Human Immune Response to a Plague Vaccine Comprising Recombinant F1 and V Antigens

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The human immune response to a new recombinant plague vaccine, comprising recombinant F1 (rF1) and rV antigens, has been assessed during a phase I safety and immunogenicity trial in healthy volunteers. All the subjects produced specific immunoglobulin G (IgG) in serum after the priming dose, which peaked in value after the booster dose (day 21), with the exception of one individual in the lowest dose level group, who responded to rF1 only. Three subjects, found to have an anti-rV titer at screening, were excluded from the overall analysis. Human antibody functionality has been assessed by quantification of antibody competing for binding to rV in vitro and also by the transfer of protective immunity in human serum into the naïve mouse. Human and macaque IgG competed for binding to rV in vitro with a mouse monoclonal antibody, previously shown to protect mice against challenge with plague, suggesting that this protective B-cell epitope on rV is conserved between these three species. Total IgG to rV in individuals and the titer of IgG competing for binding to rV correlated significantly at days 21 (r = 0.72; P < 0.001) and 28 (r = 0.82; P < 0.001). Passive transfer of protective immunity into mice also correlated significantly with total IgG titer to rF1 plus rV at days 21 (r² = 98.6%; P < 0.001) and 28 (r² = 76.8%; P < 0.03). However, no significant vaccination-related change in activation of peripheral blood mononuclear cells was detected at any time. Potential serological immune correlates of protection have been investigated, but no trends specific to vaccination could be detected in cellular markers.

Plague is a potentially fatal infection in humans caused by the bacterium *Yersinia pestis*. The existing vaccine comprises a suspension of heat-killed whole bacteria. Killed whole-cell vaccines have been in use in humans since 1946, and there is evidence that these formulations provided some protection against the flea-acquired bubonic plague which is endemic in parts of the world (5). However, there is evidence in mice, guinea pigs, and nonhuman primates that such a formulation provides little protection against the most severe pneumonic form of the disease caused by exposure to wild-type F1+ *Y. pestis* and no protection in mice challenged with an F1- *Y. pestis* strain (1, 2, 22, 28, 32).

Although *Y. pestis* produces a variety of potentially protective antigens (including F1 antigen, V antigen, and other *Yersinia* outer proteins and lipopolysaccharide), most workers consider that antibody against the F1 antigen is the key protective response induced by killed whole-cell vaccines, and no response to V antigen has been detected in mice immunized with these formulations. The killed whole-cell vaccine formulations have a high degree of heterogeneity with variable endotoxin content as well as a high incidence of transient local and systemic side effects, and they require frequent boosting to maintain immunity (3, 15, 19, 24, 25).

The killed whole-cell vaccines are known to be reactogenic in humans (18, 26), with malaise, headaches, local erythema, and induration or mild lymphadenopathy reported in approximately 10% of vaccinees. Allergic reactions induced by immunization with killed whole-cell vaccines, evidenced mainly as urticaria, occur infrequently (22). A live attenuated vaccine (EV76) has also been used in humans. However, in a study in the former USSR, a febrile response was reported in 20% of vaccinees, accompanied by headache, weakness, and malaise. Erythema surrounding the site of vaccination which could reach dimensions of 15 cm² was frequently reported. Some severe systemic reactions required hospitalization. Numerous unsuccessful attempts were made to reduce the incidence of side effects by administering the vaccine by different routes, including scarification, inhalation, and even intraocularly (18).

Although the killed or attenuated vaccines described above have several shortcomings, they do indicate that protection against both the bubonic and pneumonic forms of plague is achievable. The protective efficacy of F1 antigen alone has been recognized for many years (17), and the immunization of human volunteers with F1 purified from *Y. pestis* was reported to produce protective immune responses, as assessed by passive transfer into mice (20). The forward development of a subunit vaccine is supported by two observations. First, the major antibody response to *Y. pestis* (in sera from either vaccinated or convalescent individuals) is known to be directed against the F1 antigen (27). Second, the V antigen has attracted attention as a subunit vaccine component (14) and the superior efficacy of the EV76 vaccine over killed whole-cell vaccines may be explained by the induction of an immune response to V as well as to F1 antigen by the live vaccine only (28).
The side effects of the killed whole-cell vaccines and live attenuated vaccines can be avoided by using F1 and V in a subunit vaccine, thus harnessing the combined protective benefits (28) of the F1 and V subunit antigens. Such vaccines comprising two recombinant proteins, designated rF1 and rV, which are administered as an injectable formulation adsorbed to alhydrogel, are in research (11) and development (31). Experimental evidence indicates that the combination of rF1 plus rV protects immunized animals against plague pneumopathy (29). In general, Y. pestis is not endemic in the normal population, and hence it is not possible to carry out phase II/III efficacy clinical trials normally required for licensure. In addition, it is neither practical nor ethical to trial this vaccine for efficacy directly in humans.

