Development status and future prospects for a vaccine against *Chlamydia trachomatis* infection

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Abbreviations

EB – Elementary body

INF-g – Interferon gamma

NHP – Non-human primate

MOMP – Major outer membrane protein

RB – Reticulate body

Abstract

Chlamydia trachomatis continues to be the most commonly reported sexually transmitted bacterial infection in many countries with more than 100 million new cases estimated annually. These acute infections translate into significant downstream health care costs, particularly for women, where complications can include pelvic inflammatory disease and other disease sequelae such as tubal factor infertility. Despite years of research, the immunological mechanisms responsible for protective immunity versus immunopathology are still not well understood, although it is widely accepted that T cell driven IFN-g and Th17 responses are critical for clearing infection. While antibodies are able to neutralize infections *in vitro*, alone they are not protective, indicating that any successful vaccine will need to elicit both arms of the immune response. In recent years, there has been an expansion in the number and types of antigens that have been evaluated as vaccines, and combined with the new array of mucosal adjuvants, this aspect of chlamydial vaccinology is showing promise. Most recently, the opportunities to develop successful vaccines have been given a significant boost with the development of a genetic transformation system for *Chlamydia*, as well as the identification of the key role of the chlamydial plasmid in virulence. While still remaining a major challenge, the development of a successful *C.trachomatis* vaccine is starting to look more likely.

Chlamydial infection and disease

Tubal factor infertility (TFI) is a globally significant public health problem caused by several microbial agents, including untreated genital infections with *Chlamydia trachomatis* [1]. *C.trachomatis* remains the most commonly reported infectious disease in many countries. It is estimated that in 2008, there were 106 million new cases of *C.trachomatis* in adults (15 – 49 years) with an estimated 100 million people infected at any one time [2]. These acute infections translate into significant downstream health costs with an estimated 714,000 disability-adjusted life years (DALYs) lost as a result of *C.trachomatis* infections [3]. In the United States alone, direct medical costs for chlamydial infections exceed US$500 million annually, excluding costs for screening programs and indirect costs like lost productivity [4].

The largest burden of disease from *C.trachomatis* is in women where untreated genital infections can lead to pelvic inflammatory disease (PID) and, in some cases, sequelae including TFI (18% cases following symptomatic PID) resulting from fallopian tube scarring [1,5]. Infections during pregnancy may cause premature labor and may also cause neonates to develop conjunctivitis or pneumonia [6]. The high prevalence of infections among women of child-bearing age exposes an estimated 100,000 neonates to *Chlamydia* annually in the United States [7]. In men, *C.trachomatis* is the most commonly reported sexually transmitted infection (STI) and the leading cause of non-gonococcal (non-specific) urethritis [8, 9]. Following upper genital tract ascension, *C.trachomatis* may cause acute infectious epididymitis [10]; *C.trachomatis* infections have been reported in 40-85% men with epididymitis [11]. However, up to 90% of chlamydial infections in females and 50% in males are asymptomatic. This indicates that the incidence of reported chlamydial infections from surveillance data is likely a gross global under-estimate and that screening of asymptomatics would detect even more infections [12-14].

The need for chlamydial vaccines

Potential interventions for reducing the incidence of infection and disease sequelae associated with *Chlamydia* include; (i) educational-based behaviour change promotion (e.g. increasing condom use or reducing partner numbers); (ii) increased

screening, treatment and contact tracing / partner notification; (iii) the development of new biomedical prevention or therapeutic technologies (such as vaccines) (see review by Gottlieb et al. in this issue) [15]. However, it is not feasible to implement behaviour change campaigns to a sufficient scale and efficacy to result in population-level impacts.

Since a *Chlamydia* vaccine is not currently available, the only viable public health strategy is the scale-up of screening for chlamydial infection coupled with the administration of a course of antibiotics and counselling or follow up for partner notification or contact tracing and also rescreening. *Chlamydia* screening may be cost-effective and partner notification is an effective adjunct, with treatment using azithromycin evaluated to be cost-effective [16]. Screening is generally considered to be acceptable and feasible among most target populations [17,18]. However, uptake is likely to be the limiting factor, even in ideal study conditions with specific invitations for screening, with less than 45% of populations at risk of *Chlamydia* being routinely screened [18-22]. Modelling studies have indicated that at least 45-60% screening levels are required to have noticeable epidemiological impacts [22-25] and these coverage levels, or greater, must be sustained at least annually, indefinitely. It is unlikely that the coverage and frequency of screening and treatment interventions could reach sufficiently high levels to result in epidemic declines approaching elimination. Not only are there issues of limited coverage and frequency which reduces effectiveness, but treatment efficacy is not perfect [26-28], drug resistance is possible, re-infection is extremely common,[29,30] and there is no end to the need to continue regular rescreening.

