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Chlamydia vaccines: where are we and how far is there to go?

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Abstract

Attempts to protect against trachoma, an ocular disease caused by *Chlamydia trachomatis,* started a 100 years ago. The realization in the 1960's that *C. trachomatis* was also a common sexually transmitted pathogen further stimulated research to implement a vaccine. However, it is only recently that a phase 1 clinical trial of a *C. trachomatis* vaccine to protect against genital infections was successfully completed. Here, we discuss the most significant advances that have occurred in Chlamydia vaccinology, focusing mainly in the last 5 years, and provide advice on what steps can be taken to expedite the formulation of a successful vaccine. For in depth review of previous work in Chlamydia vaccinology we recommend the following references (1-9).

Clinical manifestations

C. trachomatis is the most common sexually transmitted bacterial pathogen in the world with a global prevalence estimated at 4.2% (10, 11). Worldwide approximately 130 million new infections occur annually with a high proportion of them affecting young, sexually active individuals. More than 1.7 million *C. trachomatis* infections in the USA were reported to the CDC in 2018 (12). In addition, asymptomatic infections occur in ~50% of males and ~80% of females (12, 13). In 2016, the World Health Assembly adopted a global strategy aimed at reducing by 90% curable sexually transmitted infections (STI) by 2030 (14).

Cervicitis and urethritis are the most frequent acute *C. trachomatis* presentations in females. Long-term sequelae include pelvic inflammatory disease (PID), chronic abdominal pain, ectopic pregnancy, and tubal factor infertility (TFI) (13, 15). Based on an analysis in the United Kingdom, Price et al. (13) concluded that for every 1,000 *C. trachomatis* infections in women, aged 16-44 years, there are on average, 171 episodes of PID, 73 of salpingitis, 2 of ectopic pregnancy and 5.1 women with TFI at age 44 years. Individuals with PID are at risk of primary fallopian tube carcinomas and infertility is a risk factor for epithelial ovarian cancer. *C. trachomatis* also facilitates the acquisition of other STIs and is an independent predictor of cervical cancer (16).

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In males, urethritis, epididymitis, and orchitis are the most common acute clinical presentations (17). No long term-sequelae are definitely associated with a *C. trachomatis* genitourinary infection in males but proctitis, particularly in HIV-1 positive men that have sex with men (MSM), prostatitis, and prostate cancer have been linked to *C. trachomatis* (18, 19).

Among randomly tested pregnant women in the USA, 3.5% where found to be infected with *C. trachomatis* with higher prevalence rates in those between 16-24 years of age (6.6%) (20, 21). Infants born from mothers with genital *C. trachomatis* infections can develop conjunctivitis, pneumonia, and gastrointestinal infections shortly after birth (22). Other pregnancy complications associated with *C. trachomatis* include spontaneous abortion, preterm birth, and premature rupture of membranes (22).

Rare clinical manifestations that affect both males and females as a result of a *C. trachomatis* infection include conjunctivitis, respiratory and gastrointestinal infections, reactive arthritis, and perihepatitis (16, 23, 24). In countries with poor sanitary conditions trachoma is the main cause of preventable blindness (25). Lymphogranuloma venereum (LGV) infections can result in highly debilitating chronic diseases with bubo formation, fistulas, fibrosis, and rectal stenosis. Among MSM, LGV infections are on the rise (26).

Immunity to *Chlamydia* infections

The mouse model has extensively been used to analyze immune responses to *C. muridarum* (previously called *C. trachomatis* mouse pneumonitis) primary and secondary genital infections (27). The results indicate that MHC-II restricted CD4+T-cells are necessary for protective immunity while MHC-I restricted CD8+T-cells do not appear to be essential for resolution of a primary infection, or a reinfection. The role of CD8+T-cells however is still controversial. For example, Johnson et al. (28) have recently demonstrated that a Class II-restricted $CD8_{\gamma 13}$ T-cell clone protected against a *C. muridarum* genital infection. Th1 cytokines, in particular IFN-γ, are needed to protect against *C. muridarum*. In contrast IL-10, a Th2 cytokine, is associated with pathological responses. Morrison and Morrison (29) were the first to propose that an effective *C. trachomatis* vaccine may need to elicit tissue-resident memory cells (Trm) in the genital tract.

