IMMUNOLOGIC AND PROTECTIVE EFFECTS OF VACCINES FOR *MYCOBACTERIUM MARINUM* IN *MORONE* SP.

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(ABSTRACT)

Recombinant and DNA vaccines utilizing *Mycobacterium* sp. antigen 85A (Ag85A) were assessed for immunostimulatory and protective effects against *M. marinum*. Because of their known susceptibility to piscine mycobacteriosis, *Morone* sp. were utilized as the models for these studies.

The first study evaluated a recombinant vaccine with a *Brucella abortus* strain RB51 vector expressing the *Mycobacterium bovis* Ag85A. Striped bass (*M. saxatilis*) were inoculated at doses equivalent to 10^6, 10^7, 10^8, 10^9, and 10^{10} colony-forming units/fish. Vaccinated fish demonstrated significant specific humoral and cell-mediated immune responses towards the Ag85A in a dose-dependant manner. However, vaccinated fish failed to demonstrate cross-protective responses after live *Mycobacterium marinum* challenge 70 days post-vaccination.

A DNA vaccine was constructed utilizing the *Mycobacterium marinum* Ag85A gene and a commercially-available eukaryotic expression vector. Hybrid striped bass (*M. saxatilis* x *M. chrysops*) were immunized by intramuscular (i.m.) and intraperitoneal (i.p.) injection at doses of 5 µg, 25 µg, or 50 µg plasmid. These fish produced significant Ag85A-specific antibody and lymphoproliferative responses over those of control fish injected with saline or empty plasmid. Non-specific macrophage phagocytic and respiratory burst functions failed to exhibit significant upregulation after vaccination. Fish receiving the DNA vaccine developed protective responses to high-dose *M. marinum* challenge 90 days post-vaccination, as demonstrated by increased relative percent survival and by reduced splenic bacterial counts over control fish. Furthermore,

specific immunostimulatory and protective effects were significantly increased using higher vaccine doses and using the i.m. injection route.

Given these promising findings, the protective responses induced by the DNA vaccine were further investigated. Hybrid striped bass were injected with 25 μg or 50 μg plasmid i.m. and developed specific protective responses to high-dose *M. marinum* challenge 120 days post-vaccination. The 25 μg and 50 μg groups both developed more rapidly and significantly increased immune responses post-challenge over those of the control groups. The vaccination groups also demonstrated increased survival, reduced splenic bacterial counts, and reduced granuloma formation compared to the control groups. However, though the vaccination groups did not demonstrate the same acute effects post-challenge as the control groups, the vaccination groups ultimately developed increased splenic bacterial counts and granuloma formation, and eventually experienced 100% mortalities.

Because piscine mycobacteriosis can affect virtually any species of fish, a vaccine against this disease could be widely beneficial to the aquaculture and ornamental fish industries. The vaccines in these studies exhibited significant immunostimulatory capabilities in *Morone* sp., but only the DNA vaccine showed promise for conferring protection against *M. marinum* challenge. Though the DNA vaccine only provided limited protection against high challenge doses, future studies may likely find enhanced protective effects against lower, more natural exposure doses.