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REVIEW

Chlamydia trachomatis: quest for an eye-opening vaccine breakthrough

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ABSTRACT

Introduction: *Chlamydia trachomatis,* commonly referred to as chlamydia (a bacterium), is a common sexually transmitted infection, and if attended to early, it can be treatable. However, if left untreated it can lead to serious consequences. *C. trachomatis* infects both females and males although its occurrence in females is more common, and it can spread to the eyes causing disease and in some case blindness.

Area covered: With ongoing attempts in the most impoverished regions of the country, trachoma will be eradicated as a blinding disease by the year 2022. A prophylactic vaccine candidate with established safety and efficacy is a cogent tool to achieve this goal. This manuscript covers the vaccine development programs for chlamydial infection.

Expert opinion: Currently, the Surgery Antibiotics Facial Environmental (SAFE) program is being implemented in endemic countries in order to reduce transmission and control of the disease. Vaccines have been shown over the years to protect against infectious diseases. Charge variant-based adjuvant can also be used for the successful delivery of chlamydial specific antigen for efficient vaccine delivery through nano delivery platform. Thus, a vaccine against *C. trachomatis* would be of great public health benefit.

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1. Introduction

C. trachomatis is an obligate, gram-negative intracellular bacterium that causes disease in the eyes and genital tracts of humans [1] (Table 1). Infection of the genital tract occurs via sexual transmission and is a leading cause of pelvic inflammatory disease worldwide. In addition, repeated *C. trachomatis* infection may lead to tubal factor infertility and ectopic pregnancy in women [2]. Infection of the eyes leads to a disease known as trachoma, which is spread via direct and indirect contact with infected ocular and nasal discharge; trachoma often spreads via contaminated flies and bedding [3]. The disease is the leading cause of infectious blindness worldwide and is characterized by repeated infection of the conjunctiva with particular *C. trachomatis* strains [4]. This repeated infection leads to scarring of the conjunctiva and trichiasis, the abrasive rubbing action of which may cause damage to the cornea and, eventually, blindness [3,4].

According to the World Health Organization (WHO), trachoma is responsible for blindness of approximately 1.9 million people. According to data collected in March 2020, 137 million people live in trachoma-endemic areas [5]. The disease is a public health concern in 44 countries and is on the WHO's list of neglected tropical diseases, a list of 20 diseases and conditions, which the foundation has identified as being neglected by local governments and healthcare authorities [6]. In certain trachoma-endemic areas, infection rates are high among pre-school aged children [7]. In addition, the economic burden is significant, with the economic cost of lost productivity from trachoma and trachoma caused sight-impairment and blindness at an estimated USD $8 Billion annually [5,8].

1.1. C. trachomatis *life cycle*

C. trachomatis has a life cycle with two distinct phases: (i) the infectious elementary body phase and (ii) the replicative reticulate body phase [9] (Figure 1). *C. trachomatis* enters the body and while in the infectious stage it is known as the elementary body; previously thought to be metabolically inactive, this phase involves some biosynthesis and metabolic activity [10]. Principally, the infective stage encompasses entrance into the host via the eyes or genital tract, and enters the cytoplasm of the host cell, via endocytosis and combination of vacuoles form an intracytoplasmic inclusion [10,11]. It is in these inclusions that *C. trachomatis* transform into reticulate bodies and replicate by binary fission – the second phase of *C. trachomatis* life cycle. In this stage, *C. trachomatis* utilizes nutrients and adenosine triphosphate from the host cytoplasm, and when these resources run out, the reticulate bodies transform back into elementary bodies and are secreted into the extracellular environment to infect neighboring cells [12].

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Article highlights

- *Chlamydia trachomatis* is a gram-negative bacteria that causes disease in the eyes and genital tracts of humans
- The major outer membrane proteins (MOMP) have been recognized as antigenic targets and are being considered in vaccine design
- The SAFE program is designed to reduce and eliminate disease
- A number of vaccine candidates show promise in pre-clinical animal studies
- The inability of routine screening has led to the professional opinion that an effective vaccine will be the better method of controlling the myriad of chlamydia-caused ocular, genital, and respiratory diseases
- A vaccine containing a version of a MOMP mixed with CAF01 or aluminum hydroxide adjuvant, stimulated antibodies, and mucosal immune responses is in a phase 1 human clinical trial

