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Status of vaccine research and development of vaccines for *Chlamydia trachomatis* infection

Taylor B. Poston [a], Sami L. Gottlieb [b], Toni Darville [a,*]

[a] Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[b] Department of Reproductive Health and Research, World Health Organization, Geneva, Switzerland

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ABSTRACT

Genital infection with *Chlamydia trachomatis*, a gram-negative obligate intracellular bacterium, is the most common bacterial sexually transmitted infection globally. Ascension of chlamydial infection to the female upper genital tract can cause acute pelvic inflammatory disease, tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Shortcomings of current chlamydia control strategies, especially for low- and middle-income countries, highlight the need for an effective vaccine. Evidence from animal models, human epidemiological studies, and early trachoma vaccine trials suggest that a *C. trachomatis* vaccine is feasible. Vaccine development for genital chlamydial infection has been in the preclinical phase of testing for many years, but the first Phase I trials of chlamydial vaccine candidates are underway, and scientific advances hold promise for additional candidates to enter clinical evaluation in the coming years. We describe the clinical and public health need for a *C. trachomatis* vaccine, provide an overview of *Chlamydia* vaccine development efforts, and summarize current vaccine candidates in the development pipeline.

1. About the disease and pathogen

Chlamydia trachomatis, a gram-negative obligate intracellular bacterium capable of infecting genital tract, ocular, and lung epithelium, is the most common bacterial sexually transmitted infection (STI) globally. Sexually transmitted genital infection and associated disease is caused by *C. trachomatis* serovars D-K. Other serovars cause distinct disease syndromes such as ocular trachoma (serovars A, B, Ba, and C) and lymphogranuloma venereum (serovars L1–L3). The replicative cycle of *C. trachomatis* is made up of two distinct phases. The elementary body (EB) form is responsible for attachment and penetration of the target cell, changing to the metabolically active reticulate body (RB) form, which replicates in a protective intracellular inclusion. After hundreds of progeny are generated, the RBs transform back to infectious EBs and are released from the host cell to be transmitted to neighboring host cells or to contacts. Replication within an intracellular inclusion aids the pathogen's ability to avoid the host immune response and promotes chronic infection.

C. trachomatis is transmitted sexually via vaginal, anal, or oral sex to cause genital, anal, or less commonly, oropharyngeal infection. Infection can also be spread perinatally from an untreated mother to her infant to cause neonatal conjunctivitis or pneumonia. Lower genital tract infection is often asymptomatic, but can manifest as urethritis in males and as urethritis or cervicitis in females. The most serious sequelae of infection result from ascension to the upper genital tract in women to cause pelvic inflammatory disease (PID), an infection and inflammation of the uterus, fallopian tubes, ovaries and/or pelvic peritoneum. The inflammation and scarring of PID in the fallopian tubes can lead to long-term sequelae including tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Based on prospective studies, about 10–15% of untreated chlamydia infections lead to clinically diagnosed PID, and about 10–15% of clinical PID cases lead to tubal factor infertility [1–3]. Genital infection with *C. trachomatis* may also increase the risk of acquiring HIV infection by 2–3 fold [4,5].

Globally, an estimated 131 million new cases of chlamydial genital infection occur annually [6]. Incidence rates are high across all world regions, but the infection disproportionately affects adolescents and young adults under 25 years of age [7]. The global burden of chlamydia-associated PID, infertility, and ectopic pregnancy has not been well defined. However, about 68 million chlamydia infections are estimated to occur among women globally each year [6]. Given what is known about the natural history of infection, the number of cases of infertility and other adverse outcomes is likely sizable. If all of these infections were left

* Corresponding author at: University of North Carolina at Chapel Hill, 8340B MBRB, CB# 7509, 111 Mason Farm Road, Chapel Hill, NC 27599-7509, USA.
E-mail address: toni.darville@unc.edu (T. Darville).

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untreated, they could result in close to 1 million new cases of infertility annually. The Global Burden of Disease study (GBD) 2013 estimates that chlamydia results in 647,000 years lived with disability (YLDs) annually [8]. The global economic burden of genital chlamydial infection has not been assessed, but annual healthcare costs in the United States are estimated at $517 million [9].

