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Safety and immunogenicity of the chlamydia vaccine candidate CTH522 adjuvanted with CAF01 liposomes or aluminium hydroxide: a first-in-human, randomised, double-blind, placebo-controlled, phase 1 trial

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Summary

Background Chlamydia is the most common sexually transmitted bacterial infection worldwide. National screening programmes and antibiotic treatment have failed to decrease incidence, and to date no vaccines against genital chlamydia have been tested in clinical trials. We aimed to assess the safety and immunogenicity, in humans, of a novel chlamydia vaccine based on a recombinant protein subunit (CTH522) in a prime–boost immunisation schedule.

Methods This phase I, first-in-human, double-blind, parallel, randomised, placebo-controlled trial was done at Hammersmith Hospital in London, UK, in healthy women aged 19–45 years. Participants were randomly assigned (3:3:1) to three groups: CTH522 adjuvanted with CAF01 liposomes (CTH522:CAF01), CTH522 adjuvanted with aluminium hydroxide (CTH522:AH), or placebo (saline). Participants received three intramuscular injections of 85 μg vaccine (with adjuvant) or placebo to the deltoid region of the arm at 0, 1, and 4 months, followed by two intranasal administrations of 30 μg un-adjuvanted vaccine or placebo (one in each nostril) at months 4·5 and 5·0. The primary outcome was safety and the secondary outcome was humoral immunogenicity (anti-CTH522 IgG seroconversion). This study is registered with Clinicaltrials.gov, number NCT02787109.

Findings Between Aug 15, 2016, and Feb 13, 2017, 35 women were randomly assigned (15 to CTH522:CAF01, 15 to CTH522:AH, and five to placebo). 32 (91%) received all five vaccinations and all participants were included in the intention-to-treat analyses. No related serious adverse reactions were reported, and the most frequent adverse events were mild local injection-site reactions, which were reported in all (15 [100%] of 15) participants in the two vaccine groups and in three (60%) of five participants in the placebo group (p=0·0526 for both comparisons). Intranasal vaccination was not associated with a higher frequency of related local reactions (reported in seven [47%] of 15 participants in the active treatment groups *vs* three [60%] of five in the placebo group; p=1·000). Both CTH522:CAF01 and CTH522:AH induced anti-CTH522 IgG seroconversion in 15 (100%) of 15 participants after five immunisations, whereas no participants in the placebo group seroconverted. CTH522:CAF01 showed accelerated seroconversion, increased IgG titres, an enhanced mucosal antibody profile, and a more consistent cell-mediated immune response profile compared with CTH522:AH.

Interpretation CTH522 adjuvanted with either CAF01 or aluminium hydroxide appears to be safe and well tolerated. Both vaccines were immunogenic, although CTH522:CAF01 had a better immunogenicity profile, holding promise for further clinical development.

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Introduction

WHO estimates that more than one million new infections with the four curable sexually transmitted diseases—chlamydia, gonorrhoea, syphilis, and trichomoniasis—are acquired each day. With around 131 million annual incident infections, chlamydia remains the most common sexually transmitted bacterial disease.[1] The prevalence of chlamydia is age-dependent, with highest incidence of laboratory-confirmed *Chlamydia trachomatis* infections observed in adolescents and young adults. However, as three in four infections remain asymptomatic, the actual incidence is likely to be underestimated.[1]

Untreated or repeated infections are the main drivers of chlamydia-associated morbidity,[2] which is estimated to cause 370 000 disability-adjusted life years annually.[3] One in every six infected women develops ascending infection and pelvic inflammatory disease, which contributes to chronic pelvic pain and is a leading cause of tubal factor infertility and ectopic pregnancy, especially in the developing world.[4] *C trachomatis* infection is strongly

Research in context

Evidence before this study

We searched PubMed using the terms "chlamydia vaccine" and "clinical trial", with no restrictions on publication dates (from Jan 1, 1966, to Jan 31, 2019) or language, and identified no reported studies. This study is, to the best of our knowledge, the first clinical trial of a genital chlamydia vaccine, and the first of a vaccine against *Chlamydia trachomatis* since the 1960s, when various studies assessed the efficacy of live attenuated bacteria against ocular chlamydia infection (trachoma).

Added value of this study

In this phase I, first-in-human, double-blind, parallel, randomised, placebo-controlled trial, we found that intramuscular administration of CTH522 adjuvanted with either CAF01 or aluminium hydroxide, as well as intranasal administration of un-adjuvanted CTH522, was well tolerated and immunogenic in healthy adult women. The vaccines induced high titres of serum antibodies and cell-mediated immune responses, measured as interferon-γ release. The antibodies were neutralising and were detectable in both the nasal cavity and genital tract. Notably, the CAF01 adjuvant induced higher antibody titres and cell-mediated immune responses than aluminium hydroxide. Intranasal booster vaccination tended to increase IgA titres in both the nasal and genital tract secretions.

Implications of all the available evidence

The promising safety and immunogenicity profile of CTH522 adjuvanted with CAF01 encourages continued clinical development of this vaccine against genital chlamydia. A phase 2 dose optimisation study is planned to start in autumn 2019.

associated with increased susceptibility to, and co-infection with, other sexually transmitted diseases, particularly gonorrhoea and HIV.[5] Infection during pregnancy poses a risk of adverse outcomes such as miscarriage, stillbirth, and preterm birth by either direct foetal infection, placental damage, or severe maternal illness.[6] More than half of infants born to infected mothers become infected during birth, of whom one in six will develop pneumonia and around half will develop conjunctivitis.[7] In men, *C trachomatis* mainly causes epididymitis, and in both men and women *C trachomatis* infection can trigger reactive arthritis in a minority of cases.

Despite the availability of both sensitive non-invasive tests and effective treatment, targeted screening and treatment programmes have, to a large degree, failed to curb the epidemic.[8,9] Thus, an effective preventive vaccine might be the best solution. Nevertheless, no vaccine against *C trachomatis* has entered clinical trials since a series of trials done against ocular chlamydia in the 1960s.

Studies of natural immunity suggest that infection can lead to partial and transient immunity to *C trachomatis* characterised by both local humoral and cellular responses.[10] Data from animal models point to a key role, preferably combined, for interferon-γ-secreting T-helper-1 cells and functional antibodies.[11] However, it remains incompletely understood which mechanisms are necessary to target for a vaccine to confer protective immunity*C trachomatis*.