B cells and/or antibodies have been found to be as protective as CD4+T cells against reinfection (30). Following a primary genital *C. muridarum* infection, mice are resistant to a rechallenge if CD4+T-cells and/or antibodies are present (30). Passive immunization with a neutralizing IgA monoclonal antibody protected mice against vaginal shedding and infertility following a *C. muridarum* genital infection (31). Naglak et al. (32) showed that the protective effects of antibodies during murine genital infection were primarily mediated by enhanced opsonophagocytosis and required the presence of IFN-γ and phagocytes. However, severe combined immunodeficiency male mice can be protected by passive immunization with a neutralizing monoclonal antibody indicating that antibodies can protect independently of T-cells (33).

Farris et al. (30) first performed a detailed analysis of the protective immune components elicited by a subunit vaccine. Female mice, vaccinated by the intramuscular and subcutaneous routes with *C. muridarum* native major outer membrane protein (nMOMP), adjuvanted with CpG-1826 plus Montanide ISA 720, were challenged vaginally with *C. muridarum*. As determined by the duration of vaginal shedding, number of IFU recovered and incidence of hydrosalpinx, vaccinated mice were found to be protected. Depletion of CD4+ T-cells, but not depletion of CD8+ T-cells, diminished vaccine-induced protection. Importantly, B-cell deficient vaccinated mice were significantly protected following passive transfer of anti-nMOMP serum. Importantly, this protection was more robust than that obtained with convalescent serum from infection-immune mice. The authors concluded that protection induced by a subunit vaccine depends on contributions from both CD4+ T cells and antibodies.

Our understanding of the immune responses to a *C. trachomatis* infection in humans is quite limited. There is evidence that cellular immunity, with Th1 responses and IFN-y production, is needed to recover from infection and correlates with protection against reinfection (34). In humans, mucosal antibodies, especially IgA, show a relationship with reduced bacterial shedding (35). Darville et al. (36) determined that in *C. trachomatis* infected women, serum and cervical IgG and IgA levels inversely correlated with cervical bacterial burden.

Poston et al. (37) determined levels of 48 cytokines in females with cervical infection only or with both cervical and endometrial *C. trachomatis* infection. Cytokines involved in Th1 polarization, recruitment and activation were found to correlate with protection from reinfection and ascension of *C. trachomatis* to the endometrium. In contrast, cytokines involved in humoral, type I interferon and Th-17 responses, were associated with susceptibility. Zheng et al. (38) compared blood mRNA levels in women with *C. trachomatis* infection limited to the cervix, to those of women with endometrial infection and endometritis and found that ascension and disease correlated with high expression of type I IFN gene pathways and elevated type I IFN-induced chemokines in cervical secretions.

To conclude, findings in mice indicate that protection against chlamydia infections requires CD4 Th1 cells producing mainly IFN-γ. CD8 T-cells, by producing IFN-γ, may also contribute to protection. The presence of mucosal and/or systemic antibodies complements the protective role of T cells. In humans, the role that T- and B-cells and antibodies play in protection will only be established following clinical trials.

Chlamydia vaccine antigens

Whole-cell vaccines

Live wild type and attenuated *C. trachomatis* and *C. muridarum* vaccines are highly protective against genital challenges in mice (1-9). Enhanced susceptibility to infection was demonstrated when killed *C. trachomatis* was inoculated directly into the uterus of mice, through induction of tolerogenic *C. trachomatis*-specific regulatory T cells induced by uptake of dead bacteria into CD103+ dendritic cells producing IL-10 (39). The possibility of using whole-cell vaccines to protect humans against genital tract infections is, in our

opinion, unlikely. Their use in prevention against trachoma is still under debate. The negative effects observed in humans and non-human primates (NHP) during the trachoma vaccine trials, including the risk of delayed hypersensitivity and increased susceptibility to reinfection, needs to be addressed before implementing whole-cell vaccines in humans (25). Safety concerns, in addition to difficulties manufacturing whole cell *Chlamydia* vaccines using Good Manufacturing Practices (GMP), has stimulated the search for a subunit vaccine.

Subunit vaccines

Major outer membrane protein (MOMP)—Results from mouse experiments and from the trachoma vaccine trials in humans and non-human primates, using whole organisms, indicated that protection against disease and shedding was serovar/serogroup specific (25). DNA sequencing of the *C. trachomatis* genome and phylogenetic analysis established that MOMP likely accounted for the serovar/serogroup-specific protection observed during the trachoma vaccine trials (40, 41).

