ct vaccine for young females in the United States concluded that a Ct vaccine could be cost effective even in the context of ongoing screening programs [4]. In settings with >6% Ct prevalence, vaccinating 14 year-old girls and continuing to screen females (15–24 years), assuming 30% Ct vaccine coverage and vaccine costs of $547 (as for human papillomavirus [HPV] vaccine), the vaccine was cost saving. At 3.2% prevalence, as in the United States, a Ct vaccine would cost approximately $35,000 per quality-adjusted life-year (QALY), similar to HPV vaccine. Vaccine cost, duration of immunity, vaccine efficacy and risk of sequelae also influenced cost-effectiveness. Better data on Ct infection and disease burden in different settings will inform future models.

Given the magnitude of Ct rates, the complexity of control efforts and preliminary models showing a vaccine would be cost

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effective, now is the time for serious consideration of Ct vaccine development by leveraging decades of basic and preclinical research studies. In May 2015, the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, sponsored a workshop entitled "Chlamydia Vaccines: the Way Forward". The goals of the workshop were to gather Ct experts to discuss key questions on the research status and path forward to a licensed Ct vaccine. Representatives from academia, WHO and U.S. Government agencies attended. This review summarizes key areas of focus discussed during the workshop and provides next steps for chlamydial vaccine development.

2. Goals of a vaccine

Meeting participants discussed the goals of a Ct vaccine in terms of reducing infection and/or disease and how these goals shape research and development, trial design and evaluation, and measures of vaccine efficacy. The ultimate goal of a Ct vaccine is to reduce the burden of upper genital tract sequelae in women. A vaccine may achieve this by preventing infection, preventing ascension of infection to the upper genital tract, or reducing the duration or bacterial load of infection. However, the ultimate goal of a vaccine may differ from the clinical endpoints that can be measured in trials, which could be infection, disease (such as PID) or both. The choice of trial endpoint is influenced by several considerations, including the natural history and timing of clinical events following infection, the proportion of PID associated with Ct, the accurate ascertainment of PID, and other clinical trial design considerations. For example, with respect to timing, a vaccine trial would need to consider the time to incident Ct infection and then to incident disease. The precise timing of PID following incident Ct infection is unknown, but generally occurs within 1 year and rates may be greater soon after infection. Even if tubal damage occurs relatively soon, infertility may not be apparent until years later when attempting pregnancy.

Acute PID is a clinical syndrome with multiple etiologies [5]. The proportion caused by Ct varies by setting, but typically Ct is involved in about one third of cases [6]. Attribution of PID to a particular etiologic agent is difficult, as cervical and fallopian tube microbiologic tests do not always agree and co-infection is common. Measurement of PID as a trial outcome may be difficult in part because current diagnostic criteria are nonspecific and many cases of upper genital tract infection are subclinical. A critical question will be whether a decrease in clinical PID parallels a decrease in subclinical PID. Magnetic resonance imaging (MRI) may be the most useful diagnostic test but has not been widely evaluated and is not uniformly available [7]. Inaccuracy in PID diagnosis will bias findings toward the null hypothesis. Using PID rather than infection as an endpoint will require larger sample sizes, as PID incidence in screening trials has been about 2% per year whereas infection incidence is several-fold higher; however, such sample sizes are not out of the question. If both endpoints are used, frequency of follow-up Ct testing will have implications for PID assessment, as positive tests require treatment.

To use only Ct infection as a clinical trial endpoint, several questions will need to be answered. (1) Does a decrease in infection precisely parallel a decrease in sequelae? If protection is limited, are the infections prevented the ones that would lead to PID? (2) Conversely, could ascension to the upper tract and thus PID still be prevented even with breakthrough infection? (3) Could breakthrough infections lead to an enhanced risk for PID? Now is the time to carefully consider and develop consensus around vaccine trial endpoints. Even if infection is the primary endpoint, assessment of PID as a secondary endpoint or during phase IV postlicensure trials will likely be needed. Research needs to inform these discussions include: better ways of measuring upper genital tract ascension, inflammation, and damage; improved knowledge of which infections cause PID and longer-term damage; and a package of evidence to confirm a vaccine would not increase tubal immunopathology on breakthrough infection.