The vaccine antigen, CTH522, is a recombinant, engineered version of the *C trachomatis* major outer membrane protein (MOMP), comprising heterologous immuno-repeats from four genital *C trachomatis* serovars (D, E, F, and G).[12] Preclinical research on this vaccine led to selection of the cationic liposomal adjuvant CAF01, which has been designed for the induction of a strong cell-mediated immune response combined with antibody induction. The vaccine has been evaluated in mice, pigs, and non-human primates, where T-cell responses and high titres of broadly neutralising antibodies were induced. Protection following genital *C trachomatis* challenge was found in both mice[12] and guinea pigs (unpublished).

Since the genital mucosa does not have immune inductive sites, other mucosal sites have been explored for induction of local genital immunity, especially intranasal immunisation, which has been shown to induce mucosal immunity in both the respiratory and genital tract. Immunisation schedules with the adjuvant CAF01 have also highlighted how systemic priming followed by mucosal boost is highly efficacious in inducing mucosal immunity and induction of IgA.[13–15]

The aim of this trial was to assess the safety and immunogenicity of three intramuscular doses of CTH522 adjuvanted with CAF01 liposomes (CTH522:CAF01) or aluminium hydroxide (CTH522:AH), followed by two intranasal boosts with un-adjuvanted CTH522.

Methods

Study design and participants

This study was a phase 1, first-in-human, double-blind, parallel, randomised, placebo-controlled trial done at the National Institute for Health Research (NIHR) Imperial Clinical Research Facility at Hammersmith Hospital in London, UK. The study protocol was approved by the London–Chelsea Research Ethics Committee, the Research and Development department at Imperial College Healthcare National Health Service (NHS) Trust, and the Medicines and Healthcare products Regulatory Agency (EudraCT number 2015-004330-10). The study was done in accordance with the International Conference on Harmonisation's Good Clinical Practices guidelines, and is registered with ClinicalTrials.gov, number NCT02787109.

The study population comprised healthy women aged 19–45 years, who were not pregnant and agreed to use two approved forms of contraception or to completely abstain from sexual intercourse during the trial period. The enrolled participants had a body-mass index lower than 35 kg/m², no history of pelvic inflammatory disease or other significant gynaecological diseases, negative serological testing for HIV, hepatitis B, hepatitis C, and syphilis, and negative urine PCR testing for *C trachomatis* and gonorrhoea. Participants were excluded if they used an intrauterine device, were currently participating in another clinical trial, had clinically significant abnormality of haematological or biochemical parameters, received immunosuppressive treatment, or had received a vaccine within 2 weeks of the trial period. Participants were recruited through the Imperial Clinical Research Facility's healthy volunteers database, posters at NHS and university sites, and advertisements on social media. All participants gave written informed consent before enrolment.

Randomisation and masking

The trial comprised three treatment groups, each with three intramuscular injections of adjuvanted vaccine (CTH522:CAF01 or CTH522:AH) or placebo (saline), followed by two intranasal administrations of un-adjuvanted CTH522 vaccine or placebo. Enrolled participants were randomly assigned to the treatment groups (3:3:1), via an electronic case report form (eCRF) system provided by a clinical research organisation (Biostata, Allerød, Denmark) with a block size of 7. The randomisation module in the eCRF was set up by an unmasked person who was not otherwise involved in the clinical trial. Unmasked trial staff members, who were not involved in any trial assessments, prepared and administered the vaccines. During trial drug administration, a masked member of staff was also present to monitor any adverse events during or after vaccination. Participants, investigators, study nurses, laboratory personnel, and outcome assessors were all masked to vaccine group allocation until database release.

Procedures

The investigational recombinant protein vaccine CTH522 (batch number 528001), was produced under good manufacturing practice at Statens Serum Institut (Copenhagen, Denmark). The intramuscular dose of 85 µg CTH522 was administered to the deltoid region of the arm in a volume of 0·6 mL, containing either the liposomal adjuvant CAF01 (625 µg N,N' -dimethyl-N,N' -dioctadecylammonium [DDA] stabilised with 125 µg of the synthetic mycobacterial immunomodulator α,α' -trehalose-6,6' -dibehenate [TDB]) or 425 µg aluminium hydroxide, both manufactured at Statens Serum Institut. The three intramuscular vaccinations were scheduled for day 0, day 28 (month 1), and day 112 (month 4). The intranasal dose of 2×30 µg CTH522 was administered to each nostril in a volume of 0·25 mL, with a VaxINator device (Teleflex, Wayne, PA, USA) at day 126 (month 4·5) and day 140 (month 5·0) (appendix p 9).

See Online for appendix

Safety was assessed after each vaccination as follows: daily completion of diary cards for 14 days, a telephone interview after 3 days, and a safety visit (vital signs and safety bloods) after 14 days. The solicited adverse events comprised local reactions to intramuscular vaccination (pain, erythema, tenderness, pruritus, warmth, stiffness, and swelling), local reactions to intranasal vaccination (discharge, bleeding, congestion, discomfort, sneezing, and cough) and systemic reactions to any vaccination (abnormally raised temperature [>38·3°C], chills, myalgia, malaise, fatigue, rash, headache, nausea and vomiting, and clinically significant abnormal values among full blood count, liver function test, and renal profile results). Local and systemic adverse events were evaluated by a study clinician.

Samples for assessment of immunogenicity were collected at baseline and 1·0, 4·0, 4·5, 5·0, 5·5, and 6·0 months after first immunisation for quantification of CTH522-specific IgG and IgA titres with ELISA, and at baseline and at 4·5 months for assessment of neutralising antibodies (appendix). Peripheral blood mononuclear cells were collected at baseline and at 4·5 months to assess cell-mediated immune responses with interferon-γ enzyme-linked immunospot (ELISpot) assay (appendix). Total mucosal IgG and IgA, and corresponding antibodies specific to CTH522 were quantified in nasal strips and vaginal fluid obtained by use of menstrual cup (Instead Softcup; EVOFEM, San Diego, CA, USA) samples collected at baseline and at 4·5, 5·0, and 6·0 months, by use of ELISA (appendix). Additional samples for exploratory immunogenicity assessment were also collected; data are being compiled for publication.

Outcomes

The primary outcomes (safety) were solicited systemic reactions as well as solicited local reactions to intramuscular and intranasal vaccination recorded at any visit. The secondary outcome (humoral immunogenicity) was the proportion of participants achieving anti-CTH522 IgG seroconversion, defined as a four-fold increase over baseline in specific serum IgG. Exploratory outcomes included evaluation of neutralising antibodies, mucosal antibody responses, antibody avidity, and epitope use, and interferon-γ ELISpot; only the neutralising and mucosal antibody responses and interferon-γ ELISpot responses are presented in this report.