In addition, despite continued improvements in diagnostic and screening procedures for *Chlamydia*, and although antibiotics like azithromycin are available to treat infections, notifications of infections continues to increase. Antibiotic treatment of individuals may also increase susceptibility to re-infection, which is most likely due to interrupting the natural course of protective chlamydial immunity [31]. Recently, data from an *in vivo* study reported that not only were T-helper (Th)1 immune responses against *C.trachomatis* in individual women slow to develop, but that these responses were also altered by treatment with ceftriaxone and azithromycin [32]. Taken together, these facts suggest that the current main line of defence against chlamydial

infections (ie. administration of antibiotics following screening) is far from fully effective on a population level, and hence a vaccine may be the only way to address this problem. In addition, the strategy of control programs based on screening, treatment and contact tracing is extremely costly and requires substantial societal infrastructure. This makes this approach impractical for the developing world, where the burden of disease is the greatest.

Thus, development of a safe and effective vaccine is the ultimate goal in the control of *Chlamydia*. The relative uptake of a vaccine versus screening is difficult to quantify at present, but it is likely that a vaccine would be more widely accepted as evidenced by uptake of the HPV vaccine in settings where it is available and supported [33,34]. Costing of a *Chlamydia* vaccine is not possible at this stage. However, based on experience from other vaccines, prices could be negotiated to levels that are cost-effective. The most important issue of all is whether a vaccine actually works, that is, has high efficacy and prevents acquisition of infection, transmitting infection or developing disease. This can only be ascertained through clinical research after the development of suitable vaccine candidate(s). With no other long-term strategy available, investment in *Chlamydia* vaccine design, development and evaluation is the most appropriate way forward.

Our objectives in this review are to discuss infections and diseases of the genital tract caused by *C.trachomatis* with a focus on the complexities and challenges of chlamydial vaccine development. These include considerations such as how to; (i) better understand the range of immunological responses elicited by / to this organism, and therefore to subsequently define effective vaccine antigens and suitable biomarkers of protection, (ii) interpret the results obtained from animal models of infection, (iii) optimally choose, combine, and present vaccine antigens (surface and/or internal antigens, mucosal adjuvants) and, (iv) interpret mathematical models to define effective vaccine goals for preventing acquisition of infection, interrupting transmission, and/or preventing tubal disease.

The immunological challenges

C.trachomatis is a small (0.5μm) bacterium that elicits inflammatory cytokine responses following infections of epithelial cells and macrophages. The complex, two-stage developmental cycle of *Chlamydia* is described in Figure 1 (a). The extracellular infectious elementary bodies (EB) avoid lysosomal fusion to survive and differentiate into metabolically active reticulate bodies (RB) [35,36 and reviewed in [37]). The chlamydial RBs then replicate by around 500-fold, and subsequently re-differentiate into EBs inside a membrane-bound parasitophorous vacuole ("inclusion") eventually being released by extrusion and/or cytolysis after 40-72 hours to infect new cells or hosts [38]. *Chlamydia* can also enter a persistent growth state if exposed to molecular and cellular stresses such as inadequate antibiotic treatment or host cytokines, particularly IFN-g. The persistent form is characterised by large viable, non-infectious aberrant bodies (AB) (reviewed in [39]). In this form chlamydiae are refractory to killing by azithromycin [40] and this may allow for *in vivo* persistence of the pathogen.

In humans, immune responses to resolve *C.trachomatis* genital tract infections apparently develop over months to years. In uncomplicated, productive chlamydial genital infections, a myriad of host immune responses are elicited that include innate and adaptive immune mechanisms acting to clear infection and to resist re-infection [41](summarized in Figure 1 (b) and reviewed in [42]). *Chlamydia* can, however, also grow inside macrophages and dendritic cells (DCs) to produce persistently-infected cells (reviewed in [43]). In both productively and persistently-infected chlamydial host cells inflammatory cytokines are released that may induce and sustain tissue damage and host inflammatory responses [44-46]. Chlamydial infections induce both innate and adaptive cascades but it is acknowledged that the key effectors for both protection and pathology pathways are IFN-g and interleukin 17. While high levels of IFN-g are chlamydicidal, low levels can actually result in persistence and this may lead to worse pathology. This highlights the critical nature of the correct balance between mechanisms of protection (as will be required for effective vaccines) versus triggering adverse pathology.

