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Delivery of *Chlamydia* vaccines

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The plethora of ocular, genital and respiratory diseases of *Chlamydia*, including nongonococcal urethritis, cervicitis pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility, conjunctivitis, blinding trachoma and interstitial pneumonia, and chronic diseases that may include atherosclerosis, multiple sclerosis, adult onset asthma and Alzheimer's disease, still pose a considerable public health challenge to many nations. Although antibiotics are effective against *Chlamydia* when effectively diagnosed, asymptomatic infections are rampart, making clinical presentation of complications often the first evidence of an infection. Consequently, the current medical opinion is that an effective prophylactic vaccine would constitute the best approach to protect the human population from the most severe consequences of these infections. Clinical and experimental studies have demonstration that *Chlamydia* immunity in animals and humans is mediated by T cells and a complementary antibody response, and the completion of the genome sequencing of several isolates of *Chlamydia* is broadening our knowledge of the immunogenic antigens with potential vaccine value. Thus, major advances have been made in defining the essential elements of a potentially effective subunit vaccine design and parameters for evaluation. However, the challenge to develop effective delivery systems and human compatible adjuvants that would boost the immune response to achieve long-lasting protective immunity remains an elusive objective in chlamydial vaccine research. In response to evolving molecular and cellular technologies and novel vaccinology approaches, considerable progress is being made in the construction of novel delivery systems, such as DNA and plasmid expression systems, viral vectors, living and nonliving bacterial delivery systems, the use of chemical adjuvants, lipoprotein constructs and the codelivery of vaccines and specific immunomodulatory biological agonists targeting receptors for chemokines, Toll-like receptors, and costimulatory molecules. The application of these novel delivery strategies to *Chlamydia* vaccine design could culminate in timely achievement of an efficacious vaccine.

Keywords: *Chlamydia*, delivery, immunomodulation adjuvants, vaccine

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1. Introduction

1.1 The need for a vaccine to control chlamydial infections

Of the four major species of the obligate intracellular Gram-negative-like bacteria of the genus *Chlamydia* (*C. trachomatis, C. psittaci, C. pneumoniae* and *C. pecorum*), *C. trachomatis* causes the most clinically relevant infections, comprising oculogenital diseases with considerable morbidity in humans [1,2]. It is composed of ~ 15 serovars (serotypes) or genovars (genotypes), designated A – K and L1 – L3, which are distinguished by the antigenic or sequence variation in the major outer membrane protein (MOMP; OmpA) [3-5]. Trachoma, the world's most common preventable blinding disease, is caused by ocular infection by serovars A, B, Ba and C, which is of endemic proportions in several developing nations, including Africa, southeast Asia and the Middle East, with an estimated 150 million people affected,

of whom 6 million are irreversibly blinded or severely visually impaired [6]. Serovars D – K and the *Lymphogranuloma venereum* (LGV) strains L1, L2 and L3 are the oculogenital biovars of *C. trachomatis* that cause sexually transmitted diseases (STDs), primarily cervicitis and urethritis. Genital infection by *C. trachomatis* constitutes the most common bacterial STD in the US and several other industrialised nations, including the UK, Germany, Japan and France. Epidemiological data from the WHO revealed that *C. trachomatis* infections account for > 90 million of the 500 million annual new STDs worldwide, and the US alone spends > $2 billion annually on 4 million reported cases [7-10]. Pelvic inflammatory disease (PID) and tubal factor infertility are major complications of genital infection, occurring respectively in ~ 40 and 10% of untreated infections, and constituting an enormous morbidity and socioeconomic burden of *Chlamydia* infections [11-14]. The LGV diseases are invasive and sometimes include ulcerative chlamydial STDs involving lymphatic tissue (e.g., inguinal bubo). These are caused by the L1, L2 and L3 serovars [15,16], which are endemic in certain areas of Africa, Asia, South America and the Caribbean [17]. In general ulcerative STDs are a major risk factor for HIV acquisition. A recent outbreak of LGV among men who have sex with men in Western Europe has attracted public health attention in several countries [18]. Furthermore, reports suggesting that genital chlamydial infection may be on the rise [9,19] or could predispose to HIV-related AIDS [18,20-26] and human papilloma virus-associated cervical dysplasia have heightened these concerns [8]. *C. pneumoniae* (also known as *Chlamydophila pneumoniae*) causes mild to sublethal acute respiratory infections, including pharyngitis, bronchitis and even pneumonia; however, it was recently associated with atherosclerosis, adult-onset asthma and certain other chronic diseases [15,27,28]. The zoonotic *C. psittaci* constitutes an occupational hazard for workers in the poultry and farming industry, and persons exposed to infected avian species [29]. The diseases caused by *C. psittaci* in man include psittacosis infection that may in rare cases become systemic and fatal, but in animals and birds the infections are psittacosis, hepatitis, mastitis, conjunctivitis, pneumoniae, abortions and diarrhoea. Finally, *Chlamydia*, *C. pecorum* is an animal pathogen that has not been associated with any human disease.