Statistical analysis

We considered a sample size of 15 participants per vaccine group and five participants in the placebo group to be adequate for a review of the safety profile of the described interventions. This study was not powered to detect differences between vaccine groups. All participants who had received at least one dose of the vaccine were included

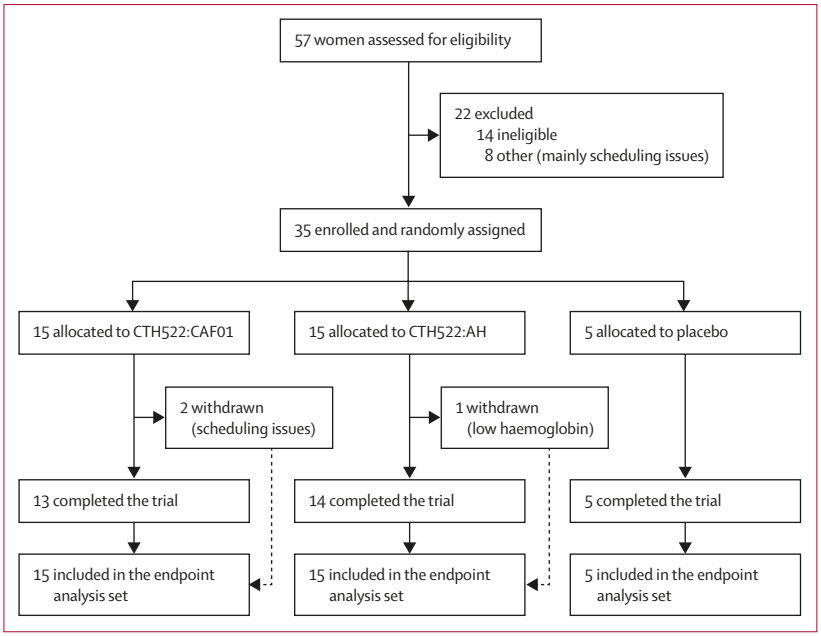

Figure 1: **Trial profile**
AH=aluminium hydroxide.

	CTH522:CAF01 (n=15)	CTH522:AH (n=15)	Placebo (n=5)
Age (years)	24 (19–42)	26 (19–43)	23 (22–45)
Ethnicity or race			
White	9 (60%)	10 (67%)	4 (80%)
Asian	3 (20%)	3 (20%)	..
Black	2 (13%)	2 (13%)	1 (20%)
Other	1 (7%)	..	..
Body-mass index (kg/m²)	23·0 (18·6–34·9)	23·1 (18·7–27·9)	22·1 (20·0–31·3)
Baseline anti-CTH522 IgG (U/mL)	1·0 (0·4–25·5)	1·2 (0·3–35·0)	2·6 (0·6–8·5)

Data are n (%) or median (range). AH=aluminium hydroxide.

Table 1: **Baseline characteristics of the per-protocol population**

in the analyses of the primary and secondary outcomes (termed the endpoint analysis set). Safety results were expressed as the proportion of participants in each vaccine group with adverse events, in the three categories—local injection site reactions, local nasal reactions, and systemic reactions—judged to be related or not related to study treatment, and compared with Fisher's exact test. The proportions of seroconverted participants in each group were compared with Fisher's exact test. Confidence intervals for point estimates of effect size are presented as 95% CIs unless otherwise stated. A post-hoc analysis of the amount of neutralising and mucosal antibodies as well as interferon-γ ELISpot results was presented as median and IQR, and compared with the Mann-Whitney *U* test. Correlation analysis was done with Spearman's rank correlation coefficient. Cell-mediated immune responder rates were defined as interferon-γ ELISpot responses higher than the mean baseline response of all volunteers plus 3 SD, and were compared between groups by use of Fisher's exact test. The safety and seroconversion results were analysed with SAS, version 9.4, following a predefined statistical analysis plan. The exploratory outcomes were assessed with R, version 3.5.1, with R studio, version 1.1.463. An independent data safety monitoring board was established to review and evaluate the trial data for participant safety and trial conduct.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The sponsor of the study (Statens Serum Institut) participated in the study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 15, 2016, and Feb 13, 2017, 35 women were randomly assigned to receive CTH522:CAF01 (n=15), CTH522:AH (n=15), or placebo (n=5; figure 1, table 1). Of the 35 participants, 32 (91%) received all five vaccinations described in the study protocol. Because of scheduling issues, two participants in the CTH522:CAF01 group withdrew from the study (one after three vaccinations and the other after five). One participant in the CTH522:AH group withdrew after the second intramuscular vaccination because of low haemoglobin concentrations caused by a combination of a menorrhagia and study-related blood sampling (figure 1).

The primary outcome was safety (appendix p 4). No related serious adverse events occurred during the trial (table 2). The most frequently reported local reactions were injection-site pain, tenderness, and movement impairment, with 88–93% of events being reported as mild in each of the groups, lasting a median of 2–4 days in all groups (range 1–11 days). All participants recovered from all related adverse events. One unrelated serious adverse event occurred in a participant in the CTH522:CAF01 group (fracture of fibula following fall from a climbing wall).

All 15 (100%) participants in the two active treatment groups had a local injection-site reaction, which—although not significant—seemed to occur at a higher frequency than in the placebo group (three [60%] of five participants, p=0·0526 for both comparisons; table 2). Intranasal vaccination was not associated with a higher frequency of related local reactions (seven (47%) of 15 in each of the active treatment groups versus three [60%] in the placebo group; p=1·000), with the most frequent local reactions being sneezing, nasal congestion, and rhinorrhoea. All but one events (moderate rhinorrhoea in the CTH522:CAF01 group) were of mild intensity.

The frequency of systemic adverse reactions did not differ significantly between the three groups, although a

numerically higher proportion of participants had systemic adverse reactions in the two active treatment groups (ten [67%] of 15 in the CTH522:CAF01 group and 13 [87%] of 15 in the CTH522:AH group; p=0·3473), than did those in the placebo group (two (40%) of five; p=0·0726). The most frequently reported systemic reactions were headache, fatigue, malaise, and myalgia.

13 unsolicited treatment-emergent adverse events were reported (appendix p 10): five in the CTH522:CAF01 group, six in the CTH522:AH group, and two in the placebo group. Among these were two cases of musculoskeletal stiffness (in the CTH522:AH group), two cases of oropharyngeal pain (one in the CTH522:CAF01 group and one in the placebo group), and two cases of nasopharyngitis (one in the CTH522:CAF01 group and one in the CTH522:AH group).

