Vaccine xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

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

Louise M. Hafner[a], David P. Wilson[b], Peter Timms[a,*]

[a] Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
[b] The Kirby Institute, University of New South Wales, Sydney, Australia

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form 4 July 2013
Accepted 11 August 2013
Available online xxx

Keywords:
Chlamydia
Vaccines
WHO special issue

ABSTRACT

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

1. Chlamydial infection and disease

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

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

2. The need for chlamydial vaccines

Potential interventions for reducing the incidence of infection and disease sequelae associated with *Chlamydia* include; (i)

Abbreviations: EB, elementary body; INF-g, interferon gamma; NHP, non-human primate; MOMP, major outer membrane protein; RB, reticulate body.

* Corresponding author at: Institute of Health and Biomedical Innovation (IHBI), 60 Musk Avenue, Kelvin Grove Urban Village, Kelvin Grove, Queensland 4059, Australia. Tel.: +61 73138 6199; fax: +61 73138 6030.
E-mail address: p.timms@qut.edu.au (P. Timms).

0264-410X/$ – see front matter.
http://dx.doi.org/10.1016/j.vaccine.2013.08.020

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

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

In addition, despite continued improvements in diagnostic and screening procedures for *Chlamydia*, and although antibiotics like azithromycin are available to treat infections, notifications of infections continues to increase. Antibiotic treatment of individuals may also increase susceptibility to re-infection, which is most likely due to interrupting the natural course of protective chlamydial immunity [31]. Recently, data from an in vivo study reported that not only were T-helper (Th)1 immune responses against *C. trachomatis* in individual women slow to develop, but that these responses were also altered by treatment with ceftriaxone and azithromycin [32]. Taken together, these facts suggest that the current main line of defence against chlamydial infections (i.e. administration of antibiotics following screening) is far from fully effective on a population level, and hence a vaccine may be the only way to address this problem. In addition, the strategy of control programmes based on screening, treatment and contact tracing is extremely costly and requires substantial societal infrastructure. This makes this approach impractical for the developing world, where the burden of disease is the greatest.

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

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

3. The immunological challenges

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

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

During active primary infections in women, serum and genital mucosal IgA and IgG antibodies to chlamydial EBs and specific chlamydial proteins including heat-shock (HSP) and plasmid proteins, are usually detected [47]. In patients with current genital infections, the predominant serum responses are maintained for at least 6 months and are mainly IgG1 and IgG3 antibodies [48]. Local IgA antibodies correlate with reduced shedding of the chlamydial organism from the genital tract [49]. However, high titres of local IgA antibodies do not correlate with resolution of infection, but can act as markers of prior chlamydial infections. The major role antibodies appear to play in clearance of infection is in the enhancement of Th1 activation with CD4+ T cells secreting IFN-g correlating primarily with the resolution of infections. Of note however, is the

Please cite this article in press as: Hafner LM, et al. Development status and future prospects for a vaccine against *Chlamydia trachomatis* infection. Vaccine (2013), http://dx.doi.org/10.1016/j.vaccine.2013.08.020