Although several prevention strategies have been proposed against *Chlamydia* (i.e., mass screening and treatment, early diagnosis and treatment, mass antibiotic treatment, health education, and the administration of an effective prophylactic vaccine), the vaccine strategy is considered the most reliable and cost effective to achieve the greatest impact in controlling *Chlamydia* infections, transmission and the associated complications in the human population [15,30]. This medical opinion is derived from the medical and scientific findings that: although if detected early chlamydial infections are treatable with antibacterial agents, such as tetracycline derivatives (especially doxycycline), and the macrolides or azalides including erythromycin and azithromycin [15], the high proportion of asymptomatic infections lead to severe and sometimes irreversible complications, which usually present as the first symptoms of an infection, specifically as genital infections in women [31,32]. In addition, whereas 10 – 40% of untreated chlamydial genital infections lead to sequelae such as PID and tubal factor infertility [9,13,14], evidence is accumulating that a significant proportion of treated genital or ocular infections may lead to persistence [33-36]. In fact, the recognition of the significance of persistent chlamydial infection in the pathogenesis of the disease is casting doubt on the long-term value of certain chemotherapies in clinically diagnosed cases of chlamydial infections [33-35,37-41]. Moreover, whereas contemporary diagnostic methods are reliable for identifying infections [9,31,42-44], there are economic, convenience and acceptance issues surrounding certain intervention strategies involving frequent community-wide screening for early detection and treatment of at-risk populations, and/or mass antibiotic prophylaxis in trachoma endemic populations to prevent silent, persistent or inapparent infections [45-48]. Besides, it has been suggested from computer modelling and prediction of the impact of a protective chlamydial vaccine that considering the urgency and enormity of the challenges, a partially protective vaccine that prevents certain severe sequelae in a partial vaccination programme would constitute an acceptable short-term goal with a remarkable global impact in reducing infections, disease prevalence and associated expenditure [49]. Furthermore, the present need for a vaccine to prevent the spread of *Chlamydia* in the population makes a prophylactic vaccine that prevents infections a better antichlamydial strategy than a therapeutic vaccine to prevent the development of sequelae in infected persons. More research efforts and support are needed to broaden the frontiers of our knowledge of *Chlamydia* biology, mechanisms of host defence against *Chlamydia* and modern vaccinology approaches to boosting protective immunity. Research areas of particular interest include: a better understanding of immunoregulation and immunomodulation of host immunity against *Chlamydia*; genomic, proteomic and bioinformatic approaches to the identification of stable vaccine candidates; and the development of effective delivery systems and potent adjuvants. All of these will furnish the blueprints for designing an efficacious chlamydial vaccine.

1.2 *Chlamydia* vaccine delivery challenges

It has been unequivocally demonstrated in both clinical studies and experimentation in animal models of genital, ocular and respiratory infection that *Chlamydia* immunity correlates with a strong T helper cell type 1 (T_H1) response and a complementary antibody response that fosters a rapid and robust memory T-cell-mediated immunity [50-54]. The obligatory requirement for cell-mediated immunity (CMI) effectors to control certain intracellular pathogens such as *Chlamydia* has been reinforced by recent developments in human genetics and disease susceptibility, where genetic evidence for a crucial role of T_H1-related cytokines in protective immunity against

