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NOTE

Innate immune response of channel catfish *Ictalurus punctatus* mannose-binding lectin to channel catfish virus (CCV)

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ABSTRACT: The channel catfish virus (CCV) is a pathogenic herpesvirus that infects channel catfish *Ictalurus punctatus* in pond aquaculture in the southeastern USA. Mannose-binding lectin (MBL), an innate immune protein, could play an important role in the innate response of channel catfish by binding to CCV. Cell cultures of CCV were grown in channel catfish ovary cells (CCOC). A dot-immunoblot enzyme-linked immunosorbent assay was done to determine the binding ability of 5 mo old channel catfish serum MBL (26.2 µg ml^{-1}) to CCOC infected with CCV. Two separate nitrocellulose membrane blotting techniques were done using uninfected and infected CCOC. The uninfected CCOC decreased by 29.3 and 33.4 % in their binding of channel catfish MBL when compared with infected CCOC using the 2 membrane procedures. The combined average binding ability of channel catfish MBL towards infected CCOC was therefore 31.4 % greater when comparing the infected and uninfected CCOC. Normalization equation values of MBL for the 5 mo old catfish were compared for the 2 membrane binding procedures. The 2 normalization values were very close (142 and 150) in binding ability of MBL to the infected CCOC. The 5 mo catfish serum had twice the concentration of MBL (26.2 µg ml^{-1}) compared to 7 mo catfish serum (13.2 µg ml^{-1}), and the binding percentage of 5 mo serum was 2.4 times greater in infected than in uninfected cells. This demonstrates that the binding of channel catfish serum MBL to CCV is concentration dependent and is related to serum concentrations of MBL.

KEY WORDS: Catfish mannose-binding lectin · Catfish herpesvirus · Immune protein · ELISA

INTRODUCTION

The channel catfish virus (CCV), a pathogenic virus of channel catfish *Ictalurus punctatus*, was first described by Fijan (1968) and was later characterized as a herpesvirus (Wolf & Darlington 1971). CCV generally infects catfish less than 6 mo old, and epizootics can result in 95 % losses of catfish fry and fingerlings in pond aquaculture (Fijan et al. 1970, Plumb 1978, Thune 1993, Stingley & Gray 2000). Channel catfish mannose-binding lectin (MBL) and blue catfish MBL can bind to *Edwardsiella ictaluri* (Ourth et al. 2007), a Gram-negative bacterium that causes enteric septicemia of catfish. However, the binding ability of blue catfish MBL to *E. ictaluri* was greater than that of channel catfish. In the present study, we found that channel catfish MBL can also bind to CCV and thus could potentially provide innate immunity to this virus. Our study provides insight for a better

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understanding of the early innate immune response of channel catfish MBL to CCV.

MATERIALS AND METHODS

Channel catfish serum

Two separate serum pools, each containing serum pooled in equal amounts from 10 channel catfish (Mississippi Delta Select Strain), were obtained from 5 and 7 mo old catfish at the Warmwater Aquaculture Research Unit, USDA, ARS (Stoneville, MS). The catfish were maintained in flow-through well water indoor tanks in a 12:12 h light:dark photoperiod at a mean temperature of 26.8°C.

Growth of CCV in channel catfish ovary cells

Cell cultures of CCV (University of Arkansas Pine Bluff-strain) were grown in channel catfish ovary cells (CCOC) at 28°C using L-15 media (Sigma-Aldrich) formulation (Plumb & Bowser 1983, Silverstein et al. 1998). Data for the infected and uninfected CCOC were obtained using the same growth lot of cells (Fig. 1). Half of the cell lot was infected with CCV, while the other half remained uninfected. CCOC inoculated with the CCV were confirmed positive by the polymerase chain reaction (PCR) technique (Kancharla & Hanson 1996), and uninfected CCOC were PCR negative. Fathead minnow cells used as a negative control were also PCR negative.