For the secondary outcome of humoral immunogenicity, all 15 (100%) participants in the CTH522:CAF01 group, 14 (93%) of 15 in the CTH522:AH group, and none in the placebo group achieved the predefined outcome of higher than four-fold IgG seroconversion after the three intramuscular immunisations (appendix p 11). The nasal booster immunisations did not increase systemic antibody concentrations. For the CTH522:CAF01 group, all 15 seroconversions (100%) occurred after the second immunisation, and were sustained to the last timepoint (appendix p 11).

The magnitude of IgG titres was assessed in a post-hoc analysis (figure 2A). Both vaccines generated strong responses after the first immunisation and responses increased with each intramuscular administration. CTH522:CAF01 induced a 5·6-fold higher median titre than CTH522:AH after the third intramuscular immunisation (p=0·0091), and remained 2·5-fold higher than CTH522:AH throughout the study.

Exploratory outcomes included assessment of neutralising antibody titres, mucosal antibodies, serum IgA, and cell-mediated immune responses. Both CTH522:CAF01 and CTH522:AH significantly increased the concentration of neutralising antibodies after the three intramuscular immunisations (p=0·00024 for both groups; figure 2B). Although CTH522:CAF01 induced a higher median neutralising antibody titre than CTH522:AH (254·1 *vs* 107·4), no statistical difference was observed between the two vaccines for this outcome measure. Anti-CTH522 serum IgA responses were significantly increased after intramuscular vaccination, which continued after intranasal boost (appendix p 12), and highly correlated with serum IgG at month 6 (Spearman's correlation coefficient 0·78, p<0·0001; appendix p 13).

Measurement of mucosal antibody concentrations is difficult because of low antibody concentrations and sampling variability; therefore, CTH522-specific IgG and IgA concentrations were normalised relative to total IgG and IgA concentrations in the sample. Antigen-specific vaginal IgG concentrations increased 16·4-fold in the CTH522:CAF01 group after the intramuscular vaccinations and increased further following intranasal boost (p=0·027, figure 3A). Antigen-specific IgG increased in the nasal samples of both groups (2·0-fold in the CTH522:CAF01 group and 2·7-fold in the CTH522:AH group) after intramuscular vaccinations, and increased further following intranasal boost in the CTH522:CAF01 group (p=0·040, figure 3C). No increase in mucosal IgG by intranasal boosting was seen in the CTH522:AH group (p=0·17). Mucosal IgA responses were only seen after intranasal boosting in the CTH522:CAF01 group (figure 3B, 3D). CTH522:AH did not promote IgA concentrations above baseline at any timepoint.

Mucosal IgG titres correlated strongly with serum concentrations (Spearman's correlation coefficient=0·89, p≤0·0001), whereas no such correlation was found between mucosal and circulating IgA levels (0·18, p=0·43) suggesting some local production of IgA (appendix p 13).

	CTH522:CAF01 (n=15)	CTH522:AH (n=15)	Placebo (n=5)
Any related adverse event*	15 (100%)	15 (100%)	4 (80%)
Solicited injection-site reactions	15 (100%)	15 (100%)	3 (60%)
Pain	14 (93%)	9 (60%)	1 (20%)
Tenderness	14 (93%)	14 (93 %)	2 (40%)
Impaired movement	14 (93%)	13 (87%)	2 (40%)
Redness	6 (40%)	7 (47%)	1 (20%)
Warmth	5 (33%)	5 (33%)	2 (40%)
Swelling	4 (27%)	5 (33%)	1 (20%)
Itching	2 (13%)	6 (40%)	0
Muscle reaction	0	1 (7%)	0
Solicited local reactions after intranasal vaccination	7 (47%)	7 (47%)	3 (60%)
Sneezing	2 (13%)	5 (33%)	1 (20%)
Nasal congestion	4 (27%)	3 (20%)	0
Rhinorrhoea	6 (40%)	1 (7%)	0
Epistaxis	2 (13%)	1 (7%)	0
Nasal discomfort	1 (7%)	1 (7%)	1 (20%)
Throat irritation or oropharyngeal pain	2 (13%)	0	1 (20%)
Cough	1 (7%)	1 (7%)	0
Ear discomfort or ear pain	1 (7%)	1 (7%)	0
Solicited systemic reactions	10 (67%)	13 (87%)	2 (40%)
Headache	9 (60%)	9 (60%)	2 (40%)
Sinus headache	0	1 (7%)	0
Fatigue	5 (33%)	8 (53%)	1 (20%)
Malaise	6 (40%)	4 (27%)	0
Myalgia	2 (13%)	4 (27%)	0
Nausea	0	4 (27%)	0
Rash	1 (7%)	3 (20%)	0
Chills	1 (7%)	2 (13%)	0

Data are n (%), indicating the number and proportion of participants having an adverse event in each treatment group. None of the comparisons differed significantly between treatment groups; p values are reported in the main text. AH=aluminium hydroxide. *See the appendix for details of the 13 unsolicited related adverse events.