mycobacterial infections was identified and established [55]. In addition, the possibility that the intact chlamydiae harbour pathogenic components [30,56-59], and the absence of tools to genetically modify chlamydiae to produce safe attenuated strains with vaccine prospects make subunit vaccines the current focus of chlamydial vaccine research. In addition, the requirement for both T-cell and humoral immune responses to achieve a protective immunity would suggest that a subunit vaccine should possess both T-cell and antibody epitopes. The potential chlamydial proteins that could form the basis of a subunit vaccine, as predicted by immunobiochemical analysis and functional genomics, include the 40-, 60- and 15-kDa outer membrane proteins (OMPs), which are encoded by the Omp-1 (*omc A*), Omp-2 (*omp C*) and Omp-3 (*omp B*) genes, respectively [60-64]. These have been serologically defined and molecularly characterised. The 40-kDa Omp-1 antigen (the MOMP), has received an enormous amount of attention as a promising subunit vaccine due to its immunogenicity (T and B cells), immunoaccessibility, abundance (60% of OMP mass), contribution to the structural integrity of the elementary body [65], function as a porin [66], possession of both species- and genus-specific epitopes [56,67] and expression in all phases of the developmental cycle of *Chlamydia* [56,68]. However, the efficacy of MOMP-based vaccines has been limited, due in part to poor immunogenicity, and consequently producing only partial protective immunity, as measured by either a reduction in infectious burden or pathology. The lack of satisfactory protective immunity with MOMP-based vaccine regimens would suggest that either MOMP alone is inadequate as a vaccine (calling for multisubunit vaccines), or that more effective delivery systems are needed to optimise the effect of MOMP or other emerging single subunit vaccine candidates. Interestingly, the immunogenicity and protective immunity induced by a MOMP DNA vaccine was enhanced by the use of an adjuvant [69], suggesting that better adjuvants and delivery systems are needed to enhance the efficacy of potential subunit vaccines. Further efforts in chlamydial genomics and proteomics are identifying additional vaccine candidates [60,62-64,70-72], particularly the polymorphic outer membrane proteins (POMPs or pmp) and the conserved PorB family of membrane [62,64,73-75], as well as an ADP/ATP translocase of *C. pneumoniae*, which was identified as a protective antigen in a DNA delivery and mouse lung infection model [76]. In addition, a *C. trachomatis* plasmid protein (pgp3) that is immunogenic after a natural infection in humans [77] was shown to confer partial protection (56%) against ascending genital infection in mice in an experimental DNA vaccine [78]. Furthermore, the chlamydial proteasome/protease-like activity factor (CPAF) [79], chlamydial toxin mapped to the plasticity zone of several strains [64], and certain members of the chlamydial type III secretory machinery [80] have also been proposed as likely vaccine candidates. A provisional list of patent claims on chlamydial vaccine candidates was recently published [15]. Comparative structural and immunological analyses of these antigens should lead to the judicious selection of a combination of immunogens for a multisubunit vaccine. A major advantage of the multiple subunit approach is the potential synergistic immunological benefit of a combination of epitopes from multiple antigens, inducing a higher frequency of immune effectors that ensures an effective long-lasting immunity. The continuing progress of research in chlamydial genomics and proteomics is likely to expand the pool of available candidate antigens. In addition, the role of conformation in the vaccine efficacy of candidate protein antigens is yet to be fully established [81], although most of the preparations of MOMP used so far were not refolded. As effectors of CMI are indispensable for chlamydial immunity, antigen conformation is less likely to affect T-cell epitope compared with antibody epitope. However, protein folding may control the availability of crucial peptides containing relevant T-cell epitopes during antigen processing for T-cell activation, and vaccine delivery media could potentially affect vaccine conformation during delivery and processing as well. Additional study is needed to clarify the role of antigen conformation in vaccine efficacy. Finally, the mouse and guinea-pig models of oculogenital and respiratory infections are commonly used for experimental vaccine evaluation and testing due to the availability of immunological reagents, the similarity of the disease profiles and certain aspects of the human disease, costs and the relative ease of handling [82-85]. Considering the recent study in the guinea-pig model of the genital infection, which revealed that a dose equivalent to 200 infection-forming units was sufficient for sexual transmission of the *C. psittaci* agent of guinea-pig inclusion conjunctivitis [86], it may be instructive to re-evaluate the higher doses previously used for vaccine evaluation in these models.

Vaccine delivery systems are carrier media, vectors and other delivery vehicles used to package an immunogen as a vaccine for administration. Although a delivery system may or may not possess intrinsic adjuvant properties that boost the immune response against the vaccine, the combination of the vehicular and adjuvant functions of a delivery system increases its attraction for use in modern vaccines that are commonly poorly immunogenic without an adjuvant. Delivery systems, therefore, include delivery vehicles such as vectors, as well as carrier media and adjuvants used to formulate the vaccine regimen for administration. They are used to ensure vaccine stability and availability to the immune system, targeting of vaccine to the desired site(s), and to achieve an optimal induction of the desired immune effectors that confer protective immunity against an infection or tumour. The delivery of a vaccine can have a great impact on vaccine efficacy as it affects both the quality and magnitude of the immune response induced. Thus, effective vaccine delivery ensures the aggregate mobilisation of the immune system for an optimum response, effectively boosting the induction of specific immune effectors against the vaccine, and targeting the effectors to the desired anatomical location(s) to confer protection against infections (**Box 1**). The significance of vaccine delivery vehicles can be

Box 1. Significance of optimal vaccine delivery.

- Impact the quality and magnitude of the immune response to the vaccine

- Boost immune response; adjuvanticity
- Skew immune response (predominant T_H1 versus T_H2 response)
- Targets vaccine to the appropriate site for optimal immune induction and/or target effectors to site of action

- Vital for subunit vaccines
- Ensures effective mobilisation of the immune system for optimum response

$T_H1/2$: T helper cell type 1/2.

appreciated by two facts: first, the most promising vaccine formulations may fail to establish the desired protective immunity due to inadequate delivery; second, although the era of epitope or subunit vaccines has obviated the concerns inherent in inactivated or live-attenuated whole pathogens [30], modern vaccinology has also encountered a major challenge associated with the relatively poor immunogenicity of candidate subunit vaccines.

