Micro-immunofluorescence antibody responses to trachoma vaccines

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Key words: antibody, micro-immunofluorescence, monkey, trachoma, vaccine

Abstract

We evaluated antibody responses to trachoma vaccine in Taiwan monkeys using the micro-immunofluorescence assay. In the experiment with B/TW-5 gradient and genetron vaccines, the level of antibody titers could be correlated with the vaccine dose administered. Immunotype-specific protection from disease or modification of disease severity was associated with higher homologous antibody titer. In the experiment with A/G17, A/SA-6, and C/TW-3 genetron vaccines, an extraordinary high antibody response was demonstrated. Again, the high homologous antibody titers were associated with complete or partial protection from eye disease caused by the eye challenge inoculations. Cross reactions between immunotype A and C in the micro-IF test were also reflected in the vaccine protection observed in this experiment. It is concluded that there is an excellent correlation between the micro-IF antibody responses, the mouse toxicity prevention test, and vaccine prevention of monkey trachoma eye disease. Study of micro-IF antibody responses should be a useful tool for development of effective trachoma vaccine.

Introduction

In the 1960s, a number of human and animal experiments with whole elementary body particle trachoma vaccines were performed [1-7, 12]. Because they could be well controlled and carefully observed, several of our experiments in monkeys offered opportunities to determine the protective capability of such vaccines. At the time these experiments were carried out, there was no effective way to measure humoral antibody against the trachoma organisms. With the development of the micro-immunofluorescence (micro-IF) test [13, 14] in the early 1970s, an effective method became available. We have chosen two of the best monkey vaccines experiments to test stored sera retrospectively for micro-IF antibodies against the Chlamydia trachomatis strains used for immunization of the monkeys and for experimental inoculation of their eyes. It was found that the micro-IF antibody titers closely correlated with the antigenic mass in the vaccines administered, and with the observed protection or modification of experimental disease.

Materials and methods

Trachoma strains

Trachoma strains A/G-17/OT, A/SA-6/OT, B/TW-1/OT, B/TW-5/OT, and C/TW-3/OT representing major endemic trachoma immunotypes were used.

Monkeys

Taiwan monkeys, Macaca cyclopis were used. Adult monkeys of either sex with a body weight of 1.5 to 3.5 kg indigenous to Taiwan were captured in
the mountainous area of Taiwan. Only those free from severe spontaneous follicles in their eye conjunctivae and virgin to any experimental inoculation were used. Each monkey was kept in an individual cage during the experiments.

**Trachoma vaccines**

Trachoma strains were propagated in the yolk sac of embryonated eggs. Gradient vaccine was prepared by particle purification with sonication or trypsin digestion of the infected yolk sac suspensions in three cycles of high- and low-speed centrifugation, and then one or two cycles of sedimentation in tubes of 0% to 40% lineal sucrose gradient in 1.0 M KCl. The purified organisms were still alive at the end of this preparation. Genetron vaccine was prepared by particle purification with sonication or trypsin digestion and three cycles of high- and low-speed centrifugation, and then treated with two cycles of extraction with trifluorotrichloroethane (Genesolv-D). The organisms were inactivated by this preparation. The purified vaccines were concentrated to 2000% (V/W) in terms of original infected yolk sac in phosphate-buffered saline (pH 7.0) containing 0.02% formalin. A particle count was determined for each purified vaccine by an electron microscopic method [10].

**Adjuvant**

Incomplete Freund adjuvant (oil adjuvant) was prepared by mixing 85% purified Bayol F (Drakeol 6 UR) with 15% Arlacel A (mannide mono-oleate). Equal portions of purified vaccine and adjuvant were emulsified by circulating from one syringe to another [12]. Emulsification was continued until a flow rate of three to five minutes was obtained with stability time of 16 days at 37°C.

**Immunization of monkeys**

Vaccine was given intramuscularly 1 ml in 4 divided sites (0.25 ml per site) at each immunization.

**Potency test of vaccine in MTPT**

Tenfold dilutions of purified vaccine, 10% to 0.001%, were made for intravenous (IV) immunization to mice. 0.5 ml of each dilution for each of 10 mice, 2 doses 1 week apart, and mice were intravenously challenged with 1.5 LD50 of a toxic dose three days after the last immunization. The method has been described [10]. The ED50 is expressed by dilution of vaccine or number of organism particles.

**Monkey eye inoculation**

Monkeys were inoculated in both eyes simultaneously with challenge materials of different strains. The eyelid was everted by an eye retractor, and a cotton swab soaked with infectious material (approximately 0.2 ml) was rubbed into the upper conjunctivae by 20 back and forth strokes [12]. The inoculum used in each experiment was simultaneously titrated in eggs [9] to determine the egg infectivity dose (EID50) in 0.2 ml.

**Follow-up of eye infection**

The methods of eye examination, recording of symptoms, and scoring of disease severity have been described [11]. The appearance of trachomatous follicles is one of the most reliable characteristics in monkey trachoma. More weight was allocated to follicles in the scoring system. Together with other clinical symptoms, the highest score or most severe disease was 19.5. The strains utilized in these two experiments exhibit characteristically milder disease in Taiwan monkeys. Monkeys were observed for 10 weeks after eye infection. The average disease score from the 10-week observation was used to express disease response of each monkey eye to the challenge inoculation. Each monkey was bled at monthly intervals. Sera obtained prior to challenge were analyzed by the micro-IF test for antibody responses to vaccine in this study.