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Vaccines to Prevent Systemic Mycoses: Holy Grails Meet Translational Realities

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In this issue of the *Journal*, Spellberg et al. [1] report that immunization with a recombinant protein (rAls3p-N) mixed with aluminum hydroxide gel (Alhydrogel) can protect mice from a lethal candidal infection. This is an extension of their original finding of protection resulting from this and related Als recombinant proteins when used with Freund's adjuvant. From a purely immunologic perspective, their finding that protection can also be achieved using Alhydrogel in place of Freund's adjuvant might be viewed as a modest advance. However, because Alhydrogel has been widely used as an adjuvant in US Food and Drug Administration (FDA)-approved vaccines, these studies represent a milestone in the movement of the vaccine toward clinical testing. Thus, on the occasion of this report, it may be useful to review the potential value of vaccines for systemic mycoses in general, using vaccines for coccidioidomycosis as a specific example, and to review the challenges these vaccine candidates will face before becoming realities in the marketplace.

There is substantial reason to believe in the possibility of using vaccines to prevent systemic fungal infections [2–9]. In the case of coccidioidomycosis, untreated infection, regardless of whether it produces clinical illness, results in life-long immunity from future infection [10]. This is likely true for histoplasmosis and blastomycosis as well. Attempts to mimic the protection afforded by coccidioidal infection with a vaccine have been ongoing for over half a century. Indeed, a formalin-killed whole-cell (spherule) vaccine, shown to be highly effective in mice [11;12], was submitted to a clinical trial and was found not to be effective [13]. The failure of the whole-cell vaccine may have been the result of an exaggerated dose-related irritation that it engendered at the injection site, which, in human subjects, limited the vaccine dose and the immunogens that it contained to quantities thousands of times less than those used for mice, relative to body mass. Were that the case, then the development of a subcellular vaccine composed of specific immunoprotective spherule proteins might be more effective. This approach has been taken for the past decade by a collaboration between 5 laboratories, coordinated through the University of California at San Francisco, and largely underwritten with approximately \$15 million of funding from the state of California and the California Health Care Foundation [14]. As a result of this effort, several recombinant antigens have shown promise [15–19]. One chimeric antigen, composed of sequences from 2 independently protective antigens, has shown sufficient immunogenicity in mice to protect >95% of immunized animals from an infection that would otherwise be lethal [20]. Corroborating evidence for the efficacy of vaccination with this chimeric antigen has also been demonstrated in studies involving nonhuman primates [21]. Although it is possible that further exploration would improve the results obtained thus far, the current vaccine candidate is arguably sufficiently promising to recommend its extension into clinical trials.

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Other fungal vaccines also have compelling rationales supporting their continued development. As Spellberg et al. have detailed [22], candidemia has a high attributable mortality. Furthermore, because the onset of candidemia usually occurs only after weeks of hospitalization [23], a vaccination strategy for at-risk patients at the time of admission is plausible.

One of the challenges of translating vaccine discovery into clinical trials is expanded vaccine production. Vaccines developed for early testing in experimental infections are prepared in relatively small quantities. The process of scaling up vaccine production typically requires extensive changes in approach, specialized equipment, and persons with experience in pharmaceutical protein expression. For example, in preclinical studies, expression of the chimeric coccidioidal antigen [20] involved a *Saccharomyces cerevisiae* host that was found to be unable to produce yields of the recombinant protein that would be typical of commercial fermentation, and, thus, another mode of expression needed to be adopted. Even if scaling up goes smoothly, process development and production would normally cost millions of dollars.

Increased quality control for manufacturing is another challenge that arises when preparing a vaccine for clinical trials. The focus of preclinical studies is typically on the design and results of experimental studies, rather than on the manufacturing process. In contrast, vaccines suitable for administration to humans must be prepared with a much greater degree of quality control and precision, sufficient to meet the FDA's Good Manufacturing Practice standards. This necessitates a program of process development in which systematic testing of a range of fermentation, purification, and other manufacturing conditions is required to find a method that results in a vaccine antigen that displays uniform characteristics from one production run to another. In the case of the current coccidioidal candidate vaccine antigen, the chimeric protein has a predilection to aggregate, presumably because its sequence includes 12 cysteines that may cross-link under nondenaturing conditions. Although this property is not inherently a deficiency in terms of efficacy, it poses a challenge for quality control to determine how to design a manufacturing process that either prevents aggregation or is able to produce aggregation in such a way that it is defined and controlled.