Both the rF1 and rV proteins, administered in alhydrogel, have been demonstrated to be highly immunogenic and protective against virulent plague in a number of animal models: mice (11), guinea pigs (12), and cynomolgus macaques (unpublished data). Further, the combination of rF1 plus rV is additive in the protection conferred on the vaccinee (28). In the mouse, the combined immunoglobulin G1 (IgG1) titer to rF1 plus rV has been shown to correlate with protection against challenge (30). Further, protection against plague in the mouse has been demonstrated by the passive transfer of antiserum specific for rF1 plus rV from immunized BALB/c mice into naïve SCID/beige mice (6).

Previously, passive transfer of vaccinee serum from a number of species into the mouse has been carried out to determine the mouse protection index as a measure of whole-cell vaccine potency (16). This passive transfer assay was a 14-day assay in which the mouse protection index was quantified as % mortality over 14 days/average time to death, with a mouse protection index of less than 10 indicative of acceptable potency. Similarly, antiserum from a clinical trial volunteer can be used to immunize mice passively, prior to organism challenge of the recipient mice. This passive transfer of immunity comprising antibody to rF1 plus rV might be expected to be protective against plague, which is predominantly an extracellular infection in which native F1 and V are produced by Y. pestis as virulence factors. A single passive transfer of antiserum will not provide indefinite protection but will serve to inhibit the toxic effects of F1 and V produced by the invading pathogen. Thus, the in vivo phase of the passive transfer assay after a single administration of antiserum will be time-bounded.

Previously, a murine monoclonal antibody (MAb) specific for rV was shown to protect mice by passive immunization against challenge with Y. pestis (8). This MAb (7.3) recognizes an epitope in a region of rV which is required for protection against infection with Y. pestis (8). If this epitope is found to be conserved in different species, it could be exploited to develop a competitive enzyme-linked immunosorbent assay (ELISA) in which antisera from clinical trial volunteers are tested for the ability to compete with MAb 7.3 for binding to the rV antigen. The assays outlined above have been applied to blood samples obtained from volunteers during a phase I clinical trial of the rF1 plus rV vaccine (designated rYP002), which was conducted in healthy adult males primarily to determine the safety of the vaccine. This phase I trial was conducted with fully ethical review of the protocol, and samples were collected with the fully informed consent of the volunteers. The rYP002 vaccine was administered in a double-blind, ascending-dose design, such that groups of six individuals received the vaccine at dose levels of 5 μg F1 + 5 μg rV, 10 μg F1 + 10 μg rV, 20 μg F1 + 20 μg rV, or 40 μg F1 + 40 μg rV. Attached to each dose group were two individuals who were administered placebo (alhydrogel in PBS), so that there were 32 subjects in the trial in total. The trial was conducted in Europe under protocol R24972 during 2001. The vaccine or placebo was administered to individuals in a two-dose intramuscular regimen, with the priming dose on day 1 and the booster dose on day 21. For any one individual, the dose level given for priming and boosting was identical. Blood samples (10 ml) were obtained throughout the schedule as follows: days −1, 8, 15, 21, 28, 35, 70, and 91. Serum was separated from these samples, by centrifugation at 1500g for 10 min, and was stored frozen (−20°C) prior to assay for specific IgG and IgG subclass, titer of competing antibody by competitive ELISA and the determination of protective efficacy by passive transfer into mice. Whole blood samples (5 ml) were collected at intervals into sodium-heparin tubes (Vacutainer CPT, no. 362753, Becton Dickinson United Kingdom Ltd.) and transported at ambient temperature (18 to 25°C) for analysis, within 24 h of collection, of cellular responses by flow cytometry.