Diagnosis of chlamydia relies on nucleic acid amplification tests (NAATs) of specimens obtained by vaginal or cervical swabs in women or urine collection in men and women. A course of doxycycline or single-dose azithromycin offers effective curative treatment. Because the vast majority of chlamydial infections are asymptomatic but can still lead to adverse sequelae and ongoing transmission, several high-income countries (HICs) have relied on screening programs to diagnose and treat chlamydia to prevent PID [10–14]. In low- and middle-income countries (LMICs), lack of affordable, feasible laboratory tests means most genital chlamydia infections are not diagnosed. However, even in HIC settings with long-standing chlamydia screening recommendations, these programs have been costly and difficult to bring to scale [15,16]. In addition, although screening has likely reduced the incidence of PID, it has not resulted in clear reductions in chlamydia transmission [17,18]. One of the main reasons for ongoing transmission is that management of sexual partners of index cases is logistically difficult and repeat infection rates are high: approximately 10–20% in the months after treatment [19]. It has been hypothesized that screening programs might increase the frequency of re-infection through reductions in population-wide protective immunity, or arrested immunity [20]. Barrier methods of contraception, including condom use, are effective at preventing chlamydial transmission, however utilization rates are low [21]. Shortcomings of current chlamydia control strategies highlight the need for an effective vaccine.

2. Overview of current efforts

2.1. Biological feasibility for vaccine development

Currently no licensed vaccine exists for *Chlamydia trachomatis*, but evidence from animal models and human studies suggests that a vaccine is feasible. Animal challenge studies, including mouse, guinea pig and non-human primate models, demonstrate that partial and sterilizing natural immunity can develop from a primary infection, however this protection is short-lived and not sufficient to provide long-term immunity [22]. In animals, partial immunity can reduce bacterial burden and duration of secondary infection but does not necessarily prevent upper genital tract pathology. In humans, epidemiologic studies reveal a decreased prevalence of infection and decreased bacterial load with increasing age despite continued exposure [23]. In addition, infection concordance between sex partners decreases with increasing age of the partners, and bacterial loads are lower among individuals with a history of infection [24]. Furthermore, in a prospective study of 200 women in the US, those whose chlamydial infections cleared spontaneously between testing and treatment were less likely to become re-infected on follow-up [25]. The ability of natural infection to induce partial immunity is promising for vaccine development.

The first *C. trachomatis* vaccines, evaluated in the 1960s, were live or formalin-fixed whole bacteria that focused on ocular infection causing trachoma, rather than genital infection [26–28]. Multiple studies demonstrated some protection from active (inflammatory) trachoma in vaccinated individuals [29–31]. However, these benefits were short-lived, often waning within one to two years [32]. Non-human primate studies of these same vaccines showed effective but short-lived protection as in human trials when high doses of organisms were used. However, when low doses were used, more severe disease was observed upon challenge with heterologous serovars [33]. Concern for exacerbated disease upon challenge of immunized hosts also arose because of the way data were initially interpreted from live trachoma vaccine studies among Gambian children (27). At the time, trachoma severity scores were reduced when conjunctival scarring was present, as scarring was considered a sign of healing, despite being the undesired sequelae of inflammation. The prevalence of scarring was lower two years post vaccination, suggesting the vaccine reduced longer-term disease sequelae, but the scoring system led to an erroneous conclusion that vaccinated children had enhanced inflammatory disease relative to unvaccinated children. Experts reinterpreting these trials in the context of current trachoma grading systems and knowledge about disease pathogenesis concluded that concerns about vaccine-induced exacerbation of disease in Gambian children were unfounded [34–36]. In addition, ocular inoculation of non-human primates with a live-attenuated trachoma serovar did not worsen disease upon challenge [37].

Overall, the short-term protection observed in human trachoma vaccine trials implies that an effective vaccine for *C. trachomatis* is feasible. Initial concerns about an enhanced pathologic response pushed the field towards development of subunit vaccines to enhance safety. This goal remains because a subunit vaccine would contain only essential antigens for protection and not all the other molecules that make up the chlamydial microbe, reducing the chances of adverse reactions. Induction of complete immunity to infection is the ideal goal, and will require augmentation of protective immune mechanisms at the mucosal site. Recent data indicate mucosal delivery of a chlamydia vaccine may be required to induce resident memory T cells that act as sentinels to protect the mucosa [38]. Advances in understanding the immunobiology of *C. trachomatis* infection over the past several decades have markedly increased the likelihood of developing a safe and effective vaccine.