1.2. Treatment – management

There are very few treatment options for trachoma that are limited to antibiotics, in particular azithromycin, and surgery for trichiasis [13]. Preventative measures include mass distribution of azithromycin and hygiene education and implementation, including reduced face touching [14]. Currently, the WHO 'SAFE' program (**S**urgery **A**ntibiotics **F**acial, **E**nvironmental) is being implemented in endemic countries in order to prevent and control the spread of trachoma [5]. The SAFE program involves: Surgery for treatment of blinding, Antibiotics for the infectious stage, Facial cleanliness and Environmental hygiene, in particular sanitation and clean water. The SAFE program was implemented in 1993 with as a means of eradicating trachoma by 2020; however, this was not achieved [15]; a new target date has now been set to 2030. In the Amhara region of Ethiopia, 124 million doses of azithromycin were distributed between 1997 and 2015, despite these efforts trachoma remained highly prevalent in the region [16]. In Senegal, 1613 children, less than 9 years, were assessed by questionnaires for facial cleanliness in order to determine factors to reduce infection rates of the eye. It was noted that a high number of children did not have clean faces throughout the day [17]. In order for the SAFE strategy to be successful, it needs to rely on the continued support and coordination of a number of contributors, including local governments of endemic areas, manufacturers of azithromycin, and the WHO [5,18].

The goals set, especially for sanitation and clean water, require not only sanitation development but also socioeconomic development. Successful deployment of the strategy as a whole will continue to involve epidemiological surveys, monitoring and surveillance, resource mobilization, and regular evaluation of the outcomes [18]. A vaccine against *C. trachomatis* would offer an alternative management of the disease. Rather than continuous antibiotic administration, a vaccine could be administered, which would give protection against infection and disease.

2. Genetic trait and their involvement

The mechanisms underlying persistent immunopathology in the absence of clinical conjunctival *C. trachomatis* infection are likely to be multifactorial [19]. There is strong evidence to suggest that an individual's genetic traits play a key role in the individual's immune response to *C. trachomatis*, both protective and immunopathological, thereby governing disease progression. Assessing the characteristics of these immune responses is critical for infection comprehension and vaccine engineering efficiency [20]. The particular genotype and antigen expression of the human leukocyte antigen plays a key role in the magnitude of conjunctival scarring [21]. Further studies reveal that genetic modification of matrix metalloproteinase 9 is the contributing factor for activating trachoma risk haplotype; this leads to increased interleukin 10 (IL10) transcription [22]. With the application of whole-genome sequencing and next-generation sequencing, scientists were able to identify the polymorphic outer membrane protein family (Pmps) as a predominant virulence factor of chlamydial infection [23]. A toxin produced by the chlamydial plasticity zone is responsible for the interferon gamma (IFNý) resistance of *C. trachomatis* and is therefore a target for extensive genetic variation detection and documentation [24,25]. In addition, genetic variation of the type III secretion system (T3SS) leads to a change in the pathogenic ability of the bacteria and its virulence propensity [26]. Infection-induced epigenetic changes, related to the innate immune response, cause changes in histone methylation, which results in hyper-inflammation within the host conjunctival epithelium cells [27,28]. Recent studies also reveal the involvement of the micro RNA (miRNA; miR-146, miR-155, miR-125, let-7, and miR-21) in the pathogenesis and subsequent inflammatory reaction regulations [29]. Polymorphic changes in such miRNA leads to chronic inflammation and fibrotic proliferation. The epigenetic regulation of stage-specific host genes' including mRNAs, lncRNAs, and miRNAs, controls the perennial regulation of essential signaling pathways required for pathogen survival [30]. The gene-by-gene framework has been developed to a well-defined

Table 1. Various types of *C. trachomatis* infections and their causal serovars.

Types of C. trachomatis Infection			
Causative Organism	Affected Organ(s)	Causal Serovar(s)	Mode of Transmission
Trachoma	Eyes	A to C	Direct or indirect contact with nasal and ocular discharge
Chlamydia	Genital tract	D to K	Sexual transmission
Lymphogranuloma Venereum	Lymph nodes	L1 to L3	Sexual transmission

