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High throughput screening of recombinant antibodies against infectious hematopoietic necrosis virus from a combinatorial antibody library

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ABSTRACT

Infectious hematopoietic necrosis virus (IHNV) is a significant rhabdoviral pathogen of salmonid fish. In this study, a single chain variable fragment (scFv) antibody library derived from rainbow trout (*Oncorhynchus mykiss*) and a glycoprotein fragment (named G4, 540 nt, 180 aa) of IHNV-Sn1203 isolate were co-expressed by a bacterial display technology. The library was subjected to three rounds of screening by flow cytometry (FCM) to select IHNV specific antibodies. Seven antibody clones with different mean fluorescence intensities (MFI) were obtained by picking colonies at random. The antibody clone with the highest MFI was expressed and purified. The purified IHNV-specific scFv antibody was used successfully in Western blotting, enzyme linked immunosorbent assay and an immunofluorescence antibody test. This method provides a high throughput means to screen an antibody library by flow cytometry and isolate an antibody that can be used as a potential universal reagent for the detection and confirmation IHNV strains that are prevalent throughout China.

Statement of relevance: Outbreaks of infectious hematopoietic necrosis caused severe economic losses to salmon and trout aquaculture in China every year. In this study, a panel of recombinant antibodies against Chinese IHNV isolates was obtained. The isolated antibody was proven can be used as a universal diagnosis regent for IHNV prevalent in China. The study provides a novel method for rapid development of antibodies to emerging diseases.

1. Introduction

Infectious hematopoietic necrosis virus (IHNV) is a rhabdovirus that causes acute, systemic disease in salmonid fish and also occurs asymptomatically. As one of the most important viral diseases in aquaculture, outbreaks of IHN result in losses approaching 100% depending on the species and size/age of the fish, the virus strain and environmental conditions. Since the first outbreak of IHN in Washington and Oregon fish hatcheries during the 1950s, IHNV is now found in many countries (Breyta et al., 2013; Enzmann et al., 2005; Jia et al., 2014; Kim et al., 2007; Kolodziejek et al., 2008; Nishizawa et al., 2006; Rudakova et al., 2007; Troyer et al., 2000). In 1985, the first outbreak of IHN was recorded in hatcheries for juvenile rainbow trout in Liaoning Province, China (Niu, and Zhao, 1988). This was followed by a series of IHN outbreaks in cultured juvenile rainbow trout in various districts of China (Jia et al., 2014; Xu et al., 2013).

In previous studies, a bacterial display technology was established and successfully applied to antibody library screening (Xu, Li, Zhou, Guo, Liu, Zhao, Cao, Li, 2014). However, by using the bacterial display technology, purified antigen must be obtained before the screening process. This is time consuming and labor intensive. In this study, an antigen-antibody co-expression display technology was established. The present study was designed to isolate IHNV-specific single chain variable fragment (scFv) antibodies from an antibody library assembled from rainbow trout using an antibody-antigen co-expression system combined with flow cytometry (FCM).

2. Materials and methods

2.1. Materials

Escherichia coli DH5α was used for displaying the scFv library, while *E. coli* Rosetta and pET27b(+) were used for expression of scFv's that were isolated. The pCoex vector was used for scFv library display and was generated in our laboratory. The glycoprotein gene sequence of the IHNV isolates used in this study was submitted to the GenBank database and referred to as follows, IHNV strain Sn1203 (KC660147), LN12 (KF871194), SD-12 (KF871193), GS-12 (KF871194), YN13 (KF871192) and XJ-13 (KF871191). A rabbit anti-IHNV glycoprotein polyclonal antibody (described below) and the cell line *Epithelioma papulosum cyprini* (EPC) were from lab stocks. An FITC antibody labeling kit was purchased from Thermo (California, USA). CY3-mouse anti-His tag antibody was purchased from eBioscience (Shanghai, China).

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2.2. Construction of antigen-antibody co-expression vector pCoex

A previously constructed vector pBFD (Xu, Liu, Zhao, Cao, Yin, Liu, Lu, 2014) was used as a basic frame to construct the antigen-antibody co-expression vector. The ribosome binding site (RBS) and pelB leader peptide were amplified from plasmid pET-27b and inserted into the pBFD by *Eco*RI and *Xho*I to construct antigen-antibody co-expression vector pCoex. The fragment of the IHNV glycoprotein G4 (540 nt, 180 aa) (Xu, Liu, Zhao, Cao, Yin, Liu, Lu, 2014) was fused with the pelB leader peptide, and the antibody library (described below in Section 2.3) was fused with the NlpA leader peptide. Both of these were co-expressed and transported to the periplasm. If the anchored antibody could bind to the antigen an antibody-antigen complex was formed. Upon removal of the outer membrane by spheroplasting, fluorescent antibodies against the glycoprotein entered the periplasmic space where they were recognized by the membrane-tethered antibody-antigen complex. As a result, specific antibody-expressing spheroplasts became fluorescently labeled and could be readily enriched and screened by a fluorescence activated cell sorter (FACS) (Fig. 1).

2.3. Construction of scFv library

Head kidney and spleen were isolated from rainbow trout (mean weight, 20 g ± 2.5) that survived an outbreak of IHN that caused approximately 80% cumulative mortality. Tissues from 6 rainbow trout were harvested and pooled two month after the outbreak of IHN, and total RNA was extracted using Trizol. cDNA was synthesized from the total RNA sample using Superscript II (Invitrogen) and random hexamer oligonucleotide primers. Primers used to amplify the VH and VL gene fragment for construction of the antibody library were designed according to a previous study (Zhou and Xie, 2015). PCR products of the VH and VL genes were linked by overlapping PCR to construct the scFv antibody library. The linker peptide used in the study were typical $(Gly_4Ser)_3$ sequences. The fragment of IHNV glycoprotein G4 (Xu, Liu, Zhao, Cao, Yin, Liu, Lu, 2014) was fused with the pelB leader peptide, and the antibody library was fused with the NlpA leader peptide. The recombinant plasmids were electroporated into *E. coli* DH5α according to standard procedures, and the library was labeled pCoex-G-scFv. The colonies were counted to calculate the transformation efficiency of electroporation. Library diversity was determined by sequencing 5 random clones obtained from the scFv library.