Table 2: **Related solicited adverse events 14 days after each vaccination**

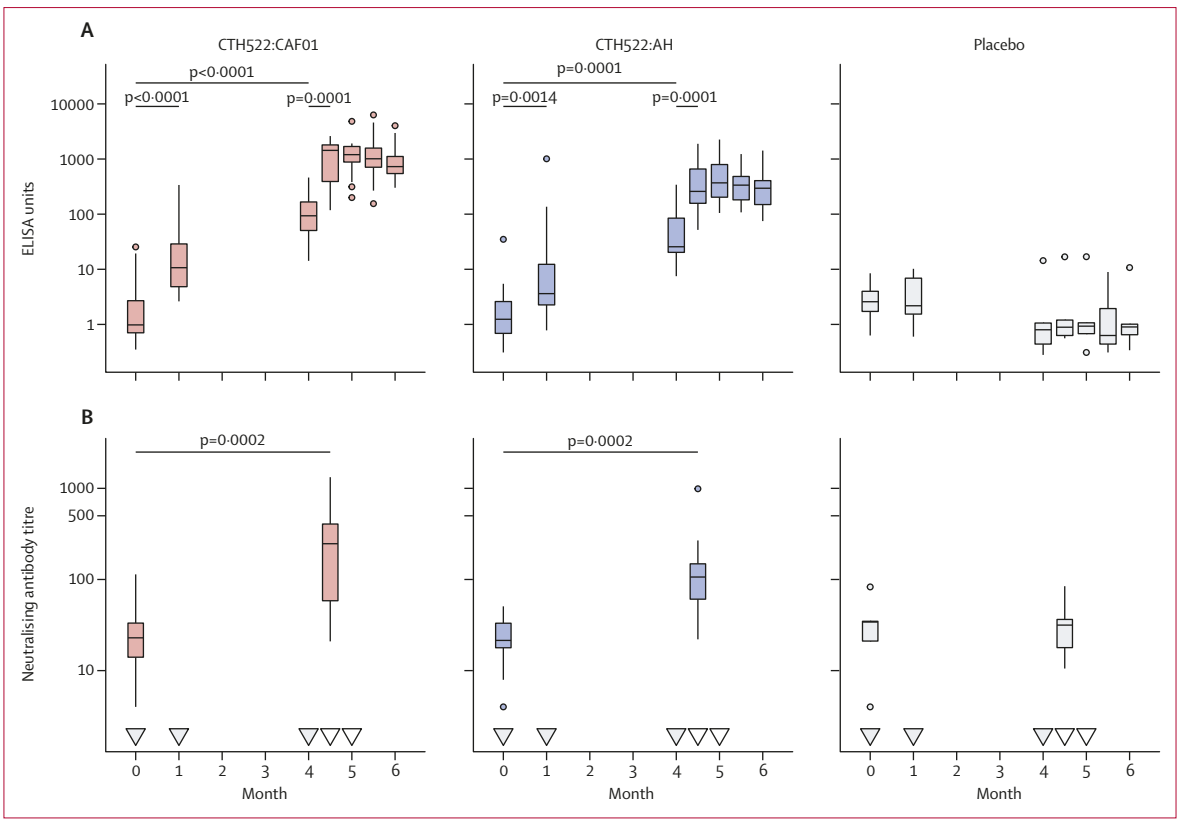

Figure 2: **Serology measurements**
Change in (A) anti-CTH522 serum IgG ELISA units and (B) neutralising antibody titres over time. The box illustrates the IQR, with a horizontal line at the median value; whiskers show 1·5 × IQR, and dots represent outliers. Wilcoxon signed rank test p values are shown. For serum IgG, the titres remained significantly higher than baseline for the duration of the study for both active vaccines, but for clarity only selected comparisons are indicated with asterisks. The vaccine schedule is shown above the x-axis, with grey triangles indicating intramuscular immunisations, and white triangles indicating intranasal immunisation. AH=aluminium hydroxide.

Vaccine-specific cell-mediated immune responses were assessed with interferon-γ ELISpot at baseline and 2 weeks after three intramuscular vaccinations (figure 4). All participants had low baseline responses and in particular CTH522:CAF01 induced strong increases, with median values of 252 spot-forming units (SFU) per 1×10^6 cells (IQR 123–424), which was higher than the cell-mediated immune response induced by CTH522:AH (111 SFU per 1×10^6 cells (IQR 70–269), although this difference did not reach statistical significance at the 95% level (p=0·05523 in a Wilcoxon rank sum test). All participants receiving the CAF01-adjuvanted CTH522 vaccine were classified as responders (13 [100%] of 13), significantly more than the CTH522:AH group, where only eight (57%) of 14 were classified as responders (p=0·0101). No significant correlation was observed between the interferon-γ ELISpot results and serum IgG titres (Spearman's rank correlation coefficient=0·38; p=0·051 at month 4·5; appendix p 14).

Discussion

We report the principal findings from a first-in-human clinical trial of the novel chlamydia vaccine CTH522. Results show that the CTH522 vaccine adjuvanted with CAF01 liposomes or aluminium hydroxide administered with three intramuscular vaccinations and two intranasal boosts is both safe and immunogenic. No vaccine-related serious adverse events were reported and local reactions were mild and comparable with the safety profile of licensed recombinant subunit vaccines such as the hepatitis B vaccines.[16] Intranasal boosting was not associated with a higher frequency of local reactions compared with placebo for any of the vaccines. The CAF01 adjuvant promoted higher antibody and cell-mediated immune responses than aluminium hydroxide. Furthermore, in contrast to aluminium hydroxide, the CAF01-adjuvanted vaccine primed individuals for increased mucosal IgA after intranasal boost, albeit concentrations were low.

Given the impact of the chlamydia epidemic on women's health, reproductive health, infant health through vertical transmission, and increased susceptibility to other sexually transmitted diseases, a global unmet medical need exists for a vaccine against genital chlamydia.[17,18] Unfortunately, no surrogate endpoint for protection against chlamydia disease exists to guide development. However, based on studies of protection after natural infection, as well as various animal models, the prevailing view is that an effective chlamydia vaccine

