Intranasal Protollin™/F1-V vaccine elicits respiratory and serum antibody responses and protects mice against lethal aerosolized plague infection

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Abstract

F1-V is a recombinant plague antigen comprising the capsular (F1) and virulence-associated (V) proteins. Given intramuscularly with Alhydrogel, it protects mice against challenge, but is less effective in non-human primates against high-dose aerosolized Yersinia pestis challenge, perhaps because it fails to induce respiratory immunity. Intranasal immunization of mice with F1-V formulated with a Proteosome-based adjuvant (Protollin™), elicited high titers of specific IgA in lungs whereas intranasal F1-V alone or intramuscular Alhydrogel-adsorbed F1-V did not. The Protollin-adjuvanted F1-V vaccine also induced high serum titers of specific IgG, comparable to those induced by intramuscular Alhydrogel-adsorbed F1-V. Mice immunized intranasally with Protollin–F1-V were 100% protected against aerosol challenge with 170 LD50 of Y. pestis and 80% against 255 LD50.

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1. Introduction

In the last two millennia there have been three major plague pandemics, which killed huge numbers of people. During the second pandemic in the middle ages for example, plague killed an estimated 30 million people in Europe—about a third of the population [1,2]. Natural outbreaks still occur in endemic areas throughout the world where pest control and/or hygiene measures are inadequate but efficient diagnosis and availability of effective antibacterials result in few cases of severe illness from the disease and fewer deaths [3]. Bubonic and septicemic plague spread to humans through bites from fleas infected with Yersinia pestis and are relatively easy to diagnose and therefore treat with antibiotics [3]. Pneumonic plague, which is transmitted person-to-person by the respiratory route or as the result of the spread of bubonic or septicemic plague by an untreated person, is the most fatal form of plague [3]. Treatment of pneumonic plague after exposure is problematic, in part because the initial symptoms are insidious and resemble those of many severe respiratory illnesses, and also because of its rapid course—unless antibacterial treatment is started within a very narrow therapeutic window, most people exposed to inhaled Y. pestis will die [3]. The outbreak of pneumonic plague at a diamond mine in Northern Congo in the first 8 weeks of 2005 caused 114 confirmed infections amongst miners, half of whom died [4]. Most of the miners fled the scene in panic, serving as a reminder of the deadliness of this form of plague, the difficulties in rapidly and correctly identifying the causative agent(s), and the fear instilled by the specter of contagious diseases.

Exploiting this fear, plague used as an agent of biological warfare or bio-terrorism is qualitatively different from naturally-occurring plague. Its intentional dissemination would likely occur as an aerosol, inhalation of which would cause primary pneumonic disease. In the absence of any other indicators, the first evidence of release of such a
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**14. ABSTRACT**
F1-V is a recombinant plague antigen comprising the capsular (F1) and virulence-associated (V) proteins. Given intramuscularly with Alhydrogel, it protects mice against challenge, but is less effective in non-human primates against high-dose aerosolized Yersinia pestis challenge, perhaps because it fails to induce respiratory immunity. Intranasal immunization of mice with F1-V formulated with a Proteosome-based adjuvant (Protollintrade mark), elicited high titers of specific IgA in lungs whereas intranasal F1-V alone or intramuscular Alhydrogel-adsorbed F1-V did not. The Protollin-adjuvanted F1-V vaccine also induced high serum titers of specific IgG, comparable to those induced by intramuscular Alhydrogel-adsorbed F1-V. Mice immunized intranasally with Protollin-F1-V were 100% protected against aerosol challenge with 170 LD(50)

**15. SUBJECT TERMS**
Yersinia pestis, plague, F1-V vaccine, adjuvants, Alhydrogel, Protollin, laboratory animals, mice, nonhuman primates

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- **c. THIS PAGE** unclassified
bioweapon would be clusters of similar severe respiratory symptoms followed in quick order by deaths [3]. High priority has consequently been given to the development of safe and effective vaccines, which could protect against the most likely biothreat pathogens, including a vaccine against the virulent, pneumonic, form of plague.

