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Molecular immune response of channel catfish immunized with live theronts of *Ichthyophthirius multifiliis*

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ABSTRACT

The parasite *Ichthyophthirius multifiliis* (Ich) has been reported in various freshwater fishes worldwide and results in severe losses to both food and aquarium fish production. The fish surviving natural infections or immunized with live theronts develop strong specific and non-specific immune responses. Little is known about how these immune genes are induced or how they interact and lead to specific immunity against *Ichthyophthirius multifiliis* in channel catfish *Ictalurus punctatus*. This study evaluated the differential expression of immune-related genes, including immunoglobulin, immune cell receptor, cytokine, complement factor and toll-like receptors in head kidney from channel catfish at different time points after immunization with live theronts of *I. multifiliis*. The immunized fish showed significantly higher anti-Ich antibody expressed as immobilization titer and ELISA titer than those of control fish. The vast majority of immunized fish (95%) survived theront challenge. Expression of IgM and IgD heavy chain genes exhibited a rapid increase from 4 hour (h4) to 2 days (d2) post immunization. Expression of immune cell receptor genes (CD4, CD8-α, MHC I, MHC II β, TcR-α, and TcR-β) showed up-regulation from h4 to d6 post immunization, indicating that different immune cells were actively involved in cellular immune response. Cytokine gene expression (IL-1βa, IL-1βb, IFN-γ and TNF-α) increased rapidly at h4 post immunization and were at an up-regulated level until d2 compared to the bovine serum albumin control. Expression of complement factor and toll-like receptor genes exhibited a rapid increase from h4 to d2 post immunization. Results of this study demonstrated differential expression of genes involved in the specific or non-specific immune response post immunization and that the vaccination against Ich resulted in protection against infection by *I. multifiliis*.

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1. Introduction

Ichthyophthiriasis caused by the parasite *Ichthyophthirius multifiliis* (Ich) has been reported in various freshwater fishes worldwide and results in severe losses to both food and ornamental fish production [1,2]. In the US, 42% producers of channel catfish *Ictalurus punctatus* suffered more than 2000 lb losses per producer due to the parasite in 2002 [3,4], and Ich infections in fingerling fish affected close to four percent of total channel catfish producers in 2009 [5]. The life cycle of the parasite includes 3 stages: an infective theront, a parasitic trophont and a reproductive tomont [1,2]. There are some treatments or combinations of treatments available for the parasite [1]. However, it is difficult to control the parasite using chemotherapy after its theront penetrates into fish skin and gills [6].

Immune protection has been successfully demonstrated in fish surviving natural and experimental Ich infections or following immunization with Ich antigens [1,7,8,9]. To date, one of the most effective ways to immunize against Ich is by intraperitoneal (IP) injection of live theronts. The fish immunized with live theronts developed strong specific and non-specific immune responses

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[8,9,10]. Both systemic and mucosal immune responses are involved in protection against *I. multifiliis* [9,11,12]. Systemic and mucosal antibodies were demonstrated to agglutinate the parasites *in vitro* [7,9,10]. There are three major types of immunoglobulins in teleost fish: IgM, IgD and IgT/IgZ, but only IgM and IgD have been identified in channel catfish [13,14,15]. IgD includes a membrane bound form (IgD2) and a secreted form (IgD3) [13]. Functions of IgD in immune responses are largely unknown and need further study. The fish infected with theronts also induced localized cellular infiltration, including lymphocytes, granulocytes and macrophages [10,16].

Sigh et al. [12] detected an increase in gene expression of IgM, major histocompatibility complex (MHC) and complement component C3 in rainbow trout after exposure to Ich. In a study of the initial response of rainbow trout fry to infection with *I. multifiliis*, Heinecke and Buchmann [17] found that IL-1β, IL-6, and IL-8 were up-regulated after fish were exposed to the Ich theronts. Von Gersdorff Jørgensen et al. [18] found that complement factors C3, C5 and factor B were up-regulated in liver and head kidney of rainbow trout following immunization with a live vaccine against Ich. Olsen et al. [15] also noted that significant up-regulation of genes encoding IgM, IgT, C3, and IFN-γ were induced by immunization with Ich theronts in rainbow trout. The gene expressions of toll-like receptors TLR-1 and TLR-2 were observed to be significantly up-regulated in the head kidney of channel catfish after infection by Ich [19]. Little is known about how these immune genes are induced, how they interact and how they lead to specific immunity against *I. multifiliis* in channel catfish. The objective of this study was to evaluate molecular immune response and investigate differential expression of immune-related genes in head kidney from channel catfish over a time course after immunization with live theronts of *I. multifiliis*.