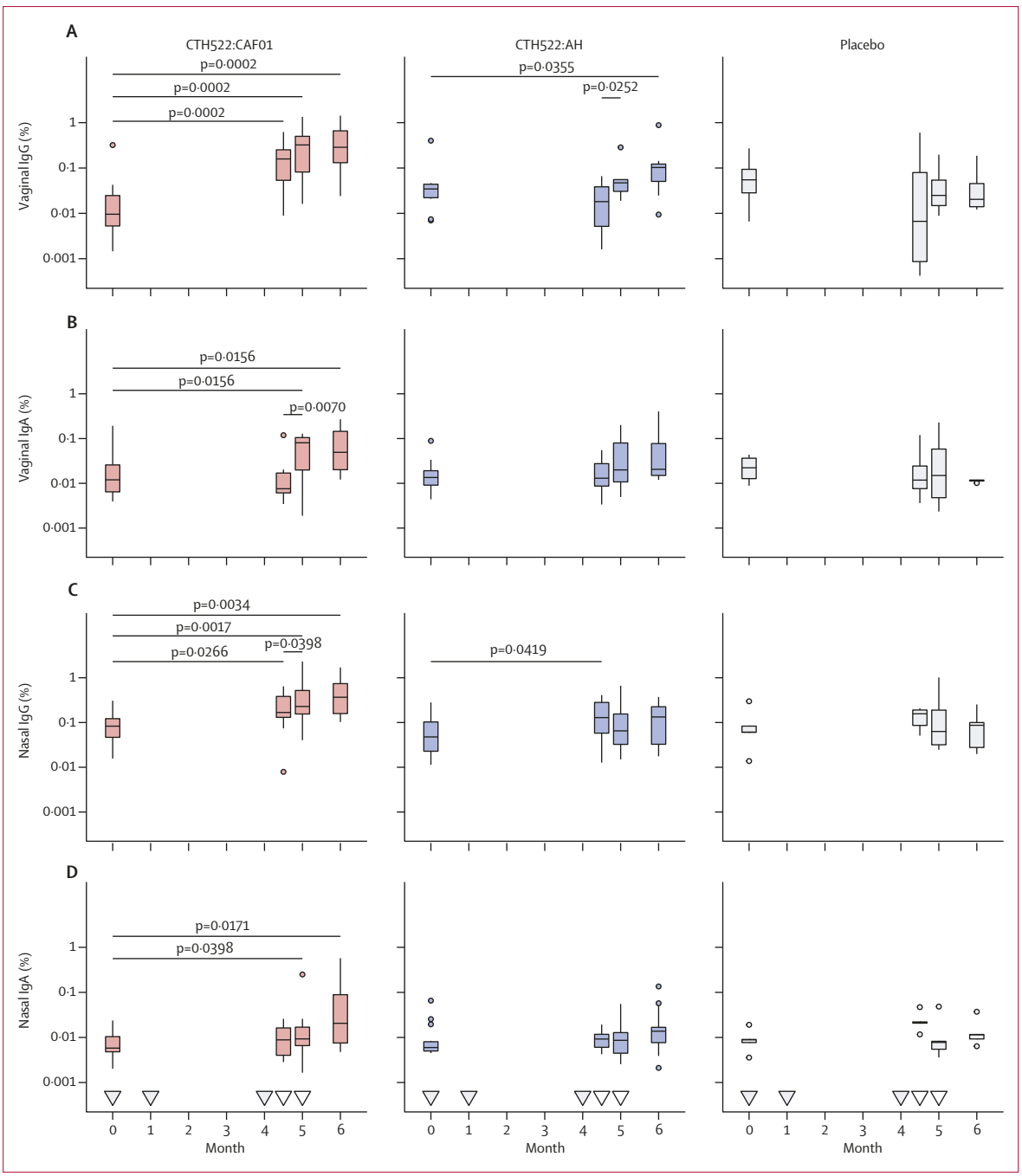

Figure 3: **CTH522-specific mucosal antibody responses**
Change in vaginal IgG (A), vaginal IgA (B), nasal IgG (C), and nasal IgA (D) from baseline to 2 weeks after the third intramuscular immunisation (month 4·5), 2 weeks after the first intranasal immunisation (month 5·0), and 4 weeks after the second intranasal vaccination (month 6·0). Values are shown as CTH522-specific IgG or IgA as a proportion of corresponding total IgG or IgA. Boxes show IQR, with a black line at the median value; whiskers show 1·5 × IQR, and dots represent outliers. Wilcoxon signed rank test p values are shown for nasal antibodies, and because of missing values at some time points Wilcoxon rank sum test p values are shown for vaginal antibodies. The vaccine schedule is shown above the x-axis, with grey triangles indicating intramuscular immunisations, and white triangles indicating intranasal immunisations. AH=aluminium hydroxide.

ideally should generate a combined antibody and T-cell response targeting genital epithelial cells.[11]

Some of the key features of this trial were the parallel assessment of two markedly different adjuvant systems, intranasal boost and assessment of both systemic and mucosal immunogenicity. The trial was designed with an accelerated schedule of three intramuscular vaccinations given at 0, 1, and 4 months followed by two intranasal

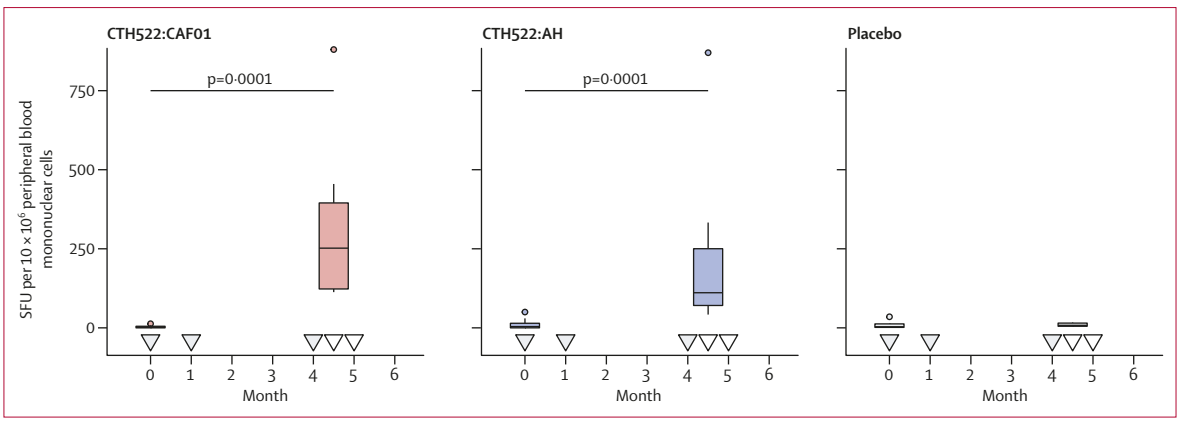

Figure 4: **Cell-mediated immune responses**

Interferon-γ spot-forming units (SFU) for each participant at baseline and at month 4·5 were assessed by use of enzyme-linked immunopot (CTH522:CAF01 [nine of 13 participants], CTH522:AH [12 of 14], and placebo [four of five]). 0·2 × 10⁶ peripheral blood mononuclear cells were stimulated in triplicates with either medium alone or 5 μg/mL CTH522 for 24 h. Presented values are spot counts after protein stimulation, which have been subtracted from the spot counts after medium stimulation. Boxes show IQR, with a black line at the median value; whiskers show 1·5 × IQR, and dots represent outliers. Wilcoxon rank sum test p values are shown. The vaccine schedule is shown above the x-axis, with grey triangles indicating intramuscular immunisations, and white triangles indicating intranasal immunisations. AH=aluminium hydroxide.

boosts with un-adjuvanted vaccine. In continuation of this trial, we are currently preparing a phase 2a trial, where this accelerated schedule will be changed into the classical schedule (0, 1, and 6 months), developed for optimal B-cell maturation and differentiation.[19] This approach will also have the added benefit of aligning with the schedule for the human papilloma virus (HPV) vaccine, which targets the same age group.

CTH522:CAF01 was consistently more immunogenic than CTH522:AH, inducing a 5·6-fold higher IgG titre after the third intramuscular immunisation, as well as stronger mucosal and cell-mediated immune responses. The IgG titres induced by CTH522:CAF01 are therefore similar to those induced by other licenced recombinant protein vaccines, including the adjuvanted hepatitis B vaccine,[16] although the absence of a correlate of protection renders such comparisons speculative. The ability of CAF01 to facilitate antibody responses has been assessed in other clinical trials, with varying outcomes. A CAF01-adjuvanted recombinant tuberculosis vaccine candidate H1 induced no antibodies, but this vaccine contained considerably less antigen than CTH522 in the present study.[20] A malaria vaccine, GMZ, however, generated a strong antibody response on par with aluminium hydroxide.[21] Aluminium hydroxide is considered the gold standard for antibody-inducing vaccines,[22] and it was thus unexpected to see CAF01 surpass aluminium hydroxide on all serological parameters.

