Trachoma Transmission-Blocking Vaccine in Nonhuman Primates: Implication for a Protection

Membrane Protein Induces Partial Protection

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Chlamydia trachomatis Native Major Outer Membrane Protein Induces Partial Protection in Nonhuman Primates: Implication for a Trachoma Transmission-Blocking Vaccine

Laszlo Kari,* William M. Whitmire,* Deborah D. Crane,* Nathalie Reveneau,2* John H. Carlson,* Morgan M. Goheen,* Ellena M. Peterson,† Sukumar Pal,† Luis M. de la Maza,† and Harlan D. Caldwell3*

A vaccine is likely the most effective strategy for controlling human chlamydial infections. Recent studies have shown immunization with Chlamydia muridarum major outer membrane protein (MOMP) can induce significant protection against infection and disease in mice if its native trimeric structure is preserved (nMOMP). The objective of this study was to investigate the immunogenicity and vaccine efficacy of Chlamydia trachomatis nMOMP in a nonhuman primate trachoma model. Cynomolgus monkeys (Macaca fascicularis) were immunized systemically with nMOMP, and monkeys were challenged ocularly. Immunization induced disease in mice if its native trimeric structure is preserved (nMOMP). The objective of this study was to investigate the immunogenicity and vaccine efficacy of Chlamydia trachomatis nMOMP in a nonhuman primate trachoma model. Cynomolgus monkeys (Macaca fascicularis) were immunized systemically with nMOMP, and monkeys were challenged ocularly. Immunization induced high serum IgG and IgA ELISA Ab titers, with Abs displaying high strain-specific neutralizing activity. The PBMCs of immunized monkeys produced a broadly cross-reactive, Ag-specific IFN-γ response equivalent to that induced by experimental infection. Immunized monkeys exhibited a significant decrease in infectious burden during the early peak shedding periods (days 3–14). However, at later time points, they exhibited no difference from control animals in either burden or duration of infection. Immunization had no effect on the progression of ocular disease. These results show that systemically administered nMOMP is highly immunogenic in nonhuman primates and elicits partially protective immunity against ocular chlamydial challenge. This is the first time a subunit vaccine has shown a significant reduction in ocular shedding in nonhuman primates. A partially protective vaccine, particularly one that reduces infectious burden after primary infection of children, could interrupt the natural trachoma reinfection cycle. This would have a beneficial effect on the transmission between children and sensitized adults which drives blinding inflammatory disease.


Trachoma, a chronic ocular disease caused by Chlamydia trachomatis, is a leading cause of preventable blindness (1). The disease has largely disappeared from Europe and North America, but it continues to be hyperendemic in many of the poorest areas of Africa, Asia, Australia, and the Middle East. Trichiasis is a painful sequel of trachoma where, due to conjunctival scarring caused by chlamydial infection, eyelashes turn inward to touch the cornea. This condition leads to decreased visual acuity, and eventually blindness, through corneal abrasion. The World Health Organization has proposed a four-element approach to eliminate trachoma as a cause of incident blindness, called the SAFE strategy (2). The acronym stands for Surgery to correct trichiasis, Antibiotics to treat infection, and Facial cleanliness and Environmental improvements to reduce chlamydial transmission. In humans, infection is asymptomatic in many individuals; thus, treating only those with clinical symptoms will not control the spread of infection. A vaccination program would have greater impact on decreasing the prevalence of blinding trachoma.

C. trachomatis is also the causative agent of the most common bacterial sexually transmitted diseases and lymphogranuloma venereum. C. trachomatis strains are divided into 15 serogroups (serovars), of which A–C cause trachoma, D–K cause genital STDs, and L1–L3 cause lymphogranuloma venereum (3–6). Chlamydiae are obligate intracellular parasites exhibiting a unique biphasic life cycle. The infectious, but metabolically inactive, elementary bodies (EBs) attach to cells and enter to form intracellular inclusions. Inside cells, EBs transform into noninfectious, metabolically active reticulate bodies which divide by binary fission and eventually convert back to EBs (7). During the extracellular EB stage, Abs present in genital tract or ocular secretions can inhibit infection both in vivo and in tissue culture (8–10). However, the reticulate bodies, residing within the intracellular inclusion, remain inaccessible to Abs. Resolution of infection at this stage requires a cell-mediated immune response likely controlled by IFN-γ-secreting Th1 cells. Thus, an ideal C. trachomatis vaccine should induce both local neutralizing Abs to prevent infection by EBs, and a Th1 response to limit infection once it is initiated. The intracellular lifestyle of the bacteria, where they reside in a well-protected inclusion, makes the production of either an effective natural or an artificial immune response difficult.