Biomarkers that indicate upper genital tract tissue damage would be useful for trial design and other aspects of Ct vaccine development [8], and may result from insights into the human immune response. Using a Ct whole proteome array [9], humoral immune responses in women were mapped for identifying biomarkers to aid diagnosis of tubal infertility [10,11]. Antibody responses to Ct antigens CT147 and CT875 were associated with acute Ct infection while those to Ct HSP60, CT557 and CT443 were associated with tubal infertility. A recent analysis of 225 high-risk young women showed that serum antibodies were associated with reduced cervical Ct bacterial load, however systemic CD4 + T cell IFN-γ responses to Ct antigens correlated with immunity to cervical infection [12,13]. Data from studies like these will be critical in designing clinical trials to test Ct vaccines, as well as better understanding the burden of Ct-associated sequelae.

Based on these discussions, meeting participants outlined clear goals for a Ct vaccine, but raised questions about how best to evaluate these goals in trials given current data. Participants agreed that clinical testing of a Ct vaccine is currently feasible, but exploring more novel methods to measure outcomes could expand options for vaccine evaluation.

3. Animal models

3.1. Mouse models

For several decades the *C. muridarum* (Cm) cervicovaginal infection mouse model has been successfully used to study chlamydial pathogenic mechanisms and host immune responses due to the high genomic similarity between Cm and Ct and the ability of Cm to induce pathologies in the upper genital tract that mimics pathologies observed in Ct-infected women. Both macroscopic and microscopic evaluations of genital tract pathologies such as uterine horn dilation [14] and hydrosalpinx [15,16] have become standardized in the murine model. Using this model combined with genetic tools for *Chlamydia* and the host, significant progress has been made in defining roles of the pathogen, host, and environmental factors in bacterial ascension and tubal inflammation [17,18]. For example, it has recently been shown that plasmid gene product 3 (Pgp3)-deficient Cm was attenuated in ascending infection and no longer able to induce hydrosalpinx [19]. Possible mechanisms for Pgp3 involvement in upper genital tract pathology include neutralization of mucosal antimicrobial peptides and modulation of TNF signaling pathways [20,21]. Further defining the molecular basis of chlamydial upper genital tract pathogenicity using the Cm murine model should provide novel information for identifying potential vaccine targets.

Researchers have also utilized the Ct model of genital infection of female C3H/HeJ mice, which have a toll-like receptor 4 mutation making them resistant to endotoxin. Primary infection in this model results in ascending infection with delayed clearance. Inflammatory salpingitis is observed, but not hydrosalpinx. Secondary infection shows a lower burden of organisms in the genital tract, but little difference in duration compared to primary infection. Virulence in this model is partially dependent on an intact or nearly intact CT135 gene, and laboratory-passaged Ct strains (as opposed to low-passaged clinical isolates) tend to accumulate disruptive and therefore attenuating mutations in this gene [22].

Newer mouse models include transcervical infection where bacteria are administered directly into the uterine horns and result

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in upper genital tract infection, albeit with lower burden and reduced duration compared to ascending Cm infection. Other model modifications include immunity-related GTPases 1 and 3 knockout mice expressing human indole oxidase, and TCR transgenic mice harboring naïve T cells with single specificity for Ct antigens [23].

Meeting participants did not conclude which model constituted the standard for testing preclinical vaccine candidates. Cervicovaginal infection of mice seems more straightforward than transcervical infection as it mimics the natural infection route in women. Both Ct and Cm vaginal inoculation models have advantages and disadvantages for testing candidate vaccines. For initial testing of vaccines the Cm model may prove superior given the acute nature of infection and strong protective immunity developing from primary infection, while the Ct C3H/HeJ mouse model may prove more useful in studying Ct pathogenesis given the often chronic indolent nature of Ct infection in women. Overall, it was concluded that investigators should evaluate candidate vaccines with respect to likely clinical trial endpoints and/or licensing indications and then employ an animal model that most closely replicates these criteria.

3.2. Non-human primate (NHP) models

Meeting participants discussed the potential use of NHP models of Ct infection to test vaccines, vaccine formulations and explore mechanisms of immunity and pathogenesis. Female pigtail macaques share many similarities with human females including the length of the menstrual cycle, reproductive tract anatomy, cervical tissue cellular structure, and vaginal microflora. However, pigtail macaques have displayed variable susceptibility to cervical inoculation with Ct and limited genital tract inflammatory pathology [24]. One attempt to increase the percentage of animals infected and generate more robust pathology was reported where animals were inoculated in both the endocervix and fallopian tubes with Ct. Inflammatory and pathological responses in the fallopian tubes and fimbriae were confirmed. If developed further such a model may prove useful for vaccine development, particularly for safety testing where vaccinated animals could be surveyed for upper genital tract pathology upon challenge with Ct.