Early attempts to produce effective plague vaccines focused on attenuated or killed whole cells. A live attenuated vaccine (EV76) was effective in mice and was used extensively in humans in Africa and the USSR, but there is little clinical data supporting its efficacy against pneumonic plague, and there were concerns about its reactogenicity [5]. It was never licensed nor commercially available in the west. A licensed, killed whole cell vaccine [6], given to servicemen stationed in endemic areas, was highly effective in protecting against bubonic plague [7], but mice immunized with the same vaccine were not protected against inhalational challenge [5] and the vaccine is no longer made. The success of attenuating other bacteria such as Salmonella has led to the employment of new strategies to attenuate Y. pestis [8], but these efforts have largely been superseded by the safest option—that of a subunit vaccine.

Subunit vaccines are being developed based on the two components of Y. pestis, which have been shown to consistently protect immunized animals against aerosol challenge, namely the F1 and V proteins [9–12]. F1 is a Y. pestis capsular protein found on most virulent strains, and is believed to play a role in preventing uptake of the organism by macrophages [13]. The virulence factor, V, is found on all virulent strains and is thought to be involved in modulating the host cytokine responses as well as translocating outer surface proteins into the host cell cytosol, also to prevent macrophage uptake [14]. Either antigen, expressed as a recombinant protein, was capable of protecting mice against subcutaneous or inhalational challenge but a combination of the two antigens acted synergistically to enhance protection [12,15].

While there is a consensus that protection is likely to be maximized with a vaccine containing both F1 and V (which would also protect against both F1− as well as F1+ strains of Y. pestis), two different approaches have been adopted. One uses separately purified components, which are mixed prior to administration [12]; the second uses an F1-V fusion protein in which the F1 is fused at its carboxyl terminus to the amino terminus of the V antigen [11]. Adsorbed onto Alhydrogel and given intramuscularly as an injected vaccine, the F1-V fusion protein was shown to be highly effective, protecting immunized mice against high dose inhalational challenge of both F1+ and F1− Y. pestis strains [16]. However, there is anecdotal evidence that this injectable vaccine was less effective in protecting non-human primates against high dose aerosol challenge, perhaps because it failed to efficiently induce appropriate immune responses in the respiratory tract (JIA, personal communication).

Administration of vaccines by mucosal routes has been shown to be an efficient way of inducing mucosal immune responses both at the site of administration and at distant mucosal surfaces [17]. Antigens formulated with ProteosomesTM or Proteosome-based adjuvants and given intranasally, have demonstrated an ability to induce potent mucosal and systemic immune responses in numerous preclinical animal studies as well as in nearly 2000 subjects in nine phase I and II clinical trials [18]. One such vaccine comprised Shigella-derived LPS complexed with Proteosomes (ProtollinTM), which when co-administered intranasally with a number of protein antigens, was shown to possess strong adjuvant activity [19]. In an attempt to induce protective plague-specific immune responses in the respiratory tract as well as in serum, mice were immunized intranasally with F1-V combined with Protollin and the resulting responses were compared with those elicited by injected, Alhydrogel-adsorbed F1-V. Immunized mice were also challenged to determine whether they were protected against lethal aerosolized challenge with live Y. pestis. The results are presented below.

2. Methods

2.1. Reagents

OMP lots of ProtollinTM containing approximately equal weights of ProteosomeTM proteins and Shigella flexneri LPS were prepared by diafiltration as previously described [20] and stored at −80 °C in aliquots. F1-V (USAMRIID, Ft Detrick, MD) was provided in 50 mM CH3COONa, 105 mM NaCl, pH 5.5. Buffer was exchanged for PBS using an Amicon Stirred Cell, the protein was concentrated in the same apparatus and then stored at −80 °C in aliquots. Alhydrogel (3% Al(OH)3) was purchased from Brentmag Biosector, Denmark.

