Vol. 94: 9–16, 2011
doi: 10.3354/dao02311

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published March 16

Identification of a 27.8 kDa protein from flounder gill cells involved in lymphocystis disease virus binding and infection

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ABSTRACT: *In vitro*, lymphocystis disease virus (LCDV) infection of flounder gill (FG) cell cultures causes obvious cytopathic effect (CPE). We describe attempts to isolate and characterize the LCDV-binding molecule(s) on the plasma membrane of FG cells that were responsible for virus entry. The results showed that the co-immunoprecipitation assay detected a 27.8 kDa molecule from FG cells that bound to LCDV. In a blocking ELISA, pre-incubation of FG cell membrane proteins with the specific antiserum developed against the 27.8 kDa protein could block LCDV binding. Similarly, antiserum against 27.8 kDa protein could also inhibit LCDV infection of FG cells *in vitro*. Mass spectrometric analysis established that the 27.8 kDa protein and β-actin had a strong association. These results strongly supported the possibility that the 27.8 kDa protein was the putative receptor specific for LCDV infection of FG cells.

KEY WORDS: Lymphocystis disease virus · Receptor · Virus entry · Flounder gill cells · Co-immunoprecipitation

INTRODUCTION

Lymphocystis disease virus (LCDV) is the causative agent of lymphocystis disease (LCD), an infectious virosis of fish manifested by hypertrophy of connective cells in the integument of the fish's body and fins (Tidona & Daral 1999, Sheng & Zhan 2004). The disease has affected over 125 different wild and cultured fish species worldwide, including species of particular commercial importance, e.g. Japanese flounder *Paralichthys olivaceus* (Garcia-Rosado et al. 2004). Great advances have been made with respect to the epidemiology, pathology and diagnosis of LCDV (Basurco et al. 1990, Bowden et al. 1995, Dixon et al. 1996, Sheng & Zhan 2004, Cheng et al. 2006, Cano et al. 2009); however, viral replication and pathogenesis of LCDV are not clearly understood (Tidona & Daral 1999). Viral receptors are defined as cellular surface proteins that, in addition to their normal physiological role, specifically bind viruses, resulting in attachment of viral particles to the cell surface. Thus, they mediate a physical interaction between virions and target cells. Research on host cell receptors of LCDV will contribute to the understanding of viral replication and pathogenesis.

To date, several cell lines, such as bluegill fry-2, gilthead seabream fin-1, Japanese flounder embryo, flounder gill, grass carp ovary and grass carp kidney, have been reported to support LCDV isolation and replication (Wolf & Carlson 1965, Perez-Prieto et al. 1999, Iwamoto et al. 2002, Zhang et al. 2003, Xing et al. 2006). Cultures of the flounder gill (FG) cell line, derived from gill tissue of Japanese flounder (Tong et al. 1997), develop obvious cytopathic effect (CPE) 1 to 2 d after inoculation with supernatants derived from LCDV cell cultures, demonstrating that the FG cell line is highly susceptible to LCDV infection. The dynamics of FG cell infection by LCDV *in vitro* studied by indirect fluorescent antibody test (IFAT) and immunocytochemical assay (ICA) using LCDV-specific monoclonal antibodies demonstrated that the virus replicated in the cytoplasm and reached its highest level after 48 to 72 h (Xing et al. 2006). Electron microscopic observa-

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tions showed that the virus could attach to the cell surface and then penetrate the cells by membrane fusion or by endocytosis, suggesting that there were virus-specific receptors located on the FG cell surface (Lv et al. 2003), and that the FG cell line is a good candidate for studying LCDV–cell receptor interaction.

Several protein–protein interaction methods can be used to study the virus receptor, such as the the yeast 2-hybrid system, glutathione S-transferase (GST) pull down, virus overlay protein binding assay (VOPBA), and co-immunoprecipitation (Melegari et al. 1998, Ryu et al. 2000, Imajoh et al. 2003, Li et al. 2003). Co-immunoprecipitation has become a widely accepted method for identification of receptors for viruses such as mouse mammary tumor virus, fish rhabdovirus, SARS coronavirus and dengue 2 virus (Golovkina et al. 1998, Bearzotti et al. 1999, Li et al. 2003, Liu et al. 2004).

To date, the cellular receptor involved in the attachment and entry of LCDV to target cells is unknown. Thus, the present study attempted to isolate and characterize the putative receptor molecule that is responsible for binding LCDV. The identification and molecular characterization of the cellular receptor molecules is of importance in understanding viral replication, pathogenesis and tissue tropism of LCDV in the host.