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2 Current address: Sanofi Pasteur Ltd., Toronto, Ontario M2R 3T4, Canada.

3 Address correspondence and reprint requests to Dr. Harlan D. Caldwell, Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Disease, National Institutes of Health, 903 South fourth Street, Hamilton, MT 59840. E-mail address: hcaldwell@niaid.nih.gov

4 Abbreviations used in this paper: EB, elementary body; MOMP, major outer membrane protein; nMOMP, native-MOMP; SPG, sucrose phosphate glutamate; IFU, inclusion-forming units; RT, room temperature; VD, variable domain.

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Development of a vaccine against *C. trachomatis* is a high priority. Computer modeling has indicated that even a partially protective vaccine would substantially reduce infections worldwide (11, 12). Efforts to create a vaccine have been unsuccessful to date. In fact, humans vaccinated with killed EBs present more severe disease than nonvaccinated individuals after naturally acquired infection (13–15). This suggests that dead intact chlamydiae harbor immunopathogenic components, thus arguing against the use of either inactivated or live-attenuated vaccines. Hence the major effort in the development of a chlamydial vaccine has focused on subunit immunogens capable of evoking protective immunity without sensitization to damaging immunopathogenic Ags.

The major outer membrane protein (MOMP) is regarded as one of the most promising subunit vaccine candidates. Highly immunogenic and immunoaccessible, it elicits both neutralizing Abs and T cell immunity (10, 16–21). MOMP is the dominant surface protein (contributing to 60% of the total protein mass in the outer membrane) and consists of four variable domains interspersed between five constant domains (22, 23). The four variable domains contain serovar-specific epitopes; the five constant domains are highly conserved between the different serovars and contain several conserved CD4 and CD8 T cell epitopes (24–26). MOMP has been used in several vaccine studies, together with various adjuvants and delivery systems. Still, attempts to induce protection using MOMP, MOMP peptides, or plasmids expressing MOMP yielded disappointing results, in both small animal models (27–32) using MOMP, MOMP peptides, or plasmids expressing MOMP and cynomolgus monkeys (33, 34). These studies demonstrated either no protection or limited protection against *C. trachomatis* infectious challenge.

An important exception is the recent study by Pal et al. (35) that showed systemic immunizations with MOMP purified in native conformation (nMOMP) induced protection against genital challenge in the murine model. The protective immune response, as measured by postchallenge infectious burden, duration of shedding, and disease (infertility), was equal to that induced by experimental infection. Currently, this remains the most successful attempt of using a chlamydial subunit vaccine to mimic natural immunity. Because of these very encouraging results, we have extended these studies to nonhuman primates. Here we describe the immunogenicity of nMOMP subunit vaccination and the resulting partially protective immunity achieved in the nonhuman primate ocular trachoma model.

Materials and Methods

**C. trachomatis strains**

*C. trachomatis* serovar A strain A2497 (A2497), serovar A strain A/HAR-13 (A/HAR-13), serovar B strain B/TW-5/OT (B), serovar Ba strain Ba/AP-2/OT (Ba), and serovar C strain C/TW-3/OT (C) were grown in HeLa 229 cells with DMEM (Mediatech) containing 10% (v/v) FCS, 4.5 g/L glucose, 2 mM glutamine, 10 nM HEPES, 1 mM sodium pyruvate, 55 M 2-ME, and 10 μg/ml gentamicin. Density gradient-purified EBs were stored in 0.2 M sucrose, 20 mM sodium phosphate and 5 mM glutamic acid buffer (SPG) at −80°C.