2.2. Immunogenicity studies

The immunogenicity of ProtollinTM formulated F1-V was assessed by immunization of groups of 20, 6–8-week-old female Swiss-Webster mice (Charles River, St-Constant, Quebec). Freshly thawed aliquots of Protollin and F1-V solutions were mixed in predetermined amounts (see text), no more than 16 h before immunization of mice. For intranasal (i.n.) immunizations, mice were lightly anesthetized by isoflurane inhalation, 25 µl of vaccine or control material was applied to the nares (12.5 µl per nare) and the mice allowed to inhale the droplets. Intramuscular (i.m.) immunization was achieved by injection of 25 µl of F1-V adsorbed to 0.5% (v/v) Alhydrogel into mouse hind limbs. Control groups received PBS, Protollin or F1-V alone intranasally.

Mice were immunized on day 0 and boosted on day 21. Ten mice from each group were euthanized either on day 35 or 55 by asphyxiation with CO2 and exsanguination. Serum was separated from clotted blood and stored at −80 °C until assay. Lung lavage was performed as previously described [21] and fluid stored at −80 °C until assay. Spleens were removed
aseptically and processed for in vitro restimulation and assessment of released cytokines (see below).

The remaining 10 animals from each group were challenged on day 35 or 55 by inhalation of 170–250 LD₅₀ of aerosolized *Y. pestis* (Colorado 92 strain) to assess protection. Mice were monitored for 28 days after challenge and any deaths recorded. The significance of survival was assessed by Fisher’s exact probability test.

2.3. Antibody assays

IgG and IgA antibody titers were determined on individual samples by ELISA using plates coated with pre-determined concentrations of F1-V (or F1 or V). Bound antibody was detected with HRP conjugated anti-mouse IgG, IgG₁, IgG₂a, or IgA as appropriate. Antibody concentrations were calculated from a standard curve run on each plate, using purified mouse antibodies (IgG from Sigma, IgA from Bethyl Labs, Montgomery, TX). Values were expressed in micrograms or nanograms of specific antibody per milliliter of serum or lung wash fluid (µg or ng/ml) and data was expressed as the geometric means of antibody concentrations in individual mouse sera within a group, the significance of which was assessed by ANOVA analysis of log-transformed titers using Tukey–Kramer pair-wise comparisons.

2.4. Splenic cell culture and cytokine determination

At sacrifice on day 35, spleens from the each of the mice in selected groups were removed, pooled, processed into single cell suspensions and then incubated with different concentrations of F1-V in a modification of the methods described in detail elsewhere [21]. The amounts of TNF-α and IL-10 released into culture supernatants were determined by quantitative ELISA using OptEIA kits (BD Biosciences).

3. Results

3.1. Immunogenicity of intranasal F1-V + Protollin™

Mice were immunized with various doses of F1-V plus Protollin™ and the titers of elicited antibodies in serum and lung wash fluids were compared with those from mice immunized intranasally with the same doses of unformulated F1-V or intramuscularly with 20 µg of F1-V adsorbed onto Alhydrogel. The results are shown in Fig. 1.

At all concentrations tested, all combinations of Protollin™ and F1-V were highly immunogenic and elicited F1-V specific serum IgG titers of between 1 and 9 mg/ml (Fig. 1a). On both sampling days there was a trend towards lower titers elicited by the lower F1-V and/or Protollin concentrations, but there were no significant differences in the specific IgG titers elicited by any combination of F1-V plus Protollin or those elicited by intramuscular injection of 20 µg of Alhydrogel-adsorbed F1-V (P > 0.05). All specific serum IgG titers elicited by formulated F1-V vaccines were significantly higher than those elicited by intranasal administration of unformulated F1-V (P ≤ 0.001), and no specific antibodies...
Table 1