Initial testing with vaccines using recombinant MOMP (rMOMP), MOMP peptides and DNA plasmids expressing MOMP yielded disappointing results. The first MOMP vaccine that elicited robust protection against a genital challenge was reported by Pal et al. (42). Mice were vaccinated by the intramuscular and subcutaneous route with the intact, or the denatured trimer of native MOMP (nMOMP), using Freund's adjuvant and were challenged with *C. muridarum* in the ovarian bursa. As determined by the number of positive vaginal cultures, duration of shedding, number of IFU recovered, number of pregnant mice and number of embryos, mice vaccinated with the nMOMP were significantly protected. Mice immunized with denatured nMOMP showed a weaker level of protection indicating that the structure of nMOMP was important to elicit a robust protection. Subsequently, mice vaccinated with nMOMP, adjuvanted with CpG-1826 plus Montanide ISA 720, were shown to be as protected against vaginal shedding and infertility as those immunized intranasally with live *C. muridarum* EBs (43, 44).

Several investigators have used *C. muridarum* rMOMP as the antigen . For example, Berry et al. (45) vaccinated mice with rMOMP and showed enhanced clearance and decrease in upper genital tract pathology following a *C. muridarum* vaginal challenge. Carmichael et al. (46) using *C. muridarum* rMOMP and a combination of mucosal and systemic routes for immunization, were the first to demonstrate protection against vaginal shedding and infertility using a recombinant chlamydial antigen. Subsequently, the same vaccine formulation was shown to elicit long-term protection against vaginal shedding, upper genital tract pathology and infertility in mice (47). Vaccinated mice were challenged at 60, 120 or 180 days after the last immunization. Significant protection against vaginal shedding and infertility was observed in mice challenged up to 180 days. Animals that had the most robust protection were vaccinated by the colonic followed by intramuscular and subcutaneous routes. Interestingly, mice vaccinated only by systemic routes had similar levels of protection and had the longest lasting levels of neutralizing antibody titters in serum. A vaccine to protect against upper genital tract pathology should be effective for approximately the 30 years females are fertile. Protection in mice for 180 days,

corresponding to one third of the life span of a mouse, suggests that this vaccine formulation could protect women during their child-bearing years.

O'Meara et al. (48) vaccinated female and male mice four times intranasally with *C. muridarum* rMOMP and the adjuvant Iscomatrix. Male mice were infected with 10^6 *C. muridarum* IFU. Female mice were challenged using prostate ejaculates (containing ~50 *C. muridarum* IFU) collected from *C. muridarum*-infected male mice. Immunization of female and male mice reduced the chlamydial burden and disease development in both sexes following a vaginal or a meatal urethral challenge, respectively. Importantly, only immunized female mice challenged vaginally with prostate ejaculates from immunized, infected males were 100% protected against infection and inflammatory disease of the upper genital tract. O'Meara et al. (48) concluded that vaccination can induce partial protection against infection in females, but a vaccine targeting males may be required to protect females against upper genital tract pathology. This synergism between partial immunity elicited by vaccination in both females and males supports the need to implement a vaccine in both sexes.

Tifrea et al. (49) have reported promising results using the *C. trachomatis* mouse model. Mice vaccinated with *C. trachomatis* serovar D (UW-3/Cx) rMOMP, were significantly protected against vaginal shedding and infertility, when challenged in the ovarian bursa with serovars D (UW-3/Cx), D (UCI-96/Cx) or E (IOL-43), but not with serovar F (N.I.1). These results indicate that a vaccine formulated with rMOMP, from the three major immune groups, will elicit protection against all the *C. trachomatis* genital serovars.

A vaccine formulated with MOMP variable domain 4 (VD4) and surrounding constant regions from *C. trachomatis* serovars D, E and F (Hirep1), with all cysteines replaced with serines, was tested in mice using CAF01 as adjuvant (50, 51). Serovar-specific immune responses to the conserved VD4 epitope (LNPTIAG) and to other MOMP regions were observed. Sera from vaccinated mice neutralized *in vitro* serovars D, E, and F (52). Based on the number of *C. trachomatis* serovar D IFU recovered from the vagina and the inflammatory responses in the upper genital tract, mice were partially protected. $CD4^+$ T cells and antibodies were found to be critical for protection (51). Vaccine-induced neutralizing antibodies transferred to naïve and Rag1 mice (T and B cell deficient) significantly reduced shedding compared to control mice (51, 52). A vaccine combining Hirep1 and the CT043 and CT414 antigens elicited neutralizing antibodies and weak protection against vaginal shedding in mini-pigs challenged with serovar D (53-55). Importantly, the multivalent MOMP-based construct has successfully completed a phase 1 clinical trial and was safe and highly immunogenic (51, 56).

