Oral Immunization With Chlamydial Major Outer Membrane Protein (MOMP)

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The effect of vaccination to stimulate mucosal immunity with a purified subunit vaccine of chlamydial major outer membrane protein (MOMP) on subsequent ocular challenge with *Chlamydia trachomatis* was studied in cynomolgus monkeys. Monkeys were immunized with MOMP by intraperitoneal priming plus oral boosting, with or without ocular boosting, or by ocular immunization alone. Cholera toxin was used as an adjuvant with the oral doses of MOMP vaccine. Animals were challenged with viable purified elementary bodies 35 days after the first immunization. The immunizing schedules used provided a transient decrease in the potentially deleterious inflammatory response in the eye but no reduction in duration of infection. Immunoblotting studies showed that anti-MOMP antibodies were induced by oral vaccination in some animals, but the antibody response following ocular challenge as determined by microimmunofluorescence (MicroIF) serology was similar in vaccinated and nonvaccinated animals. This study demonstrates that the stimulation of mucosal immunity by a vaccine of purified chlamydial MOMP was only partially effective in protecting against chlamydial eye infection. The lack of clear protection in these studies may be due to the failure of the various immunizing regimes to induce an antibody response prior to challenge. Invest Ophthalmol Vis Sci 29:1847–1853, 1988

An effective vaccine against trachoma will probably have to stimulate protective immunity at the mucosal surface of the eye. Previous studies have shown that the oral administration of an antigen can prime the eye to subsequent challenge with that antigen. Preliminary vaccine trials in a monkey model of trachoma have shown that protection equivalent to that seen after ocular infection can be induced by oral immunization with viable whole *Chlamydia trachomatis* L2 serovar elementary body (EB) vaccines. However, some of these oral preparations, as well as some systemic vaccines, can also induce a hypersensitivity response with more severe disease occurring after ocular challenge. It is thus clear that the immune response to *C. trachomatis* can be both protective and deleterious and that different chlamydial antigens may be responsible for the different types of immune response. Vaccination with subunit of *C. trachomatis* may offer the most appropriate mechanism of stimulating protective immunity without inducing harmful sensitization. Previous studies have shown that oral immunization with chlamydial lipopolysaccharide (LPS) does not induce protection. Using immunoblot analysis, we have recently shown that the tear IgA response to major outer membrane protein (MOMP) differed from the response to MOMP of other antichlamydial isotypes and to other chlamydial antigens. Tear IgA anti-MOMP antibody was a consistent feature in each animal studied and was characterized by early appearance (day 14) and persistence beyond the resolution of ocular disease.

Recently, Zhang and coworkers have shown that monoclonal antibodies which recognize epitopes of MOMP exposed on the surface of chlamydia can neutralize the infectivity of viable EB. This was true both for in vitro assays using a cell culture system and for in vivo assays of mouse toxicity or ocular infection in monkeys. In contrast, monoclonal antibodies reactive against nonimmunoaccessible subspecies- or species-specific MOMP epitopes or against an im-

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Table 1. Summary of immunization schedules

<table>
<thead>
<tr>
<th>Vaccination group</th>
<th>Number of monkeys</th>
<th>Immunization</th>
<th>Ocular challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular A</td>
<td>4</td>
<td>Ocular MOMP days 0, 21, 28</td>
<td>Day 35</td>
</tr>
<tr>
<td>Control A</td>
<td>2</td>
<td>Ocular saline days 0, 21, 28</td>
<td>Day 35</td>
</tr>
<tr>
<td>Enteric</td>
<td>4</td>
<td>IP MOMP day 0; PO MOMP days 14, 21</td>
<td>Day 35</td>
</tr>
<tr>
<td>Combined</td>
<td>4</td>
<td>As above; plus ocular MOMP day 28</td>
<td>Day 35</td>
</tr>
<tr>
<td>Control B</td>
<td>2</td>
<td>IP saline day 0; PO saline days 14, 21</td>
<td>Day 35</td>
</tr>
<tr>
<td>Naive</td>
<td>5</td>
<td>None</td>
<td>Day 0</td>
</tr>
<tr>
<td>Ocular immune</td>
<td>5</td>
<td>Ocular infection</td>
<td>Day 126</td>
</tr>
</tbody>
</table>

munoaccessible genus-specific epitope located on LPS were not protective.

