

COMMENTARY

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## Considerations for the rational design of a *Chlamydia* vaccine

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### ABSTRACT

*Chlamydia trachomatis* is the leading cause of preventable blindness and the most common bacterial sexually transmitted infection. Remarkable progress in vaccine research over the past six decades has led to the advancement of novel *C. trachomatis* vaccine candidates into clinical trials. However, many questions regarding the role of specific cellular populations and molecular mechanisms in protective immunity against human *C. trachomatis* genital tract infections remain unanswered. Biomarkers of vaccine induced protective immunity are elusive in humans, while a cautionary message on the translatability of data obtained from current animal models has emanated from vaccine research and development efforts against other important human pathogens. In this commentary, we highlight recent advances in *Chlamydia* vaccine development and discuss their implications in the context of a rational approach to the design of a human *C. trachomatis* vaccine.

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### Introduction



*Chlamydia trachomatis* infections are the most prevalent sexually transmitted bacterial infection worldwide. The World Health Organization estimates that 130 million new cases *C. trachomatis* infection occur each year.<sup>1</sup> Alarming, up to 90% of women and 50% of men with *C. trachomatis* infections are asymptomatic, and consequently do not have an impetus to seek testing and treatment.<sup>2</sup> Untreated infections in women can lead to a number of complications including pelvic inflammatory disease, tubal factor infertility, and ectopic pregnancy.<sup>3</sup> Furthermore, studies from the 1990s have shown that chlamydial infections can facilitate the transmission and persistence of other STIs, including HIV and human papillomavirus, respectively.<sup>4,5</sup> However, public health programs, including screening for at-risk individuals, partner identification, and antibiotic treatment, have had limited success in controlling the rising incidence of *C. trachomatis* infections over the past 20 years.<sup>6</sup>

In addition to being transmitted sexually, *C. trachomatis* is the world's leading cause of preventable blindness causing 85 million ocular infections per year despite implementation of the World Health Organization's SAFE strategy (Surgery for trichiasis, Antibiotics, Facial cleanliness, and Environmental improvements) for the control of trachoma.<sup>7</sup> For these reasons the development of an effective vaccine that can protect against both genital and ocular serovars is urgently needed. However, despite decades of efforts and trials there is currently no *C. trachomatis* vaccine approved for use in humans. This commentary will focus on the desired attributes of an effective vaccine based upon insights from observational human studies and experimental animal models.

### Immune mechanisms of protection

A clear understanding of the mechanisms of protective immunity to *C. trachomatis* genital tract infection and identification of immune correlates of protection are essential for the rational design of a *Chlamydia* vaccine. However, ethical concerns regarding withholding treatment for known infections and experimental infection in humans have made it challenging to observe the natural course of *C. trachomatis* genital tract infection. Nonetheless, the available evidence from human studies has suggested that some degree of partial immunity is acquired following human *C. trachomatis* infection. A reduced risk of infection has been associated with *Chlamydia*-specific IFN- $\gamma$  and IL-13 producing peripheral blood mononuclear cells,<sup>8</sup> while a reduction in the intensity of shedding was associated with *Chlamydia*-specific IgA in the genital mucosa,<sup>9</sup> suggesting that both cell-mediated and antibody-mediated responses contribute to protective immunity.

Experiments utilizing animal models of *Chlamydia* genital tract infection have corroborated the findings in human studies. Results from the mouse model have consistently demonstrated that CD4 T cells are necessary and sufficient for clearing a primary infection,<sup>10,11</sup> and that poly-functional CD4 T cells that co-secrete a number of Th1-associated cytokines (IFN- $\gamma$  and TNF- $\alpha$ ) are associated with protection against reinfection.<sup>12</sup> However, the role of CD8 T cells in protective immunity is less clear due to conflicting results from different animal models. In the *C. muridarum* mouse model, CD8 T cells are neither necessary nor sufficient for protection against primary or secondary infection, but do contribute to infection-associated immunopathology.<sup>11,13</sup> However, in the non-human primate model, CD8 T cells seem to play an important role in protective immunity, as depletion of CD8 T cells following

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immunization with live-attenuated *C. trachomatis* significantly abrogated the protective effect of the vaccine.<sup>14</sup> This discrepancy between the two models has not yet been reconciled, and the role of CD8 T cells in human *C. trachomatis* genital tract infection has yet to be defined. More efforts are required to understand this discrepancy and to identify the best model to study the role of CD8 T cells in human *C. trachomatis* infections.

