Long-Term Observation of Subunit Vaccine F1-rV270 against *Yersinia pestis* in Mice

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Long-term protection and antibody response for the subunit vaccine F1-rV270 were determined by using the mouse model. Antibodies to F1 and rV270 were still detectable over a period of 518 days. The complete protection against lethal challenge of *Yersinia pestis* could be achieved up to day 518 after primary immunization.

Plague is a zoonotic disease caused by *Yersinia pestis* (10). Recently, *Y. pestis* has attracted considerable attention because of its use as an agent of biological warfare or bioterrorism (13).

To date, there is not an ideal plague vaccine for human use. Although EV76 was effective against bubonic and pneumonic plague, it showed side effects of varying severity and has not been used in the Western world (8, 14, 16). In contrast, subunit vaccines have potential advantages over EV76 in terms of safety of use. It has been demonstrated that the F1 and LcrV antigens used alone or in the combination F1-LcrV can protect mice against bubonic and pneumonic plague (2, 7). However, an ideal plague subunit vaccine should at least have long-term protective efficacy against *Y. pestis* infection. Anderson et al. have demonstrated that a single dose of the subunit vaccine consisting of F1 and LcrV antigens adsorbed to aluminum hydroxide can protect mice against pneumonic plague for a period up to 358 days (1). Jones et al. have determined the long-term antibody response to rF1 and rV in four strains of female mice over a time course of 383 days (6). Our previous study has demonstrated that the alum-adjuvanted subunit vaccine (100 μl) comprising F1 (20 μg) and rV270 (10 μg), designated the SV in this study, provided effective protection in
mice, guinea pigs, and rabbits against subcutaneous challenge with \(10^6\) CFU of a virulent \(Y.\) pestis 141 strain at day 98 postimmunization (12). Here, we observed both the antibody responses and the protective efficacy of the SV in mice for a period of up to 518 days.

Ninety female BALB/c mice aged 6 to 8 weeks were equally divided into nine groups and were immunized intramuscularly with 100 μl of the SV, one-tenth of the human dose (\(8 \times 10^8\) cells) of EV76, and the same dose of aluminum hydroxide, respectively. After primary immunization, on day 21, the animals were boosted with identical doses at the same injection sites. The antibody responses to F1 or rV270 over a time course of 77, 147, or 539 days are shown in Fig. 1 to 3, and the detailed data are presented in Tables S1 to S3 in the supplemental material. The analysis of variance (ANOVA) indicated that the SV elicited significantly higher titers of circulating IgG for F1 (\(P = 0.0001, P = 0.0016, P = 0.0141\)) and rV270 (\(P = 0.0007, P < 0.0001, P < 0.0001\)) than EV76 in 77, 147, or 539 days. The anti-rV270 IgG titer generated by EV76 is in agreement with our previous reports and other reports stating that animals given the EV76 or KWC (killed whole cell) vaccine had an almost undetectable titer to LcrV (12, 16, 17, 18, 19). The IgG titer elicited by the SV or EV76 had a sharp boost on the day 42 post-primary immunization and entered stationary phase starting on day 322. Therefore, we would recommend a booster dose of the SV to be given before the day 322 post-primary immunization. Such a long-term observation for the subunit vaccine against \(Y.\) pestis challenge has not been previously shown. Anti-F1 and anti-rV270 antibodies were still found in the sera of immunized mice on day 518 after the first immunization, indicating that antigen-specific long-lived antibody-secreting plasma cells are able to live for a long period of time. Interestingly, after challenge with \(Y.\) pestis on day 56, 126, or 518, no significant anti-F1 antibody titer boost was observed.
in group SV or group EV76 within 21 days. This result does not seem to be consistent with the conclusion that the memory B cells could quickly produce more antibodies when they are exposed to the same antigen (3, 4, 11). Here, we venture a hypothesis that circulating antibodies may combine with the surface antigens exposed to newly invasive *Y. pestis* to prevent the live bacteria from eliciting the immune response in a short period of time. Based on this hypothesis, we can explain our previous result (12) in which there was no significant IgG titer difference between the mice given a single dose of EV76 and those given two doses of it.

The immunized animals were challenged on days 56, 126, and 518 with 10^6 CFU of *Y. pestis* strain 141, followed by observation for 21 days. Complete protection was observed for survivors immunized with the SV or EV76, whereas all the control mice succumbed to the same dose of *Y. pestis* 141 challenge (Table 1). Rank analysis showed no survival difference between the SV and EV76. The SV and EV76 groups had significantly higher survival than the alum-adjuvanted group (P < 0.0001). The survivors were killed humanely and autopsied for postmortem analysis, for which no. of survivors/total no. of mice

| Treatment group | Challenge time (days postimmunization) | Survival (no. of mice) |
|-----------------|----------------------------------------|------------------------|
| SV              | 56                                     | 10/10                  |
|                 | 126                                    | 10/10                  |
|                 | 518                                    | 9/9                    |
| EV76            | 56                                     | 9/9                    |
|                 | 126                                    | 10/10                  |
|                 | 518                                    | 10/10                  |
| Alum adjuvanted (control) | 56                                    | 0/10                   |
|                 | 126                                    | 0/10                   |
|                 | 518                                    | 0/10                   |

The challenge dose used for all treatment groups was 10^6 CFU of *Y. pestis* strain 141.

To develop a safe and effective subunit vaccine for plague, an LcrV variant (rV270) having a reduced immunosuppressive property was used to comprise a subunit vaccine (9, 15). The aim of this study was to determine how long the antibody response and effective protection of the SV can be sustained in mice. As far as we know, this study is the first of its kind to demonstrate that effective protection against lethal challenge of *Y. pestis* can be achieved for up to 518 days and that antibody titers to F1 and rV270 were still detectable in the sera of immunized mice for as long as 518 days. These results will help determine the validity of the SV vaccine in humans and also help us to determine the booster time based on the antibody titer data over such a long period.

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**TABLE 1.** Protective efficacy of the SV and EV76 against a lethal subcutaneous challenge in female mice

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