

Serological Response of Rhesus Monkeys (*Macaca mulatta*) to Immunization and Infection with *Pasteurella pestis*¹ (35979)

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It has been accepted that the presence of antibody against the Fraction 1 antigen of *Pasteurella pestis* is evidence of either immunization against or infection with *P. pestis* (1-4). Several serological tests have been devised to measure plague antibody, *i.e.*, whole cell agglutination, passive hemagglutination (HA), complement fixation (CF), mouse protection index, and agar gel precipitin-inhibition tests (5-9). The HA and CF tests, which measure the antibody to Fraction 1, are most frequently employed due to their convenience, reproducibility, sensitivity, and specificity.

Early in their studies with attenuated *P. pestis* EV76, Girard (10) and Korobkova *et al.* (11) independently reported that they had protected guinea pigs against cutaneous plague by administration of vaccine by the oral route ("par voie buccale"). Meyer (12) demonstrated that significant protection was afforded *Macaca mulatta* by living attenuated *P. pestis* EV76(51f) administered by stomach tube.

The studies described below present data on the serological response of rhesus monkeys to both living attenuated and killed plague vaccines, as well as the comparative immunity against challenge with a fully virulent

strain of *P. pestis* and their subsequent serological response to the infection.

Materials and Methods. Vaccines. A commercial plague vaccine, USP, E medium, Lot No. K3400 (Cutter Laboratories), was used as the killed vaccine in this study. The fully competent living attenuated plague vaccine used was a freeze-dried strain of *P. pestis* EV76(51f), Lot 2 (13).

Challenge. All monkeys were challenged by the intradermal route with 2×10^5 *P. pestis* 195/P 180 days after immunization. This strain had a mouse median lethal dose (LD₅₀) of less than 10 cells.

Experimental procedure. Fourteen monkeys were assigned to 3 groups: Group 1, six monkeys were immunized intramuscularly (im) with 1.0 ml of Cutter vaccine on day 0, and given a 0.2 ml booster inoculation on day 90; Group 2, three monkeys were inoculated im with 10^9 viable EV76(51f) organisms on day 90; Group 3, four monkeys were fed 10^9 viable EV76(51f) in a banana on day 90. One unvaccinated monkey was used as a control.

Serological tests. Blood samples were collected at varying intervals, and the sera were removed aseptically. One milliliter of each serum was tested immediately and the remainder was stored at -40° for later testing. The microhemagglutination and microcomplement fixation tests of Cavanaugh (14), using purified Fraction 1 antigen of *P. pestis*, were employed.

Results. During the immunization phase of the study, no adverse reactions, *i.e.*, rise in temperature, change in behavior, regional lymphadenopathy, or gastrointestinal disturbance, were observed in any of the monkeys. Both the living and killed vaccines were well tolerated by the monkeys, regardless of the

¹ In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

² A portion of this work will be included in a dissertation to be presented by Dan C. Cavanaugh to the Graduate Faculty of the Microbiology Department, University of Maryland, College Park, Maryland 20742.

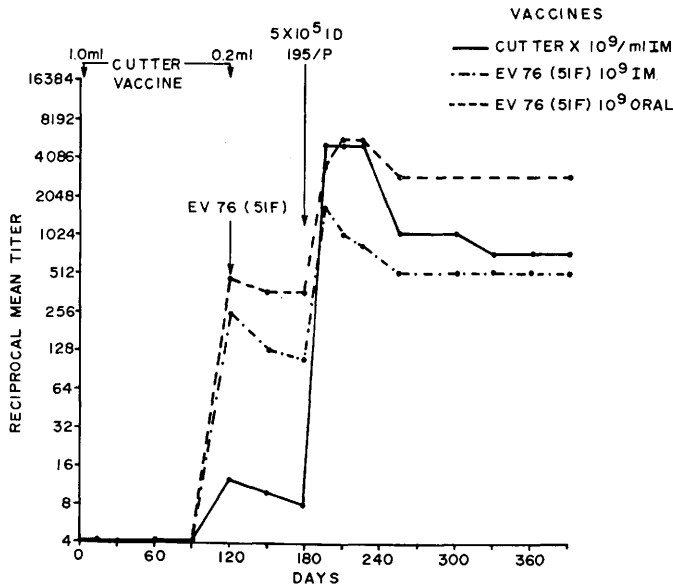


FIG. 1. Serological response of *M. mulatta* to immunization with killed plague vaccine, Cutter, Lot No. K3400, living attenuated *P. pestis* EV76(51f) and subsequent infection with virulent *P. pestis* 195/P as measured by the hemagglutination test for Fraction 1 antigen of *P. pestis*.

route of administration.

When challenged, all monkeys developed symptoms of infection, *i.e.*, fever (40° or greater), loss of appetite, and lethargy within a period of 36 hr. Three monkeys, 2 of Group 1 and the control, died of septicemic plague on days 3 and 4. *P. pestis* was isolated from heart blood, peritoneal exudate, and thoracic fluid as well as from the cut surfaces of the lungs, spleen, and liver of all 3 monkeys. There was no evidence of regional lymphadenopathy. The remaining 10 monkeys developed eschar-like lesions at the site of inoculation with enlargement of the left femoral and inguinal lymph nodes. Material taken from the lesions yielded *P. pestis* on days 4–7 only. The lesions had healed and the lymph nodes had resolved by day 28 in all monkeys.

Primary inoculation of Group 1 with 1.0 ml of killed vaccine failed to elicit demonstrable HA (Fig. 1) or CF (Fig. 2) antibodies. A second injection of 0.2 ml of the dead vaccine on day 90 resulted in the production of HA antibody by 3 monkeys and CF antibody by 2 monkeys by day 120. By contrast, all the monkeys receiving a single dose of living attenuated *P. pestis* EV76(51f)

vaccine by either the oral or parenteral routes responded by producing both HA and CF antibodies.

