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Dual route vaccination for plague with emergency use applications ☆

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ABSTRACT

Here, we report a dual-route vaccination approach for plague, able to induce a rapid response involving systemic and mucosal immunity, whilst also providing ease of use in those resource-poor settings most vulnerable to disease outbreaks. This novel vaccine (VypVaxDuo) comprises the recombinant F1 and V proteins in free association. VypVaxDuo has been designed for administration via a sub-cutaneous priming dose followed by a single oral booster dose and has been demonstrated to induce early onset immunity 14 days after the primary immunisation; full protective efficacy against live organism challenge was achieved in Balb/c mice exposed to 2×10^4 median lethal doses of *Yersinia pestis* Co92, by the sub-cutaneous route at 25 days after the oral booster immunisation. This dual-route vaccination effectively induced serum IgG and serum and faecal IgA, specific for F1 and V, which constitute two key virulence factors in *Y. pestis*, and is therefore suitable for further development to prevent bubonic plague and for evaluation in models of pneumonic plague. This is an essential requirement for control of disease outbreaks in areas of the world endemic for plague and is supported further by the observed exceptional stability of the primary vaccine formulation in vialled form under thermostressed conditions (40 °C for 29 weeks, and 40 °C with 75% relative humidity for 6 weeks), meaning no cold chain for storage or distribution is needed.

In clinical use, the injected priming dose would be administered on simple rehydration of the dry powder by means of a dual barrel syringe, with the subsequent single booster dose being provided in an enteric-coated capsule suitable for oral self-administration.

1. Introduction

Plague is an ancient disease which is endemic today in many global regions [1], with demonstrated potential for epidemic spread. Indeed, one of the worst outbreaks, featuring a high rate (70%) of confirmed and suspected pneumonic plague, occurred from August–November 2017 in Madagascar [2], with >2500 cases and a case fatality rate of 8.6% [2]. Whilst antibiotic therapy is effective, this needs to be initiated early and is not ideal in a plague-endemic area, because of the potential emergence of anti-microbial resistance, as observed previously in Madagascar and Peru [3,4].

Historically, research has shown that plague is preventable by vaccination with live attenuated strains (EV76) or killed whole cell vaccines (KWCV) [5]. Although EV76 vaccines are highly immunogenic, they also carry some risk [6]. The KWCV are hazardous to produce and whilst thought effective against bubonic plague [7], they are deficient against pneumonic plague [8]. Currently there is no readily available licensed vaccine, although a variety of plague vaccine candidates have been proposed, reviewed in [9,10]. The F1 and V proteins have emerged as the predominant protective antigens [11,12], whilst other proteins e.g. YscF [13], YadC and OppA [14,15] have demonstrated partial efficacy. Previously, using liquid formulations, we demonstrated that the recombinant F1 and V proteins were safe and immunogenic in >160 volunteers [16] and protective in a range of animal models of the disease [17,18]. The induction of functional antibody [19] and cell-mediated immunity [20] are both important for protection against plague.

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We have designed novel formulations of the F1 and V proteins, intended for emergency use authorisation (EUA) [21], inducing rapid immunity and with simplified delivery involving an injected prime and oral boost to induce systemic and mucosal immunity to counter bubonic and pneumonic plague [22,23]. This vaccine (VypVaxDuo) comprises two formulations: (i) F1 and V proteins embedded in calcium phosphate coated onto amino-acid microcrystals [24] for primary immunisation; and (ii) the same antigens dispersed in an oil vehicle [25] for oral boosting. Our aim is to provide an efficacious vaccine, easily administered in resource-poor, low- to- middle income countries (LMIC), without requirement for cold chain distribution or storage.

Here, each arm of the vaccination regimen has been optimised for eventual clinical use, entailing the selection of excipients, particularly for the oral formulation, to overcome the tolerance which protects humans from exposure to dietary antigens [26]. Enteric-coating will ensure gastric transit; subsequent uptake by Peyer's Patches (PP) in the small intestine [27] will be promoted by incorporated excipients [28] such as vitamin A.D. and E derivatives, which enhance dendritic cell (DC) activation, which in turn induces effector T-cells [29,30]. Vitamin A or retinoic acid (RA) stimulates gut-resident DC to induce T-cell expression of CCR9 and $\alpha 4\beta 7$ [31] and promote gut-homing of T-cells through binding to MAdCAM-1 and CCL25, selectively expressed on intestinal cells [32]. Gut-resident DC also actively secrete RA, whilst skin-resident DC produce the active form of vitamin D, 1,25(OH)2D3; the latter imprints CCR10 expression on T-cells and homing to skin inflammation sites through binding to CCL37, selectively expressed on keratinocytes [33]. Alpha tocopheryl acetate (ATA), a non-toxic derivative of vitamin E, also activates DC and has adjuvant properties [34].

