Proposal: "Development of Bivalent Live Recombinant Vaccine Against Brucellosis and Anthrax"

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Objective: Development of a live bivalent vaccine against brucellosis and anthrax for veterinary use.

Reviewer: Matthew Meselson

ABSTRACT.

This is a proposal to modify vaccine strains of B. anthracis (strains STI and Sterne) by introducing into them plasmids that code for fusion proteins intended to confer immunity both to anthrax and to brucellosis. The resulting live bivalent vaccine would then replace the live monovalent anthrax vaccines currently in use.

DESCRIPTION

Brucellosis is a debilitating although seldom lethal bacterial disease caused by certain strains of Brucella. Its principal reservoirs are cattle, sheep and swine. Human exposure is principally from direct contact with infected animals and animal products, including the consumption of unpasteurized milk and milk products from infected animals. Control of the disease in humans is mainly by avoiding consumption of unpasteurized milk and milk products and contact with infected animals, by sacrifice of infected animals and herds, and, in areas where brucellosis is endemic, veterinary vaccination.

Anthrax is a bacterial disease the reservoir for which is also in animals, principally cattle and other bovids. In humans, the most common form of anthrax results from contact with infected animal products, giving rise to severe but usually not fatal skin lesions. Cutaneous anthrax is effectively treated with antibiotics. Ingestion of contaminated meat and inhalation of anthrax spores, as from handling infected hides and fur, can give rise to systemic anthrax infection. This is very often fatal, even with intensive antibiotic and supportive therapy.

The present proposal is to create a live binary veterinary vaccine against brucellosis and anthrax for immunization of animals and herds at high risk. The authors state that nearly 400,000 farm animals in Russia are vaccinated each year against anthrax, using the live non-encapsulated vaccine strain STI-1. Outside Russia, similar use is made of the non-encapsulated Sterne strain. The proposed new vaccine, based on the STI or Sterne strains, would give immunity both to anthrax and to brucellosis.

The approach proposed is based on the ability of one of the components of anthrax toxin, Protective Antigen (PA), to mediate the transport into the cytoplasm of the another component of the toxin, known as Lethal Factor (LF). Collier and his associates at the Harvard Medical School have utilized this system to deliver a cytotoxic T-cell epitope into the cytoplasm of mammalian cells. This was accomplished by exposing cells, in the presence of PA, to a fusion protein consisting of a Listeria epitope linked to the 255 aa amino-terminal moiety of LF. Extremely small amounts of fusion protein were found to confer significant immunity to Listeria infection in vaccinated mice.

The present proposal is to construct and introduce into the STI or Sterne strains plasmids containing the amino-terminal moiety of the LF gene fused to a sequence that codes for a 310 aa amino-terminal segment of a *Brucella abortus* membrane protein. The recombinant plasmids would also code for PA. Vaccination of animals with STI or Stern cells containing such plasmids would therefore produce the fusion protein and PA in the vaccinated host, causing introduction of the fusion protein into the cytoplasm of host cells, including epitope-presenting cells of the immune system.

In a separate approach, it is proposed to construct similar fusions of the Brucella protein to PA itself. So far as I am aware, it is not known whether PA enters the cell cytoplasm or remains on the surface. The authors appear to expect that the PA fusion protein will remain on cell surfaces. Whether this approach is immunologically promising is unclear to me. It is also unclear why the authors mention only one cell type, the macrophage, when the anthrax toxin delivery system operates on a wide variety of cell types.

The attractions of the proposed approach, at least with the LF fusion protein, are that (1) it is modeled on a system that has been shown to confer cellular immunity in response to very low protein concentrations; (2) by incorporating the fusion-protein producing system into a live vaccine strain, greater pharmacological persistence may be achieved as compared to that obtainable by administration of purified proteins; and (3) if successful, the proposed vaccine will provide veterinary protection against brucellosis as well as against anthrax with the same expenditure of effort now devoted to vaccination against anthrax alone.

There is no inherent difficulty in constructing the proposed fusion genes. There are, however, several problems to be solved if the work is to succeed. These include: (1) stable introduction of the recombinant plasmids into STI or Sterne hosts and (2) selection of adequately immunogenic Brucella peptides. While I do not know of any other group attempting to create a veterinary anthrax-brucellosis live vaccine based on the anthrax-toxin cytoplasmic delivery system, laboratories in the United States and elsewhere are energetically exploiting the system for cytoplasmic delivery of various peptides. This provides opportunities for mutually advantageous collaboration.