Polymorphic membrane proteins (Pmps)—As vaccine antigens some Pmps have been shown to elicit protection in the mouse model (57-60). For example, immunization of C57BL/6, BALB/c and C3H/HeN mice with fragments from *C. muridarum* Pmps (E, F, G and H) accelerated the clearance of *C. muridarum* from the genital tract. A vaccine, formulated with the same four Pmp peptides from serovar D and MOMP from serovars D, F and J, adjuvanted with CAF01 was also tested in mice. Following a transcervical

challenge with *C. trachomatis* serovar D, decreased vaginal shedding and upper genital tract inflammation in C57BL/6 mice were observed (61).

The most comprehensive evaluation of the Pmps as vaccine antigens was performed in the mouse respiratory model (62). Fragments from the passenger domain of the nine *C. trachomatis* serovar E Pmps were used to immunize BALB/c mice that were then challenged intranasally with *C. muridarum.* The percentage amino acid identity between the *C. trachomatis* and *C. muridarum* fragments used, ranged from 61-81%. Based on disease burden and number of *C. muridarum* IFU recovered from the lungs, mice immunized with *C. trachomatis* serovar E PmpC were the best protected, followed by those immunized with PmpG or H. These results suggest that Pmps can be utilized to elicit *C. trachomatis* cross-serovar protection.

Chlamydial protease-like activity factor (CPAF—Vaccination of mice with *C. muridarum* CPAF, using IL-12 or CpG as adjuvants, shortened the duration of the infection, decreased oviduct pathology and elicited cross-species protection (63). Interestingly, no protection against infertility was observed following a primary vaginal challenge, but there was protection against a secondary *C. muridarum* challenge (64). A T-cell epitope from *C. muridarum* CPAF has also been found to be protective in HLA-DR4 transgenic mice suggesting that it may also be protective in humans (65). CPAF, using CpG-10109 as adjuvant, decreased vaginal shedding and genital tract inflammation in guinea pigs (66).

Plasmid antigens—Donati et al. (67) vaccinated C3H/HeN mice with a DNA plasmid expressing *C. trachomatis* serovar D Pgp3, and a control group with the same plasmid containing an irrelevant insert. As determined by the number of positive salpinx cultures, mice vaccinated with the Pgp3 plasmid were partially protected against a genital challenge with *C. trachomatis* serovar D. Intranasal vaccination of mice with a plasmid expressing *C. trachomatis* serovar D pORF5 (coding for Pgp3), were challenged vaginally with *C. muridarum* (68). Following immunization, significant Th1 responses and antibody levels were detected. Bacterial shedding, length of time of shedding and upper genital tract inflammation were reduced in the Pgp3 immunized animals.

Multi-subunit vaccines—Some multi-subunit vaccines have been designed to target dominant antigens from each developmental form of *Chlamydia* (extracellular and intracellular). For example, O'Meara et al. (69) used multistage antigens including outer membrane proteins (MOMP and PmpG), type three secretion system proteins (CdsF and TC0873) and inclusion membrane proteins (IncA and TC0500) to vaccinate BALB/c mice against a vaginal challenge with *C. muridarum*. Based on vaginal shedding and upper genital tract pathology, multistage formulations elicited greater protection than vaccines including only extra- or intra-cellular antigens.

In contrast with these results, Li et al. (63) reported that the efficacy of a CPAF based vaccine, adjuvanted with IL-12 and delivered intranasally, was not improved by addition of MOMP and/or IncA, indicating that the selection of extra and intracellular antigens is important to enhance protection. Also, Yu *et al.* (70) vaccinated mice with PmpE, PmpF, PmpG, Aasf, RplF, TC0420 or TC0825 adjuvanted with DDA-MPL and

challenged the animals in the genital tract. PmpG elicited the most robust immune response and the best protection as shown by a decrease in the number of *C. muridarum* IFU recovered. Interestingly, three combinations of antigens (PmpE+PmpF+PmpG+PmpH+Tarp; Aasf+RpIF+Rec0 and TC0420+TC0825+TC0285) only protected as well as the best individual protein in the formulation. Similar neutral findings were reported when testing combinations of antigens in the respiratory model. Mice were immunized with components of the *C. muridarum* putative ATP synthase complex, TC0580, TC0581, TC0582, TC0584, or with MOMP. In addition, TC0582 was formulated in combination with TC0580, TC0581 or MOMP. Animals immunized with combinations of two of these three antigens were protected only as well as mice vaccinated only with MOMP, the most protective protein in the formulation. These results emphasize the need to evaluate the interactions between antigens of a multi-subunit vaccine.