When administered intramuscularly in mice, CAF01 induces an immune response characterised by T-helper-1 and T-helper-17 cells, which is an ideal profile for induction of mucosal B cells and a secretory IgA response.[15] Vaccine studies in mice[14] and minipigs[13,15] have shown a cross-mucosal immunological link between nasopharyngeal and genital mucosal immunity, and the present trial was partly designed to confirm this link in humans. Although mucosal responses were low, in particular in the nasal samples, we were able to detect significant increases in vaccine-specific responses with the CAF01-adjuvanted vaccine. IgA responses were unique to the CAF01 adjuvant and seemed to be dependent on the intranasal boost, as would have been predicted on the basis of the extensive animal model data available for this vaccine.

Significant amounts of specific IgG were found in the vaginal fluid in both vaccine groups, correlating well with serum concentrations. IgG antibodies in the female genital tract are primarily thought to be derived from serum,[23] and our findings support the hypothesis that circulating IgG antibodies reach the genital tract in high titres. These results are in line with observations from the HPV vaccines, where measurable vaginal IgG antibodies are detectable at various time points after the last vaccination.[24,25] The efficacy of the HPV vaccines is well established and the major mechanism of protection is thought to be transudation of serum antibodies into cervical secretions. The relative roles of IgA and IgG for protection against *C trachomatis* are not clear, but the promising results in the present study prompt further exploration of the relative roles of these isotypes in later trials.

Neutralising vaginal antibodies are the first line of defence against *C trachomatis* infection and are thought to be key to the protective efficacy of CTH522. Adoptive transfer studies of antibodies in mice have shown that neutralising antibodies can block infection and also act in synergy with cellular immune responses.[12,26] We have developed an in vitro inhibition assay that correlates with the ability of antibodies to protect against the first phase of infection in animal models.[12,26] Significant concentrations of neutralising antibodies were found in both CTH522:CAF01 and CTH522:AH-vaccinated individuals in the present clinical trial. CTH522:CAF01 induced a

higher median neutralisation titre than CTH522:AH, and for both groups a strong correlation was observed with serum titres against CTH522 (Spearman's rank correlation coefficient=0·74). If this correlation is reproduced in confirmatory clinical trials, it would be tempting to use the plasma titres as a simple surrogate for the functional assay. However, neutralisation will most likely not capture the full picture of the protective mechanism behind CTH522-induced functional antibodies, especially the ability of antibodies to recruit the cellular immune response via Fc receptors.[27] Ongoing studies will characterise antibody function in opsonisation, complement activation, and antibody-dependent cellular cytotoxicity. These insights will aid in identification of potential correlates of protection further on in clinical development.

Comprehensive preclinical evidence supports the role of cellular immunity and in particular of interferon-γ-secreting T-helper-1 cells in the elimination of intracellular bacteria.[28] CTH522 contains numerous T-cell epitopes from MOMP,[29] and dissection of the CTH522:CAF01 protective response in animal models suggests an important synergistic role of CD4-positive T cells and neutralising antibody responses.[12] In the present clinical trial, we observed a robust cellular response measured by the number of vaccine-specific interferon-γ-secreting T cells. These results are in line with previously published interferon-γ ELISpot results with CAF01 used in a vaccine against tuberculosis; notably, in that clinical trial, CAF01 had the ability to maintain immunological memory with stable cell-mediated immune responses for more than 150 weeks.[20]

One consideration as this vaccine moves into more advanced clinical evaluation is coverage against clinically relevant strains. CTH522 incorporates a key neutralising epitope expressed in serotypes D–G, which are the most prevalent serotypes in clinical circulation, representing up to 90% of genital *C trachomatis* infections.[30] The CTH522 vaccine molecule also contains large segments of MOMP shared among all genital tract isolates, and these segments of the molecule are known to contain both shared B-cell and T-cell epitopes.[29] If the CTH522:CAF01 vaccine shows proof of concept in a future clinical efficacy trial, the vaccine might potentially provide some level of protection against the remaining 10% of clinically relevant serovars.

This study had several limitations. As with other phase 1 studies, the small sample size limited the assessment of rare adverse events and prevented well-powered immunological investigations. The accelerated schedule probably resulted in suboptimal antibody maturation, and a wider spacing between the second and third intramuscular immunisations could possibly have generated better neutralising antibody responses.[19] The chosen sampling strategy did not allow for clarification of whether the intranasal boosts were the exclusive driver of the mucosal IgA response. However, it is reassuring to see substantial induction of antigen-specific responses at the mucosal sites, which supports further clinical development; further research could establish whether a complex regimen with a mucosal boost is required.

Our trial did not enrol participants with a history of *C trachomatis* infection; however, given the high prevalence of unacknowledged infections, a potential impact of established infection or adaptive immunity on vaccine safety and immunogenicity will be a priority in future clinical assessments of this vaccine. Finally, since no established correlate of protection exists against chlamydia, whether the immune response generated by the CTH522-based vaccines correlates with protective immunity remains unknown and a priority for future study.

In conclusion, we show that CTH522:CAF01 and CTH522:AH are both safe and immunogenic. The promising immunogenicity profile of CTH522:CAF01 warrants further clinical development and preparation of a phase 2 dose optimisation study is currently ongoing.

Contributors

SA did the clinical trial, with assistance from TC. RJS, PA, and FF designed the study and analysis plans with input from MR, IK, MPK, KSK, and KM. HBJ and MR wrote the first draft of the paper, with input from FF, and analysed the immunogenicity data. HMC processed and stored all samples, and did the ELISpots and mucosal ELISAs, together with LRM and SD. PB managed the clinical trial. RBD compiled the safety and immunogenicity data. MPK and KSK qualified and did the serum ELISA, and KSK designed the ELISpot analysis and analysed the results. SK did the neutralisation assay, with input from AWO and HBJ. DL, KM, MR, IK, PA, RJS, KSK, MPK, HBJ, and FF discussed and interpreted the overall dataset. All authors have read and approved the final version.

Declaration of interests

PA, AWO, and FF are co-inventors on a patent application on vaccines against Chlamydia [WO2014146663A1]. All rights have been assigned to Statens Serum Institut, a Danish not-for-profit institute under the Ministry of health. SA, HBJ, PB, RBD, TC, HMC, MPK, KSK, DL, LRM, SD, SK, KM, IK, and RJS declare no competing interests.