We have also incorporated dimethyldioctadecylammonium bromide (DDAB), cholera toxin B sub-unit (CTB) and trehalose dibehenate (TDB) in the oral formulation. DDAB is a cationic surfactant with similar action to chitosan [35–37] promoting electrostatic contact with PP cells and vaccine uptake. TDB, a synthetic analog of the mycobacterial trehalose dimycolate, is a potent macrophage activator [38], whilst CTB is anti-inflammatory and immunomodulatory [39].

2. Materials and methods

2.1. Protein antigens, vaccine formulation and stability

Recombinant F1 and V antigens were expressed from *E. coli*, purified as previously described [39] and supplied in PBS or ammonium acetate buffer for formulation.

For initial sub-cutaneous (s.c.) testing, F1 and V were admixed in PBS and MF59 (Novartis) was added 1:1 (v/v). For subsequent studies, the injected formulations of F1 and V were prepared as calcium phosphate microcrystals, using published methodology [24]. Briefly, aqueous mixtures of F1 and V with sodium orthophosphate and glutamine (Gln) or histidine (His) were precipitated as calcium phosphate protein-coated microcrystals (CaP-PCMC), by addition into a 19- fold excess of isopropanol containing dissolved calcium chloride. The resultant suspension contained self-assembled microcrystals comprising an amino-acid core with the protein embedded in a thin surface layer of CaP. The CaP-PCMC were isolated by vacuum filtration and dried to a powder. Protein content and integrity was determined by ELISA and SDS-Page.

The preparation of oral formulations of F1 and V was adapted from published methodology [25]. In brief, F1 and V, dissolved in

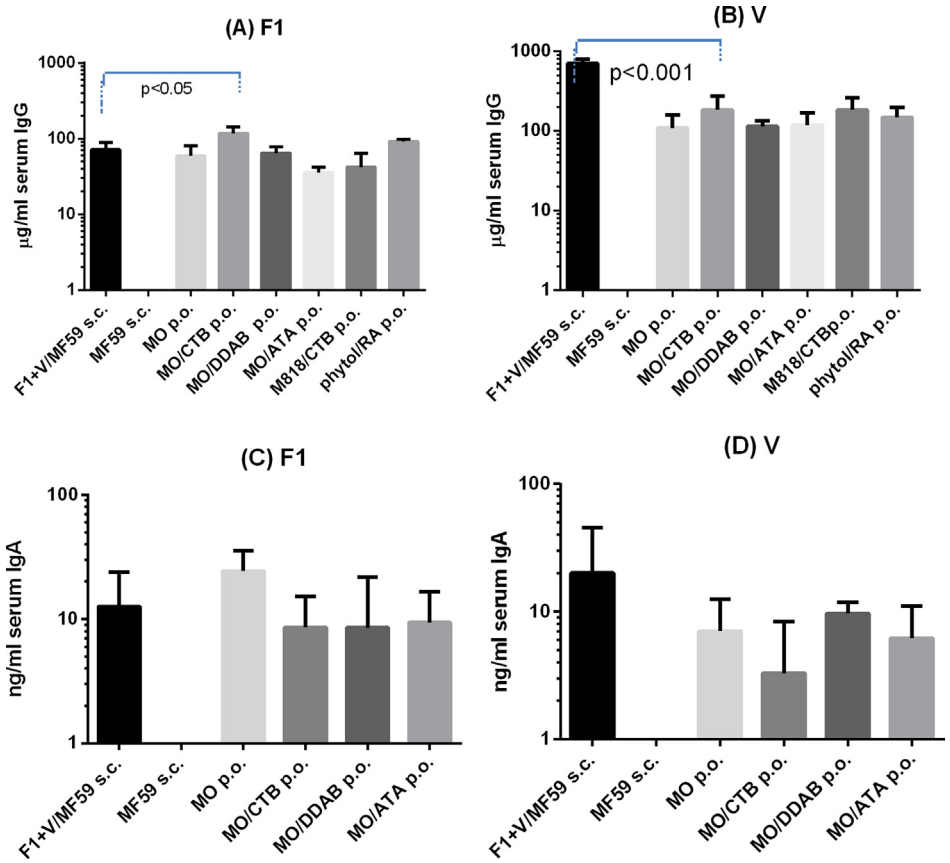

Fig. 1. Immunogenicity of F1 + V in 3 dose (days 0, 10, 31) dual route schedule. Mice were administered 10 µg F1 + 10 µg V formulated in MF59 (s.c.) and boosted with either the same formulation (s.c.) or with 25 µg F1 + 25 µg V in oil per oral (p.o.), with excipients as shown. Negative control mice received MF59 (s.c) on days 0, 10 and 31. Mean serum IgG and IgA titres per group to F1 (A) and (C) and to V (B) and (D) were determined at day 67.

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