Titration of IgG to rF1 plus rV. Human serum samples were assayed for total IgG titer specific for rF1 and rV by a good laboratory Practice-validated ELISA method modified from that previously described (29, 30) using peroxidase-conjugated antihuman antibodies (The Binding Site, Birmingham, United Kingdom), in phosphate-buffered saline (PBS). The concentration of IgG to rF1 and rV in test samples was determined from a calibration curve constructed using MultiCalc (Wallac, Milton Keynes, United Kingdom).

The curve-fitting algorithm used was a five-parameter-weighted logistical model. The lower limit of quantification was set at 97.7 U whole serum equivalents (0.977 U in assay). The IgG content of test samples was determined as an absolute value in units/ml serum. Mean values per dose level group have been determined from the calculation of standard errors of the mean. Each batch of test samples was analyzed with a set of calibration standards and quality control samples, and acceptability depended on at least four of the six quality control samples being within ±20% (±25% at the low quality control of their respective nominal values). A constant 1% (vol/vol) serum content (either nonhuman primate or human) was maintained in all calibration standards, serum controls, and test samples.

Isotyping of serum. Serum isotyping was carried out by minor modification of a previously described procedure (29) which had been qualified as for the IgG ELISA. The distribution of IgG specific for F1 and V across the subclasses IgG1, IgG2, IgG3, and IgG4 was determined and is represented in U/ml.

Mean values per dose level group have been determined from the calculation of standard errors of the mean. The lower limit of quantification was defined as the lowest calibration standard with acceptable (<20%) intra-assay (n = 6) imprecision and inaccuracy. The lower limits of quantification for antibodies to rF1 were: IgG1, 279.9 U; IgG2, 312.5 U; and IgG3, 134.9 U; and for antibodies to rV it was: IgG1, 31.3 U; IgG2, 156.3 U; IgG3, 78.1 U; and IgG4, 0.6 U).

Although 24 subjects were vaccinated in the trial, the data reported are for...
serial samples from 20 individuals, from screening to day 91. The remaining four individuals were excluded from the analysis for the following reasons: no samples were received at any time point from one individual in the 20 µg rYP002 group; serum samples from the remaining three individuals were assayed, but these data have been omitted from the overall data analysis due to the detection of an IgG titer (one individual in the 20-µg group) or IgG titer (one individual in the 20-µg group and one individual in the 40-µg group) to rV at screening.

Reference serum. The reference serum used in subsequent assays was prepared by pooling polyclonal sera collected from hyperimmune cynomolgus macaques immunized with rF1 plus rV under Good Laboratory Practice conditions (study reference MOD 051) to generate a consistent batch (unpublished data) with estimated mean IgG titer to rF1 and rV of 3631 (standard error of the mean 464) and 3433 (standard error of the mean 714) U/ml, respectively. This polyclonal macaque serum was used as the reference serum in the competitive ELISA and passive transfer assays described below.

Competitive ELISA. Individual serum samples collected in the period from days 21 to 70 were assayed by competitive ELISA in which the human serum was competed with MAb 7.3 for binding to rV antigen coated to a microtiter plate. Briefly, rV antigen was coated to 96-well microtiter plates (Dynex) at 5 µg/ml in 0.05 ml PBS (16 to 18 h, 4°C). After washing in PBS with 0.02% Tween 20, plates were blocked with 0.2 ml 5% skimmed milk powder in PBS (37°C, 1 h). After further washing, 0.05 ml MAB 7.3 (1:32,000 in 1% skimmed milk powder in PBS) was added to each well (equivalent to 80 ng/well), and the plates were incubated at 4°C for 16 h. Normal mouse serum, also diluted to 1:32,000, was added to each well (equivalent to 80 ng/ml), and the plates were incubated at 4°C for 16 h. Normal mouse serum, also diluted to 1:32,000, was added to each well (equivalent to 80 ng/ml), and the plates were incubated at 4°C for 16 h. Normal mouse serum, also diluted to 1:32,000, was added to each well (equivalent to 80 ng/ml), and the plates were incubated at 4°C for 16 h. Normal mouse serum, also diluted to 1:32,000, was added to each well (equivalent to 80 ng/ml), and the plates were incubated at 4°C for 16 h.

Cell surface marker staining was performed by adding the cell suspension (0.1 ml) to flow cytometry tubes, containing combinations of the following fluorochrome-labeled antibodies: CD4-fluorescein isothiocyanate (FITC), CD19-phycocerythrin (PE), CD3-phycoerythrin-coupled dye (ECD), CD8-phycoerythrin-coupled antibody (PE-Cy7), CD25-PE-Cy7, HLA-DR-PE-Cy7, and CD86-PE-Cy7. The positive population was identified using isotype controls.