Dot-immunoblot enzyme-linked immunosorbent assay (ELISA) for MBL and binding of MBL to CCV

The ELISA was done using a dot-blot microfiltration apparatus to determine serum concentrations of MBL in the 2 serum pools of 5 and 7 mo old channel catfish. A 1:8 dilution (50 µl) of catfish serum was spotted onto nitrocellulose membrane in the dot-blot apparatus. The primary antibody was guinea pig immunoglobulin G (IgG) antirabbit-MBL, and the secondary antibody was rabbit anti-guinea pig IgG-horseradish peroxidase conjugate (Sigma). The dot-blot apparatus (Bio-Rad) was used according to a previously described immunoassay procedure (Ourth et al. 2005, 2007, 2008). The primary antibody showed specificity to affinity-purified catfish MBL using the ELISA procedure. Image J scanning for color density of the dot-blots was done using an Epson Perfection 2400 photoscanner (Epson America) to obtain background-corrected inverse density (BCID) units (Ourth et al. 2007). MBL concentrations (µg ml^{-1}) were obtained using a standard curve of purified channel catfish MBL concentrations (µg ml^{-1}) vs. BCID units as determined by the ELISA (Ourth & Rose 2011).

Two separate experimental membrane blotting procedures were done as follows using the CCOC and channel catfish serum. In the first procedure, the dot-blot apparatus (Bio-Rad) was used. Fifty µl each of CCOC infected with CCV and uninfected CCOC were blotted onto nitrocellulose membrane in the dot-blot apparatus and washed once with 100 µl of phosphate-buffered saline (PBS), pH 7.2. Twenty-five µl of serum were added from the 5 and 7 mo old catfish, incubated for 30 min at room temperature,