MATERIALS AND METHODS

Cell culture and virus purification. The flounder gill cell line FG-9307 was obtained from the Marine Science College of Ocean University of China (Tong et al. 1997). Monolayer cultures of FG cells were grown at 22°C in Eagle's MEM (Hyclone) supplemented with 10% fetal bovine serum (Hyclone), 100 IU ml^{-1} penicillin and 100 µg ml^{-1} streptomycin. The fetal bovine serum was reduced to 2% in maintenance medium following inoculation of the cultures with virus.

Japanese flounder *Paralichthys olivaceus* with lymphocystis nodules on the body surface were obtained from a farm in Qingdao, Shandong province of China. LCDV particles were isolated and purified according to methods described by Cheng et al. (2006). Briefly, lymphocystis-infected cells were isolated from ~10 g lymphocystis-affected tissues and homogenized in 90 ml TNE buffer (0.05 M Tris-HCl, 1 mM EDTA, 0.1 M NaCl, pH 7.4) at 4°C. After 3 or 4 freeze-thaw cycles and ultrasonication, the homogenate was centrifuged at 1800 × *g* for 20 min to sediment cell debris. The supernatant was harvested, stored overnight at 4°C and ultra-centrifuged at 45 150 × *g* for 120 min at 4°C. The resulting pellet was resuspended with TNE buffer and ultra-centrifuged using a sucrose discontinuous density gradient. The virus band was harvested into TNE buffer and ultra-centrifuged at 45 150 × *g* for 120 min at 4°C. The pellet was finally resuspended in TNE buffer and stored at –80°C until use.

Preparations of cell membrane proteins and electrophoresis. Monolayer cultures of FG cells were harvested using a cell scraper. The cells were centrifuged in phosphate-buffered saline (PBS; pH 7.4), and the pellet was resuspended in ice-cold lysis buffer (0.02 M Tris-HCl, 0.137 M NaCl, 10% glycerol and 1% NP-40) in the presence of protease inhibitors (1 mM phenylmethyl sulfonyl fluoride, 2 mM EDTA, 10 mM NaF, 2 µg ml^{-1} Aprotinin and 5 µg ml^{-1} Leupetin). After ultrasonication for 3 min, nuclei and debris were removed by centrifugation at 1000 × *g* for 10 min and 10 000 × *g* for 10 min at 4°C. Membrane proteins, present in the supernatant, were sedimented by centrifugation at 100 000 × *g* for 20 min and resuspended in PBS; the protein concentration was determined by the Bradford method. The cell proteins were denatured for 5 min at 100°C in sample buffer (5% SDS, 5% 2-mercaptethanol, 0.5 M Tris-HCl, 50% glycerol, 0.05% bromophenol blue) and then subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein bands were visualized by staining with Coomassie blue R-250 (Sigma).

Co-immunoprecipitation. A volume of 20 µl Protein G agarose (Santa Cruz Biotechnology) was dispensed into a spin column and washed 3 times with PBS by centrifugation at 1000 × *g* for 5 min. Then, 1 ml of anti-LCDV monoclonal antibody, obtained according to Cheng et al. (2006), was cross-linked onto the Protein G agarose with shaking for 2 h at 22°C. The mixture was washed as above and 1 ml purified LCDV (100 µg ml^{-1}) in PBS was added and incubated with shaking for 2 h at 22°C. Following a further washing, 1 ml FG cell membrane proteins (100 µg ml^{-1}) in PBS was added and incubated with shaking for 4 h at 22°C. After the last washing, the mixture was denatured for 5 min at 100°C in sample buffer and then subjected to SDS-PAGE. PBS without LCDV and PBS without cell membrane proteins were used as 2 negative controls.

Purification of 27.8 kDa protein. Membrane proteins of FG cells were subjected to SDS-PAGE, and the 27.8 kDa band (corresponding to the cell membrane protein which showed cross-linkage to LCDV) was cut out and electro-eluted overnight at 25 V in an electro-elution apparatus (Bio-Rad). After dialysis overnight, the protein was concentrated using a freeze dryer (Heto-holten) and resuspended in PBS.

Polyclonal antiserum preparation against 27.8 kDa cell membrane protein. Three BALB/c mice were immunized intraperitoneally with 100 µg 27.8 kDa protein emulsified with an equal volume of complete Freund's adjuvant (Sigma) on Day 1. After 2 wk, the first booster immunization (100 µg protein in incomplete Freund's adjuvant [Sigma] intraperitoneally) was

given to each mouse. The other 2 booster injections (50 µg protein) were given via the tail vein at 7-d intervals. Mouse sera were obtained 7 d after the last injection and then tested by IFAT and western blot assays.