Additional Chlamydia vaccine antigen candidates—Multiple chlamydial proteins have been tested as vaccine candidates (1-9). These include, the outer membrane protein B (OmcB), putative type III secretion effector protein Tarp, macrophage infectivity potentiator (MIP), CopB, CopD, Cap1 and CT584, the inclusion membrane protein IncA, the porin protein PorB, the ribonucleoside reductase NrdB, glycolipid antigen-peptide 4, and glycogen phosphorylase. Some of these antigens are promising but additional testing is needed to reach final conclusions.

The use of chlamydial exosomes as a vaccine has been shown to provide a very robust protection (71). *C. muridarum* was grown in HeLa cells, exosomes purified and used to immunize mice that were subsequently challenged intranasally with *C. muridarum*. Exosomes, adjuvanted with CpG-1826 plus Montanide ISA 720, elicited robust humoral and cell mediated immune responses. Protection against disease and bacterial burden elicited by exosomes, was similar to that induced by intranasal immunization with live EB. Chlamydial exosomes cannot be used as vaccines because most of the mass corresponds to host cell proteins. However, exosomes can be used to identified new protective vaccine antigens. By mass spectrometry, a total of 113 *C. muridarum* proteins where identified in the exosome preparation. Interestingly, Pmps and MOMP constituted the major Chlamydia component of the exosomes.

In summary, based on the results of the human trachoma vaccine trials, and on recent experiments in various animal models, MOMP is a promising subunit vaccine antigen. MOMP is so far the only antigen that has protected against vaginal shedding and infertility and has induced cross-serovar and long-term protection in the mouse model (47, 49). Importantly, vaccinating female and male mice with rMOMP improved protection against upper genital tract pathology in females (48) suggesting that more work needs to be performed with the male mouse model (72). Protection against a genital challenge in outbred mice and against an ocular challenge in non-human primates has also been achieved with MOMP (73, 74). EB-purified nMOMP provides better protection than rMOMP but cannot be produced in any significant quantities. Attempts to refold rMOMP into its native trimeric conformation have failed. MOMP elicits serogroup protection and therefore, MOMP from two or three *C. trachomatis* senior serovars, likely E, J and G, will be necessary to elicit

broad coverage. Alternatively, making a chimeric MOMP from several serovars may also overcome the limitation.

Additional antigens, such as Pmps or CPAF, could also be included in a multi-subunit vaccine to enhance and provide broad cross-serovar protection against genital and ocular infections. Plasmid encoded proteins may also be considered. However, rare *C. trachomatis* strains lack all or part of the plasmid. Also, vaccines based only on plasmid proteins may not protect against rare *C. trachomatis* strains that lack all or part of the plasmid. However, as part of a multi-subunit vaccine, inclusion of plasmid antigens can be considered since some of them are protective and highly conserved.

Adjuvants

Unlike whole cell vaccines, subunit vaccines require the use of adjuvants to elicit coordinated innate and adaptive immune responses to the antigen. A number of studies have evaluated the use of adjuvants to protect against *C. muridarum* genital infections. Yu et al. (75) formulated PmpG-1, PmpE/F-2 and MOMP individually or in combination with three different adjuvants: CpG-1826, AbISco-100 or CAF01. As determined by vaginal shedding CAF01 was the most protective. These results were confirmed by testing four adjuvant combinations: DDA-MPL, CAF01, CAF01 and Montanide ISA 720 VG plus CpG-1826, in addition to Alum alone, using PmpG as antigen (70). The most robust immune responses and best protection against vaginal shedding were observed when DDA-MPL, or CAF01, were utilized as adjuvants. These two adjuvant combinations elicited the highest frequency of multifunctional CD4 T cells co-expressing IFN-γ and TNF-α, supporting the role of these cytokines in protection.