2.4. Spheroplast preparation and scFv screening

All colonies from the pCoex-G-scFv library-transformed DH5α were collected and cultured in lysogeny broth (LB). Expression of antigen and antibody were induced as in previous studies (Xu et al., 2015). The spheroplasts were prepared as previously described (Jeong et al., 2007). Rabbit anti-full length IHNV glycoprotein polyclonal antibody (Xu et al., 2013) was labeled using an FITC antibody labeling kit according to the manufacturer. The spheroplasts were incubated with 200 nM FITC-rabbit anti-glycoprotein antibody for 1 h at 4 °C and then screened by FACS. The library of cells was sorted on a FACSAria™ Cell Sorter (BD Biosciences, USA). scFv DNA, that was isolated by plasmid extraction from the cells collected, was electroporated into *E. coli* DH5α and subjected to another round of sorting by FACS. When the antibody-binding population reached 60%, colonies were picked at random, screened by FACS and subjected to DNA sequencing. Spheroplasts expressing random scFv of rainbow trout were used as a negative control for the FACS analysis.

2.5. Expression and purification of scFv antibodies

Expression vector pET-27b(+) was used to express the scFv-1 clone that had the highest MFI. The expression and purification of the scFv antibody was performed according to previous studies (Xu, Li, Zhou, Guo, Liu, Zhao, Cao, Li, 2014). The scFv-1 was expressed with a His 6 tag at the 3′ end.

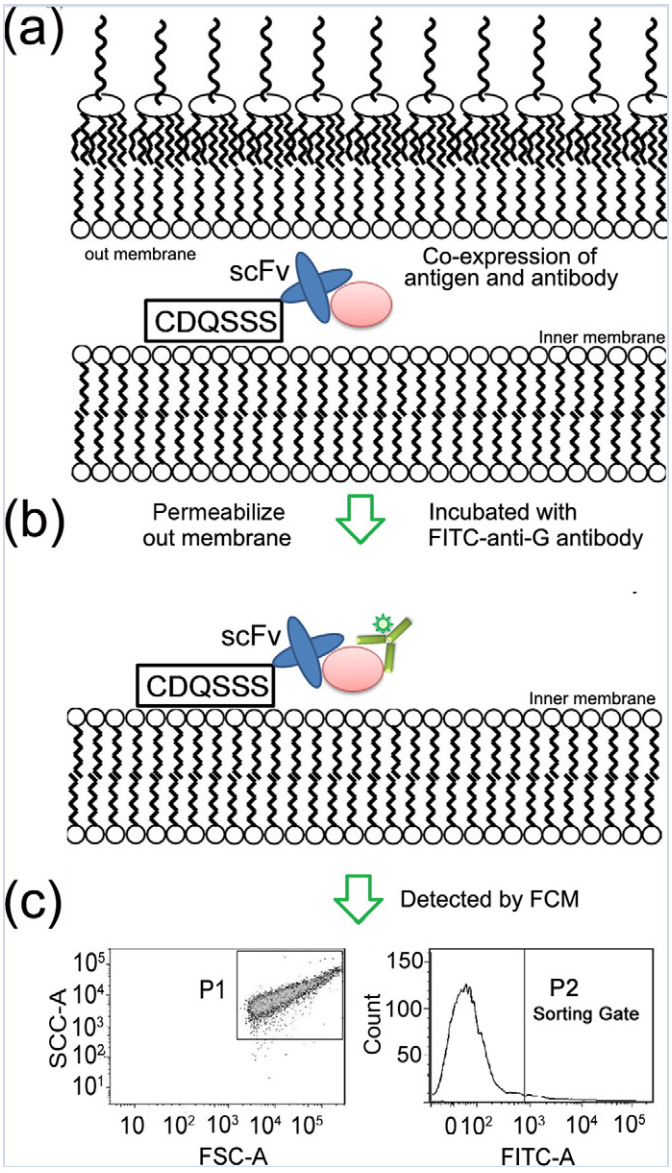

Fig. 1. The antigen-antibody co-expression display system. (a) Libraries of scFv antibodies and antigen protein are co-expressed in the periplasm of *E. coli* and antibodies that are specific to the antigen will tether the antigen to the inner membrane of the bacteria by CDQSSS. (b) After outer membrane permeabilization, the scFv antibody-antigen complexes anchored on the inner membrane bind with fluorescently labeled antigen specific antibodies. (c) Enrichment of spheroplasts expressing antigen specific antibody by gating the region defined by the distinct scatter of the spheroplasts (FSC and SSC) and the high FITC-A signal. FSC, forward scatter; SSC, side scatter; CDQSSS, NlpA amino acids 1–6.

2.6. Western blotting

Purified scFv-1 protein and bovine serum album (BSA) were electrophoresed in a native-SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% (m/v) skim milk in PBS-Tween20 (PBS-T) buffer. The blots were incubated with 100 μl of the IHNV glycoprotein (Xu et al., 2013) in PBS-T (40 μg ml^{-1}) at 4 °C overnight, followed by incubation with rabbit anti-full length IHNV glycoprotein polyclonal antibody (1:200) for 1 h. The final incubation was with HRP-goat anti-rabbit antibody (1:7500) at room temperature for 1 h. The blots were developed using the ECL Detection System.