Different vaccines can require different delivery vehicles depending on the type of immune response needed (e.g., predominantly antibodies versus CMI effectors) and the target of effectors (mucosal versus systemic or both locations). **Table 1** shows the different classes of delivery systems that have been commonly used in experimental, veterinary and human vaccine designs [87]. Adjuvanticity is a desirable property in a number of delivery systems as immonopotentiation is an important basis for their use in vaccines. The immunobiochemical mechanisms of the adjuvant property of certain delivery vehicles that are currently being studied or used have recently been reviewed [88-91]. In general, delivery vehicles target an antigen (vaccine) to the immune inductive sites to ensure that an appropriate lymphoid location drains the antigen for immune activation, initiates the Toll-like receptor (TLR) pathway that induces the maturation of antigen-presenting cells (APCs), such as dendritic cells (DCs), to process and present the antigen, and induces an ambient chemokine and cytokine environment that promotes the delivery of Signal 1 in the T-cell activation cascade. In addition, effective delivery vehicles activate an adequate costimulation to ensure that Signal 2 is available for complete and full activation of the immune system. Other desirable features of potentially safe and effective delivery vehicles include the ability to support the delivery of a subunit or multisubunit vaccine where necessary, and be devoid of toxicity. As shown in **Table 1** only alum is currently approved and licenced for human use in the US in the delivery categories shown. Most of the other delivery vehicles (e.g., the DNA, detergent-based vehicles, such as immune-stimulating complexes [ISCOMs], liposomes, the RIBI adjuvants, mutant toxins, live viral and cellular vectors), including the human compatible Montanide series and lipopeptides, have not obtained licensure for human use in the US. It is, therefore, a general challenge of vaccinology to develop universally acceptable and human-compatible delivery systems to enhance the design of vaccines against various infectious diseases and tumours.

2. Delivery systems useful for *Chlamydia* vaccine design

The development of safe and effective delivery vehicles, such as adjuvants and vectors, or biological manipulations capable of boosting T_H1 response that is targeted to the genital or ocular mucosa [62], is a major goal in chlamydial research. It is important to note that all vaccines targeted for protection against *Chlamydia* are at their experimental stages at present. Therefore, all delivery methods developed for *Chlamydia* vaccines are also experimental. The working hypothesis guiding the design of *Chlamydia* vaccine regimens is that the design and delivery of *Chlamydia* vaccines will require immunobiochemical strategies that optimise a T_H1 response and the accessory humoral immunity. Unfortunately, the vast majority of the delivery vehicles or adjuvants that have been used to deliver chlamydial antigens so far have produced mixed results in various animal models of experimental chlamydial infections [83].

As presented in **Table 2**, the current chlamydial vaccine delivery strategies include live or nonliving viral or bacterial vectors, DNA with or without cytokine genes, CpG-rich oligonucleotides, complete Freund's adjuvant (CFA), ISCOMs and DC-based cellular delivery. Interestingly, only the IL-10 knockout (IL-10KO) DC-based cellular vaccine produces a sterilising, long-term immunity against *Chlamydia* in a mouse genital infection model [92]. Unlike tumour vaccine approaches, the cellular vaccine approach may be of less practical application when considering such a widespread infection as *Chlamydia*; however, this protection system furnishes a benchmark for evaluating other potential vaccines, and its further analysis may guide vaccinologists to design a more effective delivery vehicle for *Chlamydia* vaccines. In addition, the efficacy of the system reassures us that given the optimal conditions, a protective vaccine is possible against *Chlamydia*. Certain experimental vaccine delivery systems that have either been used in experimental chlamydial vaccine design or hold promise for potential use in a future vaccine are described in Section 2.1.

2.1 Experimental vaccine delivery systems

2.1.1 Vectors and adjuvants

Vector-mediated immunisation with naked DNA has recently received a great deal of attention [69,93-99], with delivery of *MOMP* and heat shock protein 60 genes showing some of the most significant promise [95,100]; however, this approach has been mostly successful in the murine lung model, but not the genital tract model [101]. Although it is unlikely that immunity

Table 1. Common delivery systems used in experimental and clinical vaccines.