B cell and antibody-associated mechanisms of protection have not been completely elucidated. While antibodies alone are not able to protect against a primary *Chlamydia* genital infection, they are sufficient for protection against reinfection.<sup>11</sup> Interestingly, passive transfer of *C. muridarum*-immune serum into B cell deficient, T cell depleted mice provided protection only if the mice had already cleared a primary infection, suggesting the involvement of a yet undefined component of acquired immunity.<sup>15,16</sup> However, in the *C. trachomatis* mouse model, passive transfer of serum from mice immunized with major outer membrane protein (MOMP) epitopes into naïve mice was sufficient to protect against a subsequent *C. trachomatis* challenge.<sup>17</sup> While there are differences between the *C. muridarum* and *C. trachomatis* mouse models, these experiments demonstrate that sufficiently protective antibody responses against *Chlamydia* can be generated. Therefore, an ideal *C. trachomatis* vaccine should mobilize the humoral arm of adaptive immunity.

Remarkable progress has been made in the elucidation of mechanisms of protection against infection. However, there is some pessimism about whether a vaccine that induces sterilizing immunity can be developed, and the general consensus is that the primary goal of a *C. trachomatis* vaccine, at least for the time being, is to prevent infection associated pathology.<sup>18</sup> To this end, however, it is still unclear why some people develop disease but not others. As *Chlamydia* induced pathology is largely driven by aberrant immune responses to the infection rather than the infection itself, individuals who have a defect in immunity might be predisposed to developing pathology. This is important, as vaccines that aim to augment “normal” immune responses might fail to protect people with immune defects, either because they may not respond to the vaccine or because the response does not result in protection. If these are the individuals who we are trying to protect with vaccination, we need to understand why they are susceptible to infection-induced pathology in order to predict how to best protect them.

As *C. trachomatis* vaccine candidates advance into clinical trials, we should have optimism not only in their potential to be effective, but also in our potential to learn from their successes and failures. Preclinical vaccine research on other human pathogens such as herpes simplex virus (HSV) and *Mycobacterium tuberculosis* (Mtb) have generated many promising vaccine candidates that have failed to translate their efficacy into humans.<sup>19,20</sup> Lessons learned from these trials are guiding research on newer vaccines against these pathogens. Since many parallels can be drawn between the immunobiology of HSV and Mtb infections and that of *Chlamydia* infections (e.g. all three pathogens cause mucosal infections to which Th1 polarized CD4 T cells responses are essential for protection),<sup>11,19,20</sup> perhaps the *Chlamydia* field can gain insight from the successes and failures of these trials as well. For example,

how confident should we be in relying on the generation of CD4 T cells that secrete IFN- $\gamma$  for complete immunity to *Chlamydia*? Recently, a promising Mtb vaccine that generated the “correct” immune responses had failed to show any efficacy in humans.<sup>21</sup> It is unclear why the vaccine induced immune responses, which are important for protection in animal models, did not translate to protection in humans. One explanation is that the specific markers measured in the study are not reliable predictors of vaccine protection in humans. Therefore, while we should be confident in our ability to translate the findings from preclinical *Chlamydia* vaccine research into humans, we should not become complacent. It may be wise to take preemptive action to avoid costly failures at the clinical trial stage. More widespread use of the underutilized but more relevant pig and non-human primate models may help us identify useful biomarkers or immune parameters of vaccine protection that can be translated into humans. By understanding why some individuals are more susceptible to infection-induced pathology than others, we could begin to tailor the vaccine to people who need them the most.