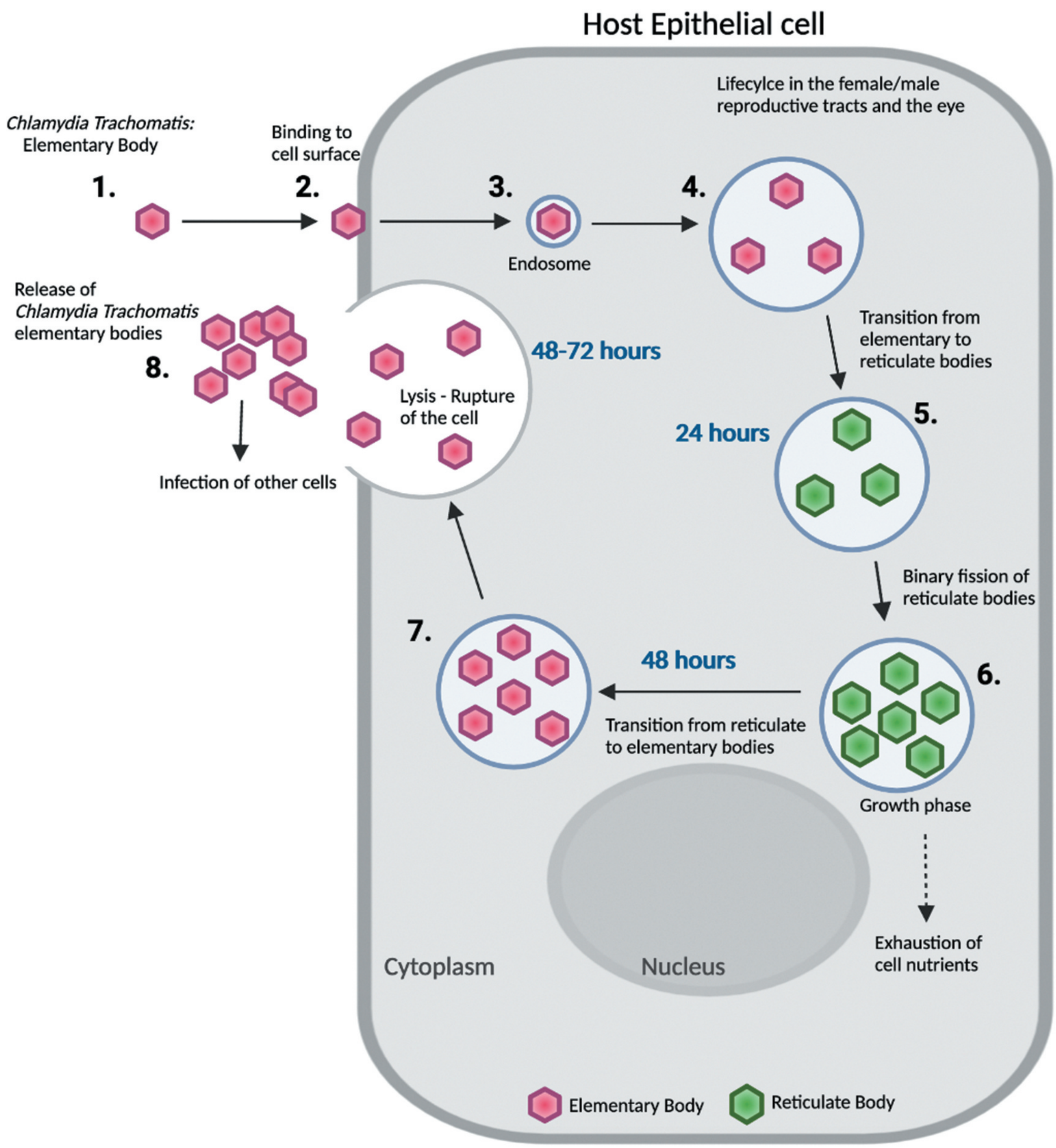

Figure 1. **Lifecycle of C. trachomatis includes the transformation of two distinct forms – the infectious elementary body and the intracellular reticulate body**. 1. Elementary body. 2. The infectious elementary body binds to the cell wall. 3. The elementary body is endocytosed and is enveloped by an endocytic vacuole inside the cell. 4. Multiple endosomes combine to form an intracytoplasmic inclusion. 5. The elementary bodies transform into noninfectious reticulate bodies. 6. Binary fission of reticulate bodies, growth phase. 7. Once the cytoplasm runs out of nutrients for reproduction, the reticulate bodies transform back into elementary bodies. 8. The elementary bodies are released from the epithelial cell by reversed endocytosis, lysis of cell membrane. Elementary bodies infect other cells.

population with established epidemiological and clinical datasets, as this will improve our understanding of *C. trachomatis* transmission and disease.

3. Clinical significance and immune response

The host immune response to *C. trachomatis* infection provides incomplete protection against subsequent infection. Human volunteer studies of trachoma, conducted over 40 years ago, have provided evidence of partial immunity against specific serovars of *C. trachomatis*; when rechallenged with the same serovar that they were previously infected with, less severe disease resulted [20]. However, protection against different serovars of *C. trachomatis* was not observed. In addition, complete protection against disease was rarely observed. Current ethical considerations prevent similar studies from being conducted in the present day [20,31,32].