Delivery system	Role/function	Approval for human use
Chemical/lipid-based adjuvants		
Alum: AL(OH)3 gel	Adjuvant (weak/moderate)	Yes
Montanide series: natural oils plus mannide monooleate as emulsifier	Adjuvant	No (human compatible)
Lipopeptides/admixture	Adjuvant	No (human compatible)
IFA (water in mineral oil)	Adjuvant	No (or uncertain)
Liposomes	Adjuvant	Uncertain
Detergent-based: ISCOMs, QS21	Adjuvant	No
Others: particulate delivery in CaPH or PLG; HSPs, cytokines, antibodies	Adjuvant/carrier	No
Microbial-related components		
RIBI adjuvants; monophosphoryl lipid A, muramyl dipeptides, CpG-rich oligonucleotides, CFA, mutant toxins (LT, CT)	Adjuvant	No
Viral/bacterial vectors		
Live: vaccinia, adenovirus, Salmonella, Listeria, BCG, canarypox virus	Adjuvant/carrier	No
Nonliving: bacterial ghosts; VLPs	Adjuvant/carrier	No
Cellular delivery		
APCs, DCs	Costimulation/antigen presentation/ adjuvant	No
DNA/RNA		
Expression DNA plasmid or RNA	Carrier/adjuvant	No

APC: Antigen-presenting cell; BCG: Bacillus Calmette-Guérin; CFA: Complete Freund's adjuvant; CT: Cholera toxin; DC: Dendritic cell; HSP: Heat shock protein; IFA: Incomplete Freund's adjuvant; ISCOM: Immune-stimulating complex; LT: *Escherichia coli* heat labile toxin; PLG: Poly-dl-lactide-co-glycolide; QS21: Quillaja saponaria 21; VLP: Virus-like particle.

Table 2. Delivery systems used in *Chlamydia* vaccine research.

Delivery system	Degree of protective immunity achieved	Ref.
Viral/bacterial vectors		
Live: poliovirus, vaccinia	Partial	[106,107]
Nonliving: bacterial ghosts	Partial	[119]
Cellular delivery		
APCs, DC	Partial (wild-type DC) Sterilising (IL-10 knockout DC)	[120] [92]
Immunomodulation via cytokines, antibodies etc.	Partial Possibly sterilising	[105] [126,127]
Detergent-based		
ISCOMs	Partial	[69,146]
Microbial-related components		
CpG-rich oligonucleotides, ospA, CT, CFA	Partial	[113,114]
DNA		
Expression plasmid	Partial	[54,76,78,95,101]

APC: Antigen-presenting cell; CFA: Complete Freund's adjuvant; CT: Cholera toxin; DC: Dendritic cell; ISCOM: Immune-stimulating complex.

to chlamydiae at different anatomical sites involves distinct mechanisms, it may reflect differences in cellular targets of infection (i.e., macrophages versus epithelial cells) in these locations, and emphasises the use of appropriate models in studying immunity and designing vaccines particularly against a pathogen with multiple infection targets. The use of DNA vectors in a prime–boost strategy, involving the sequential delivery of MOMP with DNA and ISCOMs, was also promising [69]. Using a DNA or vector delivery strategy, genes encoding candidate vaccines can be fused with T_H1 enhancers, such as immunostimulatory CpG motifs [102], or specific APC-targeting domains, such as the ligands for the costimulatory B7 [103], CD40 [104], or genes expressing specific chemokines or cytokines [105]. At present the DNA vaccine strategy constitutes a highly useful tool for rapid screening for potential vaccine candidates in experimental models [76], pending the alleviation of DNA integration and toxicity concerns. In addition, the use of recombinant viral vectors as delivery systems for chlamydial vaccines deserves serious consideration beyond earlier reports of recombinant poliovirus hybrid constructs harbouring chlamydial sequences [106] and recent experimentation with recombinant vaccinia virus [107]. Noninfectious adenovirus [108], canarypox virus [109], vaccinia virus [110] and alphavirus replicons [111] are some of the well-characterised viral delivery systems with potential in chlamydial vaccine design. In addition, experimental mucosal adjuvants, including cholera toxin, heat-labile enterotoxin, mutant toxin (LTK63 and LTR7), polymerised liposomes, microparticles and interleukins, are other potential delivery strategies for chlamydial vaccines [112]. The development of effective and acceptable adjuvants still constitutes a major challenge in chlamydial vaccine research. Recent reports of partial protective immunity by delivering MOMP with CpG, or CFA [113] or *Borrelia burgdoferi* ospA [114] may also suggest that these adjuvants should be evaluated with a multi-subunit construct that will deliver more epitopes to the immune system. Furthermore, it is likely that the ideal delivery systems for efficacious *Chlamydia* vaccines would have the capacity to harbour a multisubunit vaccine, induce an adequate cytokine and chemokine environment, boost the costimulation of the APCs, and be administered via an appropriate route that fosters the activation of a high frequency of T_H1 effectors as well as the complementary antibody response.