<table>
<thead>
<tr>
<th>F1-V + 2.5 μg Protollin</th>
<th>F1-V + 1 μg Protollin</th>
<th>F1-V + 0.25 μg Protollin</th>
<th>F1-V + Alhydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgG d35</td>
<td>0.31 (0.21–0.46)</td>
<td>0.30 (0.23–0.38)</td>
<td>0.34 (0.23–0.51)</td>
</tr>
<tr>
<td>Serum IgG d55</td>
<td>0.25 (0.18–0.34)</td>
<td>0.26 (0.19–0.36)</td>
<td>0.33 (0.26–0.42)</td>
</tr>
<tr>
<td>Lung IgG d35</td>
<td>0.40 (0.26–0.62)</td>
<td>0.53 (0.35–0.81)</td>
<td>0.44 (0.27–0.72)</td>
</tr>
<tr>
<td>Lung IgG d55</td>
<td>0.48 (0.37–0.62)</td>
<td>0.46 (0.37–0.63)</td>
<td>0.46 (0.35–0.65)</td>
</tr>
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Lung antibody titers from mice immunized intranasally with F1-V alone were too low to evaluate. 95% confidence limits are shown in parentheses.

were detected in serum from mice given Protollin alone (data not shown).

To confirm that antibodies directed against both F1 and V were elicited and to determine if either of the components of the fusion protein was immunodominant, sera from all mice immunized with the 20 μg dose of F1-V were assayed separately against F1 and V and results expressed as geometric means of the individual ratios of specific anti-F1 versus anti-V titers for the different groups of mice. In all instances and at both sampling times, antibodies against both components were elicited and relatively more of the specific serum antibodies were directed against the V rather than the F1 component of the fusion protein (i.e. anti-F1/anti-V < 1; Table 1). There were no significant differences in the geometric means of any serum anti-F1 anti-V ratios, irrespective of the formulation or route of delivery of the F1-V vaccines tested in these studies.

Lung lavage fluids were assayed by ELISA to determine the titers of specific anti-F1-V antibodies. The results (Fig. 1b) confirm that immunization by mucosal routes is the most efficient means of eliciting mucosal antibodies with all groups of mice immunized intranasally with F1-V plus Protollin responding with high titers of specific lung IgA. While the IgA responses were highest in lung lavage fluid from mice immunized with the highest dose of Protollin, ANOVA analysis indicated there were no significant differences in the IgA titers elicited by any combination of F1-V and Protollin. Unformulated F1-V, given intranasally, elicited IgA levels which were only just detectable while intramuscular injection of Alhydrogel-adsorbed F1-V did not elicit any detectable secretory IgA responses. Assay of lung lavage fluids against separate F1 and V antigens indicated that again, IgA responses against both components were elicited and that IgA responses were predominantly directed against the V component of the F1-V fusion protein (data not shown).

Lung lavage fluid also contained significant titers of specific IgG (Fig. 1c), even though the titers represented only a small percentage of the total serum titers (range 0.11–0.56%; median 0.175%). Interestingly, the anti-F1 and anti-V IgG ratios were significantly higher in lung lavage fluids than in the corresponding sera for all the vaccine formulations (P ≤ 0.05; Wilcoxon signed rank test; Table 1). The anti-F1 anti-V ratios of lung IgG responses of the intramuscular vaccine were close to unity but were significantly different (P ≤ 0.05) from the ratios for mice immunized intranasally with F1-V plus Protollin in day 55 samples but not in day 35 samples.

3.2. Cytokine release from splenocytes

To compare and contrast the responses elicited by the intranasal Protollin formulated and injected Alhydrogel-adsorbed F1-V vaccines, splenocytes from selected groups of immunized mice were restimulated in vitro with F1-V and the amounts of TNF-α and IL-10 released into culture supernatants were measured (Fig. 2).