A vaccine formulated with *C. trachomatis* serovar E rMOMP, using a combination of aluminum hydroxide and the TLR4 agonist E6020 as adjuvants, elicited high levels of neutralizing antibodies and IFN-γ producing T-cells in outbred CD-1 mice (74). Following a vaginal challenge with serovar E, as determined by the number of mice with positive vaginal cultures, number of positive cultures and number of *C. trachomatis* IFU recovered, significant protection was observed. Thus, demonstrating the ability to elicit protective immune responses in outbred animals.

Conjugating antigens with adjuvants has been used to increase the efficacy of vaccines and to decrease the amount of antigen needed, “antigen sparing” (76). To determine the effects of conjugating the antigen with the adjuvant, the *C. muridarum* nMOMP was conjugated with EP67, (YSFKDMP(MeL)aR) is a conformationally biased agonist derived from the *C*-terminal region of human complement-derived component $C5a_{65\text{-}74}$(ISHKDMQLGR), or Resiquimod (a TLR7/8 agonist), using amphipols (77, 78). Mice immunized with the conjugated adjuvants, and challenged intranasally with *C. muridarum*, based on changes in body weight, lung weight and number of IFU recovered from the lungs, were better protected than when the adjuvants were not conjugated to the antigen. Subsequently, CpG-1826 and/or Montanide ISA 720 were added to these two vaccine formulations. Increased humoral and cell mediated immune responses, with high levels of neutralizing

antibodies and IFN-γ producing T-cells, and improved protection, were observed with the addition of the two adjuvants.

To conclude, based on human and animal studies it has been found that optimal protection will require the induction of cellular and humoral immune responses. Therefore, adjuvant combinations, such as CAF01 and CpG-1826 plus Montanide ISA 720, that elicit combined Th1 and Th2 responses, will likely be needed to optimize the protection elicited by a chlamydial vaccine.

Routes of immunization

Several mucosal routes have been explored to deliver chlamydial vaccines. In general, it is thought that a vaccine against mucosal pathogens will benefit by using a mucosal route for immunization. The intranasal route has been shown to be effective for inducing mucosal immune responses (46). Safety and acceptability may, however, be a concern. For example, intranasal immunization has been associated with negative effects on the central nervous system (79). Controlling the quantity of vaccine delivered will also represent a challenge. Oral immunization with live *C. muridarum* is highly effective at protecting against genital and respiratory challenges (80, 81). Subunit vaccines, however, will have to be protected against the harsh environment of the gastrointestinal tract. The sublingual route may be a more feasible alternative and has been successful in the mouse model (46). Colonic immunization elicits higher vaginal and serum IgA levels than oral and intramuscular immunizations (46). Patient acceptance will have to be improved by utilizing oral delivery systems.

Several studies have showed the advantages of combining parenteral and mucosal routes for immunization (50, 82). Carmichael et al. (46) utilized combinations of mucosal (intravaginal, colonic, and intranasal) and systemic (intramuscular and subcutaneous) routes to vaccinate mice with *C. muridarum* rMOMP. The strongest humoral and cell-mediated immune responses were observed in mice immunized by a combination of mucosal and systemic routes. Furthermore, based on vaginal shedding and fertility rates, mice vaccinated via the combined routes were the best protected against a vaginal *C. muridarum* challenge.

Lorenzen et al. (83) vaccinated mini pigs by the intramuscular and intranasal routes with the Hirep1 antigen and CAF01 as the adjuvant, and challenged them vagino-cervically with 5×10^9 *C. trachomatis* serovar D (UW-3/Cx) IFUs. Following vaccination, strong systemic cellular immunity and IgG with local secretory IgA, were detected. An inverse correlation between levels of vaginal secretory IgA and chlamydial load were observed suggesting that, in the mini-pig model, this immunoglobulin may play an important role in protection.

Nguyen et al. (84) have shown that a vaccine formulated with the CTH522 MOMP construct, delivered subcutaneously and adjuvanted with CAF01, protects mice against vaginal shedding following a transcervical challenge with 10^3 IFU of *C. trachomatis* serovar D, but not against a higher challenge dose. Protection correlated with rapid recruitment of Th1/Th17 cells to the genital tract. Studies in mice, guineapigs and NHP support the efficacy of parenteral vaccination against *C. trachomatis* (47, 85, 86) .

To summarize, although combining mucosal and systemic immunizations may increase protection, the use of the intramuscular route will greatly facilitate delivery of a *C. trachomatis* vaccine, and public acceptance, particularly if combined with other STI vaccines.