Chlamydial elemental bodies are recognized by the hosts' innate immune system within the extracellular matrix by toll-like receptors (TLR), especially TLR2 and TLR4 [33]. All components of the innate immune system, including phagocytic and epithelial cells, bind to pathogen-associated molecular patterns (PAMPS) on the surface of the elemental bodies, which subsequently initiates the release of pro-inflammatory cytokines and chemokines [33,34]. In addition, PAMPS on the elemental bodies are recognized by the cytoplasmic pattern recognition receptor

Table 2. Some important human vaccines under development (2017 onwards) against chlamydial infection.

Antigen Type	Study Outline	Route of Administration	Stage of Development	Reference
Whole cell	Immunization with live *C. trachomatis* D	IN	Preclinical – HLA-DR4 mice and WT C57BL/6 mice	[48]
	Viable (live) *C. muridarum* elementary bodies used for immunization at a dose of 1×10^6 IFU	Per-oral	Preclinical – C57BL/6 mice	[67]
	Killed (inactivated) *C. trachomatis* elementary bodies used for immunization at a dose of 1×10^7 IFU	Per-oral	Preclinical – C57BL/6 mice	[67]
	C. muridarum elementary bodies (CMmCherryG5) oral vaccination to induce transmucosal airway protection	Oral	Preclinical – C57BL/6 J mice	[68]
Protein	Effect of CTH522 antigen with CAF01 adjuvant	IM + IN	Phase I clinical trial	NCT02787109
	Effect of CTH522 antigen with aluminum hydroxide adjuvant	IM + IN	Phase I clinical trial	NCT02787109
	Native MOMP with cationic liposomal adjuvants CAF01 or CAF09	SC + IN	Preclinical – BALB/c mice	[69]
	Recombinant MOMP with cationic liposomal adjuvants CAF01 or CAF09	SC + IN	Preclinical – BALB/c mice	[69]
	Effect of polymorphic membrane proteins (PMPs) with adjuvants CpG-1826 and Montanide ISA 720	IN	Preclinical – BALB/c mice	[70]
	MOMP based CTH522 antigen with adjuvant CAF01	SC	Preclinical – B6C3F1 hybrid mice	[50]
	MOMP based CTH522:CTH93 (1:1) antigen with adjuvant CAF01	3 SC + 2 IN	Preclinical – B6C3F1 mice	[71]
	Multivalent Hirep1 vaccine construct, based on MOMP variable domain (VD) 4 regions of *C. trachomatis* serovar D-F, with adjuvant CAF01	SC + IN	Preclinical – B6C3F1 mice	[49]
	Multi-epitope peptide of $MOMP_{370\text{-}387}$ and hepatitis B virus core antigen (HBcAg) as *C. trachomatis* vaccine candidate	SC	Preclinical – BALB/c mice	[72]
	Immuno-repeat strategy utilized to design and test immunogenicity potential of MOMP VD1 based extended regions $ExtVD1^{A}*4$ and $ExtVD1^{J}*4$	SC, SC + IN	Preclinical – A/J and C3H/HeN mice	[73]
	75 proteins from Chlamydial outer membrane complex (COMC) compared to individual and combination of recombinant outer membrane proteins (OMPs) formulated with Th1 polarizing adjuvant DDA/MPL	SC	Preclinical – C57BL/6 mice	[74]
	Mucosal administration of CTH522 using adjuvant composed of glycol chitosan coated lipid nanoparticles	IN	Preclinical – C57BL/6 mice	[75]
	Antigen Hirep2 anchored to the surface of Lactobacillus plantarum (used as a vector) co-administered with CAF01 adjuvant	SC + IN	Preclinical – B6C3F1 mice	[76]
	5 component subunit vaccine composed of 4 recombinant Pmp family members and Ctad1 from *C. trachomatis* serovar E, in combination with mucosal adjuvant cyclic-di-adenosine monophosphate	IN	Preclinical – C57BL/6 J mice	[77]
	Peptide mimic of chlamydial glycolipid antigen – Peptide 4 conjugated using an ester bond to generation 4 hydroxyl terminated PAMAM dendrimer used as a nanocarrier vaccine platform	SC	Preclinical – BALB/c mice	[78]
	Novel recombinant antigen BD584 administered with CpG as adjuvant	IN	Preclinical – C57BL/6, BALB/c, C3H/HeN mice	[79]
	Recombinant CPAF immunization administered with CpG deoxynucleotides as adjuvant	IN	Preclinical – Dunkin Hartly strain Guinea pigs	[80]
	C. trachomatis serovar E recombinant MOMP with aluminum hydroxide adjuvant SPA08 having lowest phosphate substitution	IM	Preclinical – outbreed CD-1 mice	[81]
	Chlamydial type III secretion system needle protein (T3SS) as a vaccine candidate against vaginal C. muridarum infection	IN + SC	Preclinical – BALB/c mice	[82]
	Efficacy of exosomes isolated from *C. muridarum* infected HeLa-299 cells with CpG-1826 and Montanide ISA 720 VG as adjuvants	IM + SC	Preclinical – BALB/c ($H\text{-}2^d$) mice	[83]
	Immunization with purified *C. muridarum* or *C. trachomatis* serovar D native MOMP	SC	Preclinical – C57BL/6 mice	[84]
	Recombinant VCG based chlamydial vaccine expressing PorB and N-terminal portion of PmpD from *C. trachomatis* serovar D	Rectal	Preclinical – C57BL/6 mice	[85]
	C. muridarum based PorB/VD antigen used in combination with CpG-1826 and Montanide ISA 720 VG as adjuvants	IM + SC	Preclinical – BALB/c ($H\text{-}2^d$) mice	[86]
	Immunogenicity of *C. trachomatis* serovar D derived MIP and Pgp3 (alone and combination) with CpG adjuvant against genital tract infection	IN	Preclinical – BALB/c mice	[87]
	Self-adjuvanting PLA-PEG nanoparticulate system encapsulating M278 peptide derived from chlamydial MOMP	SC	Preclinical – BALB/c mice	[88]
	Nanovaccine composed of chlamydial recombinant MOMP encapsulated in self-adjuvanting PLGA (85:15) nanoparticles	SC, IN	Preclinical – BALB/c mice	[89]