Fig. 2. Shows the amounts of TNF-α and IL-10 released from splenocytes stimulated in vitro for 48 h with different amounts of F1-V. Splenocytes were harvested on day 35 from mice immunized intranasally with 20 μg of F1-V with or without 1 μg of Protollin, or injected intramuscularly with 20 μg of F1-V adsorbed onto Alhydrogel.
Splenocytes from mice immunized intranasally with F1-V plus Protollin\textsuperscript{TM} responded to in vitro stimulation by secreting high levels of TNF-\alpha, concomitant with low levels of IL-10. In contrast, splenocytes from mice immunized by injection of Alhydrogel-adsorbed F1-V responded by secreting lesser amounts of TNF-\alpha concomitant with slightly higher levels of IL-10. Consistent with the current model that the V antigen contributes to plague pathogenicity by stimulating release of IL-10 and thereby suppressing the release of TNF-\alpha with subsequent impact on innate and adaptive immunity, splenocytes from mice given F1-V alone intranasally responded to in vitro stimulation by releasing higher amounts of IL-10 than those secreted by either of the formulated vaccines. Thus, addition of Protollin to F1-V enhanced release of the proinflammatory cytokine TNF-\alpha while simultaneously suppressing secretion of the regulatory cytokine IL-10 compared to the injected F1-V, suggesting the intranasal vaccine was more likely to facilitate the induction of adaptive immunity.

3.3. Challenge with aerosolized live \textit{Y. pestis}

To assess the protection elicited by intranasal immunization with F1-V plus Protollin\textsuperscript{TM}, mice were challenged by whole-body exposure to live aerosolized \textit{Y. pestis}, and the protection compared with that elicited by injection of 20 \(\mu\)g F1-V adsorbed onto Alhydrogel, or in control mice given intranasal F1-V or Protollin alone. For clarity, only the results for mice immunized with the 20 \(\mu\)g doses of F1-V are shown in Fig. 3.

On day 35 and at a challenge dose of 170 LD\textsubscript{50} \textit{Y. pestis}, all mice immunized intranasally with 5, 20 or 50 \(\mu\)g of F1-V and 1 or 2.5 \(\mu\)g of Protollin\textsuperscript{TM} were 100% protected against death, as were the mice injected with Alhydrogel-adsorbed F1-V (Fig. 3a). Mice immunized intranasally with 5, 20 or 50 \(\mu\)g of F1-V and 0.25 \(\mu\)g of Protollin were 90, 100 and 90% protected, respectively, while mice immunized intranasally with the same doses of F1-V without Protollin were only 30, 40 and 40% protected, respectively. None of the control mice, which received Protollin only, survived longer than 4 days post challenge. Survival for all mouse groups immunized with formulated F1-V was highly significant (\(P \leq 0.05\) or better by Fisher’s exact probability test) compared to survival in control mice or mice immunized with unformulated F1-V.

Survival following challenge on day 55 (Fig. 3b) was very similar to the day 35 data. All mice immunized with 2.5 \(\mu\)g of Protollin\textsuperscript{TM} plus F1-V were completely protected against challenge, as were mice immunized by injection of Alhydrogel-adsorbed F1-V. Mice immunized with 1 \(\mu\)g of Protollin and 50 or 20 \(\mu\)g of F1-V were also 100% protected, while all other combinations of Protollin plus F1-V elicited 90% protection. In all mice immunized with formulated F1-V, the observed protection was highly significant (\(P \leq 0.01\) or better) compared with mice immunized with unformulated F1-V (10–30% protection) or the control group of mice in which there were no survivors.

In a separate study, immunized mice were challenged on day 55 by whole body exposure to 255 LD\textsubscript{50} dose of live aerosolized \textit{Y. pestis} (Fig. 3c). Mice immunized intranasally with 50 \(\mu\)g of F1-V with or without 1 \(\mu\)g of Protollin\textsuperscript{TM},
or injected with 10 µg of F1-V adsorbed onto Alhydrogel, showed 80, 20 and 60% survival, respectively, against this higher dose lethal challenge while all the control mice given Protollin alone died. Immunization with formulated F1-V induced significant protection against death compared to control mice (P ≤ 0.001) for intranasal F1-V plus Protollin; P ≤ 0.05 for injected F1-V. There was also a significant delay in the time to death in the intranasally immunized mice compared with that in mice immunized by injection (P ≤ 0.01; Mann–Whitney rank sum test).