All monkeys that survived virulent challenge responded to the infection with a rapid rise in both HA and CF antibodies. Peak titers were observed between days 7–14 post-infection. During the next 200 days the titers, while declining somewhat from the values observed during peak period, remained higher than those observed at any time during the immunization phase of the study.

It is noteworthy that 9 of 10 monkeys surviving infection with virulent *P. pestis* had demonstrable antibody to Fraction 1 antigen prior to infection. Two of 3 vaccinated monkeys in which *P. pestis* antibody could not be demonstrated, died within 4 days following challenge. The results of previous studies have demonstrated that control unvaccinated rhesus monkeys die in 3–5 days after such a challenge.

The results obtained, when all of the sera which had been frozen at –40° were tested simultaneously, were identical to the results obtained when individual bleedings were tested prior to freezing.

Discussion. The serological response of *M.*

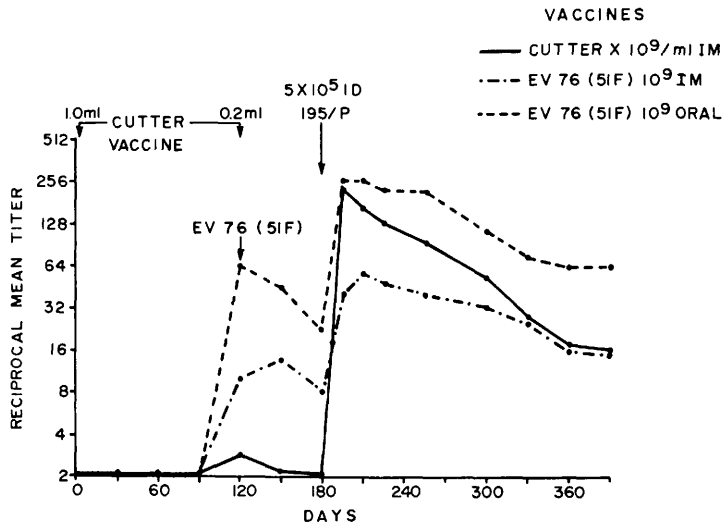


FIG. 2. Serological response of *M. mulatta* to immunization with killed plague vaccine, Cutter, Lot No. K3400, living attenuated *P. pestis* EV76(51f) and subsequent infection with virulent *P. pestis* 195/P as measured by the complement fixation test for Fraction 1 antigen of *P. pestis*.

mulatta to killed or living attenuated plague vaccines is markedly different. None of 6 monkeys, administered a single 1.0 ml injection of the killed vaccine, responded by the production of demonstrable HA or CF antibodies. A second injection of 0.2 ml of killed vaccine, 90 days after the first vaccination, elicited a low, but persistent HA response in 50% of the monkeys, and a transient CF response in 33%. By contrast, 100% of the monkeys given a single large dose of a competent living attenuated plague vaccine by either the oral or parenteral routes, responded by the production of persistent high titer HA and CF antibodies to the Fraction 1 antigen of *P. pestis*.

The presence of either type of antibody at the time of challenge proved to be an excellent indication of the subsequent fate of the experimental monkey.

The use of attenuated *P. pestis* as an oral vaccine is not new. Korobkova *et al.* (11) reported a series of experiments, using oral immunization of guinea pigs. The results of their study indicated that 3 doses of 10⁸-10⁹ were required to immunize 60-80 of the test guinea pigs by the oral route. The apparent conflict in the results of their study and our study may be attributed to the difference in species of test animals. We do not believe

this to be the explanation, as previously unreported studies in this laboratory have shown that the median guinea pig cutaneous protective dose of EV76(51f) was 23 organisms, whereas the median guinea pig protective dose of the EV strain used by Korobkova was approximately 1000 organisms. We have found that various strains labeled *P. pestis* EV76 were not similar in their competence as plague vaccines. Similar findings have been emphasized by Meyer (15). We consider that a strain of EV76 is a fully competent strain when it retains all virulence factors, except pigmentation on heme agar (16).

In the experiments conducted by Meyer (12), approximately 10 times the number of viable organisms were required to protect *M. mulatta* when administered by gastric intubation. The difference in the degree of protection afforded cannot be attributed to differences in the vaccine strains used, as both have been demonstrated to be fully competent. Studies to be reported elsewhere clearly demonstrate that the mucosal layers of the buccal cavity are the major portals of entry for both virulent and avirulent *P. pestis*.

The magnitude of the serological response to infection in the surviving monkeys of the present study was not related to the titer of preexisting antibody at the time of challenge.

Group 1, which received the killed vaccine, and had the lowest titer of both HA and CF antibody on the day of challenge, responded to infection by the greatest increase in antibody production of the 3 groups. Of the 2 groups receiving the living attenuated vaccine, Group 3 (oral) consistently had higher geometric mean titers than Group 2 (im), although there was some overlapping of titers of individuals within the 2 groups.

It was a matter of some concern whether prolonged storage of sera at -40° would alter the results of serological tests for CF and HA antibody. Therefore, each individual serum was tested for these antibodies immediately after collection, and again after varying periods of storage. A comparison of the results obtained from both the fresh and frozen specimens clearly show that no deterioration of antibody titer occurred as a result of storage for periods up to 9 months.

The persistence of both the HA and CF antibody titers after infection support Meyer's recommendations for serological surveys subsequent to the end of an epidemic as a method for retrospective epidemiological survey (1).

Summary. Living attenuated *Pasteurella pestis* vaccine EV76(51f) was an effective immunizing agent for *Macaca mulatta* by either the oral or parenteral route. As determined by serological tests and challenge experiments, a killed plague vaccine was less

effective.

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