Rabbit anti-LCDV serum preparation. Purified LCDV was suspended in TNE buffer and the protein concentration was adjusted to 1 mg ml^{-1}. A New Zealand white rabbit was injected subcutaneously at 6 locations in the hypodermis with 1.2 ml of an emulsion of inactivated LCDV and complete Freund's adjuvant (1:1). After 2 wk, 1.2 ml of LCDV was injected as an emulsion with incomplete Freund's adjuvant (1:1) as the first immunization. Then, 0.5 ml of inactivated LCDV was injected 2 times intravenously 1 and 2 wk later for booster immunization. One week after the last injection, the rabbit antiserum was collected and stored at −80°C. All studies were conducted in accordance with institutional, national and international guidelines concerning the use of animals in research.

Indirect immunofluorescence assay and western blotting. FG cells were seeded on glass slides and incubated for 2 h at 22°C, then fixed in cold acetone for 10 min and incubated with 40 µl mouse polyclonal anti-27.8 kDa protein antiserum at a dilution of 1:200 in PBS for 60 min at 37°C in a moisture chamber. After washing 3 times with PBS, the cells were incubated for 60 min at 37°C in the dark with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Sigma) at a dilution of 1:200 in PBS. Finally, the cells were washed 3 times and mounted in 90% (v/v) glycerin in PBS for observation by fluorescence microscopy. Mouse pre-immune serum was used as negative control.

Cell membrane proteins were run on 12% SDS-PAGE gels and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked overnight at 4°C with 5% (w/v) skim milk in PBS and washed 3 times for 5 min each with PBS containing 0.05% Tween-20 (PBST), then incubated with mouse polyclonal anti-27.8 kDa protein antiserum for 1 h at 37°C. After 3 washes with PBST, the membrane was incubated with alkaline phosphatase (AP)-conjugated goat anti-mouse Ig (Sigma) diluted 1:4000 in PBS for 50 min at 37°C. Finally, color was developed with substrate solution containing nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate toluidinium (Sigma). The reaction was stopped by washing in distilled water to remove excess substrate when the positive bands were visualized. Mouse pre-immune serum was used as a negative control.

Blocking ELISA (B-ELISA) and virus infection inhibition. Wells of flat-bottom 96-well plates (Costar) were coated with the membrane proteins of FG cells (100 µg $well^{-1}$) in 100 µl of carbonate-bicarbonate buffer (0.05 M, pH 9.6), incubated overnight at 4°C, and then washed 3 times with PBST and blocked with 200 µl of 3% bovine serum albumin in PBS for 1 h at 37°C. The wells were washed as above and 100 µl of mouse polyclonal anti-27.8 kDa protein antiserum, diluted 1:100, 1:200, 1:400, 1:800 and 1:1000 in PBS, was added as the primary antibody. After incubation for 1 h at 37°C, the wells were washed again and 50 µl purified LCDV (15 µg $well^{-1}$) in PBS was added and incubated for 3 h at 22°C. Following further washings, 100 µl of rabbit anti-LCDV antiserum was added as the secondary antibody and incubated for 1 h at 37°C. The wells were washed and 100 µl of goat anti-rabbit Ig-alkaline phosphatase conjugate (Sigma) diluted 1:1000 in PB-Swas added as the third antibody and incubated for 1 h at 37°C. After the last washing, 100 µl of 0.1% (w/v) *p*-nitrophenyl phosphate (Sigma) in carbonate-bicarbonate buffer containing 0.5 mM $MgCl_2$ was added to each well and incubated for 30 min at room temperature in the dark. The reaction was stopped with 50 µl of 2 M NaOH per well and absorbance at 405 nm was measured using a plate-reader (Molecular Devices). Incubation with PBS instead of polyclonal anti-27.8 kDa protein antiserum was used as positive control, and PBS instead of LCDV was used as negative control. All reactions were performed in triplicate. The blocking rate was calculated as follows: $(OD_{Pos} - OD_{Test}) \times OD_{Pos}^{-1} \times 100\%$, where OD_{Pos} and OD_{Test} are the optical density (OD) values of the positive control and test treatments, respectively. Blocking rates ≥50% showed a positive (+) result (effective blocking to LCDV), 30–49% showed an equivocal (±) result, and <30% showed a negative (–) result (non-effective blocking to LCDV).