Statistical analysis. Data were analyzed with the statistical software package (MINITAB release 13.1) to achieve analysis of variance and of regression with Pearson's coefficient. Student's t test with n − 1 degrees of freedom was used to determine the significance of the difference between paired groups of equal size. The area under the curve was determined by the trapezoid approximation method as described (http://www.duncanwil.co.uk/areacurv.html).

RESULTS

Titration of IgG to rF1 plus rV in serum. At screening, all individuals were below the lower limit of quantification except for one subject in the 20-µg dose group who was found to have an anti-rV titer prior to immunization, speculated to be due to a previous exposure to *Yersinia enterocolitica*. The immune response data for this individual has been omitted from the overall analysis. Sera from all individuals who received the placebo were below the lower limit of quantification for assay of IgG to rV and rF1, at every time point tested.

The immunized subjects produced specific antibodies against both subunits (rV and rF1) of the recombinant plague vaccine (rYP002). For each time point tested there was large variation of quantitative antibody responses between individuals within each dose level group. Therefore, group mean ± standard error of the mean values and ranges have been derived and are presented in Tables 1 and 2. Antibodies to rV were produced within two weeks of the first dose at day 1, with greater levels observed after the booster dose at day 21 (Table 1). One individual in the 5-µg rYP002 group failed to respond with a titer to rV. Across the dose range studied, at least 50% of the maximum mean anti-rV titer was retained for up to 3 months following the first dose. Antibodies to rF1 were also generated within two weeks of the first dose at day 1, with greater levels observed after the booster dose at day 21 (Table
TABLE 1. Anti-V serum IgG concentrations\textsuperscript{a}

<table>
<thead>
<tr>
<th>Day</th>
<th>5 µg rYP002</th>
<th>10 µg rYP002</th>
<th>20 µg rYP002</th>
<th>40 µg rYP002</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SEM)</td>
<td>Range</td>
<td>n</td>
</tr>
<tr>
<td>−1</td>
<td>6</td>
<td>*</td>
<td>&lt;98–136</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>155 (57)</td>
<td>98–212</td>
<td>6</td>
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<tr>
<td>15</td>
<td>6</td>
<td>1,283 (1,190)</td>
<td>98–2,508</td>
<td>6</td>
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<tr>
<td>21</td>
<td>6</td>
<td>1,956 (436)</td>
<td>98–3,104</td>
<td>6</td>
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<tr>
<td>28</td>
<td>6</td>
<td>1,506 (449)</td>
<td>98–2,700</td>
<td>6</td>
</tr>
<tr>
<td>35</td>
<td>6</td>
<td>7,573 (3,06)</td>
<td>98–21,950</td>
<td>6</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
<td>4,991 (2,712)</td>
<td>&lt;98–18,110</td>
<td>6</td>
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<tr>
<td>91</td>
<td>6</td>
<td>4,499 (2,411)</td>
<td>&lt;98–16,140</td>
<td>6</td>
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</table>

\textsuperscript{a} Rounded values are shown. n, number of subjects on which determination of the mean and range was based. Negative samples were included as zero in calculation of the mean. The lower limit of quantification (LLQ) was <98 U/ml. *, No range calculable; all samples were below the LLQ. #, No mean calculable; one or two samples positive only.

TABLE 2. Anti-F1 serum IgG concentrations\textsuperscript{a}

<table>
<thead>
<tr>
<th>Day</th>
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\textsuperscript{a} See Table 1, footnote a.
with a constant concentration of the murine MAb 7.3 for binding to rV on a solid phase. The human serum displaced the mouse monoclonal antibody, to produce a titration curve for loss of binding of the mouse antibody, with increased concentration of human serum. The percentage inhibition of binding of MAb 7.3 to rV in the presence of the test and reference serum (where each were at a 1:10 dilution) was calculated for each individual at each time point and a ratio of test:reference serum derived.

**Effect of dose level on the development of competing antibody.** When the development of an antibody titer which competed with MAb 7.3 for binding to rV was monitored by competitive ELISA at days 8 and 21 but positive at days 28 and 70. There was a significant difference between the means at day 21 ($t = 3.08, P = <0.01$) but not at days 28 or 70.