2.1.2 Bacterial delivery systems

Live attenuated bacteria, such as the *Lactobacillus* [115], *Salmonella* and *Listeria* [116] systems, are promising delivery vehicles that should be explored in chlamydial vaccine research. In fact, as part of a normal vaginal flora and integrity, the generation of recombinant *Lactobacillus*-expressing genes encoding multiple vaccine proteins for several STD agents and T_H1 chemokines under the control of a conditionally responsive promoter that initiates expression, during high oestrogen levels for example, would constitute a reliable vaccine strategy against STDs. In addition, nonliving bacterial delivery systems, such as recombinant bacterial ghosts, have the potential to direct the immune response against multiple antigens [117]. Bacterial ghosts are devoid of cytoplasmic contents while maintaining their native surface antigenic structures and cellular morphology. In the novel recombinant bacterial ghost vaccine strategy, an appropriate bacterium is transformed with the gene of interest to express high levels of the antigen in a targeted location on the cell, and ghosts are produced by controlled lysis of the cells. Certain bacterial ghost preparations possess intrinsic adjuvant properties that boost immune responses against the antigen expressed. Multiple genes can be expressed on ghosts in a deliberately controlled manner to achieve high levels of an antigen or different antigens from the same or different pathogens, which can be presented to the immune system simultaneously to produce effective combination or multi-component vaccines against multiple agents [117,118]. Recent efforts in designing experimental recombinant bacterial ghost vaccines, by expressing select chlamydial proteins on the epitheliotropic *Vibrio cholerae* ghosts (VCGs), have shown promise in inducing mucosal immunity in a mouse genital infection model [119]. Recombinant VCGs are safe, with relatively easy and cheap production, which offers a technological and manufacturing advantage for a vaccine needed on a global scale.

2.1.3 Cellular vaccine delivery

Immunisation regimens that include the use of *ex vivo* antigen-pulsed DCs to deliver and present chlamydia antigens *in vivo* have produced some of the most promising experimental protection studies so far [92,120]. Chlamydial-pulsed DCs appear to possess the necessary antigenic, costimulatory and immunomodulatory features for inducing high levels of T_H1 response and the accessory IgA and IgG effectors required for optimal protective immunity against *Chlamydia* [92,120]. Whereas the use of wild-type DCs produced a partial but significant protection against genital chlamydial infection, the application of the IL-10KO DC achieved a sterilising, long-term protective immunity that correlated with the capacity to induce a high frequency of specific T_H1 cells and elevated titres of the CMI-associated IgG2a and IgA antibodies [50,92]. It has been suggested that this high efficacy of the DC-based cellular vaccine makes them natural adjuvants or pre-eminent delivery vehicles, which are useful as tools to guide the design of effective delivery systems that mimic the action of DCs, for immunising against chlamydial infections, and to unravel the necessary vaccine machinery in terms of antigens, delivery, immunity and homing requirements [118,121]. In fact, DC-based cellular vaccines have shown that, given an effective delivery vehicle, inactivated chlamydial elementary bodies possess sufficient immunogenic epitopes to elicit a protective immunity against *Chlamydia*. The challenge for vaccinology, therefore, is to develop a delivery system that will mimic the superior immunostimulatory properties of DCs to achieve an effective chlamydial vaccine. Furthermore, the ability to confer genus-specific immunity would obviously be preferred as previous

studies have hinted that chlamydial immunity may be serovar- or genus-specific [122,123].

2.1.4 *Fc receptor-mediated delivery of antigen*

The ability of Fc receptor (FcR)-mediated uptake of antigen to induce a rapid and high level T-cell response by using specific antibodies has suggested that FcR-mediated delivery of antigen could potentially constitute an effective adjuvant system in a vaccination strategy to boost protective immunity against pathogens or tumours [124,125]. The authors recently demonstrated that this approach is valid for activating an elevated T_H1 response against *Chlamydia* [126] and that it requires specific antibodies of the IgG2a and IgA isotypes [127]. FcR delivery of antigen using specific antibody isotypes is, therefore, an additional vaccine strategy to boost protective immunity against chlamydia. The optimisation of this delivery system may require the use of specific antibody isotypes, which would benefit from the readily available monoclonal antibody production technology. In addition, the immunobiochemical mechanism by which a monoclonal anti-idiotypic antibody, a molecular mimic of the chlamydial glycolipid exoantigen, protect against chlamydial infection [128,129] and the role of FcRs in its effect are yet to be determined.

