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Brucellosis: The Case for Live, Attenuated Vaccines

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Abstract

The successful control of animal brucellosis and associated reduction in human exposure has limited the development of human brucellosis vaccines. However, the potential use of *Brucella* in bioterrorism or biowarfare suggests that direct intervention strategies are warranted. Although the dominant approach has explored the use of live attenuated vaccines, side-effects associated with their use has prevented widespread use in humans. Development of live, attenuated *Brucella* vaccines that are safe for use in humans has focused on the deletion of important genes required for survival. However, the enhanced safety of deletion mutants is most often associated with reduced efficacy. For this reason recent efforts have sought to combine the optimal features of a attenuated live vaccine that is safe, free of side effects and efficacious in humans with enhanced immune stimulation through microencapsulation. The *competitive advantages and innovations of this approach are: (1) use of a highly attenuated, safe, gene knockout, live Brucella mutants; (2) manufacturing with unique disposable closed system technologies, and (3) oral/intranasal delivery in a novel microencapsulation-mediated controlled release formula to optimally provide the long term mucosal immunostimulation required for protective immunity*. Based upon preliminary data, it is postulated that such vaccine delivery systems can be storage stable, administered orally or intranasally, and generally applicable to a number of agents.

II. Public Health Relevance

Control of zoonotic diseases in human populations has relied heavily on the control of animal disease. Over the last century human brucellosis has been controlled by vaccination and culling within cattle, goat and sheep herds [1–3]. Yet, despite past and current efforts to eradicate brucellosis as many as 500,000 new cases are reported in humans annually worldwide [2]. A direct link with economic status has been a hallmark of the disease [1]. The elevated risk in poorer countries unable to compensate the animal owner for the loss of animals confirms, in the opinion of many critics, that vaccine efforts alone are insufficient to control brucellosis [3,4]. Yet, the efficacy of vaccination-only strategies has not been seriously evaluated, and depends in large part on the quality of the vaccine employed. Finally, the potential introduction of the agent directly into the human population as a result of biowarfare or bioterrorism changes

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the dynamic and negates decades of effective public health control. In order to prevent such situations it is imperative that intervention strategies both in animals and humans be improved.

III. Public Health Strategies

An examination of the potential alternatives to eliminate brucellosis confirms the need to target improvement of vaccination strategies. Currently, antibiotic treatment of animals is systematically discouraged and highly regulated. Prolonged treatment periods and elevated levels of antibiotic required to eliminate *Brucella* infection argue against widespread use [5–11]. Antibiotic treatment in the elimination of human disease has been more successful, but relies upon the use of tetracycline or deoxycyclin in combination with rifampin and much less on streptomycin, and gentamycin [7,12–17]. The limited number of effective antibiotics and the potential for accidental or malicious introduction of antibiotic resistances into the organism emphasizes the need for alternative solutions.

The approach with the greatest promise for success is vaccination; and efforts over the past 100 years have consistently demonstrated that live, attenuated organism provides the best protection against subsequent challenge [18–20]. In contrast, trials using heat-killed organisms and various subcellular fractions have met with limited success [21–25]. However, in defense of these approaches it is not clear whether the use of live vaccine must be limited to *Brucella* alone or could be extended to replicating vectors including viruses or bacteria expressing *Brucella* antigens. In fact, DNA vaccines have provided some promising results using cocktails of up to three antigens [26]. Furthermore, the use of heat-killed organisms may be a result of antigen deformation due to the killing method rather than the absence of viable organism suggesting that other approaches such as irradiation may prove useful [27,28].

Development of a safe and efficacious human brucellosis vaccine would have a broad impact on public health. Human vaccines in endemic areas could prevent disease transmission resulting from consumption of contaminated food products as well as a potential bioterrorism or biowarfare agents, and protection against aerosol challenge [29,30]. Control of infection in animal reservoirs should also be considered to reduce zoonotic transmission.