**Estimation of the half-life of the reference serum in the mouse.** The $t_{1/2}$ of the reference serum, which comprised a polyclonal macaque IgG specific for rF1 plus rV, in the mouse was estimated to be 8 days for IgG to rV and six days for IgG to rF1, with a decline to undetectable levels by 10 days (data not shown). The duration of the passive transfer assay was therefore limited to 10 days. As for the test sera, the dominant isotype in the reference serum was IgG1.

**Establishment of passive transfer assay.** Pooled test sera from each of the vaccine dose groups on day 35, conferred some degree of protective immunity on recipient mice for the first 7 days postchallenge. The protection conferred was dose-related, so that it declined with dilution of the serum. Mice receiving human serum from the placebo groups did not survive and neither did untreated control mice. The assay was terminated at day 10, at which time pooled serum from the 40-μg rYP002 group was outperforming sera from the other dose groups (data not shown).

The protection provided by pooled test sera, collected from each of the rYP002 dose groups, was compared with that provided by the reference serum, either undiluted or diluted across the same range (75% to 25% in PBS). The data for day 35 serum samples from the 5 μg rYP002, 10 μg rYP002, 20 μg rYP002, and 40 μg rYP002 groups are shown in Fig. 3. Although sera from all the groups except the 5 μg rYP002 group conferred some partial protection at this time point, only sera...
from the 40 μg rYP002 group conferred protection equivalent to the reference serum (Fig. 3d).

**Determination of transferable protective immunity for individual serum samples.** The effect of time and dose level on the development of transferable protective immunity was studied by repeating the passive transfer experiments with individual (rather than pooled) sera collected from the 20 and 40 μg rYP002 dose groups at days 21, 28, and 70. Serum samples from all individuals receiving the placebo were tested and at each time point were negative in the passive transfer assay. At day 21, serum from only 4/8 vaccinated individuals tested was scored positive by passive transfer with a test:reference ratio ≥ 1.0; at day 28 3/8 sera from vaccinees scored positive. Passive transfer of individual sera from the 10 μg rYP002 dose group was tested only at day 70, at which time only 2/6 individuals were scored positive. At this time point, 3/5 individuals tested in the 40 μg rYP002 group were also positive by passive transfer bringing the overall total to 5/14 sera tested scoring positive by passive transfer at day 70.

Sera from the three individuals who were excluded from the analysis due to an antibody titer to rV at screening were also tested by passive transfer, in an attempt to determine whether the preexisting titer was protective. When tested at the day 21 and 28 time points, none of these sera were scored positive in the passive transfer assay. By day 70, only one of the three sera was scored positive, indicating that the preexisting IgG4 titer to rV in this individual from the 20 μg rYP002 group did not confer any cross-protection as determined by this assay.

**Correlation between total IgG to rF1 plus rV and transferable protective immunity.** For all individuals across the dose groups, the specific log_{10} values for IgG to rF1 and to rV have been determined and a regression analysis for these values against the ratio of test:reference derived from the passive transfer assay at days 21 to 70 has been conducted. Significant correlations were obtained at day 21 for IgG to rF1 and rV (r² = 98.6%; P < 0.001). At day 28, IgG to rV only was significantly related to the test:reference ratio (r² = 76.8%; P < 0.03), whereas at day 70, only the IgG to rF1 was significantly related to the ratio, but the degree of correlation was weak (r = 55.7%, P < 0.01).

The difference between the mean combined AUC_{21–70} for IgG to rF1 plus rV for all individuals with a positive score by passive transfer and the mean combined AUC for IgG to rF1 plus rV for all individuals without a positive score by passive transfer, was compared by Student’s t test but was not significant at any time point.

**Cell counts.** Prior to vaccination, leukocyte counts in peripheral blood ranged between 3.99 × 10⁸ and 1.32 × 10⁹ cells/ml and for most subjects were within the accepted normal range. There was wide variation between individuals and no significant effect of vaccination could be detected.

In contrast, lymphocyte counts which comprised 22.1 to 44.6% of leukocytes, were within the normal range for all subjects prior to immunization, and fluctuated within the normal range until immediately after the second dose was administered, when they decreased. This decrease was attributed to a loss of cells to the draining lymph nodes (9) and also occurred for the placebo group, possibly in response to the injection of alhydrogel as a mild immunostimulant. Normal levels of lymphocytes, as a percentage of leukocytes in the peripheral blood, were restored from day 35.