Taken together, these findings suggest that the tear IgA response to MOMP is likely to be important in limiting chlamydial eye infection. It seemed appropriate that a vaccine containing MOMP delivered in such a way as to specifically stimulate mucosal immunity might confer protection against subsequent infectious challenge with chlamydia. This report examines the efficacy of a purified MOMP vaccine given to stimulate mucosal immunity in the monkey model of trachoma.

Materials and Methods

Animals

Young adult cynomolgus monkeys (Macaca fascicularis) were used in these studies. The first part of the study used six wild-caught monkeys obtained from Charles River Primates, Inc. (Port Washington, NY). Four of these animals were immunized by the ocular route as described below and two provided concurrent control animals receiving saline ocular inoculations (Control group A) (Table 1). The second part of the study used ten colony-raised monkeys obtained from Hazelton Laboratories (Alice, TX). These were divided into two groups of four monkeys that were immunized as outlined below, and the remaining two monkeys provided a concurrent sham-inoculated control (Control group B). Experimental data are compared to results obtained from a previous experiment in which five colony-raised cynomolgus monkeys received a primary ocular inoculation with C. trachomatis (serovar B) and a second ocular challenge 18 weeks later.1 These animals provided additional data on the normal primary or "naive" response to infection as well as data on the secondary response seen in "ocular-immune" monkeys; ie, monkeys that have previously recovered from an ocular infection. (All procedures described herein conform to the ARVO Resolution on the Use of Animals in Research.)

Vaccination

MOMP was isolated from purified serovar B by sodium dodecyl sulfate (SDS) extraction.10 One group of four monkeys was given an ocular inoculation of 4 μg of MOMP on days 0, 21, and 28. Twenty microliters of a solution containing 200 μg per ml were instilled into each conjunctival sac. This group is referred to as the "ocular vaccine" group. They were given an ocular challenge on day 35.

A second group of four monkeys was given an intraperitoneal injection of 350 μg of MOMP on day 0, followed by the oral administration of 350 μg MOMP with 0.5 mg cholera toxin (List Biological Laboratories Inc., Campbell, CA) as adjuvant on days 14 and 21. Monkeys were fasted overnight and the gastric contents neutralized with 5 ml of sodium bicarbonate. The enteric dose, diluted to a 5 ml suspension, was delivered via a gastric tube.1 This group is referred to as the "enteric vaccine" group. They received an ocular challenge on day 35.

A third group of four monkeys was also immunized by both the intraperitoneal and oral routes as outlined above; but in addition, on day 28, they received an ocular boost of 35 μg of MOMP. This was contained in 20 μl and was instilled into each conjunctival sac. They are referred to as the "combined vaccine" group and were also ocular challenged on day 35.

Ocular Challenge With C. trachomatis

C. trachomatis serovar B (TW-5) was grown in mass tissue culture. Purified EBs were prepared by centrifugation through renograffin and resuspended in phosphate-buffered saline.1,10 Ocular inoculations were adjusted to 1 × 10^7 infection forming units (IFU) per ml. Twenty microliters of suspension were placed into each conjunctival sac, giving an ocular inoculation of approximately 2 × 10^5 IFU per eye.

Examination and Specimen Collection

The clinical response of each eye was graded as follows: the follicular response in the bulbar, limbal,
superior tarsal, superior fornix, and the inferior fornix portion of the conjunctiva; hyperemia or injection of the bulbar, superior tarsal, superior fornix, and inferior fornix conjunctiva; and ocular discharge. Each sign was graded from 0 to 3. Examinations were performed in random order without informing the examiner of the monkey allocation. The scores for each eye were added together to give an individual clinical disease score. The means of these scores were used to describe the response of a group of animals. A cumulative disease score for each animal was also calculated by summing the score for each of the ten signs on days 0, 7, 16, 21, 35, 42, 56, and 84. These scores reflect the "area under the curve" of disease.

Conjunctival swabs were collected at each examination for chlamydial reisolation cultures in a cycloheximide-McCoy cell tissue culture system and for direct fluorescent antibody cytology (DFA). The aggregate inclusion titer was determined for each animal. This score is the sum of the scores of the infectious titer determined by culture on each of the 10 examination days. The infectious titer was scored on a semiquantitative scale where 0 is negative, 0.5 is for cultures negative on first passage and positive to any degree on second passage, 1 is for 1 to 9 inclusions per well on first passage, 2 is for 10 to 20 inclusions on first passage, 3 is for 1 to 10 inclusions per ×500 field on first passage, and 4 is for more than 10 inclusions per ×500 field on first passage. At each examination, serum was obtained and tears were collected using small cellulose sponges as described in previous reports. Although both eyes were examined, specimens were taken only from the left eye to eliminate the possibility of artifactitious changes in the right eye.