A third hurdle in formulating a vaccine for clinical trials is the incorporation of an acceptable adjuvant so that vaccination results in an effective immune response. Freund's adjuvant, which was used in the discovery phase of the *Candida* rAls3p-N vaccine, is too toxic for use in a human vaccine; this led Spellberg et al. [1] to use Alhydrogel. In studies of the coccidioidal vaccine, the experimental vaccine used monophosphoryl lipid (MPL) (originally provided by the Ribi Company and later by Corixa). MPL has already been used in clinical trials of other vaccines and could be used to move our candidate vaccine into clinical trials. However, since the time we began our studies, Corixa has been acquired by another company; since then, MPL has become unavailable for further development of the coccidioidal vaccine. At present, we are investigating an alternative strategy that uses flagellin as a Toll-like receptor (TLR)-5 agonist, as in the strategy being developed by Vaxinnate [24]. We have not demonstrated that this approach will be satisfactory for our purposes. As with the *Candida* vaccine, deciding on a suitable adjuvant for the coccidioidal vaccine formulation will be critical to moving into clinical trials.

Vaccine development is a commercial undertaking. As described above, there are significant costs in the range of many millions of dollars that are involved in moving a candidate vaccine from the point of discovery to a formulation suitable for use in a clinical trial. The activities required for this to occur are collectively referred to as the "translational phase" of development. Because the product of translational work is the creation of a formulation suitable for and effective in clinical use, the work is of an applied nature, and the cost for such work is generally expected to be absorbed by the pharmaceutical manufacturer that presumably will

market and profit from its investment. Unfortunately, there is a global decline in interest in anti-infective strategies in the private sector [25]. Increasingly, private sources of capital that have traditionally supported early phase development are insisting that clinical data be available *before* an initial investment is made. This trend raises the question of how such clinical data can be generated without the initial investment.

In a 1999 Institute of Medicine report, it was estimated that the costs and benefits of research and development into a vaccine to prevent coccidioidomycosis that would be used to vaccinate infants in endemic regions and immigrants of any age would not be cost-effective, compared with savings accrued by other presently licensed vaccines [26]. A subsequent analysis that did not include the costs of development in the model estimated that an effective coccidioidal vaccine, once licensed, could be cost-effective within a fairly broad set of assumptions [27]. However, even if the latter conclusion were to be borne out, the market for a coccidioidal vaccine would be small, compared to that for most vaccines currently licensed for use. For example, the reported Arizona case rate of patients hospitalized for coccidioidomycosis in 2001 was 11.8 cases per 100,000 population, 26% of whom had infections with extrapulmonary spread [28]. Moreover, the number of reported coccidioidal infections in Arizona has grown from 2,307 in 2001 to 6,072 in 2006. Although the rate of complications for coccidioidomycosis is similar to the case rate for paralytic polio in the United States (approximately 10 cases per 100,000 population) prior to the introduction of the polio vaccine, the market for the polio vaccine is worldwide, with recipients numbering in the billions, whereas the market for a coccidioidal vaccine would at most be many millions. Obviously, this difference in scale has an enormous impact on the potential return on investment when considering marketing these 2 vaccines.

In summary, both a biologic rationale and the potential to improve the public health of targeted populations provide a strong case for pursuing vaccines to prevent coccidioidomycosis and other fungal diseases [2,3,5–7]. On the other hand, even after identifying a vaccine candidate worthy of moving from discovery to clinical trials, very substantial challenges lie ahead, and solving them requires increasingly greater financial support, even before safety and efficacy trials in human subjects can be conducted. If we are to realize the benefit from vaccines for fungal diseases, mechanisms of funding other than the conventional business model are likely to be needed.

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