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Towards a safe and effective chlamydial vaccine: Lessons from the eye

David C.W. Mabey*, Victor Hu, Robin L. Bailey, Matthew J. Burton, Martin J. Holland

Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, United Kingdom

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ABSTRACT

As well as being the most common bacterial sexually transmitted infection, *Chlamydia trachomatis* (*Ct*) is the leading infectious cause of blindness. The pathogenesis of ocular chlamydial infection (trachoma) is similar to that of genital infection. In the 1960s the efficacy of *Ct* vaccines against ocular infection was evaluated in major field trials in Saudi Arabia, Taiwan, The Gambia, India and Ethiopia. These trials showed that it was possible to induce short term immunity to ocular infection, and to reduce the incidence of inflammatory trachoma, by parenteral immunisation with killed or live whole organism vaccines. In one study, it was also shown that the incidence of scarring sequelae was reduced in vaccinated children. Detailed studies in non-human primates conducted at this time suggested that vaccination could lead to more severe inflammatory disease on subsequent challenge. Since that time there have been many studies on the immunological correlates of protective immunity and immunopathology in ocular *Ct* infection in humans and non-human primates, and on host genetic polymorphisms associated with protection from adverse sequelae. These have provided important information to guide the development and evaluation of a human *Ct* vaccine.

1. Introduction

Chlamydia trachomatis (*Ct*) is the commonest bacterial sexually transmitted infection [1]. Because a high proportion of infected people have no symptoms, screening programmes for those at risk have been the mainstay of control programmes in countries where it is prioritised and economically sustainable. However, these programmes have failed to reduce the number of reported cases, and it has even been suggested that early detection and treatment of chlamydial infection increases its incidence by preventing the development of protective immunity [2]. A vaccine against *Ct* would be of great public health benefit.

Several reviews have summarised the evidence for protective immunity to chlamydial infection, and the immunological correlates of protective immunity and immunopathology, in a variety of animal models [3–9]; but the relevance of these to human disease is not clear. The evidence for protective immunity, natural history and immunobiology of genital *Ct* infection in humans have also been extensively reviewed [10,11]. The authors concluded that more prospective studies in women with genital chlamydial infection are needed to inform development of a safe and effective chlamydial vaccine, but pointed out that these are logistically and ethically very difficult to do [5,11].

C. trachomatis also infects the human eye, causing trachoma, the leading infectious cause of blindness [12–14]. The genomes of *Ct* strains isolated from the eye and genital tract are more than 99% identical [15], and the clinical and pathological findings of ocular and genital infection are similar. Infections are often asymptomatic at both sites, and are characterised by inflammation and the presence of sub-epithelial lymphoid follicles. The damage in both the eye and genital tract results from fibrosis, which progresses slowly (over months or years) at the site of inflammation.

The eye is more accessible to examination and sampling than the urethra, cervix or fallopian tubes. There is an extensive literature on the natural history, immunology and pathogenesis of human ocular *Ct* infection. Human challenge studies, detailed studies on the natural history, pathogenesis and immune response to experimental ocular infection in humans and non-human primates, and the results of several major trachoma vaccine trials in humans were reported in the 1960s. More recently there have been many publications on the immunological correlates of protective immunity and immunopathology following ocular *Ct* infection in humans, on the genetics of susceptibility to the scarring sequelae of ocular infection, and on gene expression at the site of infection in the conjunctival epithelium [16]. The purpose of this review is to summarise the state of knowledge concerning the natural history, immunology and pathogenesis of ocular *Ct*

* Corresponding author. Tel.: +44 2079272297; fax: +44 2079272739.
E-mail address: david.mabey@lshtm.ac.uk (D.C.W. Mabey).

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infection in humans and non-human primates (NHPs), for the benefit of those interested in the development of a vaccine against *Ct*; and to suggest how a chlamydial vaccine might be evaluated in humans.

2. Natural history of human ocular *C. trachomatis* infection

Human volunteer studies showed that the follicular keratoconjunctivitis characteristic of trachoma develops within 2–15 days of inoculation, depending on the dose inoculated, and resolves over several months [17,18]. The follicles of trachoma are best seen in the conjunctiva of the everted upper eyelid (the subtarsal conjunctiva) and, according to the World Health Organisation case definition, follicular trachoma (TF) is present when more than 5 follicles of >0.5 mm diameter are seen in the central area of the subtarsal conjunctiva. In some cases the follicles are accompanied by intense inflammation which obscures the conjunctival blood vessels; intense trachoma (TI) is diagnosed when more than half the blood vessels are obscured by inflammation. *Ct* bacterial loads are highest in those with TI [19]. The presence of TF and/or TI defines active trachoma. *Ct* can often be isolated from cases of active trachoma but, because follicles can persist for months or years after the infection has resolved, even the most sensitive nucleic acid detection systems often fail to identify infection in subjects with active trachoma.

