Detection of Cell Membrane Proteins that Interact with Virulent Infectious Bursal Disease Virus

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ABSTRACT. To detect the molecules that interact with infectious bursal disease virus (IBDV), the chicken B lymphoblastoid cell line, LSCC-BK3, which is permissive for virulent IBDV infection was investigated. The sodium dodecyl sulfate-solubilized plasma membrane fraction from the cells was subjected to a virus overlay protein binding assay. The IBDV specifically bound to proteins in LSCC-BK3 plasma membranes with molecular weights of 70, 82 and 110 kDa. This is the first report to demonstrate cellular molecules that interact with virulent IBDV.

KEY WORDS: cellular molecule, infectious bursal disease virus, virus overlay protein binding assay.

NOTE Virology

Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by IBD virus (IBDV). IBDV, a member of the Birnaviridae family, in which the genome consists of two segments of double-stranded RNA [4], is a naked icosahedral particle with a diameter of 60 nm [8, 15]. Although the disease caused some mortality in 3 to 6-week-old birds, the major problem associated with IBD was that of immunosuppression following infection of younger chicks [1, 9]. The main target organ of IBDV is the bursa of Fabricius of the chicken [11]. Surface immunoglobulin M-bearing B lymphocytes are thought to be the target cells for IBDV infection [6, 7, 14]. However, the component of the target cells that is responsible for IBDV infection has not been properly defined.

The virus overlay protein binding assay (VOPBA) has been shown to be useful for determining the size and numbers of molecules that interact with a virus [2, 3, 10, 18–20]. In the present study, we attempted to detect the molecule(s) that interact with IBDV particles in the course of the virus infection by VOPBA.

A highly virulent OKYM strain of serotype I IBDV [24] was used and propagated in LSCC-BK3 cells as described [22]. LSCC-BK3 cells have been reported to be highly permissive for virulent IBDV infection [22]. IBDV was purified and biotinylated as previously described [17] for VOPBA. The purity of the virus particles was then confirmed with Coomassie brilliant blue R 250 staining, following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [12].

VOPBA was carried out according to the method as described elsewhere [10], with slight modifications. Plasma membranes of LSCC-BK3 [5] cells were prepared essentially as previously described [21]. The protein sample was separated by SDS-PAGE and then transferred electrophoretically onto a polyvinylidene difluoride membrane with a semi-dry electroblotter (Sartorius Co., Ltd., Englewood, NY, U.S.A.). To block the transferred membrane proteins, the membrane was left overnight at 4°C in 3% skim milk diluted in phosphate buffered saline (PBS). The membrane was washed with PBS, pretreated with biotinylated IBDV at room temperature for 1 hr, washed again with PBS and treated with HRPO-conjugated streptavidin (Zymed Laboratories, Inc., San Francisco, CA, U.S.A.). After another wash with PBS, the blot was developed with 3,3′-diaminobenzidine substrate solution as described [23]. The molecular weights of the detected proteins were then approximated using a Kaleidoscope prestained standard (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). To ascertain the specificities of the virus binding, a virus-binding inhibition assay was performed. A sample of 0.4 µg/ml of biotinylated IBDV was incubated with ascites fluid containing IBDV-neutralizing monoclonal antibody (MAb) GI-13 [23] diluted 500, 5,000 and 50,000-fold in 0.1% skim milk PBS for 30 min at room temperature. The VOPBA was then performed on the plasma membrane of LSCC-BK3 cells as described above and the binding inhibition was evaluated.

The biotinylated IBDV bound to proteins of 70, 82 and 110 kilodalton (kDa) in LSCC-BK3 cells which were not shown in the negative control in VOPBA (Fig. 1). The specificity of the reaction was confirmed by the virus binding inhibition assay. Pretreatment of biotinylated IBDV with serially diluted ascites fluid containing IBDV-neutralizing MAb GI-13 resulted in reduction of discernible bands of 70-, 82- and 110-kDa in a dose-dependent manner (Fig. 2).

In a previous study, Nieper and Müller [16] reported that IBDV bound to proteins with molecular masses of 40 and 46 kDa, exposed on the surface of chicken embryo fibroblast (CEF) and lymphoid cells by VOPBA. They indicated that these proteins might represent the molecules responsible for IBDV binding in both CEF and lymphoid cells. However,
in the present study, the IBDV did not bind to proteins with molecular masses of 40 and 46 kDa. This may be caused by the differences of the viruses used in VOPBA. We used a highly virulent strain of IBDV that propagates in LSCC-BK3 cells but not in CEF cells, whereas Nieper and Müller used an attenuated strain of IBDV that is propagated in CEF cells. Generally, field isolates (virulent strains) of IBDV propagate in chicken lymphocytes but not in CEF cells [13]. However, with serial passage in embryonating eggs and CEF, the virus becomes progressively adapted to growth in CEF cells [24]. The nonadapted and adapted IBDV may recognize different molecules for the virus infection.

Identification of the cellular molecules that interact with the virulent nonadapted IBDV is important for understanding the pathogenesis and virulence of IBDV. Although the function of the detected molecules was unclear in the course of the virus infection, these molecules may be responsible for the virus infection. This is the first report to demonstrate cellular molecules that interact with virulent IBDV.

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