Serology

Tears and serum were collected for microimmuno-fluorescent serologic tests against whole chlamydial EB. Each specimen was separately titered against purified preparations of serovar B (TW-5). IgG was assayed using goat antihuman IgG (Hyland Labs, Costa Mesa, CA) which cross-reacts with monkey immunoglobulin gamma chains, and IgA was assayed with rabbit antimonkey IgA (Nordic Immunological Labs, El Torro, CA).

Immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were done essentially as previously described. Preparatory SDS-PAGE was performed using whole-cell lysates of the B serovar on a 12.5% gel. After SDS-PAGE, chlamydial polypeptides were electoblotted onto nitrocellulose paper (NCP) and the NCP then incubated with "blotto" for 30 min at room temperature. NCP was then cut into 0.5 cm strips, and individual strips were incubated with samples of monkey tears followed by 125I Protein A. In following this technique, tear antibody was diluted approximately 1:5, and 1.0 ml of the diluted specimen was incubated overnight at room temperature with an NCP strip containing chlamydial antigens. NCP strips were washed in 25 mM sodium phosphate, 0.15 M sodium chloride, pH 7.2 (PBS), and incubated with a 1:200 dilution of rabbit Fc-specific antimonkey IgA (Nordic) for 2 hr at room temperature. NCP strips were washed extensively in PBS and then incubated with 5 ml of 125I Protein A (5 × 10^4 cpm/ml) in PBS for 2 hr at room temperature. The NCP strips were washed thoroughly, dried, and subjected to autoradiography. Immunoblotting was performed on serum and tears collected from the ten animals in the second part of the study at the time of ocular challenge.

Results

Clinical and Microbiologic Response

Ocular challenge of the naive control monkeys with viable chlamydia induced a brisk, self-limited episode of inclusion conjunctivitis with resolution of disease, essentially complete by 12 weeks. The clinical response of each of the three groups of vaccinated monkeys showed an initial reduction in the duration of disease (Fig. 1A-C). The clinical response of the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the duration of disease for the first 6 weeks after ocular challenge. After this, the clinical disease score in the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the severity of disease for the first 6 weeks after ocular challenge. After this, the clinical disease score in the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the duration of disease for the first 6 weeks after ocular challenge. After this, the clinical disease score in the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the duration of disease for the first 6 weeks after ocular challenge. After this, the clinical disease score in the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the duration of disease for the first 6 weeks after ocular challenge. After this, the clinical disease score in the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the duration of disease for the first 6 weeks after ocular challenge. After this, the clinical disease score in the three vaccinated groups did not differ from that of the naive animals, and there was no overall reduction in the duration of disease for the first 6 weeks after ocular challenge.
Fig. 1. Mean clinical disease score of: (A) four monkeys that received ocular vaccination with MOMP; (B) four monkeys that received enteric vaccination with MOMP; (C) four monkeys that received combined vaccination with MOMP; (D) five ocular-immune monkeys challenged 18 weeks after primary infection (derived from ref. 1). The shaded area shows the normal response in naive monkeys receiving primary infection. It is the mean score of nine animals, the four reported in this study and the five previously reported.1

Serologic Response

Immunoblotting studies at the time of ocular challenge showed the presence of serum anti-MOMP antibodies in only two animals, one each in the enteric and the combined vaccine groups. No anti-MOMP-specific IgA antibodies were detected in either sera or tears at this time. The monkey in the enteric group that had antibodies on immunoblotting at the time of ocular challenge had a MicroIF serum IgG titer of 1:16. No IgM or IgA antibodies were detected in the serum of this monkey, and its tears were negative by MicroIF. This monkey had the mildest disease of its group with a cumulative disease score of only 21, less than half of that of the other monkeys in that group. The monkey in the combined vaccine group that was positive by immunoblot had developed serum IgM antibodies detectable by MicroIF 14 days after the initial intraperitoneal vaccination. These persisted through the time of challenge, but no serum IgG or IgA antibodies could be detected and no tear antibodies of any class were found. This monkey also had relatively mild disease after infection with a cumulative disease score of 46, but its response was similar to two other monkeys in its group which had scores of 43 and 48.