Some, but not all cases of active trachoma develop conjunctival scarring, but this process usually takes several years. *Ct* cannot usually be isolated from subjects with scarring trachoma. In human volunteer studies, and in experimental infections in non-human primates, scarring sequelae were not seen following a single infection [20–24]. In trachoma endemic communities, the prevalence of scarring increases with age. It is more common in women, who are more frequently in contact with young children (the main reservoir of infection). People with intense inflammatory trachoma and persistent or recurrent *Ct* infection are more likely to develop scarring [25,26]. As the scarring progresses and the scars contract, the lashes may turn inward and rub against the cornea (trachomatous trichiasis, or TT), which is painful and causes corneal damage that may result in blindness.

3. Protective immunity to *C. trachomatis* in humans

Experimental studies in humans and NHPs showed that re-challenge with the same strain of *Ct* results in an attenuated clinical response compared to primary infection, with a lower bacterial load [17,20,21]. In trachoma endemic communities the prevalence of ocular *Ct* infection decreases with age, and the highest bacterial loads are found in young children, suggesting that a degree of protective immunity develops following natural infection. A study in a trachoma endemic community in The Gambia, in which members of affected households were examined and tested for ocular *Ct* infection every two weeks over a 6-month period in the absence of treatment, showed that the duration of episodes of disease and of infection was age dependent. The duration of untreated infection was estimated to be approximately 15 weeks in children aged 0–4 years, and 8 weeks in older children and adults [27,28]. The estimated incidence of infection was also lower in older individuals. The conclusion from this study is that protective immunity develops following natural infection, and is associated with both a reduced incidence and a reduced duration of infection.

4. Trachoma vaccine trials

Experiments in baboons and in the Taiwanese monkey (*Macaca cyclops*) in the 1960s evaluated the protective efficacy of whole organism chlamydial vaccines, delivered parenterally, against ocular infection [21,29]. In both species it was shown that vaccines can provide short term, strain-specific protection against ocular *Ct* infection, which is of relatively short duration (less than 2 years). In the Taiwan monkey exposure to a different serotype led to more severe disease in vaccinated than unvaccinated animals, suggesting that vaccination could lead to immunopathological damage on subsequent exposure [21].

Large placebo-controlled human trachoma vaccine trials, using whole organisms administered by intramuscular injection, were completed in Saudi Arabia, Taiwan, The Gambia, India and Ethiopia in the 1960s [30–36]. In Saudi Arabia, two doses of a bivalent killed whole organism vaccine, or placebo, were given to children aged less than 3 years, some of whom already had trachoma. Three vaccine groups were included, who received high or low dose aqueous vaccine, or low dose vaccine with adjuvant. Less active trachoma was seen at 6 and 12 months in children receiving the low dose aqueous vaccine compared to placebo, but a higher incidence was found in those who received a higher dose. There was no difference in active trachoma or ocular *Ct* infection between vaccine and placebo arms when the results were pooled, though a reduced bacterial load (determined by counting chlamydial inclusions in conjunctival scrapings) was found in children receiving high dose aqueous vaccine and vaccine with adjuvant [30,31].

In the first trial in Taiwan four doses of a formalin-inactivated, alum-absorbed elementary body vaccine made from a local serovar C isolate, or placebo, was given to pre-school siblings of children with active trachoma over a two year period. There was less active trachoma in vaccinated children (8% vs 18%), but the protective effect was no longer seen one year after the final dose. Two subsequent trials used killed whole organism vaccine in mineral oil, given to primary school children. A bivalent vaccine, containing a Taiwanese serovar B isolate in addition to the serovar C isolate used previously serovars, reduced the incidence of active trachoma from 8.8% to 5.1%, but this difference was not significant. In a second trial, of a monovalent vaccine containing only serovar C, there was a significantly higher incidence of active trachoma in the vaccinated group, but no difference between the groups in disease severity [32,33].

In The Gambia, live vaccines were used [34]. In the first trial, the therapeutic effect of vaccination with a Gambian isolate was assessed by randomising children with clinical signs of active trachoma to receive vaccine or placebo [35]. Eight and 17 weeks after vaccination there was a significant clinical improvement in the vaccinated but not the placebo group, and the prevalence of *Ct* infection (determined by isolation in eggs) was also reduced in the vaccinated group. The protective effect was no longer seen at one year. In the second and third Gambian trials the prophylactic effect of vaccination was determined [37]. In the second trial two doses of a monovalent vaccine, made from a local isolate with a mineral oil adjuvant, were given 6 months apart. Six months after the second dose 17/118 children in the placebo group had acquired trachoma, compared to 7/117 in the vaccinated groups ($p = 0.053$). At 12 months there was no difference between the groups [37]. In the third trial two doses of a bivalent vaccine, containing two "fast killing" isolates, was given 3 weeks apart. One of these was an ocular isolate from Saudi Arabia, and the other from the USA. At 12 and 24 months there was no significant difference in the proportion of children who had acquired active trachoma between the vaccinated and placebo arms. However, at 24 months the proportion of children in the placebo group with conjunctival scarring was higher than in the vaccinated group (18/47 vs 9/55, $p = 0.034$) [37].

In the Indian trial two doses of a bivalent, formalin inactivated vaccine or placebo were given to children aged less than 5 years without signs of clinical trachoma [36]. Twelve months after the second dose 26/182 vaccinated children had developed clinical