**Flow cytometric analysis.** The response to immunization involves activation at the cellular level. Flow cytometric analysis was carried out to investigate whether immunization resulted in changes in markers of cellular activation or gross changes in cell counts and whether either of these represents an immune correlate. However, large intra- and interindividual variation existed in all treatment groups including placebo, and thus the median was used to describe group tendencies.

Within the lymphocyte population, changes in the number and percentage of CD 19⁺ B cells, CD3⁺ CD4⁺ T helper cells and CD3⁺ CD8⁺ cytotoxic T cells in peripheral blood were monitored at several time-points following immunization in comparison with preimmunization levels. Large natural interindividual variation existed prior to immunization, in all lymphocyte parameters investigated. No significant differences were observed in the number or percentages of B-cell and T-cell populations between placebo and any dose group at any
time-point following immunization and in comparison with preimmunization levels.

The expression of the memory and activation markers CD45RO, CD25, and HLA-DR was investigated on CD4+ and CD8+ lymphocytes. High expression of CD45RO was determined to examine memory lymphocyte populations. CD25 and HLA-DR were examined on CD45ROhi lymphocytes and CD45RO negative cells. Again, large variation existed between

FIG. 3. Survival of mice passively immunized with day 35 serum pooled from the 5-, (a), 10-, (b), 20-, (c), and 40-μg (d) dose groups prior to challenge. Values are survival rates postchallenge for up to 10 days. Negative control mice received human placebo serum (human) or were naive.
FIG. 3—Continued.

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individuals and no significant differences were detected in the expression of activation or memory markers between placebo and any dose group. No significant differences were found in expression of activation or memory markers between baseline and any time-point postimmunization.

**DISCUSSION**

This is the first reported clinical trial for safety and immunogenicity of a recombinant subunit vaccine for plague. None of the formerly licensed killed whole-cell plague vaccines have been subjected to a randomized clinically controlled field study in humans (10). Although controlled clinical studies are desirable, the sporadic incidence of plague in endemic areas of the world means that such studies would be difficult to conduct. Thus, an assessment of efficacy for the rYP002 vaccine will depend on the determination of immune correlates of protection which this small study has started to investigate.

The measurement of specific IgG titers to rF1 and rV in groups of immunized individuals revealed that the vaccine was immunogenic, but that there was a wide range of responses in each dose group, as expected for the human immune response. The dominant subclass was IgG1, with some IgG2 and IgG3 to both antigens, while IgG4 was detectable to rV antigen only. This subclass profile in humans is indicative of a Th2 response, which is the anticipated response to this vaccine since this has also been seen in the mouse (11, 30) and nonhuman primates (unpublished data).

The serological data reported are for 20 of the 24 individuals in the trial. While no samples were received from one individual, three further individuals were excluded from the analysis. Serum from one individual had an IgG titer to rV at screening and competed with MAb 7.3 for binding to rV as early as day 8, but conferred no protection by passive transfer at day 21 or later. A prior environmental exposure to *Yersinia enterocolitica* could have induced an anti-V titer which cross-reacted with the *Y. pestis* sequence rV. The fact that serum from this individual did not score positive by passive transfer at any time point, despite high IgG titers to rV, suggests that the preexisting anti-V titer conferred no protective benefit. On the other hand, two individuals with an apparent IgG4 titer to rV at screening but who were negative by competitive ELISA at days 8 and 21 appeared to respond to the vaccine at day 28 with positive scores by competitive ELISA; one of these individuals had a positive score by passive transfer at day 28, the other was negative at all time points tested. This sample size (n = 3) is too small to draw any sound conclusions about the effect of preexisting titers to V, possibly due to exposure to another species of *Yersinia*, on protection against plague.

Some relationship between serum IgG titer specific for rV and the ability of this antibody to compete with MAb 7.3 for binding to rV has been demonstrated in the period days 21 to 70 for the 20 subjects included in the data analysis. The number of individuals determined to have a titer of competing antibody increased to a maximum of 18/20 at day 28, 7 days after administration of the second dose. Regression analysis of serum IgG titer to rV with the test:reference ratio by competitive ELISA indicated a significant correlation across the vaccine dose groups, at days 21 and 28. However, no significant correlation was found at day 70, when IgG titers in serum were in decline for all the dose groups.