2.2. General approaches to vaccine development for low and middle-income country markets

A genital chlamydia vaccine would ideally target adolescents before sexual debut to maximize immunity during the period of highest transmission risk. As most of the adverse sequelae of chlamydial infection occur among females, an argument could be made for vaccinating adolescent girls only, as has been done with chlamydia screening and for HPV vaccination in many settings [39,40]. However, mathematical modelling and cost considerations can inform whether a vaccine should target both adolescent males and females to optimize reductions in population transmission. The vaccine should ideally be combined with other adolescent vaccines to improve uptake and marketability. The market profile for a *Chlamydia* vaccine might emulate currently licensed HPV vaccines. Complete immunity to infection is the best-case scenario for *Chlamydia* vaccine development, but may be difficult to achieve. However, even a partially protective vaccine that inhibits upper genital tract infection and damage or reduces ongoing transmission could have significant impact and provide individual-level or population-level benefits [41]. Mathematical modelling demonstrates that a partially protective vaccine added to current screening and treatment efforts could be cost-effective compared to screening and treatment alone [9].

An effective chlamydial vaccine would have public health benefits in both HICs and LMICs. However, a chlamydial vaccine would probably provide the greatest benefits in LMIC settings, where lack of medical infrastructure and resources preclude chlamydia screening programs and the burden of chlamydia-associated sequelae is likely greatest. In LMICs, up to 186 million couples report being unable to have a child over 5 years [42]. Although

infertility is a global problem, the proportion that is tubal factor, and thus primarily caused by scarring from genital infection such as chlamydia, varies widely by population. In the United States, the proportion of infertility that is tubal factor ranges from 10% to 40% [43,44]. However, in sub-Saharan Africa, tubal infertility is the dominant cause for women, present in up to 65–85% of infertility cases [45,46]. In addition, the consequences of chlamydial sequelae such as ectopic pregnancy can be life-threatening in resource-poor settings. In African developing countries, ectopic pregnancy has case fatality rates that are 10 times higher than those reported in high-income countries [47]. Additional, updated, and more precise data on the attributable fraction of chlamydia to PID and longer-term sequelae in LMICs will be essential for better defining the potential impact of a chlamydial vaccine in these settings. Given the link between chlamydial infection and acquisition of HIV infection, a chlamydia vaccine could also have added benefits in areas of high HIV prevalence [5]. In addition, what is learned from chlamydial vaccine studies targeted to prevention of genital infection can be used to inform vaccine development for prevention of trachoma, which would expand the benefits for LMIC settings.

3. Technical and regulatory assessment

The critical role of T cells in chlamydial immunity was first demonstrated 30 years ago [48]. CD4 T cell IFN-γ production likely confers protection against *C. trachomatis*. Control of *in vivo* infection is not fully understood, since IFN-γ induces the expression of over 200 different genes in target cells [49]. However, *in vitro* studies indicate it controls chlamydial growth in part by inducing indoleamine-2, 3-dioxygenase (IDO) production [50]. IDO prompts tryptophan degradation and ultimately microbial starvation. IFN-γ producing Th1 cells are essential and sufficient for resolution of infection, but a polyfunctional response including IL-2 and TNF-α may be optimal for clearance [51,52]. Tissue-resident memory T (TRM) cells have emerged as an important T cell subset in mucosal immunity. TRM are long-lived non-circulating memory cells able to respond to infection independent of systemic T cells. After vaginal HSV-2 infection, CD4 TRM cells are maintained in the vaginal mucosa by a chemokine network facilitated by local macrophages [53]. Mucosal Th1 cells could be instrumental to vaccine success, as the intensity of mucosal CD4 T cell responses is a correlate of protective immunity [54]. Antibodies boost chlamydial protection, but the mechanism remains unclear and may be multifaceted, including enhancement of Th1 effector responses [55]. Only recently has there been convincing data on the effect of neutralizing antibodies. Immunization with an extended major outer membrane protein (MOMP) VD4 region containing the conserved LNPTIAG region elicited neutralizing antibodies in mice [56]. This protection was attributed to chlamydial neutralization and CD4-T cell mediated immunity. Studies demonstrating protective adaptive immune responses to *Chlamydia* have recently been reviewed [57].