Rhesus macaques have also been used to model Ct infection [25]. After repeated cervical inoculation with a Ct serovar D isolate, some animals developed infection in the fallopian tubes as revealed using fimbrial swabs while others resisted ascending infection. However, the limited disease produced with lab-passaged Ct hampers its utility for evaluating vaccine prevention of disease.

Despite availability of several NHP models, no regulatory requirement exists for testing vaccines in NHPs. NHPs may play some role in Ct vaccine development given their more human-like IFN-γ response, and some vaccines may need safety testing in a human-like reproductive tract. Further, NHP studies should continue as a research tool to shed light on mechanisms of immunity and upper genital tract pathology. However, it is unlikely these studies will be included in the critical pathway of Ct vaccine development.

4. Correlates of protective immunity and pathogenesis

Participants discussed data on protective immunity derived from both *in vitro* and *in vivo* Ct and Cm studies including several on trachoma, the major form of infectious blindness globally caused by ocular infection with specific Ct serovars. Emphasis was placed on the type(s) of immunity a putative vaccine should elicit and potential immunological markers needed to judge vaccine efficacy, safety and/or disease progression.

For ocular Ct infection, increasing age correlates inversely with bacterial burden, duration and prevalence of ocular infection, suggesting protective immunity can be induced [26,27]. A role for T cell-mediated protection was revealed by elevated peripheral blood mononuclear cell proliferative responses in subjects whose clinical signs resolved spontaneously versus those with persistent signs of trachoma [28]. A recent reexamination of some of the primary data from human trachoma whole cell vaccine trials from the 1960s [29] concluded that the vaccine preparations either resulted in no difference in incident infection or short-lived protection in vaccinated children, with no evidence for adverse outcomes [30,31]. In some studies, scarring had originally been considered a sign of healing and trachoma severity scores were lowered when conjunctival scarring was present. The prevalence of scarring was lower two years post vaccination, thus the original scoring system led to an erroneous conclusion that vaccinated children had enhanced inflammatory disease. Other studies from Saudi Arabia [32] and Taiwan [33–35] evaluated whole cell killed vaccines at different doses in children. At 6 months to 1 year of follow-up, no difference in disease severity was observed [36]. However, some meeting participants were still concerned about potential side effects of whole cell-based vaccines since in other studies in both humans and NHPs there was evidence of a hypersensitivity reaction and in a few cases, an increase in infection rate in vaccinated individuals [32–35,37–41].

Studies of conjunctival gene expression in children with active trachoma and adults with scarring disease provide information related to disease pathways. Microarray analyses reveal enhanced inflammation and disease in persons with increased expression of pro-inflammatory cytokines/chemokines IL1, IL17, CXCL5 and S100A7 [42–45] involved particularly in neutrophil chemotaxis and activation. A recent longitudinal study explored the pathogenesis of progressive scarring [44]. In Tanzanian and Ethiopian adults with established trachomatous conjunctival scarring followed for two years, progressive scarring was found in 308/1162 (27%) of participants. A strong relationship existed between progressive scarring and numbers of inflammatory episodes, but few episodes of Ct infection were detected. Thus, scarring progressed in the absence of detectable Ct. These data suggest that prior Ct infection may lead to epigenetic changes in conjunctival tissue such that other bacteria could then stimulate preprogrammed inflammation and pro-fibrotic pathways. A similar phenomenon could contribute to development of tubal factor infertility in women with previous Ct genital tract infection.

Using Cm mouse genital infection models, immune responses resolving primary infection can be distinguished from responses contributing to protection from reinfection. Studies with mice genetically deficient for or postneonately depleted of specific immune cells indicate that MHC Class II-mediated responses, αβ TCR + T cells, CD4 + T cells and IFN-γ-producing CD4 + T cells are necessary for resolution of primary infection [17,46–49] while MHC Class I-mediated responses, CD8 + T cells, Th2 cytokines, and antibody are dispensable. However, antibody is a highly efficacious in preventing secondary infection in the genital tract [50–53]. Antibody seems to protect through an antibody-cellular interaction. Independently, antibody and CD4 + T cells confer equivalent levels of protective immunity, but in combination, reduce shedding of infectious bacteria in mice an additional 100-fold. Given the combined benefit of antibody and CD4 + T cells, meeting participants noted that a vaccine targeting both CD4 + T cell and antibody responses is highly desirable.

