CVI
Journals.ASM.org

Demonstrated Efficacy of a Pilot Heterologous Whole-Spore Vaccine against Microsporidial Gill Disease in Rainbow Trout

J. E. Harkness, N. J. Guselle, D. J. Speare

Department of Pathology & Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada

Intraperitoneal vaccines using whole viable spores of the microsporidian *Glugea anomala* or *Glugea hertwigi* reduced the numbers of branchial xenomas by 80% and 91%, respectively, after a standard experimental infection of juvenile rainbow trout with the microsporidian *Loma salmonae*. Similar significant results were obtained when killed-spore preparations were used.

Microsporidial gill disease (MGDS) of salmon (*Loma salmonae*) is a leading cause of mortality in farmed Pacific salmon in Canada (1). During the disease, large spore-filled xenomas (cysts with contributions from host and parasitic cells) develop within the gills; when the xenomas rupture, they evoke a severe inflammatory response in the gills which can lead to death (1). Previous work has demonstrated a strong protective immune response in those fish which recover from initial infection (1); the prophylactic efficacy of an nonadjuvanted whole-spore vaccine based on a low-virulence strain of *Loma salmonae* has also been demonstrated, with vaccinated fish developing up to 93% fewer gill lesions than control fish following experimental challenge with virulent *L. salmonae* (2). A factor limiting commercial production of this vaccine is the availability of *Loma* spores in commercially relevant numbers to support a vaccination program encompassing millions of fish. One of several options is to investigate a heterologous vaccine based on more readily available spores from a related microsporidian. In the current study, based on the degree of genetic similarity of *L. salmonae* to two species of *Glugea* (*G. anomala* and *G. hertwigi*) (3), we assess the potential to develop a heterologous whole-spore vaccine against MGDS using *Glugea* spores.

For the preliminary trial, spores of *G. hertwigi* were obtained from the ovaries of wild-caught smelt (*Osmerus mordax*); spores of *G. anomala* were obtained from the peritoneal wall of three-spined sticklebacks (*Gasterosteus aculeatus*). Both species of fish were captured within estuaries of Prince Edward Island, Canada. Spores were liberated from xenomas dissected from these fish and suspended in 0.85% sterile saline by means of centrifugation at 1,000 rpm for 10 min. Pelletized material was resuspended in 0.85% saline solution to form a 1:10 dilution, yielding a prototype vaccine containing 10^6 spores per dose (2). Trout were vaccinated 6 weeks prior to subsequent exposure to *L. salmonae*, as this has been shown to be the induction time for protective immunity (2, 4).

The following four groups of juvenile rainbow trout were used to assess the efficacy of a *Glugea*-based whole-spore vaccine: (i) a group of 20 fish that had recovered from a standard experimental infection with *L. salmonae* (5), (ii) a group of 30 fish intraperitoneally (i.p.) vaccinated 6 weeks prior with *G. anomala*, (iii) a group of 30 fish i.p. vaccinated 6 weeks prior with *G. hertwigi*, and (iv) a group of 30 unvaccinated control fish. The fish, each weighing between 30 and 40 g, were then housed together within a 250-liter tank. Treatment groups were identified by patterns of fin clip. Minced gill tissue containing 4,500 xenomas was added to the tank as a standard infective oral challenge dose (5). All fish had equal access to this infective material, and it was observed that all fish participated in consuming it; the exact infective dose per fish is therefore not known, but prior studies have shown that this method of infection yields the least variability compared to individual oral or i.p. dosing (5). Five weeks following exposure, fish were euthanized in a water bath containing 100 mg/liter benzocaine. The first left gill arch was removed, and the numbers of spore-filled xenomas were counted (1, 5). Infection levels are expressed as xenoma counts per gill arch (XCPGA) (mean values and standard deviations [SD] are shown below) (2). The Kruskal-Wallis nonparametric test followed by a Bonferroni multiple-comparison test was used to assess the null hypothesis that the XCPGA were no different in vaccinated and control fish. Differences were considered significant at the more stringent alpha level of probability of 0.0125 to account for the multiple comparisons.

Whereas 87.5% of naive fish developed xenomas (XCPGA = 39.4 ± 40.1), those with previous Loma exposure showed no xenomas. In the *G. anomala*-vaccinated group, 71.4% of fish developed xenomas but the mean XCPGA was low (7.6 ± 9.9) and significantly less than in controls. Similarly, in the *G. hertwigi* group, although 75.9% of fish developed xenomas, the mean XCPGA was also very low (3.4 ± 5.4) and significantly less than in controls.