HLA: human leukocyte antigen; WT: wild type; IFU: inclusion-forming units; Hirep: heterologous immune-repeat DDA/MPL: dimethyldioctadecylammonium liposomes with monophosphoryl lipid-A; Pmp: polymorphic membrane proteins; CPAF: chlamydial protease like activity factor; PorB: porin B; VD: variable domains; MIP: macrophage inactivity factor; PLA-PEG: poly (lactic acid)-poly (ethylene glycol); PLGA: poly (D, L-lactide-co-glycolide) known as nucleotide-binding oligomerization domain protein 1 (NOD1), leading to proinflammatory gene activation. Following the host bodies initial recognition and phagocytosis of the bacteria, expression of discrete antigens on the cell surface allows for T- and B-cell activation and the generation of an antigen-specific humoral immune response [1].

The resolution of Ct infection is dependent on the Type-1 CD4 + T-helper lymphocyte (Th1), mediated by IFNγ. IFNγ has many anti-chlamydial actions, including the induction of expression of indoleamine-2,3-dyoxygenase (IDO) and up-regulation of inducible nitric oxide synthase (iNOS) [35]. IDO lowers the level of tryptophan in the extracellular environment, an amino acid that is crucial for chlamydial metabolism. iNOS may protect against chronic sequelae in murine models of disease. In addition, in vitro studies have shown IFNγ to decrease chlamydial growth via iron depletion [20,31,32].

4. Vaccines against *C. trachomatis*

Absence of an approved vaccine and the drawbacks of antibiotic treatment for Chlamydial infections has put pressure on scientists to develop a vaccine as soon as possible. Chlamydial infection affects the ocular and genital regions and is the second most prominent blindness causing disorder, beaten only by cataracts [36]. *C. trachomatis* is a very common sexually transmitted disease (STD) with an estimated 1 in 20 sexually active women in the age group of 14–24 years being infected, with an overall global infected population of 4 million in 2018, according to the CDC [37]. Therefore, generation of a protective immune response in cervical mucosa is considered a prime approach while devising vaccine candidates.