The competitive ELISA assay measured antibody competing with MAb 7.3 for binding to rV only, whereas the passive transfer assay measured the protective capacity of antibody directed to both antigens. Evidence of transferable protective immunity by passive transfer was detected in individuals in the two highest dose groups (20 μg and 40 μg) at days 21 and 28, with individuals in the 10-μg group also becoming positive by day 70, so that a maximum of 5/14 individuals tested were positive in the passive transfer assay at day 70. Nevertheless, a significant correlation was found between total IgG to rF1 and rV and transferable protective immunity by passive transfer at day 21; at day 28, the IgG titer to rV only correlated significantly with the test:reference serum ratio determined in the passive transfer assay. In contrast, the IgG titer to rF1 only, correlated with protective immunity measured by passive transfer at day 70.

In the 6 weeks (days 28 to 70), titers were in gradual decline (estimated at 2% per week); however protection by passive transfer was maximal at day 70. Thus, it is not surprising that statistical correlation between the two indices was lost. It is likely that although circulating antibody titers were in decline from peak values around day 35, residual antibody was still functional and indeed would have been undergoing affinity maturation which would off-set the decline in titer. The macaque reference serum was very protective when passively transferred into the mouse. Both the macaque and human sera were predominantly of the IgG1 isotype and would be expected to have a similar half-life in the mouse (4).

In the competitive ELISA, the human sera were able to displace MAb 7.3 from rV as effectively as the reference serum, suggesting that each source of serum was of similar binding affinity to rV. Thus, the finding that only 5 of 14 human sera tested at day 70 were more protective than the reference serum by passive transfer, would suggest that the human response to this vaccine has not plateaued at the 40-μg maximum dose level used here and that higher dose levels of vaccine should be considered for future clinical trials. However, the application of the competitive ELISA and passive transfer assays has demonstrated that individuals enrolled in the phase I clinical trial have developed antibody competing with MAb 7.3 for binding to rV and transferable protective immunity which correlates at early time points with the serum IgG titer to rF1 and rV.

The immune correlates of protection investigated here rely on measuring the function of specific serological antibody. The assays suffer the disadvantage that the natural decline in functional antibody with time (in the absence of a further booster dose) may render the assays less accurate and predictive at later time points. The data presented above indicate that beyond day 28, the competitive ELISA and passive transfer assays no longer give significant correlation with total IgG to rV and/or rF1. However, it is expected that cellular memory to the vaccine will be maintained for many months. Exposure of an immunized individual to plague, even in the presence of only a low antibody titer, would stimulate memory B and T lymphocytes, triggering rapid antibody production. An assay of cell-mediated immunity would be advantageous in determining the degree of memory recall response which prevails in the individual after serum antibody titers have declined.
To this end, whole blood samples from individuals in the phase I clinical trial were subjected to flow cytometric analysis to determine activation status and recall response of their immune effector cells, principally B- and T-cell repositories of immunological memory. Large variations in cell counts and surface markers were expected and observed between individuals within each dose group. A decrease in the numbers of lymphocytes as a percentage of the PBMC population was observed at 24 h after the booster dose of rYP002 at all dose levels of vaccine and also of placebo. This temporary depletion may be due to migration, activation, and subsequent antibody secretion in the spleen and/or lymph nodes (9).

The natural variation in display of cell surface markers by individuals within each vaccine dose group as well as the placebo group meant that no significant changes could be detected that were related to vaccination. However, neither were any pathophysiological alterations observed in lymphocyte subsets as a result of vaccination. Assays of cell-mediated immunity are under development in which immune effector cells are derived from a vaccinee’s whole blood sample and their activation on reexposure to the rF1 and rV antigens in vitro is determined in order to assess the degree of memory an individual has for the vaccine. They would be particularly valuable to apply when antibody titer has declined to low levels, in order to determine the need for a booster dose.

In summary, this is the first report of the human immune response to this recombinant vaccine for plague. The data gained indicate that the vaccine was immunogenic in recipients at all dose levels tested, although data from only three subjects were available at the 20-μg dose level and the lowest dose level (5 μg) was suboptimal. The titer of specific IgG developed by individuals at 21 and 28 days post-initial vaccination and 7 days postboost correlated with the development of a titer of anti-Yersinia pestis V antigen that contribute to protection against plague identified by passive and active immunization.

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