Since replication of genital serovars of Ct is limited to reproductive tract epithelium, and MHC Class II expression and CD4 + T cells are essential for controlling infection, the most straightforward mechanism for clearing genital tract infection would involve Ct-specific CD4 + T cell interactions with infected epithelial cells.

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IFN-γ-inducible MHC-II expression on oviduct epithelial cells correlated with inhibition of Cm replication by Cm-specific CD4 + T cell clones [54]. IFN-β blunted the IFN-γ induction of MHC-II on epithelial cells, resulting in inhibition of T-cell activation, suggesting a major role of IFN-γ in upregulating MHC-II on epithelial cells during infection.

Different murine CD4 + T cell clones rely on different primary mechanisms for Cm inhibition including nitrous oxide production, perforin-mediated cytolysis, and T cell degranulation [55]. Two genes, *Plac8* and *Casd1* were highly expressed by CD4 + T cell clones that terminated Cm replication by an iNOS-independent mechanism. Plac8-deficient mice had delayed clearance of infection, and when treated with the iNOS inhibitor N-monomethyl-l-arginine were largely unable to resolve infection. These results demonstrated two independent and redundant T cell mechanisms for clearing Cm genital tract infections: one dependent on iNOS, and the other dependent on Plac8 [56]. Thus, putative vaccine antigens might be evaluated for their ability to induce Plac8/Casd1-positive CD4 + T cells in vaccinated mice. An important goal is to determine if similar molecules play a role in inhibition of Ct by human CD4 + T cells.

Despite >99% conservation in the open reading frames between Ct and Cm, these two species have evolved specific mechanisms to resist inhibition from human and murine IFN-γ, respectively. The canonical mechanism for inhibitory effects of human IFN-γ on Ct is via activation of indoleamine 2,3-dioxygenase (IDO), which catalyzes conversion of tryptophan to N-formyl-kynurenine, which depletes tryptophan stores and undermines pathogen growth [57–59]. Human genital tract strains possess a functional tryptophan synthase, which can generate tryptophan needed for chlamydial growth and replication using indole as a substrate, thus evading IFN-γ induction of IDO. Since ocular strains of Ct lack a functional tryptophan synthase, they may be compromised in comparison to genital strains.

Murine IFN-γ drives induction of p47 GTPases in murine cell lines, which can restrict Ct growth. The importance of murine p47 GTPases in clearing *C. psittaci* and Ct infection in mouse models has been documented [60–62]. Resistance to these effects is observed with Cm and may be due to a full-length cytotoxin gene in Cm [61,63]. The human genome does not encode IFN-γ-inducible homologues of p47 GTPases. Although human IFN-γ can induce human cells to express guanylate-binding proteins that harbor GTPase activity, data are lacking regarding the potential for human IFN-γ to inhibit Ct via a GTPase-driven ubiquitin-centered mechanism in ocular or genital epithelial cells. Such data would strengthen the utility of testing Ct vaccine candidates in the murine Cm model.

A relative consensus was reached that putative Ct vaccines should generate Ct-specific CD4 + T cells targeting genital epithelial cells, combined with a strong antibody response. Markers such as Plac8/Casd1 and specific GTPase activity may prove useful in designing vaccines and could possibly be incorporated in potency assays for clinical development. Connection of the molecular basis of conjunctival scarring in trachoma to development of tubal factor infertility in women with previous Ct infection may prove important for the design of future clinical trials and the evaluation of genital Ct vaccine safety.

5. Vaccine development and approaches

Efforts to develop vaccines started soon after the isolation of Ct from ocular tissue. Early concerns about enhanced pathologic responses, as discussed above, pushed the field toward development of subunit vaccines to enhance safety (Table 1). Additionally, efforts have been made to optimize whole cell-based vaccines by removing pathogenic factors. The goal has been to induce long-lasting protection with minimal side effects, and in large part, testing has utilized the Cm or Ct mouse models.