### Immunization strategies

Evidence from studies on immunological mechanisms of protection provides insight on the type of immune response that an ideal *C. trachomatis* vaccine would need to generate, which is determined by the route of immunization and the adjuvant/delivery system used. Since *Chlamydia* is a mucosal pathogen, vaccine research has been focused on the development of immunization strategies that can generate effective mucosal responses. The available evidence suggests that an ideal mucosal *C. trachomatis* vaccine would likely be administered intranasally or sublingually, as immunization at these two routes have been shown to induce robust mucosal immunity at the genital tract.<sup>22</sup> Stary et al. demonstrated that establishment of tissue resident memory T cells (Trm), memory T cells that permanently reside in tissues, was necessary for optimal protection against genital *C. trachomatis* challenge.<sup>23</sup> Importantly, Trms only seeded the genital tract if the vaccine, which consisted of UV-inactivated elementary bodies (EBs) combined with charge switching adjuvant particles (cSAP), was administered mucosally (intranasal or intrauterine) but not parenterally (subcutaneous). While the concept of Trms is relatively new in the *Chlamydia* field and more research is needed to define their role in protective immunity, Trms highlight the potential of mucosal immunization and may help to explain many discordant results within and between animal models and human studies. Readers are redirected to an excellent review and commentary by Brunham et al. for an insightful discussion of Trms in the context of *Chlamydia* immunobiology and vaccine development.<sup>24</sup>

While mucosal immunization may represent the most direct way to establish immunity at the genital tract, the inherent difficulties associated with mucosal immunization (e.g., epithelial barrier and antigen degradation) have hindered the development of mucosal adjuvants, and alternative immunization strategies have been explored. Yu and colleagues immunized mice subcutaneously with different adjuvants and found that cationic adjuvant formulation 1 (CAF01), which is not a mucosal adjuvant, produced one of the best protective immune

responses that reduced bacterial load by 99%.<sup>12</sup> While mucosal immune responses were not assessed in that study, another group has shown that an intramuscular prime and intranasal boost (using CAF01 for the prime only) was sufficient to induce protective mucosal immunity in minipigs.<sup>25</sup> This is an interesting finding that may have direct implications for immunization strategies in humans – that sufficient mucosal immunity may be achieved without the need for a mucosal adjuvant. Therefore, a *C. trachomatis* vaccine may utilize one of many strategies to direct immune responses to the genital tract.

Overall, animal models have been invaluable for *Chlamydia* vaccine research. The challenge now is to extrapolate the findings from animal models into humans, as the identification of correlates of protective immunity will greatly facilitate the advancement of potential *C. trachomatis* vaccine candidates in human trials. To this end, investigators should consider the possibility that multiple, independent mechanisms of protection and correlates of protective immunity may exist, which may also depend on the choice of antigen. For example, antibodies are largely dispensable in chlamydial protease like activity factor (CPAF) induced protective immunity while antibodies toward conserved regions of MOMP are sufficient for MOMP-mediated protection.<sup>17,26</sup> Therefore, adjuvants should be tailored to the choice of antigen, and measuring multiple correlates of protective immunity in humans could help identify effective antigen and adjuvant combinations that would otherwise be overlooked if only one correlate is used.

### Choice of antigen

Remarkable progress in genetics, immunology, and cell biology have led to the discovery of a large number of potential vaccine antigens for *Chlamydia*.<sup>27,28</sup> A majority of these antigens are surface-associated and thus amenable to antibody effector functions such as direct bactericidal activity, enhancement of opsonization, and neutralization. However, as *Chlamydia* is able to circumvent humoral immunity by residing and replicating inside the host cell, an ideal *Chlamydia* vaccine should include both B cell and T cell antigens.