During active primary infections in women, serum and genital mucosal IgA and IgG antibodies to chlamydial EBs and specific chlamydial proteins including heat-shock (HSP) and plasmid proteins, are usually detected [47]. In patients with current genital

infections, the predominant serum responses are maintained for at least 6 months and are mainly IgG1 and IgG3 antibodies [48]. Local IgA antibodies correlate with reduced shedding of the chlamydial organism from the genital tract [49]. However, high titres of local IgA antibodies do not correlate with resolution of infection, but can act as markers of prior chlamydial infections. The major role antibodies appear to play in clearance of infection is in the enhancement of Th1 activation with CD4+ T cells secreting IFN-g correlating primarily with the resolution of infections. Of note however, is the fact that CD4+T cell immunity is slow to develop and therefore infections frequently are repeated and chronic.

Chronic infections are characterised by genital tract inflammation and infiltration of innate immune cells along with inflammatory mediators to the genital mucosa [for a summary of chemokines and cytokines produced during chlamydial infections see [50]. High levels of IFN-g are found in the cervix and fallopian tubes in women with *C.trachomatis* genital tract infections [51]. IFN-g delays the developmental cycle of *Chlamydia* which may result in persistent and in-apparent infections that might contribute to promoting inflammatory damage of the genital tract [52].

The inability of immune responses to clear infections and prevent ascension of bacteria to the oviduct is also due to several strategies used by *Chlamydia* to evade the immune system [53]. Mechanisms used by *Chlamydia* to subvert host innate immune responses include blocking transcription factor NF-kB activation directly through the proteolysis of the p65/RelA subunit of NF-kB [54]. Virulence associated genes of *Chlamydia* have also recently been reported to be transcriptionally regulated by the Pgp4 protein encoded by the highly conserved 7.5kB cryptic plasmid of *Chlamydia trachomatis* [55].These genes include *pgp3* that encodes a protein to which immune responses are elicited in patients with *C.trachomatis* infection (see Table 1) . *Chlamydia* also inhibit IFN-g-inducible major histocompatibility complex (MHC) class II expression expression [56], down-regulate MHC class I heavy chain (HC) presentation [57], and in human endocervical cells this is mediated by direct and indirect (soluble) factors [58]. The multiple potential mechanisms used by *Chlamydia* dampen immune responses have recently been well summarized [50].

The consequent development of chlamydial disease following genital tract infections in humans is multifactorial and involves not only chlamydial factors such as virulence via different *C.trachomatis* strains but also host and environmental factors. For example, a recent prospective study of African-American women with clinically suspected mild to moderate cases of PID showed that gene polymorphisms in several innate immune receptors (including Toll-like receptors [TLR] 1 and 4) were associated with increased genital tract *C.trachomatis* infections [59]. The female genital tract is also a unique mucosal site in that it is influenced by fluctuating hormone levels and the polymicrobial environment. Hormone changes directly affect cell type and indirectly affect both the innate and adaptive immune responses to chlamydial genital infections [60]. Changes in bacterial flora and genital tract inflammation are both suggested cofactors for persistence of *Chlamydia* at this site and affect vaginal pH, which may be associated with the risk of acquiring *C.trachomatis* infection [61] [62]. The reproductive tract microbiome, sex hormones and immune responses are challenges for development of vaccines against genital tract pathogens and are discussed in detail in a paper in the current issue [63].

The value of animal models

What the mouse model has, and has not, told us

While animal models are useful and convenient, they must provide data about vaccination that will eventually be transferrable to the human situation. In the case of chlamydial STIs, the mouse model is the most widely used model for infection, pathogenesis and vaccine studies. Primary genital tract infections of female mice with elementary bodies of the mouse-adapted *C.muridarum* strain are enough to cause tubal dilatation since a consistent observation is the development of hydrosalpinx shortly (1-2 days) after initial chlamydial infection in this model [64]. Hydrosalpinx characteristically is also associated with tubal factor infertility in humans [65], making this model useful in this respect. However this observation in the murine model contrasts with documented evidence from the guinea pig *C.caviae* model of a primary genital tract infection in which chronic oviduct pathology was reported in only 12% of the animals, even though almost 80% were infected[66]. In humans, long-term chronic infections can develop after the primary infection [67] and the risk of pathology is known to increase after repeated infections [5]. Thus the

guinea pig model, with observed pathology following primary chlamydial infections and anatomy, and physiology similar to the human female genital tract, more closely resembles human chlamydial disease than the murine model.