Delivery Systems

Several delivery systems, including DNA plasmids, poliovirus, adenovirus, hepatitis B virus, HPV, influenza virus, modified vaccinia Ankara, *Vibrio cholera* ghosts, *Lactobacillus plantarum*, *Neisseria lactamica* porB, vaults, exosomes, nanoparticles and edible plants have been used to vaccinate mice with various chlamydial antigens (71, 87-97). Although some of these delivery systems offer advantages, such as the ability to carry antigens from several pathogens, so far the results obtained do not appear to be better when compared with well-established formulations. Furthermore, inclusion in a vaccine of not-relevant antigens, from the delivery system, introduces unknown safety issues and may divert immune responses.

Conclusions and advice

The main goal of a *C. trachomatis* vaccine should be to elicit protection against long-term sequelae in females, e.g., PID, chronic pelvic pain, ectopic pregnancy and infertility. The possibility that a vaccine will induce “sterilizing immunity” is highly unlikely since few vaccines, if any, elicits this type of protection. The successful completion, of a phase 1 clinical trial of a *C. trachomatis* vaccine, marks a turning point in a long road to control the pandemic caused by this pathogen (56). This vaccine formulation, tested in three separate animal models, elicited protection against vaginal shedding but there is no experimental data supporting that it protects against long-term sequelae. The expectation that a decrease in vaginal shedding will correlate with protection in the upper genital tract is reasonable but not proven. This type of vaccine will certainly decrease transmission. That, by itself, will have a major impact on the number of new genital infections and long-term sequelae.

Based on the trachoma vaccine trials, and current experimental data in animal models, MOMP is the leading protective antigen. Optimizing protection with this protein is going to be challenging because, like with other subunit vaccines, the conformation of the antigen appears to be critical. The tertiary structure of MOMP is complex and refolding a recombinant construct into the correct native conformation is technically difficult. The presence of numerous cysteines in MOMP further compounds the problem. To facilitate formulation of the CTH522 construct, currently undergoing clinical trials, all the cysteines were replaced with serines. The conformation of the antigen affects both B- and T-cell responses and therefore, the ultimate goal to optimize a chlamydial antigen should be to formulate a construct that mimics, as much as possible, the structure of the protein in its native conformation. Other proteins that can be considered for inclusion in a Chlamydia vaccine include the Pmps, CPAF and Pgp3. The Pmps, in addition to having many cysteines, have a complex tertiary structure and therefore, it is also likely that it will be difficult to refold a recombinant protein into its native structure. CPAF and Pgp3 may not have the same limitations.

Although not as a clear distinction in humans between Th1 and Th2 responses as there is in mice, the need for humoral and cellular immune responses to optimize protection favors the use of adjuvant combinations. CAF01 is a good example of this type of adjuvant. CAF01 is a cationic dimethyldioctadecylammonium/trehalose 6,6,-dibehenate (DDA/TDB) liposome, that elicits robust humoral and cell-mediated immune responses and is used in the Chlamydia vaccine undergoing clinical trials. In our experience, combinations of CpG-1826 (TLR-9 agonist) plus Montanide ISA 720 (non-TLR adjuvant) or CpG-1826 plus Pam_2CSK_4 (TLR-2 agonist) have also been shown to be strong inducers of both humoral and cell mediated immune responses in mice.

Mucosal pathogens are thought to be best prevented by mucosal delivery of the vaccine. This paradigm may have to be reassessed based on the results of the HPV vaccine. HPV and *C. trachomatis* both infect the transition zone of the uterine cervix. Vaccine induced protection against HPV appears to be mediated by circulating neutralizing IgG antibodies that can be generated by intramuscular immunization. Protection against Chlamydia on the other hand is dependent on cell mediated and humoral immune responses. The possibility of delivering a multi-pathogen vaccine against STIs by the intramuscular route will greatly facilitate its implementation. Finding the antigen(s)/adjuvant(s) combination that can elicit robust protection when delivered by the intramuscular route is a key current challenge in the field of Chlamydia vaccinology.

We expect that the chlamydial vaccine in clinical trials will propel the field to quickly move forward. There is a light at the end of a long tunnel. However, the journey is not over yet. Humankind needs and deserves a chlamydial vaccine. Collaboration between governments, private companies and research laboratories is needed to achieve this goal.

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