IV. Vaccine Design and Platform

The most promising of the vaccine approaches based historically on published results and effective use in the field is live, attenuated agent. Vaccines currently in use are derived from spontaneously occurring attenuated forms that arise randomly with little means of controlling the combination of defects that attenuate survival [31,32]. In contrast, current and future vaccine development dovetails nicely with studies determining the role of individual genes in the survival and virulence of the organism [33–38]. These approaches generally involve the inactivation or removal of a gene using either targeted or random approaches and testing immune protection once the vaccine candidate has cleared from the system. However, although straightforward in concept the approach requires a great deal of experimental trial and error. First, mutations having a drastic effect on survival may attenuate the organism so that the level of protective immunity provided is insufficient. Combination of mutations often completely eliminates the induction of protective immunity and is especially problematic when trying to combine mutations in an effort to enhance safety [39,40]. In contrast, organisms that persist for an extended period at a low level may be of questionable safety and often induce symptoms associated with full-blown disease. Thus, the level of attenuation requires fine-tuning to provide a protective immune response while maintaining safety. The use of live attenuated vaccines takes advantage of the natural properties of the organism, including cell invasion and tissue tropism while presenting a full complement of immunogens, and is supported by a history of success. In contrast, the lack of knowledge concerning protective immunogens and the apparent failure of non-viable vaccines suggests that much more time will be required to

identify and develop delivery platforms for subunit vaccines capable of stimulating immunity sufficient to prevent disease.

Combining attenuated organisms with severely compromised survival with a delivery platform that extends exposure to boost the immune response offers a superior approach by enhancing both safety and efficacy. Persistence of highly attenuated strains is artificially extended by the use of a microencapsulation delivery platform. The choice of delivery platforms for these vaccines is limited to microcapsules due to the large size of the bacteria. *Brucella* range in size from 0.5–0.7μm by 0.6–1.5μm while liposomes range in size from 15–60 nm and microcapsules can range in size from 1–900 μm. The size of capsule used will depend on the delivery method of choice. As a depot larger particles may be employed, but when considering intranasal delivery or any approach designed to target cellular uptake, particles 3–10 μm in size are taken up by Peyer's patches [41]. It is important to note that current research has suggested a strong relationship between the stimulatory effect of adjuvant and particle formation; and the improved protection observed using microencapsulated vaccine is due in part to this phenomenon [42–44].

V. Animal Models

Although the goal of vaccine development is to provide immune protection directly in humans using a safe, self-administered and storage-stable vaccine, preliminary testing requires a number of in vitro and in vivo models. First, attenuation is most often established in vitro using cultured macrophage [45–50]. Of course, the overall complexity of the immune response prevents in vitro evaluation so, historically, the mouse model has been used to evaluate survival, virulence and the immune response. Although a good predictor of virulence and immune protection for larger species, the mouse model does not permit evaluation of symptoms associated with disease in humans, such as fever, lethargy, weight loss, etc. As a result the next step in evaluation of vaccine has been in the target species for example, cattle, sheep, goats, bison or other ruminants. For human vaccine development this might suggest the use of nonhuman primates at this stage of evaluation. However, evaluation of vaccine candidates may be performed in the goat model to eliminate candidates that produce symptoms associated with disease [51,52]. Furthermore, the pregnant goat provides a model of safety via protection against abortion, a symptom that is unique to ruminant species, but provides an ultimate evaluation of safety [52]. The sensitivity of this model reflects the tissue tropism of the agent that targets the female reproductive tract.

Finally, safety and efficacy may be examined in the nonhuman primate model. As a close relative of humans the symptoms, tissue colonization and persistence of the organism is identical to human infection [53–55]. However, the use of such animals should be seriously considered on ethical grounds and only with the safest candidates.