2.2 Vaccine delivery using agonists that activate the innate immune response

It is now appreciated that adjuvants function at least partly by stimulating the innate immune response, including selectively modifying the cytokine and chemokine environment, activate TLRs or upregulate co-stimulatory molecules on APCs. Thus, the recognition that certain non-toxic and human compatible lipid structures act as powerful adjuvants by binding to and activating specific TLRs on APCs such as DCs [130] has led to the design of lipopeptides as potential vaccines [131]. Lipopeptide vaccine constructs produce effective protective immunity against microbial pathogens (viral and bacterial), and tumours, which was characterised by the elicitation of elevated T-cell and antibody responses [132]. As certain lipid structures selectively target T_H1- or T_H2-associated TLRs, their application in designing peptide vaccines against *Chlamydia* may lead to interesting results.

2.3 Delivery systems for targeting and optimising mucosal immunity

The induction of mucosal immunity by a vaccine is required for protective immunity against *Chlamydia*. Therefore, effective vaccine delivery may require practical immunomodulatory techniques relating to a choice of appropriate routes of vaccine administration that optimise relevant mucosal immune response. Modern vaccinology strives towards the exploitation of the cooperative interaction between immune inductive sites (i.e., draining lymphoid tissue[s] containing the primary APCs, such as DCs, where an immune response is initiated) and immune effector sites (e.g., site of infection) to produce an optimal vaccine efficacy. Using this approach, the induction of optimal mucosal immunity requires the targeting of antigens to the specialised APCs of the mucosa-associated lymphoid tissues (MALT) in specific mucosal inductive sites [133]. MALT includes the nasal-associated lymphoid tissue (NALT), gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT). As the inductive and effector sites of the common mucosal immune system (CMIS) are compartmentalised, certain inductive and effector sites interact effectively to produce an optimal immune response [133]; hence, during vaccine delivery it is important to select a route of immunisation that favours an effective cooperation between a given mucosal inductive site and a targeted mucosal effector site of infection.

To optimise chlamydial vaccine efficacy, it is crucial to both develop mucosal-compatible delivery vehicles and select a route of administration that targets the inductive sites that produce high levels of T_H1 response in bronchial and oculogenital mucosae. Systemic immunisation routes are not effective for inducing significant protective immunity in mucosal tissues [134,135]. Intranasal immunisation with live chlamydiae or acellular outer membrane complex preparation induced protective chlamydial immunity [136,137], which correlated with rapid elicitation of a genital mucosal T_H1 response and the CMI-associated IgG2a and secretory IgA [138]. In addition, intranasal delivery of an experimental DNA-based vaccine protected against *C. pneumoniae* in a lung infection model [93]. Nasal immunisation caused rapid generation of effector lymphocytes detectable within days of exposure [139] and was superior to vaginal, gastric, peritoneal, or rectal immunisation for inducing mucosal anti-HIV or anti-herpes simplex viruse immune responses [140,141]. Therefore, in terms of compartmentalisation within a CMIS, there is a strong link between NALT, BALT and the genital mucosa [133]. The cellular and molecular basis for this cooperation involves, among others, adhesion molecules, cytokines and chemokines that direct cell trafficking by distinct homing pathways [142]. The specific biological processes include the induction and retention of T cells in the genital mucosa [143] via the $\alpha_4\beta_1$-vascular cell adhesion molecule and the $\alpha_4\beta_7$-mucosal addressin cell adhesion molecule in the leukocyte adhesion pathways [144,145]. In addition to intranasal delivery, the intramuscular route has been used successfully in experimental DNA immunisations against chlamydial respiratory infections [94]. Moreover, intramuscular delivery of MOMP-ISCOMS or VCGs expressing MOMP induced a genital mucosal T_H1 response and protected mice against genital challenge [119,146]. Intranasal and intramuscular routes are, therefore, well established vaccine delivery routes that lead to the induction of protective immunity against *Chlamydia*. Current vaccine delivery and testing should focus on these routes among others, including intravaginal delivery for vaccinating against genital infections [138]. A potential vaccine delivery strategy that could exploit the immune cooperation between NALT and the genital mucosa is the use of live attenuated respiratory viral vaccines as vectors for STD vaccines. Thus, it is conceivable for a future *Chlamydia* vaccine

Box 2. Lessons from experimental *Chlamydia* vaccine delivery.

- Protection induced by existing methods is partial

- Need to boost immune response during immunisation
- Need to increase the frequency of primed T cells

- Immunity is temporary

- Need to increase the frequency of primed cells
- Need to maintain local mucosal immunity

- Multisubunit vaccines or "special delivery" needed

- Need to deliver sufficient epitopes to induce high frequency of effectors
- Need to use more potent delivery systems and adjuvants

Box 3. Conditions favouring protective immunity.

- Rapid elicitation of high-level T_H1 response

- Leads to rapid clearance of *Chlamydia*
- Prevents ascending infection (genital)
- Prevents persistant infection
- Eliminates injurious antigens and chronic inflammation
- Prevents immunopathology

- Role of chlamydial antigens and individual health status and genetics

T_H1: T helper cell type 1.

regimen to be delivered with the live attenuated cold-adapted influenza virus vaccine [147,148] as the vector. The continued progress in influenza virus genetics and the recent report of methodologies for expressing foreign genes in these viruses [149] is likely to facilitate the use of the vaccine strain (already licenced for human use) as a vector for delivering vaccines against certain STD agents, including *Chlamydia*.