Choosing the most informative animal model to investigate CD4+ effector cell subsets elicited to combat *C.trachomatis* genital tract infections in humans will require prudence. Rather than using the mouse strain, *C.muridarum*, several groups have used human *C.trachomatis* and shown that intravaginal inoculation of mice with this strain results a mild, self-limiting, lower reproductive tract infection with minimal ascension to the upper genital tract [68]. The eradication of *C.trachomatis* in the mouse is reportedly independent of indoleamine 2, 3-dioxygenase (IDO) [69] and yet this is a principle mechanism of protection against *C.trachomatis* infection in human cells, where IDO-catalyzed tryptophan degradation starves the chlamydial inclusion of this amino acid [70]. Nevertheless, using this murine model, it has been established that to resolve genital chlamydial infection an influx of IFN-g-producing CD4+ Th1 cells is required [71,72] along with numerous host factors including matrix metalloproteinases (MMPs) such as MMP9 [73].

The host response to chlamydial infection is also proposed to directly damage mucosal tissues of the female genital tract. One hypothesis states that infected epithelial cells secreting pro-inflammatory cytokines/chemokines to initiate pathogenesis (the cellular paradigm) whilst the second (immunological paradigm), as mentioned earlier in this paper, proposes that T-cell responses that are essential to resolve infection can also cause tissue damage [46, 53,74]. The immunological paradigm for pathogenesis is supported by observations from both the guinea pig [75] and the non-human primate (NHP) [76] models of genital infection in which repeated oviduct infections cause rapid infiltration of CD4 and CD8 T cells to the infection site.

The value of the non-human primate model

Despite the fact that the majority of vaccine studies have been undertaken using the mouse model, there has also been a long history of non-human primate (NHP) models used in the study of chlamydial disease (dating back to 1936). The value of using NHPs as a model lies in their evolutionary closeness to *Homo sapiens*. NHPs have been particularly effective in the study of tubal pathology (pelvic inflammatory

disease) (reviewed in [77]). However, the use of NHPs in vaccine evaluation has been less effective. Currently there are no studies that have evaluated the protective efficacy of a vaccine targeting urogenital infections (the closest simply measuring immune responses at multiple mucosal sites following immunisation [78]). Nevertheless, recent studies have shown the NHP model to be a promising platform for the evaluation of trachoma vaccines [79, 80], including one recent study showing promise with a live, plasmid-free, attenuated vaccine [81]. Although NHP models offer a biological system much more comparable to that of the human they are not without limitations. Currently there is no known natural NHP strain of *Chlamydia*. High inoculum doses of *C.trachomatis* are required to establish an infection (and pathology) [81, 82], as well as the fact that differences in immune responses and disease states have been found with different infecting serovars [82,83], as well as the NHP species used [78]. Therefore, for the successful use of NHPs in vaccine evaluation, it is essential to define the immunological mechanisms behind clearance of the human strains, and to compare that to the paradigm associated with clearance in humans. If this can be done, then NHP models will indeed be valuable in the development of *C.trachomatis* vaccines for humans.

Update on current vaccine progress

Given the global importance of *C.trachomatis* STIs, and the strong case for a vaccine to curb increasing infection rates, how are we progressing towards the goal of an effective vaccine? The critical questions to ask are, (i) why doesn't natural infection result in strong protection? and (ii) how successful have past vaccination attempts been, or at least, what can we learn from these trials? The answers to both of these questions are actually quite promising.

Natural infection can induce partial immunity but may also result in adverse pathology : Natural infection does lead to a degree of protection. In the mouse model this is certainly the case, with animals given a live infection being very solidly protected against a second (challenge) infection in that they shed very low levels of organisms [64]. A similar effect was observed in the early trachoma vaccine trials in which inactivated *C.trachomatis* organisms offered some degree of protection [84]. Indeed, there are some valuable lessons that can be learned from the early trachoma trials as well as more recent studies of ocular *C.trachomatis* natural