Preclinical vaccine development utilizes well-established animal models for candidate testing. Mouse models offer convenient manipulation and research tools for analysis of the immune response, but differ from humans with respect to many facets of infection, disease, and adaptive immune responses. *Chlamydia muridarum* is a mouse-specific strain that shares extensive homology with *C. trachomatis*. However, *C. muridarum* induces a more acute infection with complete resolution compared to the often quiescent, chronic infection of *C. trachomatis* in humans. Further, mechanisms of IFN-γ mediated chlamydial clearance differ in mice and humans. The guinea pig model utilizing *Chlamydia caviae* elicits disease more similar to humans, but the relative lack of immunological reagents detracts from its use for vaccine studies [22]. Female minipigs that have a reproductive cycle and genital tract similar to humans are being used for chlamydial vaccine studies but also suffer from reduced availability of reagents [58,59]. Non-human primate (NHP) models are often employed prior to human testing, but infection of the eye or genital tract in NHPs demonstrates a shorter, self-limiting infection compared to humans. Despite this limitation, NHP testing could play an important role in assessing cellular and humoral responses after infection or vaccination to identify correlates of protective immunity. Animal and human studies could provide insight into a protective transcriptional blood signature that might be translated to a biomarker of efficacy for use in human clinical trials [60].

The ultimate goal of a chlamydia vaccine is to reduce the burden of upper genital tract sequelae in women. However, the use of disease as a clinical endpoint in vaccine trials is influenced by several considerations, including the natural history and timing of clinical events such as infertility following infection, the measurement of PID, the proportion of PID associated with *C. trachomatis*, and factors related to trial design. The clinical diagnosis of PID is notably insensitive and nonspecific, and the previous gold standard laparoscopic diagnosis is invasive, not widely available, and no longer routinely performed. In addition, PID is a clinical syndrome that has multiple causes. Typically, *C. trachomatis* is involved in about one third of cases; however, attribution of PID to a particular pathogen may be difficult. More precise, feasible, non-invasive measures of chlamydia-specific upper genital tract inflammation and damage are a critical priority for the design of practical and informative clinical studies. Additional studies are required to define the role of radiologic techniques such as MRI and power Doppler in PID diagnosis [61]. In women, endometrial biopsies via minimally invasive sterile endometrial sampler have been increasingly used to yield data on ascension of infection and presence of upper tract inflammation [62,63]. Current efforts are focused on identification of a blood biomarker for less invasive sampling [64]. Cervical bacterial burden may also be an appropriate surrogate for upper tract ascension [65].

Use of *C. trachomatis* infection as a clinical endpoint is relatively straightforward and would involve interval *C. trachomatis* NAAT testing via urine samples for men and vaginal or cervical swabs for women in placebo-treated versus vaccinated subjects. Reflex quantitative NAAT could be used to evaluate bacterial load. However, given that complete immunity may be hard to achieve, it will be important to build consensus around the most appropriate primary and secondary vaccine trial endpoints as *C. trachomatis* vaccine development moves forward. Including disease endpoints will be most valuable if only partial immunity is achieved, since a vaccine might still limit ascension and protect from PID. Choice of endpoints also has implications for trial design, such as sample size considerations and whether frequency of follow-up testing and treatment affects assessment of PID outcomes. Discussion will be aided by better measures of upper genital tract infection and damage, predictors of ascension, and a package of evidence to confirm a vaccine would not increase tubal immunopathology on breakthrough infection.

4. Status of vaccine R&D activities

Vaccine development for *C. trachomatis* has been in the preclinical phase of testing for many years, but the first Phase I trials of chlamydial vaccine candidates are underway, and scientific advances hold promise for additional candidates to enter clinical evaluation in the coming years (Table 1). Current strategies hinge on a variety of different platforms and are supported by academic, government, and corporate institutions.

A major focus is development of vaccines prepared with *C. trachomatis* MOMP. MOMP vaccination utilizing cationic liposomes (CAF01) induced robust antibody responses, type-1 immunity,

Table 1
Development status of current vaccine candidates

Candidate name/identifier	Preclinical	Phase I	Phase II	Phase III	References
MOMP-VD4 neutralizing antibodies [Statens Serum Institut]		X			[56,66]
Intranasal MOMP nanoemulsion [NanoBio Corporation]	X				Unpublished study by Beagley et al., reported online at [67]
MOMP + Pmps [Pan-Provincial Vaccine Enterprise Inc. (PREVENT) and British Columbia CDC]	X				[68]
cSAP TLR7 agonist with UV-killed *Chlamydia* [Selecta Biosciences]	X				[38]
Vaxonella platform (*Salmonella* vector) [Prokarium]	X				[72]
Live attenuated (plasmid-deficient) trachoma vaccine [NIH/NIAID]	X	Planned for 2017			[37]

and partial protection from infection in minipigs, and significant protection from upper tract disease in mice [56,66]. A second MOMP formulation prepared with a novel oil-in-water nanoemulsion (Nanostat™) and delivered intranasally purportedly decreased oviduct pathology in mice by 80 percent [67]. Protection was associated with high levels of serum and vaginal antibodies and robust IL-17/IFN-γ responses. An immunoproteomics approach identified *Chlamydia* polymorphic membrane proteins (PMPs) preferentially loading MHC Class II, and vaccination with three MOMP and four PMP alleles emulsified with DDA/MPL adjuvant significantly reduced bacterial shedding in a transcervical *C. trachomatis* mouse model [68]. Current investigation is centered on development of an outer membrane protein based vaccine for Phase I testing.

