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Recombinant (F1 + V) vaccine protects cynomolgus macaques against pneumonic plague

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ABSTRACT

Cynomolgus macaques, immunised at the 80 μg dose level with an rF1 + rV vaccine (two doses, three weeks apart), were fully protected against pneumonic plague following inhalational exposure to a clinical isolate of *Yersinia pestis* (strain CO92) at week 8 of the schedule. At this time, all the immunised animals had developed specific IgG titres to rF1 and rV with geometric mean titres of 96.83 ± 20.93 μg/ml and 78.59 ± 12.07 μg/ml, respectively, for the 40 μg dose group; by comparison, the 80 μg dose group had developed titres of 114.4 ± 22.1 and 90.8 ± 15.8 μg/ml to rF1 and rV, respectively, by week 8. For all the immunised animals, sera drawn at week 8 competed with the neutralising and protective Mab7.3 for binding to rV antigen in a competitive ELISA, indicating that a functional antibody response to rV had been induced. All but one of the group immunised at the lower 40 μg dose-level were protected against infection; the single animal which succumbed had significantly reduced antibody responses to both the rF1 and rV antigens. Although a functional titre to rV antigen was detected for this animal, this was insufficient for protection, indicating that there may have been a deficiency in the functional titre to rF1 and underlining the need for immunity to both vaccine antigens to achieve protective efficacy against plague. This candidate vaccine, which has been evaluated as safe and immunogenic in clinical studies, has now been demonstrated to protect cynomolgus macaques, immunised in the clinical regimen, against pneumonic plague.

1. Introduction

Plague is caused by the Gram-negative organism *Yersinia pestis* and although an ancient disease associated with epidemics from the Middle Ages onwards, it is still endemic in parts of the world today [1]. Bubonic plague is primarily a disease of small rodents and mammals that is spread by fleas in endemic areas, to establish enzootic foci which occasionally erupt as an epizootic outbreak, particularly after major geological disturbance such as earthquake [2]. Humans can be infected either by flea bite or by inhalational exposure through a secondary host, for example wild rabbit, prairie dog or domestic cat and this leads to several thousand WHO-reported cases of plague per annum globally [1,3]. The consequences of infection in man are serious and the infection needs to be detected and treated promptly to prevent serious morbidity leading to death. Transmission to man, by feeding fleas, leads to the characteristic swelling of the draining lymph nodes, to form buboes, which may develop into a septicaemic illness or secondary pneumonic plague. However, plague is also highly transmissible by the coughing of patients with bubonic or septicaemic plague who have developed pulmonary lesions [4,5]. An outbreak of pneumonic plague at a diamond mine in Northern Congo in 2005 caused 54 deaths and was limited only by the dispersal of miners fleeing from the mine in panic [1]. Pneumonic plague would also be the most likely form of disease if *Y. pestis* were to be used as a biowarfare agent; it is both the most serious and most feared manifestation of this disease, protection against which is the paramount requirement to prevent epidemic spread.

There is accumulated epidemiological and experimental evidence that existing vaccine formulations, comprising sterile suspensions of killed whole bacteria, provide little protection against the pneumonic form of the disease caused by exposure to wild type *Y. pestis* [6,7] and this is strongly supported by experimental observation in animal models [8–10]. Additionally, whilst killed whole cell vaccines (KWCVs) have been demonstrated to protect mice against bubonic plague arising from exposure to $F1^+$ *Y. pestis*, they do not protect against an $F1^-$ *Y. pestis* strain [11,12]. Renewed research effort in the last two decades has led to the development of a recombinant vaccine comprising the two protein antigens, rF1 and rV [10,13,14]. In combination, these antigens are potently

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immunogenic in mouse, guinea-pig, macaque and human [15] and to date they have been demonstrated to induce protective immunity against plague in the mouse and guinea-pig models, leading to the identification of potential immune correlates of protection [15]. Unlike KWCV, the experimental evidence indicates that the rF1 + rV formulation can induce protective immunity against pneumonic plague in the mouse model [10,16]. Furthermore these proteins have been expressed as a genetic fusion to yield a single recombinant protein (rF1–V) in which the N-terminus of the V antigen is fused to the C-terminus of the F1 antigen [17] and which has similar protective efficacy against pneumonic plague in the mouse [13] and macaque [18,19] as do the combined antigens.

In previous studies in the cynomolgus macaque, we have demonstrated that the rF1 + rV vaccine formulated by adsorption to alhydrogel in the dose range 5–40 μg of each sub-unit and used in the same dosing regimen as used in a Phase 1 clinical trial [14], was highly immunogenic [15]. Immune macaque sera from this study inhibited the cytotoxic effect of Yersinia-delivered V antigen on macrophages in culture, competed with the protective monoclonal antibody Mab7.3 for binding to rV *in vitro* and conferred protection against plague in mice by passive transfer [15]. The current study was designed to extend the immunising dose range to 80 μg each of rF1 and rV and to determine whether the functional serological assays used previously represent immune correlates of protection by challenging the immunised macaques with aerosolised *Y. pestis*. The identification of immune correlates of protection will be essential for the licensure of such a vaccine according to the FDA's Animal Rule, and equivalent guidance from the European Medicines Agency [20,21].