For the virus infection inhibition assay, FG cells were grown in a 96-well plate at 22°C until confluency, then washed gently with PBS and incubated for 4 h at 22°C with polyclonal anti-27.8 kDa protein antiserum at dilutions of 1:50, 1:100, 1:200 and 1:500 in PBS. After incubation, the cells were washed twice with PBS and then infected with LCDV at 4 $TCID_{50}$ at 22°C for 1 h. Following viral adsorption to the cells, unattached virus was removed by washing with PBS and 100 µl maintenance medium was added. The cultures were incubated for 5 d at 22°C and development of CPE in FG cells was monitored by phase-contrast microscopy. Pre-immune serum diluted 1:50 was used as a positive control and PBS instead of LCDV as a negative control. The experiment was performed in duplicate.

Mass spectrometry analysis and western blotting. The 27.8 kDa protein band was excised from gels, minced and allowed to dry before trypsin digestion. The dehydrated gels were reduced by incubation with 10 mM dithiothreitol (DTT) and 25 mM $(NH_4)_2CO_3$ at 56°C for 1 h, and then alkylated by incubation with 55 mM iodoacetamide and 25 mM $(NH_4)_2CO_3$ in the dark at room temperature for 45 min. Protein digestion was carried out in 25 mM $(NH_4)_2CO_3$ containing

0.05 µg ml^{-1} trypsin solution at 37°C overnight. The digested products were desalted by POROS R2 (Applied Biosystems) and then co-crystallized with a matrix of α-cyano-4-hydroxycinnamic acid spotted on the target wells. The dried matrices were subjected to Bruker Autoflex matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The mass spectrometer was operated at 19 kV accelerating voltage in the reflection mode and the *m*/*z* range was from 700 to 3500. All peptide mass fingerprints (PMF) were externally calibrated using standard peptide mixtures and internally calibrated using the masses of trypsin digestion products. The monoisotopic peptide masses obtained from MALDI-TOF-MS were analyzed by *m*/*z* software and interpreted using MASCOT (Matrix Science) against the National Center for Biotechnology Information non-redundant (NCBInr) database. The protein score was a measure of the statistical significance of a PMF match. The algorithm of protein score was described previously by Pappin et al. (1993).

Western blotting was carried out as described above. Cell membrane proteins were run on SDS-PAGE and transferred to PVDF membranes. After blocking, β-actin monoclonal antibody (AA128, Beyotime Biotech) was added as primary antibody and AP-conjugated goat anti-mouse Ig as secondary antibody.

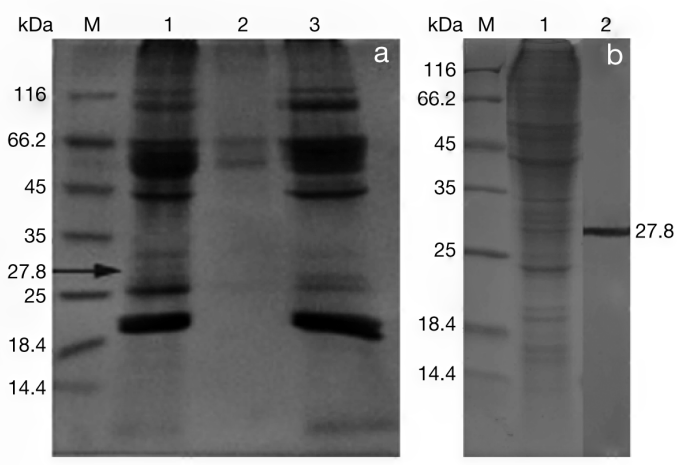

Fig. 1. Analysis of lymphocystis disease virus (LCDV) binding proteins. (a) Co-immunoprecipitation. Lane M: molecular mass marker; Lane 1: co-immunoprecipitation carried out with flounder gill (FG) cell membrane proteins showed LCDV bound specifically to a protein of 27.8 kDa; Lane 2: the first negative control carried out with FG cell membrane proteins in the absence of virus; Lane 3: the second negative control carried out with LCDV in the absence of FG cell membrane proteins. (b) SDS-PAGE profile of FG cell membrane proteins and purified 27.8kDa protein. Lane M: molecular mass marker; Lane 1: FG cell membrane proteins; Lane 2: purified 27.8 kDa protein

RESULTS

LCDV-binding protein in FG cells

The co-immunoprecipitation results showed that LCDV could bind to only one protein from the FG cell preparation with a molecular mass of 27.8 kDa (Fig. 1a, Lane 1), compared with 2 negative controls (Fig. 1a, Lanes 2 and 3). This result suggested that the 27.8 kDa protein was a binding protein that specifically interacted with LCDV. Therefore, the purified 27.8kDa protein (Fig. 1b, Lane 2) was used in subsequent experiments.