The ability to generate vaccine-induced resident memory T cells in the mouse genital mucosa is a major advancement in the field [38]. Mucosal immunization with ultraviolet light (UV)-inactivated *C. trachomatis* complexed with novel, charge switching synthetic adjuvant particles (cSAPs) incorporating the TLR7-agonist resiquimod conferred significant protection against chlamydial infection in mice. Uterine vaccination induced mucosal resident and systemic T cell responses that induced optimal chlamydial clearance compared to intranasal and intramuscular vaccine delivery.

Another major advancement is the use of high-throughput technology for determination of T cell-specific epitopes. Examination of T-cell IFN-γ responses in a cohort of 141 subjects led to identification of eight CD4 and eighteen CD8 antigens associated with clearance or resistance to infection [69]. Another group assessed 120 *Chlamydia* proteins and identified seven novel antigens that conferred partial protection in mice [70]. Recent analysis demonstrated chlamydial proteins recognized by highly exposed women that limit or resist genital tract infection [65]. These proteins were primarily involved in protein synthesis, central metabolism, and type III secretion. Ongoing research is focused on *in vitro* screening of PBMC responses from previously infected subjects to chlamydial proteins. These efforts will help identify protective antigens broadly expressed by human leukocyte antigen (HLA) haplotypes to better guide an effective vaccine strategy.

A Vaxonella® platform for chlamydia immunization is being investigated for immunogenicity and efficacy in animal models. The oral delivery system utilizes an attenuated *Salmonella enterica* vector that has passed Phase II trials as the Typhella® vaccine and allows for insertion of chlamydial antigenic gene sequences. The bacteria are ingested and transverse M cells in the gut where they mount an immune response within Peyer's patches. *Salmonella* act as an immunostimulator bypassing the necessity of additional adjuvants. The vector is constructed with technology designed to generate stable attenuation and is formulated to exclude toxic bile salts during ingestion for optimal delivery [71,72].

Finally, work related to vaccine development for *C. trachomatis* ocular infection might shed light on vaccine development for the genital tract. Ocular inoculation of NHPs with attenuated, plasmid-deficient *C. trachomatis* ocular serovar A elicited partial protection against a virulent strain in a subset of cynomolgus macaques that appeared to correlate with MHC Class II haplotype [37] and CD8+ T cell responses [73]. This strategy is currently in preclinical development with the National Institute of Allergy and Infectious Diseases (NIAID). Murine genital inoculation with plasmid-deficient *C. muridarum* conferred protection against upper genital tract pathology [74]. These results were not replicated in the NHP model of genital infection; however, pathology was minimal in monkeys inoculated with wild-type *C. trachomatis* oculogenital serovar D [75]. This illustrates the need for delineating protective immune mechanisms and optimal vaccine formulations in ocular versus genital tract infections [76].

5. Likelihood for financing

The likelihood for financing a chlamydial vaccine by multilateral agencies is currently unknown, but vaccine development is likely to depend on its applicability to both HIC and LMIC markets. Gavi support of HPV vaccination provides a model for prioritization of an STI vaccine to prevent adverse reproductive health outcomes, as well as an adolescent platform for vaccine delivery in LMICs. The possibility that chlamydia prevention could lower HIV transmission may have a positive impact for other funding agencies. Developing a strong public health investment case, by obtaining better data on chlamydia-associated PID and outcomes like infertility, ectopic pregnancy, and chronic pelvic pain and their costs in LMICs, will be crucial for encouraging investment in and financing of a future *C. trachomatis* vaccine. Gaining consensus on clinical endpoints and developing more feasible and reliable measures of upper genital tract disease for clinical evaluation will also "de-risk" chlamydial vaccine development for industry and funders [77]. The global roadmap for STI vaccine development, generated jointly by WHO, NIAID, and a wide range of technical partners, outlines critical next steps to address barriers to development and encourage investment in these important vaccines for global sexual and reproductive health [78,79].

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