ANTIGENIC ANALYSIS OF FISH VIRUSES WITH MONOCLONAL ANTIBODIES

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Health control of culturing fish and prevention of infection are very important to the aquaculture business. However, maintaining fish health is practically difficult and particularly in the case of viral diseases, there are almost no prevention methods because of the rapid contagious property.

The purpose of our study is to produce monoclonal antibodies (MAbs) to fish viruses and apply them as diagnostic or therapeutic reagents. we describe here the establishment of MAbs to three fish viruses and characterization of viral antigens using these MAbs.

Table. 1. List of monoclonal antibodies reactive to fish viruses

Viral antigens	Monoclonal antibodies	Class	IgG subclass	Light chain	Cross reaction	Neutralizing activity
IHNV (ChAb)	HG59-9	IgG	IgG1	κ	-	-
	5HG-1	IgG	IgG1	κ	-	-
	HM-2	IgM		κ	HRV	-
	HM-3	IgM		κ	HRV	-
	HM-11	IgM		κ	HRV	-
IPNV (VR299)	4PG-4	IgG	IgG2b	κ	-	-
	4PG-15	IgG	IgG2b	κ	-	+
	4PG-16	IgG	IgG2b	κ	-	+
	3PM-19	IgM		κ	-	-
	4PM-7	IgM		κ	-	-
	4PM-10	IgM		κ	-	-
	4PM-17	IgM		κ	-	+
HRV (8401-H)	HRG-25A	IgG	IgG1	κ		-
	HRG-2B	IgG	IgG3	κ	IHNV	-
	HRG-5B	IgG	IgG2a	κ		-
	HRG-10B	IgG	IgG2b	κ		-
	HRG-12B	IgG	IgG1	κ		-
	HRG-18B	IgG	IgG1	κ		-
	HRG-27B	IgG	IgG1	κ		-
	HRM-3A	IgM		κ	IHNV	-
	HRM-4A	IgM		κ		-

Materials and methods

Production of murine monoclonal antibody

Purified fish viruses, infectious hematopoietic necrosis virus (IHNV, ChAb), infectious pancreatic necrosis virus (IPNV, VR299) (1) and *Rhabdovirus olivaceus* (HRV, 8401-H) (2) shown in Fig. 1 were immunized in BALB/c mice, and the immunized lymphocytes fused with mouse myeloma P3X63-U1/Ag-8 as described previously (3). Hybridomas secreting MAbs were screened by both enzyme-linked immunosorbent assay (ELISA) and indirect immunoperoxidase technique. Positive hybridomas were cloned twice by limiting dilution method.

Antigenic characterization of fish viruses

Specificity of the MAbs to each viral antigen was determined by ELISA. The viral antigens directed by the MAbs were proven by immunoblot assay (3). Neutralizing activity of the MAbs was estimated by plaque reduction test (1). Further immunoassay was also conducted by both indirect immunofluorescent and immunoperoxidase techniques.

Results and Discussion

We obtained five anti-IHNV, seven anti-IPNV and nine anti-HRV MAbs. Their characteristics are summarized in Table 1. Anti-IHNV IgG MAbs, HG59-9 and 5HG-1 specifically reacted with IHNV, while other MAbs (IgM) cross-reacted with HRV, suggesting the presence of common epitopes between both rhabdoviruses, IHNV and HRV. Immunoblot assay showed three MAbs, HG59-9, 5HG-1, and HM-2 recognized to N protein (nucleoprotein) of five IHNV structural proteins of L, G, N, M1 and M2, which were different from other reports showing G protein (glycoprotein) (4, 5).

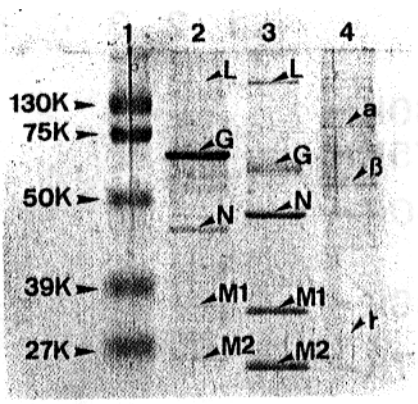

Fig. 1. Structural proteins of purified fish viruses analyzed by SDS-polyacrylamide gel electrophoresis. Lane 1, standard protein markers; lane 2, IHNV (ChAb); lane 3, HRV (8401-H); lane 4, IPNV (VR299).