4. Discussion

These studies were performed to establish whether an intranasally administered subunit plague vaccine was capable of eliciting enhanced responses compared with those elicited by the same subunit vaccine adsorbed onto Alhydrogel and injected intramuscularly. The results demonstrate that F1-V combined with the intranasal adjuvant Protollin was capable of eliciting systemic immune responses comparable to or better than those elicited by a conventionally adjuvanted vaccine, and unlike the injected vaccine, could also elicit potent mucosal responses in the respiratory tract. A range of concentrations of F1-V and Protollin adjuvant provided 90–100% protection against lethal challenge with 170LD₅₀, aerosolized Y. pestis in immunized mice as did the injected vaccine. Furthermore, intranasal immunization with F1-V at doses as low as 5 µg and adjuvanted with Protollin at doses as low as 0.25 µg or 1 µg, elicited high specific IgG titer and 90–100% protection against aerosol challenge (with 170LD₅₀ of Y. pestis), responses which were statistically indistinguishable from those elicited by intramuscular injection of 20 µg of F1-V adsorbed onto Alhydrogel. In addition, mice immunized with the intranasal Protollin-formulated vaccine were better protected against a higher dose (255 LD₅₀) of aerosolized challenge than mice immunized with the injected vaccine. Although the intranasally immunized mice were given 50 µg of F1-V while the injected mice received 10 µg, the serum IgG titers elicited were very similar (476.4 and 417.9 µg/ml, respectively) suggesting that differences in protection were unlikely to be attributable solely to the elicited serum anti-F1-V IgG titers. Thus, it is possible that by induction of mucosal immunity in the respiratory tract, the intranasal plague vaccine comprising F1-V combined with the proprietary mucosal adjuvant Protollin may offer improvements over a conventional, injected vaccine although it is equally possible that these differences in protection could be due to other immunologic factors not measured in these studies.

Much of the accumulated evidence suggests that protection against plague, at least in the short term, is mediated by induction of systemic humoral responses. For example, serum anti-F1 titers have been shown to correlate with protection [10]; passive immunization with anti-F1 antibodies protected mice against lethal challenge with F1⁺ strains of Y. pestis, [22]; the combined anti-F1 and anti-V IgG₁ serum titer was correlated significantly with protection [23]; anti-V antibodies could passively protect against infection [24,25]; a neutralizing anti-V monoclonal antibody could alone protect against challenge with live bacteria [26]; and anti-F1 and anti-V monoclonal antibodies synergistically protected mice against challenge [27]. Furthermore, challenge studies in animals immunized by injection of F1-V adsorbed onto Alhydrogel have also demonstrated that virtually complete protection was possible [11], primarily through induction of high titer systemic antibodies since Alhydrogel is known to favor the induction of predominantly type 2 phenotype immunity characterized by enhanced humoral responses [28]. Since the intranasal F1-V plus Protollin vaccines elicited high specific serum IgG titers (of the order of 1–10 mg per ml of serum), comparable to those induced by injection of Alhydrogel-adsorbed F1-V, it was not surprising, therefore, that the intranasal vaccines were also capable of fully protecting immunized mice against lethal challenge with aerosolized Y. pestis. Correlations between protection and the serum anti-(F1-V) IgG₁ geometric mean titer, as well as serum anti-(F1-V) IgG, anti-F1 and anti-V IgG titers were highly significant, consistent with previous reports.