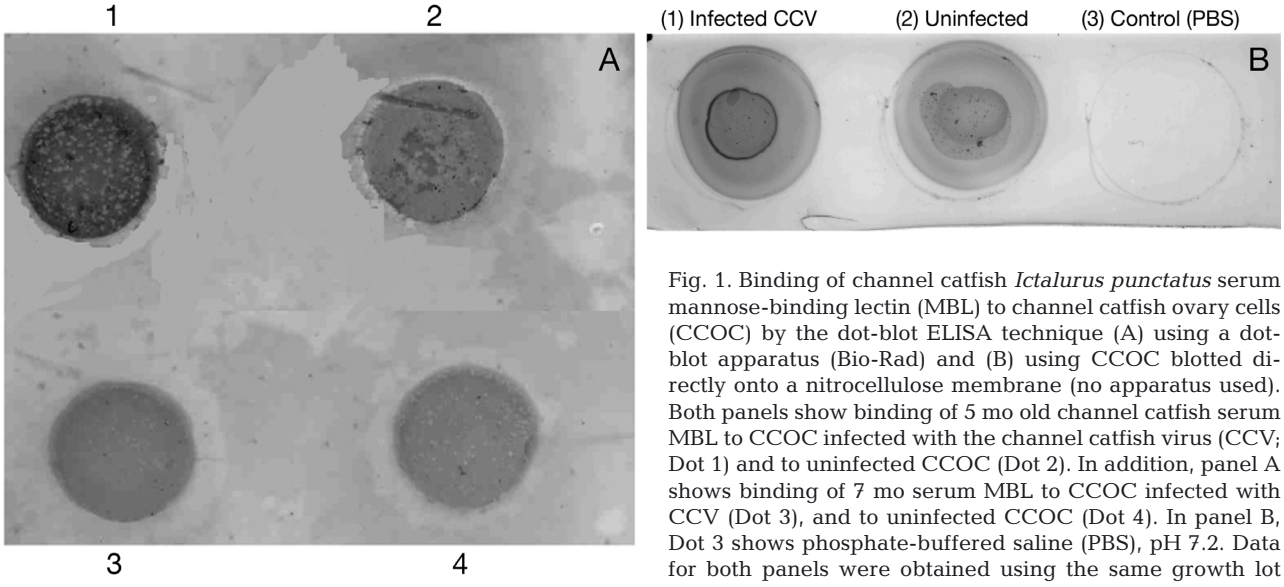

Fig. 1. Binding of channel catfish *Ictalurus punctatus* serum mannose-binding lectin (MBL) to channel catfish ovary cells (CCOC) by the dot-blot ELISA technique (A) using a dot-blot apparatus (Bio-Rad) and (B) using CCOC blotted directly onto a nitrocellulose membrane (no apparatus used). Both panels show binding of 5 mo old channel catfish serum MBL to CCOC infected with the channel catfish virus (CCV; Dot 1) and to uninfected CCOC (Dot 2). In addition, panel A shows binding of 7 mo serum MBL to CCOC infected with CCV (Dot 3), and to uninfected CCOC (Dot 4). In panel B, Dot 3 shows phosphate-buffered saline (PBS), pH 7.2. Data for both panels were obtained using the same growth lot of CCOC

and then washed once with 100 µl PBS, pH 7.2. The ELISA was done as before using guinea pig IgG antirabbit-MBL as the primary antibody (Ourth et al. 2007).

In the second procedure, 50 µl each of CCOC infected with CCV and uninfected CCOC were blotted directly onto nitrocellulose membrane and then dried with no dot-blot apparatus used. The diameter of the blot on the nitrocellulose membrane was 18 mm. Twenty-five µl of serum were added from the 5 mo old catfish, incubated for 30 min at room temperature, and then washed 2 times with PBS, pH 7.2. The ELISA was done as before using guinea pig IgG antirabbit-MBL as the primary antibody (Ourth et al. 2007).

RESULTS

Five mo old channel catfish serum (MBL: 26.2 µg ml^{-1}) gave a BCID reading of 123.6 density units (DU) when bound to CCOC infected with CCV (Table 1A, Fig. 1A). Uninfected CCOC gave a BCID reading of 87.4 DU. The uninfected CCOC therefore decreased by 29.3% in BCID units compared to the infected CCOC. This approach used the dot-blot apparatus (BioRad) for the ELISA procedure.

Seven mo old channel catfish serum (MBL: 13.2 µg ml^{-1}) gave a BCID reading of 80.1 DU when bound to CCOC infected with CCV (Table 1A, Fig. 1A). Uninfected CCOC gave a BCID reading of 70.4 DU. The uninfected CCOC decreased by 12.1% in BCID units compared to the infected CCOC. This approach also used the dot-blot apparatus for the ELISA procedure.

The 5 mo catfish serum had twice the concentration of MBL (26.2 µg ml^{-1}) than the 7 mo catfish serum (13.2 µg ml^{-1}), and the binding percentage in 5 mo serum was 2.4 times greater in the infected than in the uninfected cells (Table 1A). This corresponds with a decrease in MBL as previously seen in catfish aged 6 to 9 mo compared to catfish aged 2 to 4 mo (Raghu et al. 2016). This result also demonstrates that binding activity to CCV of channel catfish serum MBL from 5 and 7 mo old catfish is related to serum concentrations of MBL and is therefore concentration dependent.

The second approach used CCOC blotted directly onto nitrocellulose membrane with no dot-blot apparatus used (Table 1B, Fig. 1B). With this procedure, the 5 mo channel catfish serum (MBL: 26.2 µg ml^{-1}) gave a BCID reading of 68.9 DU when bound to the CCOC infected with the CCV (Table 1B). Uninfected CCOC gave a BCID reading of 45.9 DU. The uninfected CCOC decreased by 33.4% in BCID units and in binding of MBL compared to the infected CCOC.

When both nitrocellulose membrane procedures were compared and averaged for the 5 mo old catfish, the combined average percentage decrease in BCID units for uninfected vs. infected CCOC was 31.4% (Table 1), thus demonstrating the increased binding activity of channel catfish MBL to CCV when comparing uninfected vs. infected cells. With both membrane blotting procedures (Table 1, Fig. 1), the infected CCOC were always greater in BCID units than the uninfected CCOC. This demonstrates the binding ability of channel catfish MBL to the CCV and indicates that channel catfish serum MBL could provide innate immunity in channel catfish to this virus.