Seven anti-IPNV MAbs were all IPNV-specific and no cross-reaction was observed in other fish viruses tested. Among them, two IgG (4PG-15 and 4PG-16) and and one IgM MAbs (4PM-17) neutralized IPNV and, in particular, 4PG-15 and 4PG-16 neutralized IPNV more than an order of 10^2 at a concentration of 1μg/ml (Table 2).

Table 2. Neutralizing activity of anti-IPNV monoclonal antibody 4PG-16 against fish viruses

Virus	Infectivity (PFU/ml)		Neutralizing activity*3
	Monoclonal antibody*1	Control*2	
IPNV	5.1×10^5	6.5×10^7	2.1
IHNV	1.8×10^5	2.4×10^5	0.1
HRV	4.5×10^5	5.6×10^5	0.1
OMV	3.5×10^4	3.5×10^4	0.0

*1 Infectivity of virus reacted with monoclonal antibody 4PG-16 (1 μg/ml).
*2 Infectivity of virus reacted with E-RDF medium contained 10% fetal calf serum.
*3 Logarithmic value of viral infectivity divided infectivity of virus reacted with control sample by infectivity of virus reacted with 4PG-16 sample.

These neutralizing MAbs all recognized only β protein of IPNV, suggesting that β protein appeared to be related to infectivity or pathogenicity of IPNV. This indicates that β protein will be very effective to promote neutralizing anti-IPNV antibody production in immunized fish if purified β protein can be vaccinated to fish. One of the seven MAbs, 4PG-4 only recognized γ protein of IPNV (Fig. 2). These observations are similar to those

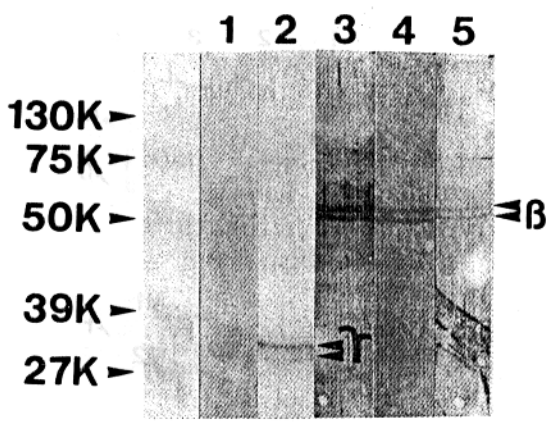

Fig. 2. IPNV proteins recognized by anti-IPNV monoclonal antibodies. Lane 1, 3PM-19; lane 2, 4PG-4; lane 3, 4PG-15; lane 4, 4PG-16; lane 5, 4PM-17.

of another report (6). IPNV-infected cells were clearly distinguishable from other fish virus-infected and noninfected cells by indirect immunofluorescent and immunoperoxidase techniques with both β and γ protein-specific MAbs (Fig. 3).

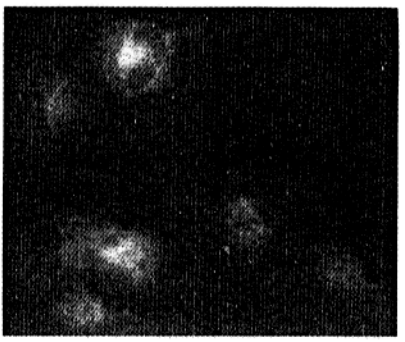

Fig. 3. IPNV-infected fish cells stained by indirect immunofluorescent technique with anti-IPNV monoclonal antibody 4PG-16.

In the case of anti-HRV MAbs, seven of nine anti-HRV MAbs specifically reacted with HRV, and the other two were cross-reactive to IHNV in ELISA. Two of them (HRG-25A, IgG and HRM-4A, IgM) only directed themselves to M2 protein of HRV. Therefore, these fish virus-specific and neutralizing MAbs would be very useful for epizootics, classification, and immunology of fish viruses and can also be applied as diagnostic and therapeutic reagents for fish viral diseases. We are in the process of large-scale production and purification of neutralizing anti-IPNV MAbs to apply the MAb to affinity chromatography for purification of IPNV particles. In the future, IPNV particles purified by such a chromatographic procedure with IPNV-specific MAb may be used as a vaccine against IPNV infection.

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