Specific antibody titers were determined in mucosal fluids from the control groups and mice immunized with 20 µg of F1-V. Following challenge with 170 LD₅₀ of aerosolized Y. pestis, there was a significant correlation between protection and lung IgG titer (correlation coefficient) (R = 0.91) for day 35 and 0.95 for day 55 data. Since the mice immunized by i.m. injection did not elicit detectable levels of secretory IgA in the lungs but instead produced high IgG titers (either through local production or by transudation) and yet were still 100% protected, the degree to which secretory IgA contributed to the protection against aerosol challenge with 170LD₅₀ of Y. pestis is unclear. In contrast, in the study in which mice were challenged with 255 LD₅₀ of Y. pestis, mice immunized i.n. with F1-V plus Protollin were better protected against aerosol challenge than mice immunized i.m. with Alhydrogel-adsorbed F1-V and the correlation between protection and lung IgA (R = 0.91) was greater than the correlation between protection and lung IgG (R = 0.85). Nonetheless, the most significant correlation was, as before, between serum IgG and protection (R = 0.99). It may be that the induction of secretory IgA at the portal of entry of the challenge, i.e. the nasopharynx, may have had some impact on reducing the burden of the inoculum but further studies would be required to determine the contribution of the local antibody response to the observed protection against inhalational challenge.

Other groups have assessed non-injectable means of administering plague vaccines in mice. In one such study, transcutaneous administration of three doses of F1 and V antigens combined with cholera toxin (CT) elicited high specific antibody titers which protected mice against challenge with live inoculum [29]. Quantitative assay of cytokines released into culture supernatants following restimulation of splenocytes from immunized mice showed that only IL-6 was produced in amounts significantly higher than those
from restimulated splenocytes from naive mice. A similar study showed that two doses of F1-V given transcutaneously in combination with Escherichia coli heat-labile enterotoxin (LT), elicited modest specific serum antibody responses [30]; ironically in this same study, an intranasal prime-boost regimen proved that two doses of F1-V plus LT were the most effective in eliciting potent specific serum responses. Neither study reported specific mucosal IgA responses following transcutaneous administration of vaccine, although one [30] preferentially looked for and detected detectable levels of specific IgG, in bronchoalveolar lavage fluids. Thus, the studies reported here demonstrate that the intranasal F1-V plus ProtollinTM vaccine was capable of eliciting systemic responses, which were at least comparable to those elicited by the transcutaneous or injected vaccines. Furthermore, the cytokine data reported suggests that this intranasal F1-V plus Protollin vaccine was capable of reducing the suppressive effect of the regulatory cytokine IL-10, in contrast to the Alhydrogel-adsorbed F1-V which failed to promote enhanced release of the proinflammatory cytokine TNF-α. The phenotype of response associated with the formulation of Protollin with antigens such as F1-V, is likely the result of its major components, por B and LPS, engaging with TLR2 and TLR4, respectively [31–34], and inducing the release of inflammatory cytokines which then promote the potent adaptive responses similar to those reported in these studies.

This latter point may have important implications for the development of a plague vaccine. It is now well established that the V antigen possesses important immunomodulatory properties enabling the bacteria to evade the host innate immune response by stimulating the production of IL-10 which then suppresses innate inflammatory responses and thereby hindering appropriate adaptive cellular immune responses [35–39]. A CD4+ T-cell epitope has recently been identified in the V protein [40] indicating that F1-V is capable of eliciting adaptive T-cell responses as well as innate immune mechanisms. The results presented above demonstrate that F1-V alone was indeed capable of eliciting CD4+ T-cell mediated release of IL-10 are consistent with these reports. But importantly the results also demonstrate that the intranasal ProtollinTM formulated vaccine was able to facilitate a switch towards an adaptive immune response as indicated by the higher TNF-α and lower IL-10 secretion in the groups given the intranasal vaccine.

At the outset, these studies were conducted to assess the potential for an easily administered plague vaccine and to this end, the data reported here suggests that F1-V given intranasally in combination with ProtollinTM is a potent vaccine which may offer benefits and improvements over an injected, Alhydrogel-adsorbed F1-V vaccine. The intranasal F1-V plus Protollin vaccine has potential for induction of both innate and adaptive immunity of a mixed phenotype which could possibly contribute to enhanced short and long term protection, and also generate valuable mucosal antibodies which might prove crucial in protecting against exposure to high challenge doses of a bacterium which presents such an insidious bioterror threat.

References


