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Cloning and expression of chromosomal DNA of vaccine strain STI of *B. anthracis*
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Chameleon Pharma AS, Norway and Bioeffect, Russia.

Earlier we have shown that the avirulent R-strain of *Francisella tularensis* (LVS R strain) can be used as a model to study the expression of heterologous DNA from gram negative intracellular pathogenic bacteria. We have now shown that the fragment of chromosomal DNA from the gram-positive *B. anthracis* STI without plasmid pXo2, responsible for forming the capsule, can be transferred to the LVS R strain. Specific binding of anthrax phage κ (B1- morphotype) identified the capsule of the recombinant microorganism. The LVS R strain has itself no defined capsule. The recombinant microorganisms have been studied and characterized with respect to stability, morphology, biology, immunology etc. We conclude that the LVS R-strain can be a universal recipient for chromosomal DNA fragments, and we speculate on the potential role of the reported recombinant microorganism as a live vaccine against *B. anthracis*

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Protection Studies Following Bronchopulmonary and Intramuscular Immunization with *Yersinia pestis* Recombinant Subunit Vaccines Entrapped in Biodegradable Microparticles

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We compared the ability of intramuscularly and intratracheally administered recombinant F1 and V subunit antigens to protect mice from a lethal systemic challenge with plague. Two intratracheal immunizations with the microsphere entrapped antigens (1 μ g V; 5 μ g F1) resulted in serum and lung antibody responses which were equivalent to, or superior than, those evoked by two intramuscular injections of the particles. A subcutaneous injection of *Yersinia pestis* bacteria (10^7 MLD_{50s}), was comparatively well tolerated by all subunit treatment groups (with survival rates between 66 and 90 %). In contrast, 80 % of the mice injected intramuscularly with soluble F1 and V were defeated by a 10^7 MLD₅₀ challenge. Groups immunized intratracheally and intramuscularly with spheres were better protected (55 and 50 % respectively) at this higher challenge dose. Such findings corroborate the thesis that introduction of appropriately formulated F1 and V into the respiratory tract may be an attractive alternative to parenteral immunization schedules for protecting individuals from plague.

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Non-invasive Immunization Strategies for Plague

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Current, killed whole cell, vaccines for plague have undesirable side effects and fail to confer satisfactory protection to pneumonic infection. Fraction 1 (F1) and V are recombinant subunit vaccines which can safeguard experimental animals against pneumonic and bubonic plague. In light of the overwhelming logistical and immunological rationales which favor the development of non-invasive vaccination strategies, we have sought to develop mucosal immunization procedures to counter plague. One approach has been to co-encapsulate the F1 and V subunits in microspheres composed of a biodegradable polyester (Poly-L-lactide). When these particles are introduced into the upper or lower respiratory tract, utilizing intranasal or intratracheal inoculations, local and systemic antibody responses in conjunction with cell mediated immunity can be engendered. Further, experimental animals can be protected from lethal (>1000 MLD_{50s}) inhalational or injected challenges with virulent *Yersinia pestis* bacilli. We have also attempted to utilize microencapsulated F1 and V to achieve enteric immunization. An overview of these, and related experiments, will be presented.

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Development of an immunotherapeutic vaccine strategy to bypass the T-helper cell defect in HIV-1-infected persons and generate systemic and mucosal immune responses.

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HIV-1 infection results, early in impaired function, and later in gradual numerical depletion of T-helper (CD4+) cells. In order to overcome the T-helper cell defect inherent to HIV-1 infection we identified a vaccine carrier that would potentially stimulate effector cells directly and bypass the requirement for CD4+ T-helper cells. Heat-inactivated *Brucella abortus* (BA) was conjugated to an 18-mer peptide CGRAAIGPGRAFYTCKNG, designated V3. Neutralizing anti-peptide antibodies and CTL were elicited in normal and in mice depleted of CD4+ T cells. Optimal systemic and mucosal responses to V3-BA were obtained using combined intranasal and intraperitoneal immunizations as determined by anti-V3 antibody titers and ability of sera and mucosal samples from these mice to neutralize HIV-1 in a syncytia inhibition assay. Intramuscular immunization of Rhesus macaques with V3-BA also resulted in production of neutralizing anti-V3 antibodies in the serum and at mucosal surfaces. Cellular responses were also elicited in the monkeys as shown by IFN γ responses. These data, coupled with previous observations showing that *B. abortus* can induce T-independent antibody production and Th1-like cytokine responses in humans, supports the notion that *B. abortus* as a carrier for HIV-1 peptides or proteins may be a suitable candidate for immunotherapy of individuals infected with HIV-1.