Although no gross anatomical evidence of *Glugea* infection was noted in any of the fish which had received vaccines, a second study was completed to assess the efficacy of a killed whole-spore vaccine prototype. Spores were exposed to two freeze-thaw cycles, which has previously been shown to kill all spores (2). Thirty juvenile trout were i.p. vaccinated with a vaccine with proven efficacy which contained killed spores (10^6) of a low-virulence strain of *L. salmonae* (2). Thirty trout received a similar i.p. dose of killed *G. hertwigi* spores, and 30 others received a similar i.p. dose of killed *G. anomala* spores. Thirty-four naive unvaccinated fish served as controls. Fish subsequently cohabited, were exposed to *L. salmonae* 6 weeks after vaccination, and were euthanized for XCPGA counts as described for trial 1. In this trial, whereas 100%

Received 22 May 2013 Returned for modification 13 June 2013
Accepted 1 July 2013
Published ahead of print 3 July 2013
Address correspondence to D. J. Speare, speare@upei.ca.

doi:10.1128/CVI.00340-13

of naive fish developed xenomas (mean XCPGA = 72.8 ± 60.5), the XCPGA was significantly reduced in all of the vaccinated groups (percentages of trout with xenomas were 86.2%, 92.9%, and 96.4%, whereas the mean XCGPA were 19.9 ± 25.6, 13.5 ± 13.9, and 24.3 ± 41.4 in *G. hertwigi*-, *G. anomala*-, and *L. salmonae*-vaccinated groups, respectively).

The current results add to a body of knowledge suggesting that MGDS may become a disease manageable through vaccination. Following infection or vaccination, a cell-mediated immune response is elicited, and it becomes protective by 4 weeks after exposure (4); in fish which are vaccinated, the life cycle of *L. salmonae* becomes ablated during a prebranchial merogonic stage within subendocardial macrophages (4). An effective vaccine prototype has been developed from virulent and low-virulence strains of *L. salmonae* (2), and herein we have demonstrated an effective response to a heterologous vaccine using two microsporidians closely related to *L. salmonae*. Specifically, the vaccine preparations were able to reduce the XCPGA by 80% and 81.5% (live-spore and dead-spore *G. anomala* vaccines, respectively) and 91% and 72.7% (live-spore and dead-spore *G. hertwigi* vaccines, respectively), compared to a 66.6% reduction when a vaccine based on a low-virulence strain of *L. salmonae* was used. It is noteworthy that although the vaccines developed with *Glugea* spores appear to outperform the vaccine developed with low-virulence *L. salmonae*, previous studies (2) have demonstrated a slightly higher level of protection from *L. salmonae* than was noted in the work described herein. Variability between trials may derive from many aspects. The xenoma expression model used herein compares fish based on the parasite's ability to develop into fully formed xenomas, which would be affected by the percentage viability of spores used in challenge models, an aspect that was not determined but which may have accounted for differences in XCPGA between trials.

MGDS is a not a "reportable" disease in Canada. Fish with light levels of infection do not need to be eradicated for regulatory or international trade requirements. Given that the pathophysiological effects of *L. salmonae* are directly linked to the XCPGA, the marked reduction in XCPGA afforded by vaccination becomes a viable management approach which could be adopted by the aquaculture industry. The XCPGA levels developing in nonvaccinated test animals in these trials would be considered moderate levels of infection (5); therefore, the vaccines have proven effective at a Loma level typically associated with clinical expression. The availability and relative technical ease of collection of *Glugea* spores, coupled with the effectiveness of *Glugea*-based prototype vaccines of live- or killed-spore preparations, suggest that some of what might otherwise constrain a spore-based vaccine from becoming commercially viable may be sidestepped. The use of whole-spore vaccines for microsporidial diseases of other animals has also been considered (6).

REFERENCES

1. **Kent ML, Speare DJ.** 2005. Review of the sequential development of *Loma salmonae* (Microsporidia) based on experimental infections with rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*O. tshawytscha*). Folia Parasitol. **52:**63–68.
2. **Speare DJ, Markham RJF, Guselle NJ.** 2007. Development of an effective whole-spore vaccine to protect against microsporidial gill disease in rainbow trout (*Oncorhynchus mykiss*) by using a low-virulence strain of *Loma salmonae*. Clin. Vaccine Immunol. **14:**1652–1654.
3. **Lovy J, Kostka M, Dykova I, Arsenault G, Peckova H, Wright GM, Speare DJ.** 2009. Phylogeny and morphology of *Glugea hertwigi* from rainbow smelt *Osmerux mordax* found in Prince Edward Island Canada. Dis. Aquat. Org. **86:**235–243.
4. **Rodriguez-Tovar LE, Markham RJF, Speare DJ, Sheppard J.** 2006. Cellular immunity in salmonids infected with the microsporidial parasite *Loma salmonae* or exposed to non-viable spores. Vet. Immunol. Immunopathol. **114:**72–83.
5. **Ramsay JM, Speare DJ, Sanchez JG, Daley J.** 2001. The transmission potential of *Loma salmonae* (Microspora) in the rainbow trout, *Oncorhynchus mykiss* (Walbaum), is dependent upon the method and timing of exposure. J. Fish Dis. **24:**452–460.
6. **Sobottka I, Igluaer F, Schuler T, Schmetz C, Visvesvara GS, Albrecht J, Schwartz DA, Pieniazek NJ, Bartschi K, Laufs R, Schottelius J.** 2001. Acute and long term humoral immunity following active immunization of rabbits with inactivated spores of various *Encephalitozoon* species. Parasitol. Res. **87:**1–6.