**Nonhuman primates**

Six healthy adult male cynomolgus macaques (*Macaca fascicularis*) maintained in the nonhuman primate wing at Rocky Mountain Laboratories (Hamilton, MT) and cared for under standard practices implemented by the Rocky Mountain Veterinary Branch were used for all clinical procedures. Once entered into experiments, animals were housed in single cages. All clinical and handling procedures were reviewed and approved by the Animal Care and Use Committee at Rocky Mountain Laboratories, and work was conducted in full compliance with the Guide for Care and Use of Laboratory Animals, as well as all applicable federal laws and regulations. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

**Vaccination**

nMOMP was prepared from A2497 as previously described by Pal et al. (35). Three anesthetized macaques (ketamine hydrochloride, 1 mg/kg body weight) received 200 μg of nMOMP divided equally between s.c. injections on both sides of the shaved neck and i.m. injections into the shaved right and left triceps. Injections contained a 3:7 ratio of CpG ODN-2395 (1 mg; Coley Pharmaceutical Group) and nMOMP to Montanide ISA 720 (Seppic) in a total volume of 1.4 ml/animal (0.35 ml/injection site). Three other macaques received 200 μg of OVA in a similar manner. Vaccinated and control animals were boosted twice at 71 and 141 days after vaccination using the same routes and doses.

**Challenge of vaccinated monkeys**

All six macaques were challenged at 175 days after vaccination with 1 × 10⁷ A2497 EBs in 20 μl of SPG, placed under the protracted upper and lower eyelids of each eye (2 × 10⁶ EBs/eye). After inoculation, the upper lids of closed eyes were briefly rotated with sterile forceps to ensure complete coverage of the inoculum.

**Culture and disease evaluation**

Three and 7 days after challenge, weekly until clearance of disease, swabs from each eye of every animal were cultured on HeLa 229 cells in 96-well microtiter plates. Sterile type 1 Calgiswabs (Puritan Medical Products) were pressed onto the inner surface of the upper and lower lid of each eye of anesthetized animals and passed back and forth 8–10 times. Swabs were placed in 2-ml microtube tubes containing 0.5 ml of SPG and three sterile glass beads, mixed on an Eppendorf Thermomixer (Brinkmann Instruments) for 2 min at 1400 rpm and 4°C, and titrated for recoverable inclusion-forming units (IFUs).

Before each swab collection, monkeys were scored for hyperemia and follicle formation on the upper conjunctival surfaces in both eyes, as described by Taylor et al. (36). Hyperemia was scored as follows: 0, no hyperemia; 1, mild hyperemia; and 2, severe hyperemia. Follicles were scored as follows: 0, no follicles; 1, 1–3 follicles; 2, 4–10 follicles; 3, >10 follicles; and 4, follicles too numerous to count. The scores recorded for the upper conjunctival surfaces of both eyes were added for each animal and termed the clinical response score. The highest possible score was 12. Statistical analyses of the clinical response and recoverable IFUs were...
done by the statistical language R and used the nonparametric Wilcoxon rank sum test. Differences were considered significant at a value of \( p < 0.05 \).

**Coomassie and immunoblot analysis**

Purified MOMP was loaded onto 10% polyacrylamide gels to view via Coomassie staining (500 ng/lane) or Western blot analysis (100 ng/lane). To view MOMP trimers, samples were kept below 37°C, whereas MOMP monomers were viewed by boiling the sample for 5 min. GelCode Blue Stain Reagent (Thermo Scientific) was used to stain proteins according to the manufacturer’s specifications. For immunoblot analysis, proteins were transferred to 0.2-μm pore size nitrocellulose membranes (Bio-Rad) and blocked at room temperature (RT) for 30 min. The membranes were rinsed three times for 5 min in hybridization buffer (3% BSA, 1× PBS (pH 7.3), 0.05% Tween 20, 0.02% NaN₃). Primary Abs were then added to aliquots of hybridization buffer at a 1/1000 dilution and incubated at RT overnight. The primary Ab solution was removed, and the membranes were rinsed

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**FIGURE 2.** Immunized monkeys recognize both trimeric and monomeric MOMP by Western blot analysis. nMOMP was purified from *C. trachomatis* serovar A strain A2497-infected cells. The purity of the immunogen and its heat-labile trimeric state were evaluated by SDS-PAGE followed by Coomassie staining or immunoblotting with or without boiling. A, Coomassie staining of nMOMP. Arrows on the left show trimeric and monomeric forms. B, Control immunoblot of a serovar A-specific mAb (A-20). C, Immunoblot using the three immunized monkey sera. D, Immunoblot using pools of OVA and prebleed controls. kD, Kilodaltons.