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References

1 Newman L, Rowley J, Vander Hoorn S, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One* 2015; **10:** e0143304.

2 Davies B, Turner KME, Frolund M, et al. Risk of reproductive complications following chlamydia testing: a population-based retrospective cohort study in Denmark. *Lancet Infect Dis* 2016; **16:** 1057–64.

3 GBD 2016 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017; **390:** 1260–344.

4 Cohen CR, Brunham RC. Pathogenesis of Chlamydia induced pelvic inflammatory disease. *Sex Transm Infect* 1999; **75:** 21–24.

5 Ng BE, Butler LM, Horvath T, Rutherford GW. Population-based biomedical sexually transmitted infection control interventions for reducing HIV infection. *Cochrane Database Syst Rev* 2011; **2011:** CD001220.

6 Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Sexually transmitted diseases and infertility. *Am J Obstet Gynecol* 2017; **216:** 1–9.

7 Adachi K, Nielsen-Saines K, Klausner JD. *Chlamydia trachomatis* infection in pregnancy: the global challenge of preventing adverse pregnancy and infant outcomes in Sub-Saharan Africa and Asia. *Biomed Res Int* 2016; **2016:** 9315757.

8 Hocking JS, Temple-Smith M, Guy R, et al. Population effectiveness of opportunistic chlamydia testing in primary care in Australia: a cluster-randomised controlled trial. *Lancet* 2018; **392:** 1413–22.

9 Lewis J, White PJ. Changes in chlamydia prevalence and duration of infection estimated from testing and diagnosis rates in England: a model-based analysis using surveillance data, 2000–15. *Lancet Public Health* 2018; **3:** e271–78.

10 Batteiger BE, Xu F, Johnson RE, Rekart ML. Protective immunity to *Chlamydia trachomatis* genital infection: evidence from human studies. *J Infect Dis* 2010; **201** (suppl 2)**:** S178–89.

11 Zhong G, Brunham RC, de la Maza LM, Darville T, Deal C. National Institute of Allergy and Infectious Diseases workshop report: "Chlamydia vaccines: the way forward". *Vaccine* 2017; published online Oct 31. DOI:10.1016/j.vaccine.2017.

12 Olsen AW, Follmann F, Erneholm K, Rosenkrands I, Andersen P. Protection Against *Chlamydia trachomatis* infection and upper genital tract pathological changes by vaccine-promoted neutralizing antibodies directed to the VD4 of the major outer membrane protein. *J Infect Dis* 2015; **212:** 978–89.

13 Lorenzen E, Follmann F, Boje S, et al. Intramuscular priming and intranasal boosting induce strong genital immunity through secretory IgA in minipigs infected with *Chlamydia trachomatis*. *Front Immunol* 2015; **6:** 628.

14 Fiorino F, Pettini E, Pozzi G, Medaglini D, Ciabattini A. Prime-boost strategies in mucosal immunization affect local IgA production and the type of the response. *Front Immunol* 2013; **4:** 128.

15 Christensen D, Mortensen R, Rosenkrands I, Dietrich J, Andersen P. Vaccine-induced Th17 cells are established as resident memory cells in the lung and promote local IgA responses. *Mucosal Immunol* 2017; **10:** 260–70.

16 Heineman TC, Clements-Mann ML, Poland GA, et al. A randomized, controlled study in adults of the immunogenicity of a novel hepatitis B vaccine containing MF59 adjuvant. *Vaccine* 1999; **17:** 2769–78.

17 Unemo M, Bradshaw CS, Hocking JS, et al. Sexually transmitted infections: challenges ahead. *Lancet Infect Dis* 2017; **17:** e235–79.

18 Brunham RC, Rappuoli R. *Chlamydia trachomatis* control requires a vaccine. *Vaccine* 2013; **31:** 1892–97.

19 Plotkin S, Orenstein WA, Offit PA. Vaccines, 6th edn. Philadelphia, PA: Elsevier, 2013.

20 van Dissel JT, Joosten SA, Hoff ST, et al. A novel liposomal adjuvant system, CAF01, promotes long-lived *Mycobacterium tuberculosis*-specific T-cell responses in human. *Vaccine* 2014; **32:** 7098–107.

21 Dejon-Agobe JC, Ateba-Ngoa U, Lalremruata A, et al. Controlled human malaria infection of healthy lifelong malaria-exposed adults to assess safety, immunogenicity and efficacy of the asexual blood stage malaria vaccine candidate GMZ2. *Clin Infect Dis* 2018; published online Dec 18. DOI:10.1093/cid/ciy1087.

22 Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. *Nat Rev Immunol* 2009; **9:** 287–93.

23 Mestecky J, Moldoveanu Z, Russell MW. Immunologic uniqueness of the genital tract: challenge for vaccine development. *Am J Reprod Immunol* 2005; **53:** 208–14.

24 Fife KH, Wheeler CM, Koutsky LA, et al. Dose-ranging studies of the safety and immunogenicity of human papillomavirus type 11 and type 16 virus-like particle candidate vaccines in young healthy women. *Vaccine* 2004; **22:** 2943–52.

25 Nardelli-Haefliger D, Wirthner D, Schiller JT, et al. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. *J Natl Cancer Inst* 2003; **95:** 1128–37.

26 Olsen AW, Lorenzen EK, Rosenkrands I, Follmann F, Andersen P. Protective effect of vaccine promoted neutralizing antibodies against the intracellular pathogen *Chlamydia trachomatis*. *Front Immunol* 2017; **8:** 1652.

27 Moore T, Ananaba GA, Bolier J, et al. Fc receptor regulation of protective immunity against *Chlamydia trachomatis*. *Immunology* 2002; **105:** 213–21.

28 de la Maza LM, Zhong G, Brunham RC. Update on *Chlamydia trachomatis* vaccinology. *Clin Vaccine Immunol* 2017; **24:** e00543–16.

29 Nunes A, Nogueira PJ, Borrego MJ, Gomes JP. Adaptive evolution of the *Chlamydia trachomatis* dominant antigen reveals distinct evolutionary scenarios for B- and T-cell epitopes: worldwide survey. *PLoS One* 2010; **5:** e13171.

30 Suchland RJ, Eckert LO, Hawes SE, Stamm WE. Longitudinal assessment of infecting serovars of *Chlamydia trachomatis* in Seattle public health clinics: 1988–1996. *Sex Transm Dis* 2003; **30:** 357–61.