4.1. Preclinical evaluation of vaccine candidates

Preclinical development of vaccines requires establishment of suitable models in order to realize the full potential of vaccine candidates. A limitation of using animal models is the inherent difference between human pathophysiology and theirs, specifically in terms of immune system functioning. Major outer membrane proteins (MOMP) have been a highly recognized antigenic target and are used as a substitute for whole cell targets. MOMP's also have the ability to be combined as a systemic and mucosal vaccine strategy. While there has been extensive research conducted toward whole cell antigenic target vaccines in mouse models, their commercial viability with respect to replication potential is compromised [38]. The Chlamydial species *C. muridarum* and *C. caviae* have been used for research in mice and Guinea pig models, respectively [39]. Both mice and Guinea pigs have been determined as suitable surrogate models for Chlamydial research due to the similarity in their disease pathology with that of humans, also making them suitable to be used in challenge trials [40]. Mice are the most convenient models; *C. muridarum* is a mouse specific Chlamydia strain that has similar homology with that of *C. trachomatis* in humans [41]. However, the intensity of infection induced by *C. muridarum* is acute whereas that of *C. trachomatis* is known to be chronic, and the IFNγ mediated bacterial clearance mechanisms of both these species also differ, thereby making the comparison inaccurate [41,42]. When *C. trachomatis* is used for inducing vaginal infection in a mouse model, it does not lead to ascending genital tract infection and fallopian tube pathology as seen in humans, making the model imperfect [43]. Recently, a trans cervical method of infection was adopted to facilitate development of an ascending infection in uterus and fallopian tubes, allowing duplication of human infection conditions [38,43]. *C. trachomatis* and *C. suis* have been found to induce homo- and heterologous IFNγ $^{+}$TNF-α^{+}CD4 T cell based immune responses in pigs, indicating possibility of cross-protection between them and humans [44]. Therefore, pigs are an excellent animal model candidate for development of human chlamydial vaccine research [44]. *C. trachomatis* serovar D strain infection of sexually mature female Göttingen minipigs was performed using transcervical and direct vaginal inoculation and intrauterine inoculation during estrus and diestrus [45]. A significant IFNγ response specific to *C. trachomatis* was observed in minipigs inoculated during estrus but caused higher clearance of infections. Intrauterine inoculation during diestrus caused a long lasting infection (10 days) and a model that bypassed the cervix was considered optimal due to their ability to retain infection for a longer duration [45]. Preclinical trials have also been conducted in order to screen antigens producing the most effective immunogenic response. A recent trial was conducted in rhesus macaque monkeys whereby a primary *C. trachomatis* serovar D genital infection was induced in order to identify new immunodominant B-cell antigens [46]. Eight antigens including CT242 (OmpH-like protein), CT541 (mip), CT681 (ompA), CT381 (artJ), CT443 (omcB), CT119 (incA), CT486 (fliY), and CT110 (groEL), were found to meet the selection criteria and can therefore be used as a diagnostic tool to identify individuals with genital *C. trachomatis* infection. In addition, these antigens may also be helpful while designing vaccine against the bacteria [46,47]. Studies are also being conducted to determine a suitable animal model for evaluating vaccine candidates. In one such study, *C. trachomatis* serovar D (strain UW-3/Cx) was administered to human leukocyte antigen DR4 (HLA-DR4) mice and wild type (WT) C57BL/6 mice in an attempt to induce infertility [48]. The HLA-DR4 mice exhibited a higher susceptibility toward transcervical *C. trachomatis* infection whereas, WT mice showed robust humoral as well as cellular responses. A further 10^4 IFU (inclusion forming units) of *C. trachomatis* serovar D was delivered to the two mouse groups, intranasally, in a challenge test and both showed significant immune protection [48]. Variable domain (VD) 4 regions of *C. trachomatis* serovar D-F have been used to design a multivalent heterologous immune-repeat 1 (Hirep1) vaccine, where Hirep1 antibodies induced short and long term immune protection preventing establishment of infection in 48% of mice, emphasizing the role of antibodies in early protection ability of vaccines [49].

MOMP-based antigen CTH522 has been tested in several models, and recently it was tested along with adjuvant CAF01, following subcutaneous administration in 6–8 week old female B6C3F1 hybrid mice [50]. The observations indicated non-mucosal Th1 and Th2 cell mediated protection against *C. trachomatis* and its chronic pathology, and conferred a protective tissue-specific immunization memory in the genital tract. A vaccine containing antigen CTH522 (a version of a MOMP of *C. trachomatis*) along with adjuvants CAF01 liposomes or aluminum hydroxide was tested in a phase 1 first-in-human trial against *C. trachomatis* [28]. In order to induce both humoral and mucosal immunization, intramuscular injections at 0, 1 and 4 month intervals were followed by an intranasal booster (without adjuvant) at 4.5 and 5 months (NCT02787109). The primary outcome of the trial was establishing the safety of the vaccine followed by induction of humoral immune response, and CTH522 with CAF01 exhibited a better immunogenicity profile than CTH522 with aluminum hydroxide [28,50].

MOMP of *C. trachomatis* constitutes almost 60% of its outer mass and functions as an antigenic trimeric porin [51]. This role makes MOMP a fantastic potential vaccine candidate, which has been shown to generate novel MOMP-binding antibody in *C. trachomatis* infected HeLa229 cells; however, it is yet to reach preclinical trials. The roadmap to optimal clinical evaluation of vaccine candidates for Chlamydia would need definite clinical endpoints and identification of specific biomarkers in order to facilitate desired vaccine development [52].