Immunofluorescence and western blotting reaction of polyclonal antibodies against the 27.8 kDa protein

Polyclonal antiserum against the 27.8 kDa protein, obtained after immunization of mice with the electro-eluted protein, could react with the FG cells as demonstrated by intense green fluorescence signal (Fig. 2a) in the immunofluorescence assay, whereas pre-immune serum yielded negative results (Fig. 2b). This result confirmed that the 27.8 kDa protein was a protein of FG cells.

Western blotting was carried out to determine whether the polyclonal antiserum against 27.8 kDa protein could recognize the respective protein from FG cells. The results indicated that the antisera were able to react strongly with the 27.8 kDa protein and weakly cross-react with the 42.7 kDa protein (Fig. 3a, Lane 1). No reaction was observed after incubation with pre-immune serum (Fig. 3a, Lane 2).

Blocking effect of the antiserum against the 27.8 kDa protein by B-ELISA

B-ELISA was used to determine the blocking effect of the mouse antiserum against 27.8 kDa protein. The results (Table 1) showed that the antiserum at dilutions of 1:100 and 1:200 produced a blocking rate to LCDV of ≥50% (+), the dilution lower than 1:800 had a blocking rate of <30% (–), and the antiserum diluted in 1:400 produced blocking rates of 30 to 49% (±), suggesting that the antiserum against the 27.8 kDa protein was able to partly block the interaction between LCDV and FG cell membrane proteins.

LCDV binding and inhibition of FG cell culture infection by antiserum against the 27.8 kDa protein

Following infection of FG cell cultures by LCDV in the presence of mouse pre-immune serum (at a dilu-

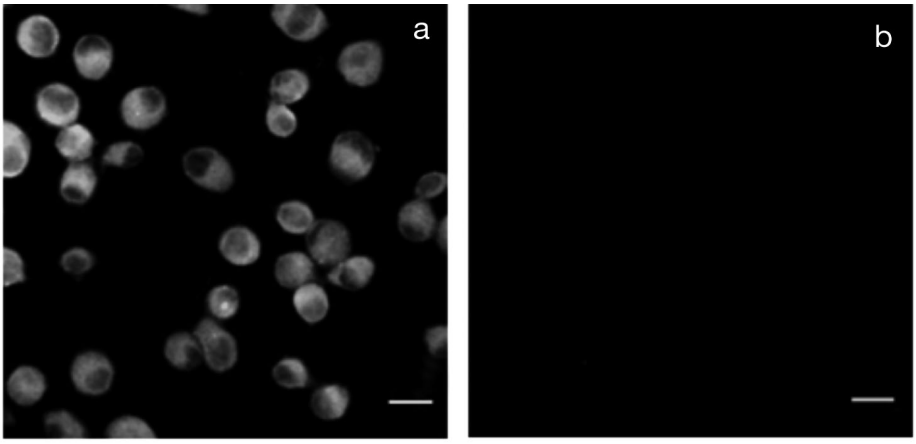

Fig. 2. Immunofluorescence assay. (a) Flounder gill cells using anti-27.8kDa protein antiserum as the primary antibody demonstrate intense green fluorescence (light grey). (b) Negative control using pre-immune serum resulted in no detectable signal. Scale bars = 5.0 µm

tion of 1:50), CPE was first observed in <25% of cells at Day 2 post-infection (p.i.), and then increased to >50% at Day 5 p.i. (Table 2). The CPE was characterized by cellular retraction, aggregation and loss of adherence to the substrate, followed by cellular disintegration. In contrast, in the presence of post-immune serum diluted at 1:100, CPE did not appear until Day 5 p.i., and it was inhibited by the post-immune serum in a concentration-dependent manner (Table 2, Fig. 4), which demonstrated that antiserum against the 27.8 kDa protein was able to inhibit the CPE induced by LCDV, indicating that the antiserum inhibited infection of the cells by LCDV.