5.1. Whole cell-based vaccines

The successful identification of Cm virulence factors in animal models has made development of a live-attenuated chlamydial vaccine possible. Both the plasmid-encoded pGP3 [19,64] and the chromosomal gene encoded TC0237/TC0668 [65,66] have been identified as key virulence factors for Cm induction of hydrosalpinx in mice. Attenuated strains lacking these genes not only failed to cause upper genital tract pathology but also triggered protective responses against subsequent challenge with wild type Cm. These findings are consistent with an earlier observation that attenuated plasmid-deficient Cm maintained the ability to induce protective immunity against challenge-induced pathology [67]. Despite promising data from mouse studies, applying a live-attenuated Ct vaccine in humans would potentially raise safety concerns. A killed whole organism vaccine would significantly reduce these concerns. However, killed Cm failed to induce any significant protection [68,69], indicating the need for adjuvants that lead to controlled inflammation for inducing a protective immune response. A recent study in mice evaluated immunogenicity of a whole cell killed vaccine formulated in charge-switching synthetic adjuvant particles (cSAPs) containing TLR ligand as adjuvant [70]. When delivered via intranasal inoculation, this formulation induced robust transmucosal immunity in the mouse genital tract, which suggests that the dependence on chlamydial viability for inducing protective immunity can be overcome by an optimized formulation delivered via a mucosal route. Importantly, only mucosal vaccination induced resident memory T cells that led to optimal Ct clearance upon challenge.

5.2. Subunit vaccines

Protective immunity generally correlates with CD4 + T cell responses during chlamydial infection [17,71]. A genome-wide Ct protein array screening of human antibody responses identified Ct antigens associated with tubal pathology but not protection [10,11]. Thus, extensive efforts have been made to identify chlamydial T cell protective antigens. By analyzing peptides eluted from pulsed dendritic cells, various chlamydial membrane proteins have been determined to have T cell epitopes including PmpG, TC0420 and PmpE. Some of these proteins induced protection against genital challenge with Cm [56,72,73]. The Ct-secreted serine protease or *Chlamydia* protease-like activity factor (CPAF) [74,75] is a dimeric complex [76,77] and an immunodominant antigen [78], which has been shown to promote Ct survival in the mouse lower genital tract [79], potentially via targeting host innate effectors [80,81]. Immunization with a recombinant CPAF alone, or in combination with other antigens, induced significant protection against infection and pathology in various mouse strains and guinea pigs [82–85]. The chlamydial major outer membrane protein (MOMP) is the serovar-typing antigen with multiple B and T cell epitopes [86–88] and has long been proposed as a vaccine candidate [89–91]. Its vaccine efficacy became obvious only when native MOMP (nMOMP) purified from chlamydial organisms was tested. nMOMP induced solid immune protective responses against both infection and infertility in mice [92,93]. The protection was dependent on CD4 + T cells while antibodies were also shown to be highly protective [53,94]. Cynomolgus monkeys vaccinated with Ct serovar A nMOMP mounted high serum IgG and IgA antibody titers with robust strain-specific neutralizing activity against the homologous serovar [95]. The PBMC of immunized monkeys produced a broadly cross-reactive, antigen-specific IFN-γ response.

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Table 1
Candidate chlamydia vaccines and vaccine concepts.

Vaccine candidate or concept	Description	Challenge model	Results	Development status	Ref.
Attenuating chromosomal and plasmid mutations in *Cm*	*Cm* strains deficient in either plasmid gene product Pgp3 or chromosomal gene TC0668	Vaginal infection of mice with virulent *Cm*	Vaccine strains showed attenuation Protective immunity against oviduct pathology upon challenge	Preclinical	[19,66]
Plasmid-deficient *Cm*	*Cm* cured of virulence plasmid	Vaginal infection of mice with virulent *Cm*	Vaccine strains showed attenuation Protective immunity against oviduct pathology upon challenge	Preclinical	[67]
UV-inactivated whole cells	UV-inactivated *Ct* or *Cm* elementary bodies formulated in nanoparticles coated with a toll-like receptor ligand adjuvant delivered transcervically or intranasally	Transcervical or vaginal infection of mice with virulent Ct or Cm	Protective immunity was induced based on reduced chlamydia load and pathology in vaccinated animals	Preclinical	[70]
Membrane protein-based vaccine	T cell epitope-containing polymorphic membrane proteins (Pmp) PmpG, TC0420 and PmpE Combination vaccine containing PmpEFGH + MOMP	Vaginal infection of mice with virulent *Cm* and transcervical infection of mice with *Ct*	Individual antigens were protective Robust protection with combination vaccine in multiple strains of mice	Preclinical	[73,103]
Soluble protein-based vaccine	Recombinant *Chlamydia* protease-like activity factor (CPAF) alone, or in combination with other antigens	Vaginal infection of mice with virulent *Cm* and guinea pigs with virulent *C. caviae*	Significant protection against challenge infection and pathology	Preclinical	[82–85]
The native major outer membrane protein (nMOMP) as a vaccine	Native major outer membrane protein (nMOMP) from *Cm* or *Ct*	Vaginal infection of mice with virulent *Cm* Ocular infection of cynomolgus monkeys with virulent *Ct*	Solid protection against both infection and infertility in mice by *Cm* nMOMP Monkeys immunized with Ct nMOMP exhibited significant decrease in infectious burden but no protection against ocular disease	Preclinical	[92–95] [53]
A bacterial ghost as a vehicle for delivering chlamydial subunit vaccine	*Vibrio cholera* ghosts as a vaccine delivery platform for antigens including PmpD, MOMP, and PorB from *Ct*	Vaginal infection of mice with virulent *Ct*	Immunized mice showed reduced IFU recovered from vaginal swabs and normal fertility rates	Preclinical	[100,101]
MOMP peptide-based vaccine	Recombinant vaccine formulated using multiple repeats of the VD4 region of *Ct* MOMP, or VD4 that included contiguous regions of the VD4 and VD3 regions, which contain B and T-cell epitopes	Vaginal infection of mice with virulent *Ct*	Immunized mice showed a decrease in the number of IFUs recovered from vaginal swabs and decreased upper genital pathology	Clinical (Phase I)	[96,97]