**FIGURE 3.** Kinetics of the serum ELISA IgG and IgA titers against serovar A EB (A2497). The serum Ab responses of the immunized monkeys were measured by ELISA using formalin-fixed EBs (A2497). A, Serum IgG; B, serum IgA. ELISA titers were measured ~30 days after the primary vaccination and the first and second boosts.

**FIGURE 4.** Titer and specificity of the serum ELISA Ab responses for different trachoma serovars. The trachoma strain specificity of the serum Ab response was evaluated by ELISA using EBs of four different strains. A, Serum IgG; B, serum IgA. ELISA titers were measured 34 days after the second boost.
Cytokine analysis

Levels of IL-4, IL-10, IL-12p70, IFN-γ, and TNF-α were determined from culture supernatants of nonhuman primate PBMCs stimulated with UV-killed EBs of A2497, B and C. Blood was collected from each anesthetized animal into a sterile heparinized cell preparation tube (BD Biosciences) and processed according to the manufacturer’s instructions. After hypotonic lysis of the remaining RBC, collected PBMCs were washed twice in PBS, counted, and adjusted to 5 \times 10^6 cells/ml in DMEM culture medium. Two hundred microliters of each cell suspension were then placed into flat-bottom microtiter plate wells in triplicate, and either 50 µl of killed EBs at a total protein concentration of 50 µg/ml or 50 µl of SPG were added to test or control wells, respectively. Plates were rocked at 37°C for 1 h and then further incubated at 37°C for 72 h. After incubation, 130 µl of culture supernatant were collected from each well, placed into polypropylene microtiter plates, sealed, frozen at −20°C, and sent for multiplex cytokine analysis at LINCO Diagnostic Services.

Serum and tear collection

Serum was obtained from venous blood, and tears were collected by absorbing onto autoclaved and dried 5 × 20-mm Whatman No. 41 filter strips. Each strip was placed under the lower eyelid and allowed to saturate with tears. Saturated strips were then placed into microfuge tubes containing 0.2 ml of PBS, refrigerated overnight, and frozen until assayed.

ELISA

Serum and tear IgG and IgA Ab titers for each animal were determined by ELISA. Two-fold dilutions of serum or tears (100 µl/well) were added to the wells of Immulon 2 HB flat-bottom 96-well plates (ThermoLabsystems) coated with formalin-fixed A2497, A/HAR-13, Ba, or C EBs (1 µg of total protein per well). After incubation at 37°C for 2 h, plates were washed three times, and 100 µl of biotin-conjugated goat anti-monkey IgA or IgG (Rockland Immunochemicals; 1/60,000 and 1/5,000, respectively) was added to appropriate wells for another 1 h of incubation at 37°C. Plates were then washed, incubated with 100 µl/well of streptavidin (1:5,000), washed, and exposed to 100 µl of p-nitrophenyl phosphate (Invitrogen) for 30 min in the dark at RT. Reactions were stopped with 50 µl of 1 N NaOH per well and analyzed on a plate reader at 405 nm.

In vitro serum neutralization assays

C. trachomatis neutralization assays were performed in HAK cells as previously described (37). Briefly, 1 × 10^6 EBs per ml were added to 2-fold dilutions of preimmune and immune sera from vaccinated macaques and incubated for 1 h at 37°C in microfuge tubes placed on a rotator. Two hundred microliters of each dilution were then inoculated (in triplicate) onto 4 × 10^5 HAK cells/well in a 24-well plate and incubated with rocking for 2 h at 37°C. Inocula were then removed, and monolayers were fed with DMEM containing 1 µg/ml cycloheximide and incubated at 37°C for

Table I. Cytokine secretion profiles of PBMCs from nMOMP-immunized monkeys pulsed with different trachoma serovars

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<th>Immunogen</th>
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*<3 pg/ml.
36–42 h. After methanol fixation, monolayers were stained with a Chlamydia-specific mAb (A-20) and counted for IFUs. Percent specific neutralization for each dilution was calculated as \[
\frac{(\text{preimmune IFUs} - \text{immune IFUs})}{\text{preimmune IFUs}} \times 100.
\]

