for external adjuvants. Challenges for development of live attenuated vaccines include achieving the correct balance between safety, a primary requirement, and immunogenicity with protective capacity. Such a vaccine must be confirmed for an inability for reversion to virulence. This can be achieved with the inclusion of two or more independently attenuating mutations.

Other Vaccine Strategies

Bacterial live vectors can be used for the delivery of heterologous antigens. For example, expression of the *Francisella* lipoprotein antigen TUL4 in a live attenuated *Salmonella* vector was shown to provide partial protection against live LVS challenge (59). Jia et al. demonstrated protection against lethal type A challenge following immunization of mice with recombinant *Listeria monocytogenes* expressing *IglC* (60). Interestingly, the level of protection was less than that following LVS immunization.

Other groups are pursuing a more typical acellular subunit approach. Belisle has demonstrated the protective efficacy of a membrane fraction of *Francisella* combined with the adjuvant CLDC against Schu S4 challenge (61). A 17-kDa lipoprotein, a 43-kDa OMP, and heat shock protein 60 have been studied in murine models (62). These proteins were not protective in murine tularemia. Purified proteins, *FopA* and a 17-kDa lipoprotein, *TUL4,* were immunogenic but not protective (63,64). The fact that only some mouse strains are protected by vaccination with LVS suggests that it possesses a limited number of MHC-restricted protective antigens (8). To develop a licensable vaccine for tularemia, it may be necessary to give several proteins to elicit full response, rather than a poorly defined cocktail.

The porin protein PorB from *N. meningitidis*, a TLR2 ligand, was able to enhance the protective response of *Francisella tularensis* LVS LPS from 25% to 70% of mice immunized, and later challenged intranasally with LVS four weeks after the last booster (65). In another study, the protective efficacy of isolated OMPs of *Francisella tularensis*, ethanol-inactivated LVS, or purified LVS LPS administered IP in Freund's adjuvant were compared in a Schu S4 pulmonary challenge model. OMP immunization provided 50% survival at 20 days with a 1000-fold decrease in bacterial loads in the liver and spleen (66).

Conjugate Vaccines.

To date, the only antigen identified as having a possible protective role in a subunit vaccine is LPS, which in *Francisella* lacks endotoxic activity (62). Immunization of mice with a vaccine comprised of *Francisella tularensis* LPS conjugated to bovine serum albumen protected against ID challenge with a strain of *subspecies holarctica* (type B), but had marginal protection against the same strain delivered as an aerosol, and had no protection with a strain of *subspecies tularensis*. (8) Since protection against virulent strains may require T-cell mediated immunity, investigators have considered conjugation to an antigen capable of eliciting T-cell immunity. A novel strategy pursued by investigators at Epivax is the use of mixtures of T-cell epitopes identified by genomics approaches delivered in a heterologous prime-boost regimen consisting of DNA vaccine prime and peptide boost (67). This vaccine was able to protect 50% of immunized mice from a lethal aerosol challenge with LVS.

While earlier literature had described a capsule, such a structure had not been definitively described. Preliminary studies with a capsule-like carbohydrate conjugated to a protein carrier are under current study.

Additional Strategies

As is the case for many subunit vaccines, those for tularemia may need the addition of adjuvants. While DNA vaccines have been examined experimentally for some select agents such as anthrax, there are no reports for such genetic vaccines for tularemia. Ultimately, it may be necessary to assess prime-boost strategies for tularemia vaccines. Such an approach may enable the priming of the immune system by delivery of a tularemia vaccine to a mucosal site, with a boost administered at the time of a suspected biological attack. Oral administration of LVS to mice induced both humoral and cellular responses as well as the induction of a short-lived protection against lethal systemic and respiratory infection with types A and B stains of *F. tularensis* compared to sham-immunized mice (68).

CONCLUDING REMARKS

Many hurdles remain before an effective vaccine for tularemia could be licensed. Despite dramatic advances in genomics that have allowed the sequencing of both LVS and Schu S4 strains of *F. tularensis*, neither the basis for LVS attenuation nor the increased virulence of the Schu S4 strain have been determined. Recent advances in the ability to prepare defined mutations in the Schu S4 strain may now enable the development of a safe, protective type A-based vaccine. Alternatively, the identification of heretofore-undefined virulence factors may lead to the development of protective subunit vaccines. In either event, the difficulty in evaluating the efficacy of these vaccines in human clinical trials will place a burden on the identification of effective animal models in which to test these vaccines. Under current FDA guidelines, for a vaccine against a select agent to be approvable, it must demonstrate efficacy in at least two different animal species. This has posed a problem for vaccines against tularemia, since mice, unlike humans, are acutely susceptible to all subspecies of *F. tularensis.* To date, there have been few animal models considered adequate for testing of tularemia vaccines that may be predictive of success in humans.

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