魚類における免疫学的不妊化技術の開発に向けた研究

[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract of Dissertation

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As the world's population grows rapidly, the demands for farmed fish as a source of protein increase every year. Sexual maturation in fish is one of the factors that reduce the production efficiency of aquaculture, because fish consume large amounts of energy for gonadal development, and sexual maturation leads to a slump in growth rates and poor meat quality in farmed fish. In order to improve the efficiency of aquaculture production, it is necessary to develop a simple and versatile sterilization technology to solve the problem of sexual maturation. Several sterilization technologies have already been developed. However, problems still remain and sterilization technologies are still under development. One sterilization technology that is different from all of the above is the immunological sterilization. This technology induces autoantibodies against sex-related hormones, thereby inhibiting the progress of sexual maturation in a target animal by the host's own immune system. However, immunological sterilization against hormones has proven to be less effective in fish than in mammals. The objective of this study was to obtain basic knowledge for the establishment of immunological sterilization technology for gonads and germ cells.

First, 40 μL of testicular and ovarian homogenate with Freund's complete adjuvant (FCA) (20 mg/mL) was injected into immature rainbow trout *Oncorhynchus mykiss*. Two negative control groups were prepared; a mixture of muscle tissue homogenate with FCA and PBS only. Blood plasma and gonads were collected 22 days after injection, and antibody titers against gonadal tissues and histologic analysis of gonadal tissues were performed. The results showed a significant increase in antibody titers in the gonad-injected group compared with the PBS group, but no statistically significant difference compared with the muscle-injected group. In this study, tissue sections of the gonad-injected group were prepared from individuals who had relatively high antibody titers in the gonad-injected group and subjected to HE staining for observation. No cell aggregation was observed, as had been reported in previous studies. However, globular structures were observed in case of the testis.

Second, 50 μL of germ cell-specific monoclonal antibodies (anti-GC mAbs) (500 μg/mL) with Montanid ISA 763A VG were injected into the body cavity of the trout. Rainbow trout in the negative control group were injected with 50 μL of a mixture of PBS and adjuvant. An additional 50 μL of antibodies (1 mg/ mL) were injected 23 and 30 days after the initial injection, and 100 μL of antibodies (500 mg/ mL) were injected on day 37. Blood and gonads were collected 40 days after the first injection. According to the analysis, antibody titers against anti-GC mAbs increased slightly in the anti-GC mAbs group. Individuals with particularly high antibody titers were selected for tissue sections and immunostained with anti-GC mAbs. No morphological changes were observed.

Next, 0.5~1.0 μL of anti-GC mAbs (215 μg/ mL, 21.5 μg/ mL) were injected into the body cavity of larval or juvenile rainbow trout. Alexa488-labeled antibodies were injected into larval trout 20 days after

fertilization. At 28 days after injection, the gonads were observed under a fluorescence microscope, and some germ cells showed Alexa488 positive signals. Observation of individuals 76 days after injection revealed the presence of distinct Alexa488-positive cells at the posterior end of the gonads in both sexes. 25 µL of Alexa488-labeled antibodies (21.5 µg/mL) were injected into the body cavity of rainbow trout 102 days after fertilization, and the gonads were observed under a fluorescence microscope three days later. Only nonspecific fluorescent signals were observed in the testis. In the ovaries, green fluorescent signals were observed in an arrangement parallel to the ovarian lamina. According to these results, it may be difficult for the antibody to reach the germ cells stored in the gonadal membrane. The Alexa488-positive cells observed in larva trout are thought to have been bound by antibodies during migration.

Finally, single-chain variable region fragments (scFv) of anti-GC mAbs were developed to improve their penetrability. First, total RNA was extracted from the hybridoma strain No. 189 producing the anti-GC mAbs, and its antibody variable region fragments were amplified by the 5'-RACE PCR method. The resulting sequences were then analyzed and confirmed to be the variable regions of mouse immunoglobulin of heavy and light chains. These heavy and light chain DNA fragments were joined by overlap PCR using GGGSx3 linker and ligated into pCold-II DNA vectors to generate recombinant proteins, anti-GC scFv. *E.coli* BL21 strain cells were transformed with these vectors and cultured in a protein production medium, and the recombinant proteins were successfully obtained as soluble fractions. On the other hand, the anti-GC scFv did not show the specificity for germ cells on the paraffin-embedded tissue section of testis.

In conclusion, immunization with gonadal homogenates and anti-GC mAbs had no critical effect on sexual maturation in rainbow trout. However, intraperitoneally injected antibodies were able to reach some of the primordial germ cells at a very early larval stage, although the antibodies could not bind to the germ cells after entering the gonadal tissues. In the future, it will be necessary to test whether small molecule antibodies, such as single-chain variable fragments can be delivered into the interior of gonadal tissues. Antibody drug conjugate (ADC) is another cell elimination technology that has been developed in recent years. This technology eliminates target cells by using toxins directly conjugated to recombinant antibodies. It is expected to be simpler and more versatile because it does not require host adaptive immunity. The most realistic strategy would be to eliminate PGCs at the larval stage by injecting anti-GC scFv as ADC.