Following an ocular challenge, immunized monkeys exhibited a significant decrease in infectious burden but no protection against ocular disease. Mice and mini-pigs immunized with a recombinant vaccine formulated using multiple repeats of MOMP variable domain 4 (VD4), or VD4 that included contiguous regions of constant domain-4 and -5, were protected against both infection and pathology following intravaginal challenge [96,97]. This vaccine is currently in Phase I human testing.

It is clear that both membrane-anchored and secreted proteins can induce protection. There are also many other chlamydial antigens that have been evaluated including the chlamydial glycogen phosphorylase [98], macrophage infectivity potentiator [69], and Pgp3 [99]. The logical next steps are to evaluate these antigens in parallel and to formulate the most effective combinations for comparing with whole cell-based vaccines in various models. Parallel efforts are also required for further improving the efficacy of promising antigens, including developing delivery vehicles and maintaining/creating structural determinants required for inducing protective immunity. Recombinant *Vibrio cholera* ghosts have been evaluated as a delivery platform for antigens from Ct or Cm [100,101] and mice immunized with these constructs showed protection against intravaginal challenge. Protection against infection by different serotypes of HIV has been achieved by immunization with optimized and stabilized antigen structural determinants targeted by broad neutralization antibodies [102]. The challenge is whether chlamydial vaccinologists can borrow similar approaches for inducing protection against infection and pathology by all relevant Ct serovars.

6. Challenges and recommendations

The NIAID Ct vaccine workshop allowed the Ct research community, public health professionals, and government agency staff to work collaboratively to define the current status of Ct vaccine research and identify gaps and challenges to catalyze further development. The workshop established that Ct vaccine development is needed and has a firm foundation in basic research, and a robust set of vaccine candidates already exists, with at least one entering clinical development. However, several challenges remain, and efforts to address these challenges could accelerate progress toward a successful Ct vaccine:

- Although preliminary modelling suggests even a partially protective Ct vaccine may be cost-effective, more data are needed regarding progression of Ct infection to upper genital tract sequelae and burden of Ct-associated disease, especially in lower- and middle-income countries, to better define the potential worldwide impact of a Ct vaccine.
- Clinical testing of a Ct vaccine is feasible; however, choice of clinical trial endpoints warrants further investigation and discussion. Blood biomarkers and other novel approaches for identifying upper genital tract infection and inflammation in women would be useful for defining endpoints for vaccine efficacy studies as well as disease burden.
- Although the immunological basis for protection from Ct infection and disease has been well studied, key issues such as the role of antibody still need to be clarified. A relative consensus

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was reached that putative Ct vaccines should generate Ct-specific CD4 + T cells targeting genital epithelial cells, combined with a strong antibody response.
- Further analysis is needed on the utility of several mouse models available to test candidate vaccines. Harmonizing these models such that candidate vaccines can be compared across labs with respect to important clinical endpoints or product indications would be valuable.
- Although intramuscular immunization has worked effectively for preventing cervical HPV infection, it is unclear whether a Ct vaccine can be similarly administered to achieve protection given the need for robust local T cell immunity. An effective *Chlamydia* vaccine may need to induce strong transmucosal immunity with resident memory T cells in the genital tract.

It is hoped that these proceedings will be used as a guide for future high-quality and thematically integrated research projects on Ct vaccine design and testing.

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