Acquired Protection and Production of Immobilization Antibody against Cryptocaryon irritans (Ciliophora, Hymenostomatida) in Mummichog (Fundulus heteroclitus)

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Cryptocaryon irritans is a pathogenic ciliate of marine fishes. Heavy infections cause mass mortalities of fishes. Although the occurrences had been known only among ornamental fishes in private or public aquaria1,2,3, it has been prevailing in cultured food fishes with the growth of mariculture4,5. In Japan, various marine fishes, e.g., red sea bream (Pagurus major), tiger puffer (Takifugu rubripes) and Japanese flounder (Paralichthys olivaceus), cultured in net cages in the sea suffer from C. irritans infections. Despite the increasing occurrence of this epizootic, few methods are available to control it, especially in maricultured food fishes. To transfer net cages to uncontaminated areas near the open sea seems to be the only feasible method for the time being.

Recently, the immunological responses of fishes to parasites have been studied by many researchers, aiming to establish measures to control parasitic diseases6,7,8,9. It is well established that fishes once infected with the freshwater parasitic ciliate Ichthyophthirius multifiliis, the life cycle of which is similar to that of C. irritans, acquired immunity against the parasite7,8,9. However, little is known on the immunological response against C. irritans. Although the presence of acquired protective immunity against C. irritans in thick-lipped mullet (Chelon labrosus) was experimentally demonstrated10, the mechanism of the protection is not clear as yet. The present study was conducted to demonstrate the presence of acquired immunity of fish against C. irritans and the production of immobilization antibodies in immunized fish, as well.

Materials and Methods

A whitespot-tail morwong (Gonostisbus zonatus) infected with C. irritans was obtained from a local pet shop and used to initiate infections. The propagation of C. irritans and collection of free-swimming tomonts and infective theronts were carried out as previously described11 with a modification that mummichogs as well as saltwater-adapted black mollies (Poecilia hybrid) were used as the host. Briefly, C. irritans was propagated by adding host fishes once or twice a week into saltwater propagation aquaria with gravel filters at 24–26°C. Fisher infected in the aquaria were removed and placed in beakers. Tomonts leaving the infected fish were collected from the bottom of the beakers and incubated at 25°C for collection of theronts. The mummichogs used were obtained from the Arasaki Marine Biological Station, National Research Institute of Fisheries Science, and reared in 1/3 strength sea water to avoid C. irritans infection for four months before use.

Mummichogs kept in 1/3 strength sea water were placed in full strength sea water for two weeks before immunization. For immunization, mummichogs weighing 8 to 12g were placed in the propagation aquaria for three days and subsequently for treatment kept in 1/3 strength sea water for more than two weeks. The infection and treatment was repeated again. The surviving mummichogs after the second infection and treatment were used as immunized fish. Control fish were kept in 1/3 strength sea water without exposure to C. irritans.

Eight immunized and seven control fish were challenged by theronts of C. irritans. Fish were placed in full strength saltwater for one day for acclimatization and placed individually into 800 ml plastic containers. Five hundred and forty theronts within 3 hours after excystment were added into each container. The fish were kept in the containers with gentle aeration at 24–25°C and the saltwater was changed every day. Tomonts that left each fish were counted.

Sera were taken from 4 immunized and 4 control fish, heat-inactivated (30 min at 56°C) and stored at -40°C. The sera were serially two-fold diluted with PBS in a 96-well multiplate (30 μl in each well). Theronts were concentrated by centrifugation (400 g, 5 min) and re-suspended in PBS. The suspension (30 μl), in which several hundred theronts were contained, was added into each well, incubated at room temperature for 1 h, and observed by an inverse microscope. Maximum dilution immobilizing almost 100% theronts was taken as the endpoint titer.

Results

When fish were challenged by C. irritans theronts, tomonts left host fish 3 to 4 days after the challenge. All of the seven control fish were infected and the numbers of tomonts recovered from each fish ranged from 19 to 284 (mean: 132). On the other hand, one of the eight immunized fish was infected, from which only one tomont was recovered. There was a significant difference in the intensity of infection between immunized and control fish groups (P > 95%, U-test).

Sera of immunized fish immobilized the theronts (Fig. 1A,B). In low dilutions, theronts were attached to each other, forming large aggregations. In high dilutions, the cilia of the theronts stuck together, stopping all movement. However, immobilization of theronts was not observed in the sera of control fish (Fig. 1C), in which theronts moved around actively. Endpoint titer in sera from immunized fish ranged from 2^4 to 2^7 (geometric mean: 2^5.7), and were less than 2 for control

Sera
There was a significant difference in the titers between immunized and control fish groups (P > 95%, U-test).

Discussion

In the present study, mummichogs which were immunized showed almost complete protection against the challenge by *C. irritans* theronts. This result is consistent with a previously report10). Sera of carps and channel cat fish immunized with *I. multifiliis* immobilized and agglutinated theronts8,9), suggesting the immobilization activity contributes to the protection against reinfection. Immobilization antibodies produced against the antigen on the surface of the parasite is involved in the immobilizing activity9). As shown in the present study, sera of fish immunized by *C. irritans* also immobilized the theronts. It is clear that the immobilization antibody was also produced in fish immunized against *C. irritans*. As previously predicted6), the mechanism of immunization against *C. irritans* seems to be similar to that of its freshwater counterpart *I. multifiliis*.

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References