4.2. Vaccines in human clinical trials

Vaccine attempts for the prophylactic treatment of *C. trachomatis* infections date back to 1913, when a group in Tunis initiated studies in non-human primates and humans [31]. Some resistance to reinfection was observed, but the results were largely inconclusive. After the isolation of Chlamydia organisms for the first time in 1957, four research groups began vaccine trials, ultimately yielding similar results to those obtained in 1913; some vaccine formulations offered protection for between 1 and 3 years, and those that did were serovar specific [32]. In addition, some immunized individuals developed more severe disease upon re-exposure to the pathogen, when compared with their control counterparts. Since these preliminary vaccine studies, no vaccine has been approved for human use [15]. One major difficulty in the development of a Chlamydial vaccine has been finding an appropriate animal model for animal studies.

Furthermore, human studies conducted in the 1960s have provided evidence of partial immunity against specific serovars of *C. trachomatis*; when rechallenged with the same serovar that they were previously infected with, less severe disease resulted [53]. However, protection against different serovars of *C. trachomatis* was not noted. In addition, complete protection against disease was rarely observed. Using the whole organism of *C. trachomatis* against ocular infection only provided short-term infection (<2 years) [54,55]. In addition, large placebo-controlled clinical trials conducted in the 1960s in Taiwan, The Gambia, Saudi Arabia, and India using whole-killed bacteria showed no difference in active trachoma although lower bacterial load (amount of chlamydial inclusions in conjunctival scapings) [56]. In Taiwan, formalin inactivated aluminum absorbed *C. trachomatis* given to pre-school children showed less active trachoma but no protection after 1 year. Another bivalent vaccine of dead vaccine in mineral oil reduced the incidence of active trachoma, but the reduction was not significant [57]. In Gambia, live vaccine formulations of *C. trachomatis* were injected in children with active signs of trachoma and showed clinical improvement 4 months after the final injection; however, the protective effect was no longer evident after 1 year [58]. In parallel, clinical trials were conducted in India using formalin inactivated vaccine in children under 5 years of age with no clinical signs of trachoma and 1 year later those that had received the vaccine 14% developed trachoma compared to 37% in the placebo group; however, the severity of disease did not differ in vaccinated vs non-vaccinated group [56,59,60].

These active human clinical trial studies in the 1960s efforts have been invested to develop highly immunogenic subunit vaccine formulations against *C. trachomatis*. Thus far, only one has shown to reduce bacterial load in non-human primates using MOMP protein [61]. As such, in the modern era, the first human phase I clinical trial was conducted in 2017 and published in 2019. The study was a double-blind, parallel, randomized, placebo-controlled trial in healthy females. The vaccine comprised of antigen CTH522 (a version of a MOMP of *C. trachomatis*) mixed with CAF01 or aluminum hydroxide [62]. In order to induce both humoral and mucosal immunity, intramuscular injections at 0, 1 and 4 month intervals were followed by an intranasal booster (without adjuvant) at 4.5 and 5 months (clinical trials registry number NCT02787109). The vaccine was shown to be well tolerated and both vaccine formulations induced humoral immune responses, with CAF01 exhibited a better immunogenicity profile compared to aluminum hydroxide adjuvant [50,62]. This study is important as it paves the way for further vaccine consideration and testing in humans.

4.3. Safety and efficacy of vaccine under development

Chlamydial vaccines have been under development for more than 70 years, and preclinical trials have been recorded in the last seven decades (Table 2). In the 1960s, immunoprotective efficacy of chlamydial vaccines against ocular infection was tested in baboons and *Macaca cyclops* (Taiwanese monkey) [63–65]. However, the protection was retained for less than 2 years and exposure to a different serotype led to severe immunopathological damage in *Macaca cyclops*, highlighting the importance of vaccine safety [64]. An effective vaccine for *C. trachomatis* should be able to prevent primary infection, reduce transmission, curb re-infection, affect disease progression post infection, and aid in reduction of bacterial load to reduce the duration of infection [20,56]. The lack of simulation ability of animal models mandates that human trials be undertaken in order to determine the exact effect of vaccine candidates. However, studying ocular infections seems more challenging than experiments involving the study of genital infection due to the availability of experimental protocols [66]. Trials in primates and humans have suggested the occurrence of severe inflammatory disease if subjected to re-challenge with different serovar of *C. trachomatis*. Another challenge is that the trials are bound by ethical endpoints wherein, the volunteers have to be provided with treatment as soon as they incur infection, making it difficult to compare treated groups with placebo [56]. Oral immunization strategies are also being explored in order to deal with Chlamydial infections, and London-based Prokarium's Vaxonella® platform is one such technology that is currently under development. This technology is being tested in a preclinical setting for its ability to impart humoral and mucosal protection [40].