Many vaccine antigens studied thus far contain, at least in part, surface exposed proteins. Antibody responses to these antigens produce wide ranging levels of neutralization, from no neutralization for CT043, to significant, cross-serovar protection for antigens like BD584 or heterologous immune-repeat 1 (Hirep1).<sup>17,29,30</sup> *C. trachomatis* can be divided into three biovars based on the tissue tropism, and 19 serovars based on the differential immune response to MOMP. Researchers should first ask themselves what are we going to try and protect against, one serovar? one biovar? or all serovars? Knowing what the vaccine is intended to protect will aid in choosing an antigen. For example, MOMP has been the most widely studied antigen in *Chlamydia*. However, its use has been limited by the inability to induce cross-serovar protection and the difficulty in producing conformationally correct MOMP from recombinant sources. Recently, Hirep1, which is a subunit vaccine consisting of the variable domain 4 (VD4) regions of MOMP from multiple serovars, addresses the concerns seen with native and

recombinant MOMP and exploits the presence of a broadly neutralizing epitope found in the VD4 domain.<sup>17</sup>

Recombinant antigens are likely to represent the best source for producing a *Chlamydia* vaccine because of their ease of production and purification as compared to native sources. To this end, choosing an antigen which is conserved and expressed similarly across serovars would offer the greatest chance of inducing cross-serovar protection. While the use of whole *Chlamydia*, live-attenuated or inactivated, was the method of choice for early vaccination studies in the 1960s-1970s, the potential for worsening secondary infections has led researchers away from using these methods of vaccination. However, newer adjuvants and plasmid-less strains of *Chlamydia* have recently reinvigorated the search for a new *Chlamydia* vaccine that utilizes inactivated or live-attenuated *Chlamydia*, respectively.

One question that may be worth asking is should a *Chlamydia* vaccine include antigens that are upregulated during the persistent stage of its developmental cycle. Under certain external stresses, such as immune pressure, *Chlamydia* can convert into what is known as a persistent form. In this non-infectious, non-replicating, but viable state, *Chlamydia* establishes a chronic infection to which the immune system responds as low grade inflammation that can eventually lead to scarring.<sup>31</sup> In this state, *Chlamydia* adopts a different transcriptome.<sup>32</sup> Many genes that encode for immunodominant antigens such as MOMP and Pmps are downregulated - presumably to hide from immune surveillance. It is unknown whether this differential transcriptome leads to the presentation of a different set of T cell antigens, and whether this temporal change in immunoproteome alters the effectiveness of immune responses primed with classical EB or reticulate body (RB) antigens. Potentially important parallels can be drawn from vaccine research against other intracellular pathogens such as Mtb. Following an initial acute infection, Mtb transforms into a non-replicating, persistent state with low metabolic activity within macrophages. In this latent stage, Mtb has a different transcription profile where genes encoding the Ag85 family (immunodominant antigens widely used as vaccine antigens) are downregulated to low levels.<sup>33</sup> Interestingly, multi-stage Mtb vaccines consisting of both early and latency-associated antigens are demonstrably better than vaccines formulated with early stage antigens alone in multiple mouse models of latent tuberculosis.<sup>34,35</sup> Whether *Chlamydia* also has a different antigen repertoire during persistence remains to be investigated, and, given the pathological implications of persistent infections, the merit of a multi-stage *Chlamydia* vaccine consisting of EB, RB, and persistence-associated antigens needs to be discussed within the field.

### Summary

Six decades of vaccine research encompassing advances in genetics, mucosal immunity, and adjuvant development have led to the evaluation of a large number of potential antigens and adjuvants for a human *C. trachomatis* vaccine. The mouse model has been, and will continue to be, essential for preclinical vaccine development and should be sufficient to move *C. trachomatis* vaccines into clinical trials. Future efforts, however,

will be required for the identification of specific immune mechanisms of protection and dependable markers of protective immunity in humans, and this could be achieved with wider use of the pig and non-human primate models which more closely resemble the human. The field is cautiously optimistic as the first wave of *C. trachomatis* vaccines enter clinical trials.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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