infections (reviewed by Mabey et al., 85] The early trachoma vaccine trials in countries such as Saudi Arabia, Taiwan, The Gambia, India and Ethiopia, showed that it was possible to induce short term immunity to ocular infection, and also to reduce the incidence of inflammatory trachoma, by administering vaccines based on killed or live whole organisms. The problem though is that these whole organism vaccines, whether infectious chlamydial elementary bodies or whole inactivated organisms, contain both protective as well as deleterious antigens. In the case of the early trachoma vaccine trials, the approach led to some enhanced immunopathology in some of the vaccinees [84]. This same enhanced immunopathology effect is observed in mouse trials in animals given a live vaccine and then challenged with live organisms at a later date [86]. Recent studies have also suggested that some women, infected naturally at the genital tract site, can mount a level of immunity against subsequent infections [87]. Indeed, these observations are extremely encouraging for the development of effective vaccines as these individuals can be utilised to identify bio-profiles of protection (for humans) and then vaccine trials can attempt to induce these protective bio-profiles. The second issue, which is often not discussed, is that any protection is usually only short lived, with most vaccine trials only evaluating protection up to 4 weeks post vaccination.

Identification and choice of vaccine target antigens : As a result of the issues surrounding crude, whole chlamydial vaccines, all efforts now involve the use of purified / cloned individual chlamydial antigens and virtually all of these studies have been conducted in the *C.muridarum* – mouse model. Indeed, while early vaccine efforts focussed very much on MOMP, and other surface antigens (eg Omp2), the past 5 years has seen a significant expansion in the number and type of antigens evaluated, including CPAF, PmpD, PmpG, CopN, IncA, NrdB and Pgp3 (many of which are intracellular proteins or at least not outer membrane proteins), in addition to the earlier favourites of MOMP, Omp2 and Hsp60 [42] (see Table 1 for a list of all antigens that have been shown to induce an immune response following genital tract infection). Figure 1 (a) provides an overview of the chlamydial developmental cycle, including points of attack as well as vaccine candidates that have, or could be, tested for each of these stages. Progress towards identifying the “holy grail” vaccine antigen has been relatively slow, with most new antigens evaluated only providing very modest, stepwise improvement in protection against live challenge. The search

for the best protective antigens though has become much more sophisticated recently, with groups directly identifying effective T cell antigens [88]. Brunham and colleagues are using an immunoproteomic screening approach to identify chlamydial antigens, or more correctly the actual T cell peptides, that are presented by *C.muridarum*-infected dendritic cells in the mouse model. Using this approach they have recently identified 13 *Chlamydia* peptides derived from eight novel epitopes presented by MHC class II molecules from bone marrow derived dendritic cells infected with *Chlamydia*. While some of the targets are new (RplF, FabG, AasF, ClpP-1, Gap, PmpE), interestingly, some overlap with previously identified antigens (PmpG).

Will a single antigen be sufficient or are antigen combinations required : In addition to searching for the most highly protective vaccine candidate antigen, several groups now believe that a combination of antigens will be required. There are several lines of thought in compiling combination antigen vaccines. *C.trachomatis* has multiple serovars and to cover the antigenic diversity that exists across the main genital tract strains (D to K) will require the vaccine to contain epitopes or whole proteins for each strain; this is certainly the case for a MOMP-based vaccine and will probably also be the case for other variable proteins, such as the Pmps. A more sophisticated strategy that is evolving, is to target several different but key proteins in the chlamydial repertoire. *Chlamydia* has evolved over its long history to have multiple mechanisms of infecting and controlling its host and hence a vaccine that does not rely on a single target has the best chance of success. To this end, the concept of targeting several surface proteins (such as MOMP, Pmps, Incs) as well as some internal or secreted regulatory proteins (such as CPAF, NrdB) has significant merit (Figure 1 (a) summarizes the antigens related to each stage of the chlamydial developmental cycle, and Table 2 shows how these might be combined effectively in multi-antigen vaccines). In addition, specifically targeting antigens that are more highly expressed in the persistent or chronic phase of infection / disease, has considerable merit. While the major goal of a chlamydial vaccine is to prevent infection in naive individuals, it may not be possible to screen all vaccinees to ensure they are negative prior to vaccination. In addition, if sterilizing immunity is difficult or impossible to achieve, then including persistence phase antigens in a vaccine would have significant merit. Such multi-target vaccines are well within the reach of current

technologies and clearly are successful with other infectious disease vaccines, such as meningococcal disease vaccines.