3. Expert opinion and conclusion

The development of effective, safe and practical delivery systems and adjuvants remains an important objective in chlamydial vaccine research. The lessons from experimental vaccine designs using current approaches reveal that the delivery of a potentially efficacious chlamydial vaccine requires a vehicle with a strong mucosal adjuvant property to boost immune response and increase the frequency of primed effector cells (**Box 2**).

The high frequency of primed immune effectors is required for long-term immunity. Moreover, specialised delivery such as the protective experimental system provided by the IL-10KO DC cellular delivery, or the lipopeptide design strategy may be required to achieve an efficacious *Chlamydia* vaccine. With

Box 4. Requirements for inducing protective immunity.

- Antigens/vaccine with sufficient T_H1 epitopes

- Multiple subunits; attenuated strains (some concerns)

- Efficient delivery, processing/presentation

- Specific antibodies (e.g., IgG2a)
- Other delivery vehicles and adjuvants; immunomodulators

- Adequate costimulation

- Upregulation of ICAM-1, B7, CD40 etc.
- Use of immunomodulators: IL-12, IL-1, chemokines etc.

- Induction of high frequency T_H1 effectors and the complementary antibodies

ICAM: Intracellular adhesion molecule; T_H1: T helper cell type 1.

respect to the antigenic requirement, a multisubunit approach seems best for the purpose of furnishing more epitopes to the immune system. If the IL-10KO DC cellular vaccine is named as a reference experimental vaccine, further analysis of the system could lead to defining the essential requirements of a practical delivery system for a human *Chlamydia* vaccine. Furthermore, as *Chlamydia* may be capable of inducing both protective and immunopathological immune responses, a challenge in chlamydial vaccinology is to clearly define the vaccination or delivery conditions that favour either response in order to skew responses against vaccines to the favourable protective pathway (**Box 3**).

In this respect, the recent finding from the experimental IL-10KO DC-based cellular vaccine [92] would suggest that vaccine delivery that produces a fast and vigorous T_H1 response after an infection will rapidly arrest chlamydial replication, clear the infection, eliminate residual antigens and prevent the establishment of a latent infection. On the other hand, an inadequate or suboptimal T_H1 response delays clearance of the pathogen, and may lead to the establishment of a latent or persistent infection, which fuels a low-grade chronic immune response that causes tissue damage. This proposition is supported by other findings in experimental chlamydial infection, as recently reviewed [50].

As shown in **Box 4**, the requirements of a vaccine to induce protective immunity against *Chlamydia* include the possession of adequate T_H1 and T_H2 epitopes, effective delivery to enhance antigen presentation, the ability to produce adequate costimulatory signals and potent adjuvanticity. The ultimate requirement is the induction of a high frequency of T_H1 cells with other CMI and humoral immune effectors. Guided by this paradigm, Igietseme *et al.* have so far designed and delivered four experimental *Chlamydi*a vaccines that have yielded encouraging results.

In conclusion, the ideal delivery systems for efficacious *Chlamydia* vaccine would have the capacity to harbour a

multi-subunit vaccine, induce an adequate cytokine and chemokine environment, boost the costimulation of the APCs, and be administered via an appropriate route that fosters the activation of a high frequency of T_H1 effectors as well as the complementary antibody response. More research efforts and support are needed to broaden the frontiers of our knowledge of *Chlamydia* biology, mechanisms of host defence against *Chlamydia* and modern vaccinology approaches to boosting protective immunity. Research areas of particular interest include a better understanding of immunoregulation and immunomodulation of host immunity against *Chlamydia*, genomic, proteomic and bioinformatic approaches to the identification of stable vaccine candidates, and the development of effective delivery systems and potent adjuvants, all of which will furnish the blueprints for designing an efficacious chlamydial vaccine. In this respect, further molecular analysis of the delivery apparatus furnished by the highly efficacious IL-10KO DC cellular vaccine system using proteomics and other functional methodologies may guide vaccinologists to design a more effective delivery vehicle for *Chlamydia* vaccines. Furthermore, the need to better define the level of immune response that is adequate for protective immunity and devoid of deleterious effects should also be determined to achieve a safe and reliable *Chlamydia* vaccine. Finally, because even a partially protective vaccine has the potential to make a remarkable impact on global infection, a relatively safe chlamydial vaccine that prevents the most serious complications of the infection would deserve the opportunity for clinical trials beyond the current laboratory status of most vaccine designs.

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