A statistical equation using a normalization value of 100 for the uninfected CCOC was used to compare the BCID units (Table 1) for binding ability of channel catfish MBL to the infected and uninfected CCOC (Dodge & Cox 2006). The uninfected CCOC were normalized to 100 for comparison of the 5 and 7 mo old catfish sera. In the 2 different membrane procedures that were used, one 5 mo serum (Table 1A) had a normalized value of 142, and the other 5 mo serum (Table 1B) had a normalized value of 150, indicating very close normalization values (142 and 150) for binding of MBL to infected CCOC when the 2 different membrane blotting procedures were compared. Both of these values were higher than that of the 7 mo serum (Table 1A), which had a normalized value of 114.

Table 1. Background corrected inverse density (BCID) units and mannose-binding lectin (MBL) serum concentrations (µg ml^{-1}) in channel catfish *Ictalurus punctatus* aged 5 and 7 mo old, determined (A) with and (B) without the use of a dot-blot apparatus. Each group represents a serum pool of 10 fish. Uninfected channel catfish ovary cells (CCOC) and CCOC infected with the channel catfish virus (CCV) were compared for the binding ability of channel catfish serum MBL; the BCID decrease in uninfected vs. infected cells is shown. Data for A and B were obtained using the same growth lot of CCOC

Catfish age	MBL (µg ml^{-1})	CCOC	BCID units	BCID decrease (%)
(A)				
5 mo	26.2	Infected	123.6	29.3
		Uninfected	87.4	
7 mo	13.2	Infected	80.1	12.1
		Uninfected	70.4	
(B)				
5 mo	26.2	Infected	68.9	33.4
		Uninfected	45.9	

DISCUSSION

Channel catfish are raised in pond aquaculture in the southeastern USA and are highly susceptible to bacterial and viral infections acquired from their pond environment. Channel catfish that are <1 yr old are especially susceptible to infections by the channel catfish herpesvirus and the bacterium *Edwardsiella ictaluri.* Viral hemorrhagic disease in channel catfish is caused by CCV, an ictalurid type 1 enveloped herpesvirus (Wolf & Darlington 1971). CCV disease occurs primarily during the summer months from June to September in the southeastern USA.

Innate immunity is the first line of defense in an animal and offers the main resistance to pathogens within the first hours and days following an infection (Fujita 2002). Innate immunity recognizes microbes and provides initial protection until an adaptive immune response takes place in addition to the innate immune response (Holland & Lambris 2002). Teleost (bony) fish and cartilaginous fish bridge the innate and adaptive immune responses and are the first jawed vertebrates with both immune systems (Whyte 2007).

MBL is a C-type lectin that activates the complement-mediated lectin pathway of the innate immune response (Hoffmann et al. 1999, Suckale et al. 2005). Serum MBL activates the lectin pathway by binding to mannose expressed on microbial surfaces. Complement activation then results in killing of microorganisms by the membrane attack complex and by opsonization to enhance phagocytosis (Takahashi et al. 1993, Sato et al. 1994, Thiel et al. 1997). Channel catfish MBL via the lectin complement pathway could therefore offer a protective function in catfish by binding to CCV.

Channel catfish MBL was studied here for binding activity to CCV. Serum pools were used, as insufficient serum was available to test each fish individually. We found that serum MBL (26.2 µg ml^{-1}) from 5 mo old catfish could bind to CCOC infected with CCV using an ELISA (Table 1, Fig. 1). The average binding activity of catfish MBL was 31.4% greater in BCID units when comparing both nitrocellulose membrane procedures for the infected and uninfected CCOC (Table 1A, B). Previous research indicated that both 2 mo (21 µg ml^{-1}) and 12 mo (19.9 µg ml^{-1}) catfish sera were very similar in their MBL concentrations (Raghu et al. 2016). The greatest increase in MBL was seen in 4 mo old catfish (26.9 µg ml^{-1}). This previous study also used serum pools of 10 catfish from 2 to 12 mo of age. Channel catfish could also produce the innate immune protein lysozyme at 2 mo equivalent to lysozyme concentrations found in 9 and 12 mo old catfish (Raghu et al. 2016). The channel catfish MBL could therefore potentially provide innate immunity to CCV in juvenile and older channel catfish (Ourth et al. 2007, Ourth & Rose 2011, Peterson et al. 2015, Raghu et al. 2016).

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