A key role for adjuvants : All candidate antigens though require effective adjuvants and the optimal delivery mechanism to be an effective vaccine. The challenge with a *C.trachomatis* STI vaccine is that the vaccine-adjuvant combination must elicit the correct balance of Th2 (neutralising antibodies) and Th1 (IFN-g and Th17 cytokines) responses and it must do this at the required mucosal sites (female genital tract). Thanks to recent progress in vaccinology and immunology more broadly, the range of adjuvants that are now available, and well advanced in human safety trials [89] is rapidly increasing and some promising results with *C.trachomatis* vaccines are emerging. The range of adjuvants and delivery systems that have been evaluated with *C.trachomatis* vaccines include immunostimulating complexes [88,90], detergent/surfactant-based adjuvants [91], live viral vectors [92], *Vibrio cholerae* ghosts [93], liposomes [[94], CpG and their more recently developed, safe derivatives [88] and cytokines.

Vaccines against infection or downstream pathology : One challenge for chlamydial vaccine development is whether it should (i) primarily aim to significantly reduce or even eliminate the infection, or (ii) should also, or perhaps only, aim to reduce or eliminate the adverse pathology, in particular upper genital tract pathology in females. The holy grail is to produce a sterilizing vaccine that would completely prevent infection in the individual and hence also prevent transmission of infection to others. However, if 100% prevention of infection is not possible to achieve, then some consideration needs to be given to a vaccine that mainly prevents ascending infections that lead to disease pathology. In fact, one argument might be to focus on the disease pathology, as this is the major consequence of infection. A vaccine that could do both would clearly be ideal. The reality though is that any vaccine needs to be evaluated in clinical trials and the measurement of reduction of infection is more readily quantifiable than immune-mediated damage, such as PID or infertility. Until recently, the majority of efforts have focused on evaluating prototype vaccines by measuring the reduction in infectious burden following live challenge of vaccinated animals, almost totally in the mouse model. As already mentioned, these vaccines are much easier to evaluate through the regulatory process. Recently though, there

have been increasing and encouraging reports of vaccine strategies that can protect against the downstream adverse pathology [95].

The other aspect of a *C.trachomatis* vaccine is the target group. All efforts to date have been directed at developing prophylactic vaccines, with the assumption that the vaccine would be administered to young girls prior to sexual activity. In reality though, a therapeutic vaccine that could be safely administered to women who either had a past or even current infection, would be very useful. There are very few published studies in this area, although the report of Carey et al. [86] in the *C.muridarum* – mouse model suggest that vaccinating either presently infected or previously infected individuals may not result in a strong immune response.

Modelling the impact of a *C.trachomatis* STD vaccine

There are no absolute criteria for the properties that a vaccine should have before it can be recommended for wide use in programs to improve the health of populations. The World Health Organization recommends vaccines which have long-term protection and high efficacy [89][100], however, vaccines which offer lower levels of protection are suggested for use in certain circumstances or populations [97-101]. When it is anticipated that only partially effective vaccines may become available, mathematical models have been used to investigate the potential epidemiological impact for the infectious disease in question, associated with different vaccine properties and implementation strategies [102]. Most theoretical vaccine modeling studies for sexually transmissible infections have been for HIV (e.g. [103-110]), but numerous vaccine modelling studies have emerged for HPV in recent years due to the availability and implementation of the cervical cancer vaccine in many countries [111-114]. These models have identified the most crucial vaccine parameters (vaccine take, efficacy and waning/duration) and demonstrated the trade-off between these factors and required frequencies and levels of boosting, coverage and intensity of scale-up among targeted population groups. It is particularly useful when comparison analyses across multiple models is done to produce a ‘consensus’ from the field (such as been attempted for aspects of HIV [115], HPV [114], and influenza [116] vaccine implementation).

A comparison of *Chlamydia* screening models has been conducted [117] but currently there is only one modeling study that has assessed the potential impact

and critical properties associated with *Chlamydia* vaccines [118]. This analysis considered not only the public health outcomes of vaccine implementation but the measurable biological properties to be assessed in vaccine design and regulation. It found that in order to have the greatest public health impact, a vaccine should primarily aim to increase the threshold of the infectious dose required to infect susceptible individuals. The next most important objective would be to decrease the peak infectiousness among infected individuals. Both these parameters are regularly measured in vaccine trials (in the mouse model) and several vaccine antigens are showing promise in this regard. The duration of vaccine efficacy was also identified to be of large importance and would influence the coverage and boosting schedule required in implementation to achieve a desired epidemiological outcome. This is one aspect that has not yet been well addressed in vaccine trials. But an imperfect vaccine can still have an impact. For example, a vaccine which reduces the peak chlamydial load among infected individuals by just 1-$\log_{10}$ could reduce prevalence of *Chlamydia* in the population by 40-50% after 20 years. In this respect, the models are very useful in that they give us an idea of just how effective a vaccine needs to be to (ie. what level of infectious load reduction) when translating mouse model data eventually across to human population studies.