VI. Candidate Selection

Research focused on the development of live, attenuated vaccines is expected to combine the optimal features of a deliverable vaccine that is safe, free of side effects and efficacious in humans with enhanced immune stimulation. Such an approach is expected to include i) the use of highly attenuated, safe, candidates; ii) oral/intranasal delivery to optimally provide the long term mucosal immune stimulation required for protective immunity; and iii) manufacturing with unique disposable closed system technologies that are storage stable, and generally applicable to a number of select agents.

B. abortus S19 has an extensive history of effective use in agricultural animals and has been used for years as the approved vaccine strain for cattle in the U.S. without reversion to virulence [56]. Due to the induction of a persistent cross-reacting immune response, the use of S19 was

discontinued during the latter stages of the brucellosis eradication program in favor of a serologically non-reactive vaccine in the late 1990s [56]. In addition to elevated antibody titers, S19 can cause abortion in some vaccinated animals, and is pathogenic to humans in its current state [19,20,57–60]. S19 displays a clearance profile in mice that when compared to wild-type strains indicates attenuation that compromises the establishment of chronic, not acute, infection [61–64].

The selection of *Brucella abortus* strain 19 (S19) as a genetic platform for introduction of specific mutations is based on previous and well-documented i) use as the cornerstone of the bovine brucellosis eradication program in the U.S [56], ii) use in numerous animal species from ground squirrels to yaks [20,65–77], iii) use following genetic modification, [62,64,78–81] iv) use in humans, [18,82] and v) cross protection against *B. melitensis* challenge [61]. The documented stability of S19 during 80 years of use in a broad number of hosts has been confirmed by recent genomic sequencing [20,83].

Despite this extensive history, only now with the use of specific genetic mutations and a microencapsulation delivery platform is it possible to provide a live attenuated vaccine that is safe for use in humans. Targeted gene deletions in S19 generated in the Ficht lab produced strains with ideal qualities necessary to achieve robust immunization free of side effects [81]. In particular, the S19Δ*vjbR* deletion strain i) persists in mice at levels comparable to S19 alone, ii) induces no detectable splenomegaly or adverse histological changes, iii) elicits a high degree of protection and iv) induces substantial IgG_1 titres when delivered in a microencapsulated format [81]. When considering the enhanced safety (as evidenced by the lack of splenomegaly) coupled with retained protective capabilities, and the factors described above, we hypothesize that S19 deletion mutants are viable and superior alternatives to *B. melitensis* deletion mutants. In addition, the use of a strain exempt from select agent regulations will expedite marketing and release of a human vaccine, and facilitate potential use in target animal species.

VII. Summary

B. abortus mutants, or in this case S19 mutants, are capable of protecting against a range of *Brucella* species in a challenge scenario. We have previously demonstrated the greatest heterologous protection from mutants of a *B. abortus* backbone against a *B. melitensis* challenge [29,84]. There are numerous precedents and ample data supporting the premise that a *B. abortus* vaccine strain will efficiently protect against various other species of *Brucella*, particularly when delivered in a microencapsulated format [61,85,86].

Recognition of S19 as a model live attenuated bacterial vaccine prompted several attempts at human use including the vaccination of over 3 million people in the USSR [18]. Human vaccinations were associated with a ≥50% reduction in brucellosis during a period in which sheep and goat populations showed no discernable reduction in *B. melitensis* infection. The primary difficulty with S19 use in humans was a localized reaction presumably caused by previous exposure to *Brucella*. In controlled experimental trials, S19 was shown to be effective against human infection, but persisted in 2 of 16 vaccinates precluding recommended usage [87]. Since further attenuation will enhance the safety of S19 while retaining the protective properties through microencapsulation, the deleterious side effects of previous S19 exposure will be minimized while inducing protective immunity against infection in humans.

Using the same preferred genetic mutations identified in previous work, it is possible to utilize an alternate genetic background that provides increased safety, greater performance flexibility, and enhanced FDA approval and licensure. The development of the new product is well under way and has required a strong team and ongoing research with bacterial genetics and testing in multiple animal models coupled with novel manufacturing and microencapsulation technologies that balance product safety, stability, costs, and potency.

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