Overall, the MicroIF antibody response of the combined vaccine and the ocular vaccine groups was the same as seen in naive animals, although the animals in the enteric vaccine group had a less pronounced antibody response following challenge. All four monkeys in the enteric group developed detect-
able antichlamydial IgM, IgG, and IgA antibodies in serum; however, the titers were relatively low and the mean titers were one to two dilutions less than those in naive animals. Only one animal in the enteric group developed tear antibodies and then only after 6 weeks. The ocular-immune animals had higher tear IgA, serum IgA and IgG responses, and a lower serum IgM response than the other groups. In general, the titers in each group tended to plateau at about 4 weeks and the titers at this time are presented (Fig. 4).

Discussion

These studies demonstrate that purified MOMP vaccine did not induce strong immunity against ocular challenge, although there was a reduction in the initial severity of disease in those that received ocular vaccines. MOMP is a potent antigen since a strong antibody response is seen following ocular infection.8 MOMP appeared to be an even more appealing vaccine candidate since it has already been shown not to induce an ocular hypersensitivity response in immune monkeys.13 When purified MOMP was administered to the mucosal surface, however, it was found to be only weakly immunogenic and produced a meager antibody response in only a few animals.

In a previous report, we have shown how the oral administration of an antigen leads to priming of the conjunctiva.2 This finding supported the notion that the conjunctiva is an integral component of the mucosal immune system (or mucosal-associated lymphoid tissue). The immunizing regimen that was used in the present studies was designed to give maximal stimulation of mucosal immunity employing purified cholera toxin as an adjuvant for the oral doses.14 Cholera toxin has been shown to be a strong adjuvant for mucosal priming15 and probably has two effects: a specific effect due to tissue binding of the B subunit to GM1 gangliosides on the surface membrane of lymphoid cells and a nonspecific pharmacologic effect in which the A subunit of the toxin is thought to activate membrane-associated adenylcyclase.16

It is possible that the dose of MOMP that was used was inadequate, although each enteric dose represents the MOMP isolated from approximately $10^9$ organisms. An alternative explanation for the poor immunogenicity of MOMP may be related to structural changes in the protein molecule. MOMP is known to have a complex folded structure that is embedded in the chlamydial membrane,17 and it is possible that conformation changes may affect the immunogenicity of certain epitopes. Certainly some epitopes are immunoaccessible in the intact EB whereas other are not.8,18 It should be noted, however, that MOMP retains its specific antigenicity when used in in vitro serological studies such as immunoblotting8 and ELISA assays,19 and this would argue against overwhelming structural alteration. MOMP might have been denatured following oral administration, although the stomach contents of the fasting animals were neutralized first with sodium bicarbonate and MOMP is known to be acid stable.20 This explanation would not account for the poor an-
The clinical disease score, which summarizes both of the previous indices. Further, we have constructed the cumulative disease score which summarizes the "area under the curve" to summarize both severity and duration of clinical disease at ten standardized times over the course of infection.

Previous immunohistochemical studies of conjunctival biopsies of monkeys immunized with whole EB showed a relative increase in the number of T helper lymphocytes and also increased proportions of IgA- and IgG-expressing cells after challenge. Similar changes were observed in the monkeys in the current study that were immunized with MOMP (unpublished observations). There was a negligible serologic response to vaccination with MOMP, although the response of those animals that received ocular boosting was altered. These animals clearly showed a blunting of their initial clinical response, even though the duration of disease was not reduced. This finding requires further investigation and suggests that the immune response to MOMP may be of more importance in preventing the establishment of infection or reducing the severity of infection rather than in the clearance of an established infection. If this were true, it would have special significance for use against trachoma where low-grade chronic disease is thought to be less damaging than periods of severe inflammation in which episodes of reinfection occur frequently. Furthermore, it may suggest that cell-mediated immunity may be more important than previously realized. The paucity of a tear antibody response in the enteric group may suggest the oral induction of tolerance by this route, and this is worthy of further investigation. However, because of the variation in response seen in the animals within each group and because of the limited number of animals, this system could only detect major differences in the response to challenge. Nevertheless, the results that were obtained indicate that better methods of stimulating mucosal immunity are required.

**Key words:** Chlamydia trachomatis, trachoma, protective immunity, mucosal immunity, major outer membrane protein, subunit vaccine

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