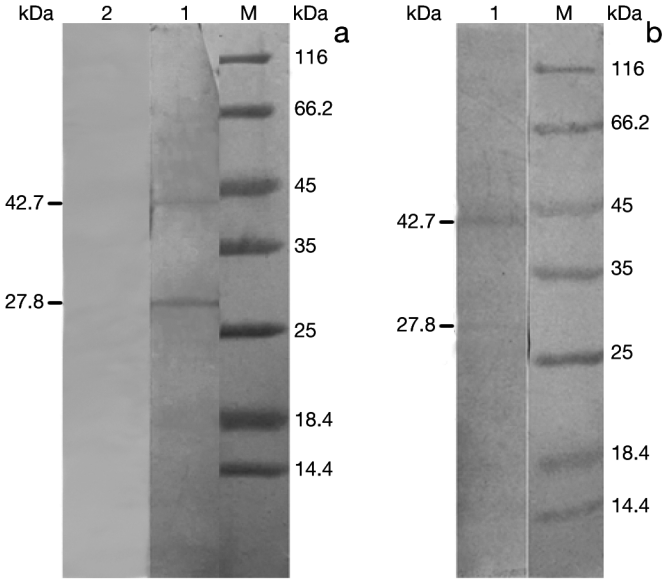

Fig. 3. Western blotting analysis of flounder gill cell membrane proteins with (a) anti-27.8 kDa protein antiserum and (b) anti-β-actin antibody. Lane M: molecular mass marker. (a) Lane 1: anti-27.8 kDa protein antiserum reacted with 27.8 and 42.7 kDa (weakly); Lane 2: negative control (pre-immune serum). (b) Lane 1: anti-β-actin antibody reacted strongly with 42.7 kDa protein and weakly with 27.8 kDa

Mass spectrometry analysis of the 27.8 kDa protein and western blotting

The 27.8kDa protein band was cut from the SDS-PAGE gel (Fig. 1b) and used for MALDI-TOF-MS analysis. As shown in Fig. 5, the PMF was analyzed using the MS-FIT system and involved a search of coincident proteins according to the MS data of bony vertebrates provided by NCBInr. The protein score greater than 73 was significant ($p < 0.05$) in this experiment. The most similar protein to 27.8 kDa protein was the β-actin protein of *Mus musculus* with a score of 123. A protein with the second highest score (108) was an unkown protein similar to cytoplasmic β-actin isoform 2 of *Macaca mulatta*.

Table 1. Antibody blocking effect analyzed by blocking ELISA. +: blocking rate ≥50%, showing positive result; ±: 30–49%, showing equivocal result; –: <30%, showing negative result. OD_{405}: optical density at 405 nm

Antibody dilution	OD_{405} absorbance	Blocking rate (%)
1:100	0.1473	73.9 (+)
1:200	0.2573	54.4 (+)
1:400	0.3474	38.4 (±)
1:800	0.4748	15.8 (–)
1:1000	0.5156	8.7 (–)
Positive	0.5639	
Negative	0.0855	

Table 2. *In vitro* lymphocystis disease virus infection inhibition assays by polyclonal anti-27.8 kDa binding protein from flounder gill cell antiserum. +++: 50–75% cells showing cytopathic effect (CPE); ++: 25–50% cells showing CPE; +: few cells showing CPE; –: no CPE

Mouse serum	Antibody dilution	Day post injection 1	2	3	4	5
Pre-immune	1:50	–	+	++	+++	+++
Post-immune	1:50	–	–	–	–	–
	1:100	–	–	–	–	+
	1:200	–	–	–	+	+
	1:500	–	–	+	+	++

The cell membrane proteins were identified by SDS-PAGE (Fig. 1b) and western blotting (Fig. 3b) using the β-actin antibody as the first antibody. The result showed that the β-actin antibody reacted strongly with the 42.7 kDa protein band and weakly with the 27.8 kDa protein band (Fig. 3b).

DISCUSSION

The entry of infectious virus into host cells is mediated by the initial interaction of a virus attachment protein (VAP) and cellular receptor molecules. The interaction of VAP and its cellular counterpart is known to contribute to viral host range, tissue tropism and pathogenesis. To our knowledge, this is the first study describing the putative cellular receptor from FG cells that mediates LCDV binding and entry.

Co-immunoprecipitation of proteins from cellular extracts is the most convincing evidence that 2 or more proteins physically interact with each other, and it was used to identify the LCDV-binding proteins of FG cells. Firstly, it was necessary to undertake a preliminary study to ensure that there was no cross-reaction between the anti-LCDV monoclonal antibody and FG cell membrane proteins by western blotting (data not shown). The subsequent results shown here identified one LCDV-binding protein, of 27.8 kDa, from FG cells.