Results

Immunized monkey sera recognize both trimeric and monomeric MOMP under Western blot analysis

A schematic diagram showing the immunization and challenge schedule of cynomolgus monkeys is shown in Fig. 1. Initially, we analyzed the serum Ab response by Western blotting under conditions that detect both trimeric nMOMP (65 kDa) and monomeric MOMP (42 kDa). Coomassie staining revealed a strong trimeric nMOMP band and a less abundant 42-kDa monomeric MOMP band. No other protein bands were detected. After boiling, only the monomeric form of MOMP was found (Fig. 2A). Western blots were then performed using sera from immunized and control animals. Sera from all three nMOMP-immunized monkeys reacted with both trimeric and monomeric MOMP (Fig. 2C), whereas sera from both the OVA-immunized animals and prebleed controls were negative (Fig. 2D). A serovar A-specific mAb (A-20) was used as a positive control. The A-20 Ab recognized both trimeric and monomeric MOMP (Fig. 2B).

Kinetics and specificity of the serum ELISA Ab responses against different trachoma serovars

The serum and tear Ab responses of the immunized monkeys were measured by ELISA using formalin-fixed A2497 EBs. Low serum IgG and IgA titers were detected post-primary immunization, but reasonably high titers were measured after the first and second boosts (Fig. 3). Tear IgG Abs were of very low titer, and tear IgA Abs were not detectable (data not shown).

The trachoma strain specificity of the serum Ab response was also evaluated by ELISA using EBs of four different trachoma strains as coating Ags. For both IgG and IgA, the highest titers were measured against the serovar A strains (A2497 and A/HAR-13), followed by the serovar Ba and C strains (Fig. 4).

Titer and specificity of the serum neutralizing Ab responses against different trachoma serovars

We next assayed the serum of immunized monkeys for neutralizing Abs. The sera of all three immunized monkeys revealed high neutralizing titers following immunization (1/20,000–1/100,000; Fig. 5A). The trachoma strain specificity of these neutralizing titers was also evaluated. Surprisingly, and unlike the ELISA Ab titers, these experiments showed highly specific neutralizing activity against strain A2497 (Fig. 5B), the strain from which nMOMP was prepared. Neutralizing titers against the other three trachoma strains, including the other serovar A strain (A/HAR-13), were relatively low (1/2400–1/4000) compared with the homotypic titers.

Cytokine secretion profiles of PBMC from nMOMP-immunized monkeys pulsed with different trachoma serovars

The Ag-specific cytokine-mediated immune response and its serovar specificity were evaluated by profiling the Th1/Th2 cytokine response of PBMCs. The cytokine profile showed that IFN-\(\gamma\), a Th1 cytokine, was consistently induced after being pulsed with Ag (Table I). The data also revealed that, unlike the serum ELISA and

![FIGURE 6. Follicular conjunctivitis in nonhuman primates. The images are examples of uninfected and infected upper conjunctiva 4 wk postchallenge. The infected conjunctiva exhibits severe hyperemia, edema, and large follicles.](http://www.jimmunol.org/)

![FIGURE 7. Chlamydial shedding after ocular challenge of nonhuman primates. The nMOMP and OVA-immunized nonhuman primates were ocularly challenged with strain A2497. Chlamydial shedding was evaluated at weekly intervals to monitor the course of infection. The nMOMP-immunized animals shed significantly fewer organisms during the first 2 wk postchallenge (\(p = 0.002–0.026\), Wilcoxon rank sum test).](http://www.jimmunol.org/)
neutralizing titers, IFN-γ induction was heterotypic, not dependent on the serovar used for stimulating the PBMCs.