2. Materials and methods

2.1. Animals

This study was conducted in compliance with the U.S. Animal Welfare Act and other Federal statutes and regulations relating to animals and it adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council 1996.

Twenty-two adult cynomolgus macaques (males and females) of bodyweight range 3–8 kg were used in this study. Telemetry devices (TA10DA-T70) were used to measure body temperature and were implanted sub-cutaneously (s.c.) in all cynomolgus macaques with appropriate sedation and analgesia, approximately 30 days prior to the study start. These transponders were placed on the dorsum of the animals, between the scapulae. The animals were offered environmental enrichment throughout the study and had access to food and water *ad libitum*.

2.2. Immunisation

A batch (03D11601-04A) of formulated vaccine comprising 120 μg rF1 + 120 μg rV in 0.5 ml 0.26% (w/v) alhydrogel (Brenntag, Fredericksaand, Denmark) was received from Avecia Billingham, UK (now Pharmathene Annapolis, USA). Each recombinant antigen had been separately expressed from *E. coli* and purified as previously described [14]. On receipt, the formulated vaccine was diluted with alhydrogel in 0.9% saline to achieve dose-levels of either 40 μg of each protein (group 1) or 80 μg (group 2) of each protein in a 0.5 ml final volume comprising 0.26% (w/v) alhydrogel, per animal.

Groups of 10 cynomolgus macaques were immunised at either dose-level. Immunisation was carried out intra-muscularly (i.m.) in 0.5 ml on two occasions, separated by 3 weeks. An additional two animals were administered a 0.5 ml placebo dose of 0.26% (w/v) alhydrogel in 0.9% saline in the same dosing regimen. Two placebo control animals were included with the objective of confirming that the level of *Y. pestis* challenge delivered was indeed capable of causing pneumonic plague as determined by lethality and histopathology, rather than with the objective of achieving a statistically valid group size. Baseline blood samples were collected from each animal immediately prior to vaccination, and these were used to check that there was no pre-existing titre to either antigen.

2.3. Blood sampling and immunoassay

The animals were bled immediately prior to immunisation and then approximately every week up to week 8. Serum antibody levels were measured by a conventional ELISA, as previously described [15] and using peroxidase-conjugated sheep anti-human IgG (The Binding Site Ltd., Birmingham, UK) as the secondary reagent. The IgG sub-class profiles at week 6 were also determined exactly as previously described [15] using human IgG standards from Sigma Aldrich (Poole, Dorset), and peroxidise-conjugated anti-human isotype reagents (The Binding Site Ltd., Birmingham, UK).

2.4. Competitive ELISA on week 8 serum samples

The detection of antibody which competed for binding to rV antigen *in vitro* with a murine monoclonal antibody (Mab7.3) was determined as previously described [14] for individual serum samples from macaques. Briefly, rV antigen was coated (5 μg/ml) to solid phase prior to the binding of 80 ng Mab7.3. Subsequently, the macaque test sera, or negative control sera (from placebo animals 99–310 and 49–470), or positive reference serum, were added in duplicate in the dilution range 1:10 to 1: 80 in 1% (w/v) skimmed milk powder in PBS. The positive reference serum was derived as previously described [14] by pooling polyclonal sera collected from cynomolgus macaques hyperimmune to rF1 + rV. The assay was developed with HRP-goat anti-mouse IgG (Serotec; 1:2000 in PBS) followed by incubation (37 °C, 1 h). Plates were washed prior to addition of ABTS substrate (Sigma) with subsequent reading of the absorbance at 414 nm. The OD_{414nm} was determined for each test and reference serum.

2.5. Challenge

The *Y. pestis* CO92 clinical isolate was prepared as described previously [22] to challenge immunised animals by the inhalational route. Immunised macaques were anaesthetised with Telazol (6 mg/kg i.m.) prior to aerosol challenge with *Y. pestis* CO92 at weeks 8–9 of the schedule. The 40 μg and 80 μg rF1 + rV dose groups were challenged on consecutive days, with 1 unvaccinated control in each cohort. Respiratory minute volumes were measured by whole body plethysmography using a Buxco Biosystem XA (Buxco Electronics, Sharon, CT, USA) immediately before challenge. The anaesthetised macaques were then immediately exposed to the bacterial aerosol, head-only, in a dynamic aerosol chamber controlled using the Automated Bioaerosol Exposure System. The aerosol (mass median aerosol diameter 1.2 μm) was generated by a three-jet Collision nebuliser and sampled continuously by an all-glass impinger (AGI-30; Ace Glass Inc., Vineland, NJ). For each animal, the aerosol concentration of *Y. pestis* organisms was calculated by plating out dilutions of a sample from the AGI onto blood agar plates (Remel). The inhaled doses were then determined.

2.6. Post-challenge monitoring

Post-infection (p.i.), the macaques were returned to their home cages where they were continuously monitored for body temperature, with data collected on an hourly basis up to 14 days post-challenge, using Dataquest A.R.T.2.3 software. Animals were