Future directions and opportunities

While progress towards an effective *C.trachomatis* vaccine has been reasonably slow, it nevertheless has moved forward in a stepwise fashion, and there are some recent events that could significantly accelerate this goal. Whole organism vaccines (whether live or inactivated) do show a significant degree of protection, usually far beyond that obtained by individual purified antigen vaccines. Therefore, if we can avoid the deleterious pathology associated with these earlier versions, perhaps we can use this general approach. In this respect, the recent findings that the chlamydial plasmid contributes, by an as yet undefined mechanism, to the adverse pathology observed in both *C.trachomatis* and *C.muridarum* infections, could be a major opportunity [119]. A plasmid-free, attenuated strain of *C.muridarum*, while it grows similarly to the isogenic wild type, plasmid containing strain, fails to activate TLR 2-independent immune responses and as a result, is attenuated during mouse genital tract infections and does not result in the upper genital tract pathology that is seen with the wild type strain [120]. The plasmid-deficient strain also functioned as a

successful live attenuated vaccine in mice, whereby infection (vaccination) with the plasmid-negative strain limited the pathology usually associated with subsequent infections [121]. Importantly, Kari et al. [80] showed a similar phenomenon with *C.trachomatis*, whereby they generated a plasmid-free, attenuated strain of ocular *C.trachomatis* and showed that it could protect against trachoma in a nonhuman primate model. These plasmid-free strains could be our best chance of a vaccine that can generate sufficiently strong immunity, involving both B and T cell responses, to an array of important antigens, in the absence of adverse pathology. Of course, the regulatory requirements involved with the use of live attenuated vaccines means that it will be essential to fully understand the molecular mechanisms underpinning these plasmid-free "vaccine" strains. In this respect, the other recent breakthrough that could significantly accelerate vaccine research is that we now have the ability to genetically manipulate *Chlamydia* [122]. This major achievement that still has some technical challenges, means that potentially we can delete, or inactivate, key genes to understand their role in pathogenesis, and this should eventually result in a controlled means to produce a live attenuated vaccine strain that is unable to cause adverse pathology. These exciting advances, combined with rapid developments in vaccine adjuvants and delivery mechanisms, means that the previously elusive *C.trachomatis* vaccine goal may soon be within our reach.

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502 Table 1: *Chlamydia trachomatis* antigens that stimulate host immune responses to
503 genital tract infection

Chlamydial antigen	**Immune response(s) elicited**	**Reference(s)**
Polymorphic membrane protein D (**pmpD**)	Genital tract Th1 cells and IgG2a mucosal antibodies	Eko et al., 2011 [123]
Chlamydial type III-secreted effector protein (**Tarp**)	Th1 dominant humoral and cellular responses	Wang et al., 2009 [124]
Protein antigens CT823 and CT144	CD8+ T cell, Th1-polarised CD4+T cell, Th1-skewed antibody responses	Picard et al., 2012 [125]
MOMP-based serovar E DNA vaccine	Serum IgM, IgG and IgA, CD8+ and CD4+ Tcells in spleen and pelvic lymph nodes	Schautteet et al., 2011 [126]
C.trachomatis serovar D strain **pgp3** gene	Humoral (serum IgG) and mucosal IgA anti-Pgp3 antibodies	Comanducci et al, 1994 [127]
Pgp4 gene	Unknown - but the mutant exhibited decreased expression levels of Pgp3, a potential virulence factor amongst others	Song et al., 2013 [55]
TC0052, TC0189, TC0582, TC0660, TC0726, TC0816 and, TC0828	IgG antibodies with both Th1 and Th2 bias	Molina et al., 2010 [128]
MOMP (CT681), **HtrA** (CT823), **OmcB** (CT443), **TARP** (CT456), **GroEL** (CT110), **Lcr-E** (CT089), **Nqr3** (TC0551/CT279), **MAC-perforin** (TC0431/CT153), **IncA** (TC0396/CT119), and the hypothetical proteins CT622, TC0284, TC0313, TC0651, TC0890, and TC0106 (CT016, CT043, CT372, CT601, and CT733).	Human serum IgG, IFN-γ–producing $CD4^+$ T cells	Finco et al., 2011 [129]
DnaK (CT396)	Human CD4+ T cell responses	Coler et al., 2009 [130]
CT043	CD4+ Th1 cells	Meoni et al., 2009 [131]
OmcB (CT443) And also CT004, CT043, CT184, CT509, and CT611, CT082, CT089, CT322, CT396, and CT681, CT110	T cells, B cells or both B and T cells (OmcB)	Follman et al., 2008 [132]
Enolase (CT587)	Human CD4+ T cells	Goodall et al., [133]
chlamydial protease-like activity factor (**CPAF)**	CD4+T cell, IFN-γ	Murthy et al. 2006 [134]
NrdB	CD4+ T cells	Barker et al., 2008 [135]
Heat shock protein 60 (cHSP60)	Cervical IgG and IgA antibodies, IFN-g	Agrawal et al., 2007 [136]
Outer Membrane Protein 2 (OMP2)	Humoral antibody responses	Portig et al., 2003 [137]