Ocular challenge of nonhuman primates immunized with nMOMP

At 34 days after the third immunization, the nMOMP- and OVA-immunized nonhuman primates were ocularly challenged with strain A2497. Chlamydial shedding and gross clinical pathology were evaluated at regular intervals to monitor the course of infection. Gross clinical pathology was scored based on hyperemia and follicle formation on the upper conjunctiva. Fig. 6 shows examples of an uninfect ed naive upper conjunctiva before challenge and an infected conjunctiva 4 wk postchallenge. The infected conjunctiva exhibits the maximum clinical score of 12, presenting with severe hyperemia, edema, and multiple large follicles. The nMOMP-immunized animals shed significantly less organisms during the first 2 wk postchallenge (p = 0.002–0.026), with 98% (70-fold) reduction at the peak shedding period observed on day 7 (Fig. 7). The total infectious burden (total bacterial shedding during the entire experiment) for these nMOMP-immunized animals was reduced by 94% (18-fold). However, the duration of bacterial shedding did not differ significantly from that in the control animals. Interestingly, monkey 139 had a delayed peak shedding period (wk 4 instead of wk 1) that corresponded to the highest serum ELISA IgG and neutralizing titers and the highest IFN-γ response. Furthermore, and surprisingly, the high nMOMP neutralizing titers, strong IFN-γ response, and reduced chlamydial shedding in the immunized animals did not manifest in a significant difference in gross clinical pathology between the two groups (Fig. 8).

Discussion

We have shown that nMOMP is highly immunogenic in nonhuman primates. Systemic immunization elicited high levels of serovar-specific serum IgG and IgA Abs, but very low levels of tear IgG and undetectable levels of tear IgA Abs, as measured by ELISA. Serum from vaccinated monkeys was shown to contain high strain-specific neutralizing Abs. Conversely, immunization induced a broad trachoma strain cross-reactive IFN-γ response. This immune response resulted in highly significant protection against homotypic ocular challenge, reducing the infectious burden >70-fold over the first 2 wk postchallenge. However, protection was restricted to early time periods postchallenge, with minimal differences observed between vaccinated and control monkeys in either infectious burden or duration at later time points. Surprisingly, these marked early differences in organism burden in the conjunctival epithelia did not reduce the severity of ocular disease. Nevertheless, this is the first time a subunit vaccine has shown a significant reduction in ocular shedding in nonhuman primates. Although two previous studies described partial protection in nonhuman primates after subunit vaccination (33, 38), this protection was limited to a transient decrease in clinical response with no significant reduction observed in shedding.

A major and perhaps important finding of this work was the high strain-specific neutralizing titers generated after immunization with nMOMP. We believe that the native trimeric structure of MOMP could be the reason for achieving such high strain-specific neutralizing titers. Neither this high titer nor strain specificity was found by ELISA when using purified formalin-fixed EBs as Ag. Serum ELISA Ab titers showed virtually identical titers against the two serovar A strains (A2497 and

FIGURE 8. Gross clinical pathology after ocular challenge of nonhuman primates. The nMOMP- and OVA-immunized nonhuman primates were ocularly challenged with strain A2497. Gross clinical pathology was evaluated at weekly intervals to monitor the course of infection. The clinical response score was determined based on the hyperemia and follicular formation of the upper conjunctiva of both eyes. 0, No disease; 12, maximum disease. There was no significant difference in the gross clinical response between the two groups.
AHAR-13), with lower but measurable titers against the heterologous Ba and C trachoma serovars. The significance of this finding is unclear, but it is consistent with previous findings that protective immunity against C. trachomatis ocular infection is serovar specific, with little to no cross-protection against different serovars (39–41). Indirectly, these findings implicate serovar-specific neutralizing Abs in ocular immunity. The exquisite degree of strain specificity found in serum neutralizing Abs of nMOMP-immunized monkeys was unexpected and unpredicted, as the MOMPs of strains A2497 and AHAR-13 differ by only four amino acids (42). Two of these differences are located in MOMP variable domains (VD; aa 80 and 153 in VDs I and II, respectively), and the other two in constant regions. According to the two-dimensional model of MOMP (43), VD I is the latching loop for the trimers, and that loop should be critical for trimer formation. Also, recombinant phage clones expressing MOMP antigenic determinants revealed that protective serotype-specific mAbs recognized epitopes in VD I and II (26). Although speculative, these findings argue the four amino acid substitutions, either independently or collectively, may change the structural properties of trimeric nMOMP. These substitutions could thus generate immunodominant determinants recognized by highly efficient infection-blocking neutralizing Abs.