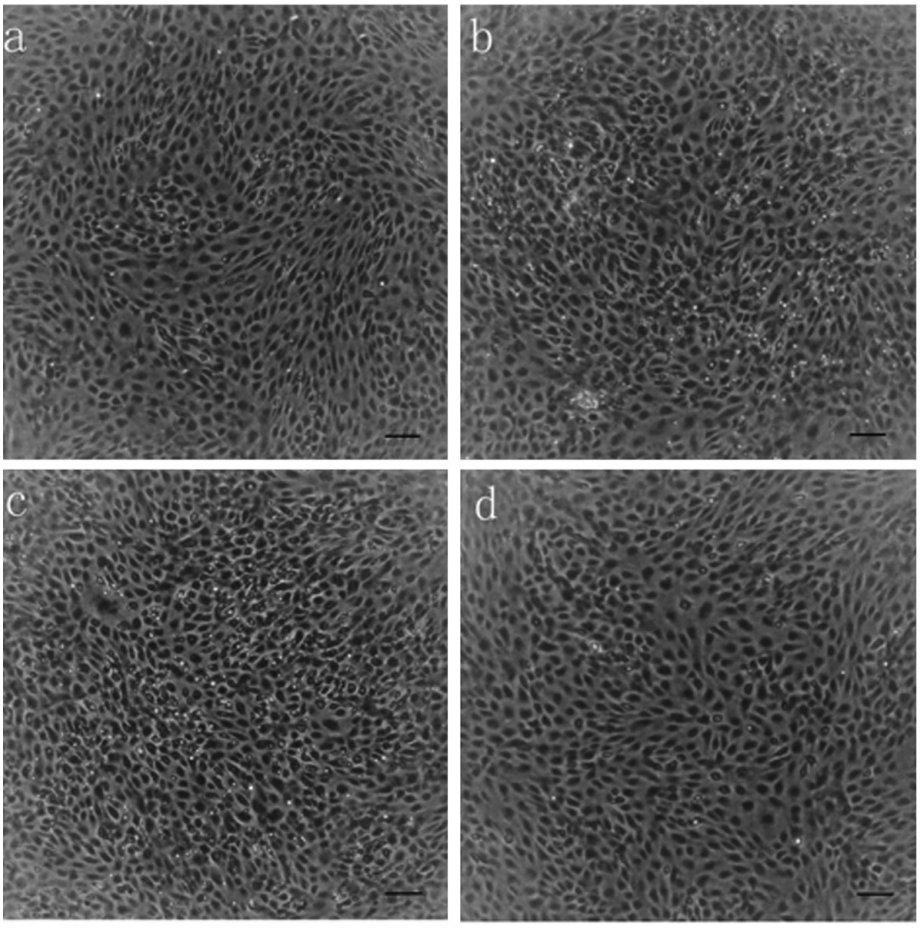

Fig. 4. *In vitro* lymphocystis disease virus (LCDV) infection of flounder gill (FG) cells showing inhibition by polyclonal anti-27.8 kDa protein antiserum on the fourth day of LCDV infection. (a) FG cells pre-incubated with post-immune sera diluted 1:100 before LCDV infection showing no obvious cytopathic effect (CPE). (b) A few of the cells pre-incubated with post-immune sera diluted 1:500 before LCDV infection showing CPE. (c) More than half of the cells pre-incubated with pre-immune sera diluted 1:50 before LCDV infection showing CPE. (d) FG cells without LCDV infection. Scale bars = 100 µm