5. Concluding remarks

It is well known that Trachoma blindness is permanent. According to WHO data from March 2020, 137 million people are affected by the chlamydial infection in endemic areas. In a similar report published by WHO in 2019, approximately 92,000 affected people underwent surgery for advanced stages of chlamydial disease, while around 95 million infected people were given antibiotic treatment. Antibiotics are presently the one and only treatment option; however, with high levels of re-infection, there is increasing interest and investment toward the development of chlamydia vaccines. Despite the fact that many protein subunit vaccines have been investigated, outcomes from whole-cell vaccine targets appear to be fractionally more convincing overall. Regardless of the fact that mucosal vaccine drug delivery has shown great promise, scientist are more focused toward systemic vaccine delivery systems [90]. Clinical experiments involving non-human primates with an emphasis on the above discussed measures and relevant adjuvant mixtures could lead to an effective human vaccine.

We believe that appropriate government wellbeing and welfare programs, financial support from multiple sources, and communication with both the water and sewage sectors are the key to trachoma eradication in these areas. In addition, we believe that with ongoing attempts in the most impoverished regions of the country, trachoma will be eradicated as a blinding disease by the year 2022. A prophylactic vaccine candidate with established safety and efficacy is a cogent tool to achieve this goal.

6. Expert opinion

Trachoma symptoms include soreness of the eyes, itchy skin, eye and eyelid irritation, eye weeping, swelling of the eyelids, eye pain, and photophobia. It is critical to ascertain the period of these symptoms and acquire a history of travel to endemic areas (e.g. North Africa, the Middle East, and India). Vaginitis, cervicitis, or urethritis may occur concurrently. Trachoma is diagnosed primarily based on the patient's history and clinical signs that can be seen on slit-lamp evaluation. While many diagnostic assays have been developed to diagnose the organism, no 'gold standard' exploration persists. Trachoma has a favorable prognosis wherein early detection and treatment are critical for preventing irreversible side effects and eye damage. In recent years, community-based SAFE strategy implementation has improved the prognosis for thousands of people at risk. A study conducted in Rural Sudan found that using this stratification resulted in massive declines in the pervasiveness of active disease [91].

Patient education should focus on avoiding urban sprawl and unhygienic conditions, and they should be trained to limit hand-eye contact. Good hygiene, particularly hand washing on a regular basis, should be encouraged. When the patient is diagnosed, they should be isolated and thoroughly educated on the disease's nature, modes of transmission, and precautions. Patients should then be cautioned about the potential consequences of noncompliance with medication or therapy failure.

Efforts to develop a vaccine to safeguard against *C. trachomatis* -induced trachoma began over a century ago and have continued for decades. Shielded responses were received using whole organisms, however, after being exposed to *C. trachomatis*, some immunized people developed disease exacerbation, preventing the vaccine from being implemented. To accelerate the enactment of *C. trachomatis* vaccines, we believe the next step should be to test the most promising vaccine formulations in parallel.

Whole-cell antigenic targets can elicit better safety and efficacy with reduced chlamydial shedding. A major problem encountered with such vaccine platforms is the lack of reproducibility. As an alternative, major outer membrane protein (MOMP) based vaccines have been proved to be equally effective as that of whole-cell vaccine with systemic and mucosal delivery in mouse models. Future trials designed with non-human primates could lead to a suitable vaccine candidate. The first human phase 1 clinical trial is currently underway marking a significant milestone in the development of chlamydial vaccines.

Vector-based vaccine delivery with promising adjuvanticity has provided an attractive niche to the vaccine research for trachoma, especially biodegradable polymer-based nanoparticle encapsulating specific chlamydial antigen [15]. Certain biodegradable polymer-based adjuvants like PLGA matrix-based Nano vaccine is already evaluated for trachoma with appropriate safety data and sufficient immunity [92 93]. Similarly, polylactic acid (PLA) and polyethylene glycol (PEG) co-polymer is evaluated as vaccine carrier with potential immune response [90,93]. Charge variant-based adjuvant can also be used for the successful delivery of chlamydial specific antigen for efficient vaccine delivery through nano delivery platform. The remarkable success of nucleic acid vaccine (DNA- and mRNA-based vaccine) for SRAS-CoV-2 has demonstrated the potential of such vaccine platform that can be explored for safe and efficacious vaccine design for chlamydial infection.

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