Immunization with nMOMP resulted in an Ag-specific production of IFN-γ by PBMCs. The response was broadly cross-reactive, given that different trachoma strains were equally effective in its induction. IFN-γ is thought to play an important role in resolving C. trachomatis infection; however, our findings indicate that it is not sufficient to significantly alter the course of ocular infection. Possible explanations for this finding are that systemic cellular immunity was ineffective mucosally or perhaps the levels of IFN-γ generated by systemic immunization were simply insufficient. A more accurate role for IFN-γ in ocular immunity against chlamydial infection may require, like neutralizing Abs, strategies capable of targeting local ocular immune responses. Typically, C. trachomatis infections generate primarily homotypic immunity, providing less protection against heterotypic challenges (39–41). Our findings suggest protective immunity is not solely reliant on the cytokine-mediated immune response; homotypic neutralizing Abs could also be important factors. Due to the limited number of primates available for experiments, we were unable to challenge nMOMP-vaccinated animals with strains other than A2497. Heterotypic challenge using other serovars could better define the role of IFN-γ in achieving the protection observed in our experiments. Similarly, heterotypic challenge with AHAR-13 would help characterize the in vivo function of the strain specificity of the neutralizing serum Abs.

The immunity induced by systemic immunization with nMOMP was equivalent to or even better than the immunity induced by experimental infection in nonhuman primates. In a previous experiment, monkeys were infected with A2497 twice, and immunity was evaluated 3 wk later (unpublished data). The serum ELISA IgG and IgA titers and the IFN-γ response of PBMCs were comparable with that induced by nMOMP immunization. However, serum neutralizing titers were ~10-fold lower (4,000–8,000 vs 32,000–76,800) than those measured after nMOMP immunization. Because the nMOMP immunizations induced chlamydial-specific IFN-γ and serum neutralizing Ab responses that correlated with a significant reduction in the level of early bacterial shedding, the lack of impact on gross pathology postchallenge was puzzling. In mice, systemic immunization with nMOMP induced protective immunity comparable with that induced by infection with live bacteria. Discrepancies between the results of the murine and nonhuman primate experiments underscore the importance of using the nonhuman primate trachoma model in preclinical studies. Aside from the inherent differences between host and chlamydial species, these discrepancies likely result from differences in infection sites. Both the ocular conjunctiva and the upper female genital tract are mucosal sites; however, the ocular mucosa is probably more regionally isolated. Lacking a practical genital model in nonhuman primates, we were unable to investigate this further. Another interesting aspect of the partial protection induced by nMOMP immunization in nonhuman primates is that it does not differ much from the protection induced by experimental infection. A single experimental infection in primates also induces only partial protection, with a 1- to 2-log decrease in shedding and limited reduction in pathology (41, 44). This suggests that an ideal vaccine of nonhuman primates and humans would need to produce immunity that actually exceeds that of natural infection. At present, the underlying mechanisms for this disparity remain unclear, but it is certain that a better understanding of human immunity and C. trachomatis virulence factors capable of altering natural and vaccine-mediated immunity are needed. Vaccines that can provide effective protection of the eye will probably require immunization strategies that target regional ocular immune induction sites.

Computer modeling studies have predicted even a partially efficacious vaccine would have a significant effect on decreasing chlamydial transmission (11, 12). Considerably reducing chlamydial shedding throughout communities could successfully interrupt the trachoma reinfection transmission cycle. This approach would in fact be similar to that of mass antibiotic treatment, which reduces transmission by temporarily reducing the total infectious burden in a community. However, the effect of mass antibiotic treatments is unsustainable, and treatment must be repeated as infections return over time (45–48). In contrast, a vaccine providing long-lasting partial protection need be administered only once, or at least less frequently, to achieve a sustainable effect on transmission. In our study, systemic immunization with nMOMP reduced the total infectious burden by 94% (18-fold). Thus, a community-wide nMOMP immunization could significantly impact the fight against blinding trachoma by interrupting the reinfection cycle. Admittedly, this vaccination campaign could face significant logistical difficulties, such as the high production cost of the immunogen and the delivery and storage of the vaccine in rural African villages. This might be overcome by expressing nMOMP in a surrogate system and combining it with other childhood vaccines. Nevertheless, it is foreseeable that nMOMP vaccination in combination with antibiotic treatment could be the most effective way to eliminate trachoma.

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