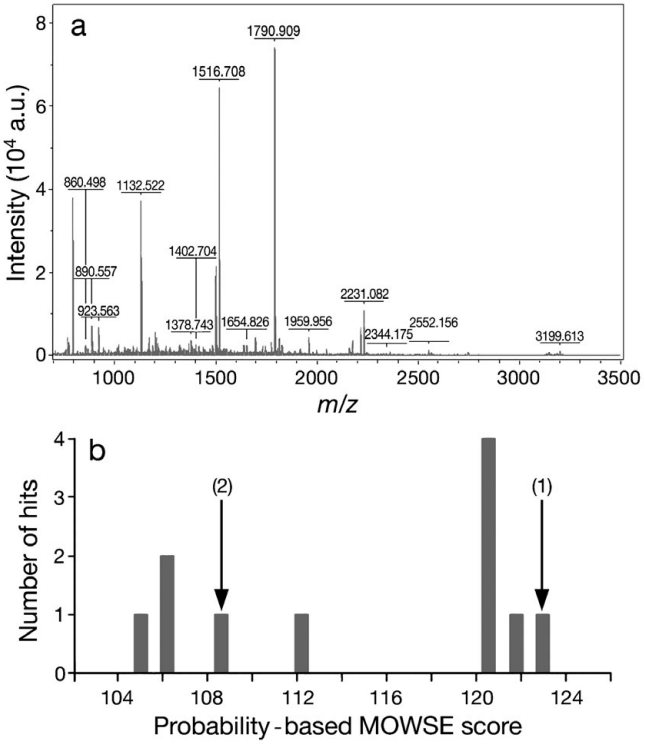

Fig. 5. Mass spectrographic analysis of 27.8 kDa protein. (a) Fingerprint of the 27.8 kDa protein. (b) Mass spectrographic analysis of the fingerprint of the 27.8 kDa protein using Mascot. Protein scores greater than 73 are significant ($p < 0.05$). Accession information is as follows. (1) gil49868; Mass: 39446; Score: 123; Description: β-actin (*Mus musculus*). (2) gil109119055; Mass: 28478; Score: 108; Description [PREDICTED]: similar to cytoplasmic β-actin isoform 2 (*Macaca mulatta*)

Interestingly, Diao et al. (2009) reported that the stomach, intestine and epidermis tissues of Japanese flounder shared a binding protein of LCDV with the same molecular weight of 27.8 kDa by VOPBA.

In the present study, blocking ELISA and virus infection inhibition assays similar to those used by Chu & Ng (2003) and Esterhuysen et al. (1995) were performed using the anti-27.8 kDa protein antiserum. The results showed that the antiserum could strongly inhibit the binding and entry of LCDV to FG cells, suggesting that the 27.8 kDa protein is a putative receptor for LCDV. Furthermore, western blotting demonstrated that the anti-27.8 kDa protein antiserum reacted strongly with the 27.8 kDa protein, as expected, and also weakly cross-reacted with a 42.7 kDa protein. The 42.7 kDa protein was not observed in the co-immunoprecipitation analysis. It is possible that the 2 proteins had some epitopes in common that were not associated with the LCDV binding.

Of interest, β-actin protein in different species has high homology as well as a molecular weight of ~42 kDa (Nakajima-Iijima et al. 1985). Xie & Yang (2005) reported that VP26 protein of white spot syndrome virus could bind to actin and became associated with the cytoskeleton of the host cell. In the present study, MS analysis indicated that the 27.8 kDa protein and β-actin had a strong association, and western blotting showed that the β-actin antibody reacted strongly with the 42.7 kDa protein band and weakly with the 27.8 kDa protein band of FG cells. This suggests that the 27.8 kDa protein might be a part of the β-actin protein or an unknown protein sharing epitopes with β-actin protein, as suggested by the cross-reactivity of the polyclonal anti-27.8 kDa protein antiserum with the 42.7 kDa protein band in western blotting.

Considering the cross-reactivity of the anti-27.8 kDa protein antiserum, the immunofluorescence assay could not demonstrate unequivocally the surface localization of 27.8 kDa protein on FG cells. Therefore, further studies such as immunogold electron microscopy using an anti-27.8 kDa monoclonal antibody are planned.

In conclusion, a 27.8 kDa protein in FG cells was found to bind LCDV using co-immunoprecipitation. Virus binding and infection were partly blocked by the anti-27.8 kDa protein antiserum, suggesting that the 27.8 kDa protein of FG cells played an important role in binding and infection of LCDV to FG cells. Moreover, MS analysis indicated that the 27.8 kDa protein and β-actin had a strong association. To confirm that this molecule is indeed the LCDV receptor, further work is needed, such as the nucleic acid sequence determination of the corresponding gene and amino acid sequence determination of the 27.8 kDa protein. Determination of the early interactions between viruses and molecules on the surface of susceptible cells will contribute to our understanding of viral infection and replication, pathogenesis and tissue tropism in hosts, and hence faciliate the development of anti-viral therapeutic agents.

Acknowledgements. This study was supported by the National Science Fund of China (grants 30771648 and 31072232) and by the National High Technology Development Program of China (863) (grant 2006AA100306) .

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Editorial responsibility: Mark Crane, Geelong, Victoria, Australia

Submitted: July 6, 2010; Accepted: November 16, 2010
Proofs received from author(s): February 20, 2011