Table 2 : Strategies for targeting antigens from various stages of the chlamydial developmental cycle

Stage of the chlamydial developmental cycle	Immune response needed / possible	Opportunities and challenges
Initial attachment of the chlamydial EB to the new host cell	Antibodies – should be neutralizing	Has been the traditional approach for antigens such as MOMP. Vaccine needs to produce antibodies to conformational epitopes. Difficult to get 100% neutralization / blocking.
Early alteration of host cell pathways to enable *Chlamydia* to successfully enter its inclusion and convert to the RB stage	Primarily T cell response but antibodies may also be effective	This phase has not been directly targeted although proteins such as TARP are involved. Many more targets are available for targeting.
RB multiplication	Primarily T cell response but antibodies may also be effective	Many targets are available on / in the RB phase. Could an ineffective response to RB targets result in pushing the infected cell into persistence?
Persistence phase	Primarily T cell response but antibodies may also be effective	This phase has not yet been specifically targeted. A multi-antigen vaccine consisting of acute and persistence phase targets could well have advantages.
Late stage development and exit	Primarily T cell response but antibodies may also be effective	For *Chlamydia* to be “infectious” for its next host cell, it must transform from the RB stage to the EB stage, including all necessary surface displayed proteins. In addition to MOMP, other surface proteins are appealing targets.

Figure 1: Overview of the chlamydial developmental cycle, stages at which potential vaccine targets have, or could be, directed, and the immune pathways triggered during chlamydial infection. Panel A describes the seven key stages in the chlamydial developmental cycle; 1 – extracellular elementary bodies (EBs) and their initial attachment to susceptible cells; 2 – internalization of EBs inside suitable host cells and immediate subversion of host cell pathways; 3 – conversion of EBs to reticulate bodies (RBs) and further subversion of host cell pathways; 4 - multiplication of RBs via binary fission; 5 – persistent phase of chlamydial development in which the RBs are still metabolically active but have altered gene expression patterns and are less susceptible to host defences and antibiotics; 6 – conversion of RBs to EBs by an unknown trigger; 7 – exit of infectious EBs from the host cell.

Panel B summarises the steps in the immune response (either following natural infection or immunisation), with a focus on the adaptive immune pathways. Red bold indicates components which have been experimentally confirmed for *Chlamydia*. Innate immune cells constitutively secrete an array of soluble antimicrobials including elafin, lysozyme and cathelicidins, amongst others. Chlamydial infection of columnar epithelial cells and local genital tract innate natural killer (NK) cells induces the production of interferon-g (IFN-g) and other pro-inflammatory cytokines and chemokines. Recruitment and activation of B cell (Humoral) and T cell (Cell-mediated/Adaptive) immunity is also coordinated by the release of these factors. Humoral immunity in the female genital tract is dominated by immunoglobulin (Ig) G although secretory IgA antibodies are also present at this mucosal site. Once *Chlamydia* have established intracellular infection, cells of the adaptive immune system, and particularly T helper 1 (Th1) type CD4+ T cells secreting IFN-g are required for effective clearance of primary infection and to protect from re-infection. Other major lineages of activated CD4+ T cells that play critical roles in chlamydial infections include the Th2 cells, Th17 cells (producing IL-17 and IL-23) and Tregulatory (Treg) cells. Additional T cells involved in adaptive immunity include intraepithelial lymphocytes (γδ T cells) and cytotoxic T lymphocytes (CD8+ T cells) that are known to induce apoptosis of infected chlamydial cells.

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