2. Materials and methods

2.1. Fish and parasite

Channel catfish (Industry pool strain) were obtained from disease-free stock from the USDA-ARS Catfish Genetic Research Unit, Stoneville, MS and reared to experimental size in indoor tanks at the USDA, Aquatic Animal Health Research Unit, Auburn, AL. *Ichthyophthirius multifiliis* (ARS 15 strain) originally isolated from infected tropical pet fish was maintained by serial transmission on channel catfish held in 50-l glass aquaria as described by Xu et al. [9].

2.2. Water quality

Dissolved oxygen (DO) and temperature were measured using an YSI 85 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH). The pH, hardness, ammonia and nitrite were determined using Hach CEL/890 Advanced Portable Laboratory (Loveland, CO). During the trial, a light:dark period of 12:12 h was maintained and supplemental aeration was supplied by air stones. The water quality was measured daily throughout the trial and expressed as mean ± standard error: dissolved oxygen 5.8 ± 0.4 mg l^{-1}, pH 7.0 ± 0.2, ammonia 0.27 ± 0.3 mg l^{-1}, hardness 95.5 ± 7.9 mg l^{-1}, nitrite concentration below detectable level and water temperature 22.1 ± 0.4 °C, measured daily. All water parameters were within acceptable levels for catfish.

2.3. Theront preparations

Fish heavily infected with mature trophonts were anesthetized with 300 mg l^{-1} tricaine methanesulfonate (MS 222), rinsed with water and the skin was gently scraped to dislodge the parasites. Trophonts were harvested by filtering through a 0.45 μm filter to remove fish skin. The isolated trophonts were placed in Petri dishes and allowed to attach. After replacing the water in the Petri dishes with fresh dechlorinated water to remove contaminating mucus, trophonts were incubated for 18 h at 24 °C. Theronts were harvested by pouring through a sieve with a pore size of 22 μm. For IP injection, theronts were concentrated by centrifugation at 100 × *g* for 3 min and suspended in phosphate-buffered saline (pH 7.2, PBS). Theronts were counted in five 10 μl samples of theront solution with the aid of a Sedgewick-Rafter cell, where average counts were used for concentration calculation. A 5% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) solution was prepared in PBS to be used in the mock-immunized control.

2.4. Experimental design and immunization procedure

A total of 110 channel catfish with an average length of 15.1 ± 1.3 cm (mean ± SEM) and weight of 30.1 ± 7.2 g were used in this trial. All fish treatment protocols were approved by Institutional Animal Care and Use Committee at the Aquatic Animal Health Research Unit. Ten catfish were sampled to verify that the fish were free from parasites and detectable serum antibody against Ich prior to initiation of the trial. All fish were negative for Ich infection (*i.e.* no visible attached theronts under microscopy) and no anti-Ich antibody was detected in fish serum prior to study.

Fish were then distributed into 4 replicated groups and immunized by IP injection as follows: groups 1 and 3 with live theronts at a dose of 15,000 theronts (0.1 ml in volume) per fish; groups 2 and 4 with 0.1 ml of 5% BSA as mock-immune control. There were 15 fish per tank in the groups 1 (immune) and 2 (control) to collect tissues for immune gene expression analysis. There were 10 fish per tank in groups 3 (immune) and 4 (control) to evaluate immune protection and fish survival 21 days post immunization.

2.5. Blood and tissue sampling

Five fish were sampled from groups 1 and 2 at h4 (4 hour), d1 (1 day), d2, d6, d10 and d20 post immunization to collect serum for measuring antibody and tissues for evaluating immune related genes. The blood was collected after fish were anesthetized with 100 mg l^{-1} MS-222. After blood collection, fish were euthanized by 300 mg l^{-1} MS-222 and head kidney was collected and immediately immersed in RNAlater (Invitrogen, Carlsbad, CA). All samples were incubated overnight at 4 °C and then transferred to − 20 °C until RNA extraction.

2.6. Anti-Ich antibodies in serum of channel catfish

Serum anti-Ich antibodies were determined by both theront immobilization assay and enzyme-linked immunosorbent assay (ELISA). The immobilization assay was conducted by placing theronts in serum serially diluted in 96 well flat bottomed microtitre plates (Corning Costar, Corning, NY) as described by Xu et al. [9]. A solution of 50% (v:v) PBS diluted with tank water was used as a diluent. Immobilization titer was the inverse of the highest dilution of serum in which all theronts were immobilized. Anti-Ich antibodies were measured in two replicates for each serum sample with ELISA as described by Xu et al. [20]. An optical density (OD) reading in a sample from experimental fish was considered positive when its value was two times greater than the OD readings in the control. The ELISA titer was the inverse of the highest dilution of serum in which a sample was positive.