African Journal of Biotechnology Vol. 7 (25), pp. 4832-4835, 29 December, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Review

The cellular receptors for infectious bursal disease virus

L. Q. Zhu [1], S. L. Wu[1], G. P. Zhang[2] and G. Q Zhu[1]*

[1]College of Veterinary Medicine, Yangzhou University, Yangzhou, 225009, China.
[2]Henan Provincial Key Laboratory of Animal Immunology, Henan Academy of Agricultural Science, Zhengzhou 450002, China.

Accepted 9 December, 2008

Virus receptors are simplistically defined as cell surface molecules that mediate binding (attachment, adsorption) and/or trigger membrane fusion or entry through other processes. Infectious bursal disease virus (IBDV) entry into host cells occurs by recognition of specific cellular receptor(s) with viral envelope glycoprotein, which comprises the initial and key step of infection. Infection can be inhibited by blockage of the process. So the interest in receptors has been stimulated in large part by the potential in the application of developing substances that show directed blocking activity. While for the purpose one should know which host cell and viral molecules are involved in the reciprocal recognition and interaction leading to the virus entry into the cell. Here, the review presents the currently available knowledge regarding the receptors or molecules that interact with IBDV.

Key words: IBDV, SIgM, cellular receptor, chicken heat shock protein 90α.

INTRODUTION

Infectious bursal disease virus (IBDV), a member of the genus *Avibirnavirus* of the family *Birnaviridae* is the causative agent of infectious bursal disease (Giambrone et al.,1977), a highly contagious immunosuppressive disease which causes substantial economic losses in commercial broiler production worldwide (Balamurugan and Kataria, 2006; Thierry and Van Den, 2000). Turkeys, ducks, and ostriches are susceptible to IBDV infection but resistant to clinical disease (Lukert and Saif, 1997; Oladele et al., 2008).

Two serotypes of IBDV (1 and 2) are described, distinguished by cross-virus neutralization test (Jackwood et al., 1985). IBDV strains of serotype 1 are pathogenic only in chickens (Oladele et al., 2008), and further classified as classical virulent IBDV (cvIBDV), very virulent IBDV (vvIBDV), antigenic variant IBDV (avIBDV) and attenuated IBDV (atIBDV) (Den Berg et al., 2004). Whereas, strains of serotype 2 are naturally avirulent for chickens (Cummings et al., 1986; Ismail et al., 1988).

*Corresponding author. E-mail: yzgqzhu@yzu.edu.cn, yzgqzhu@hotmail.com. Tel: (0086)-514-87972590. Fax: (0086)-514-87311374.

IBDV field isolates mainly infect and destroy actively dividing IgM-bearing B cells in the bursa of Fabricius (BF) and other locations (Hirai et al., 1981; Rodenberg and Sharma, 1994). While recent data show that the virus also infects and replicates in macrophages (Khatri et al., 2005; Kim et al., 1998; Palmquist et al., 2006; Khatri et al., 2008). Additionally, IBDV can also replicate in chick embryo fibroblast cells (CEF) (Yamaguchi et al.,1996), Vero cells (Kwon and Kim, 2004), DF-1 cells (a spontaneously immortalized cell line derived from primary CEF) (Lin et al., 2007), LSCC-BK3 cells (a bursal-derived lymphoblastoid cell line) (Ogawa et al.,1998) and DT40 cells (an avian leukosis virus-induced chicken B cell line) (Terasaki et al., 2008). VvIBDVs cannot be propagated directly in tissue cultures but the virus can adapt to the tissue cultures by serial blind passages and become attenuated (Muller et al., 1986).

Glycoprotein VP2 trimers from IBDV constitute the external surface of the mature virus capsid, containing the antigenic regions responsible for elicitation of neutralizing antibodies (Birghan et al., 2000; Fahey et al., 1989). Based on the atomic structure of the viral particles (Coulibaly et al., 2005) the external domain of the VP2 trimers exists as ‘protrusions’ on the capsid surface and is believed to be responsible for receptor binding. That

glycoprotein VP2, responsible for the recognition of corresponding receptor, has been certificated in Vero cells on the molecular level (Yip et al., 2007).

IBDV cell tropism may be correlated with the receptors or coreceptors required in different cells, indicating the complexity of studying IBDV receptor(s). Here we mainly review the knowledge on receptors for IBDV attachment to B lymphocytes, CEF, and Vero cells.

THE RELATION BETWEEN SIg M AND THE CANDIDATE RECEPTOR ON B LYMPHOCYTES

Immature surface immunoglobulin M (SIgM) - bearing B lymphocytes is the target cell for infection. Hirai and Calnek (1979) investigated the susceptibility of chicken lymphocytes *in vitro* to a strain of vvIBDV by using immunofluorescence assay. They found that all of the thymus-derived lymphoblastoid cell (T-cell) lines were refractory to virus exposure. However, a bursal-derived lymphoblastoid cell (B-cell) line from an avian leukosis virus-induced tumor was highly susceptible. The virus also replicated in a small percentage of normal lymphocytes prepared from lymphoid tissues and peripheral blood of chickens. Pretreatment of the lymphocytes, with heat-inactivated serum either against B cell or against fowl immunoglobulin M before IBDV inoculation can block infection; while no inhibition occurred with the serum against T cell, fowl immunoglobulin G and immunoglobulin A. This suggests that SIgM-bearing B lymphocytes were the target cells for infection, and the IBDV receptor was specifically present on the SIgM-bearing B lymphocyte. It is possible that the SIgM molecule itself is the candidate receptor for the virulent IBDV.

Additionally, Ogawa (1998) studied the binding of biotinylated IBDV of highly virulent OKYM strain to various cells using flow cytometry assay. They found that vvIBDV bound to more than 90% of LSCC-BK3 cells, and also 94% of BF lymphocytes. This result confirmed that B lymphocytes were the target cells for virulent IBDV infection (Hirai et al., 1979). Using two-color fluorescence assay, they also examined the relationship between virus binding and SIgM expression. The data showed that IBDV binding was observed in 89-100% of total SIgM bearing lymphocytes but IBDV binding was also observed in the remaining 2 ~ 21% of SIgM-negative cells. Interestingly, they found that the binding of the virus to SIgM-bearing B lymphoblastoid cells was not inhibited by the antibody against IgM, which is a contrast to the observation of Hirai and Calnek (1979). However, it is certain that a virulent IBDV infection was observed only in cell lines expressing SIgM. We speculate that for IBDV infection the first step, binding to the target cells is not mediated by a single molecule and all the associated molecules including SIgM are co-expressed to form polymers or to sustain a certain conformation which is crucial to mediate infection. We also speculate that SIgM is necessary as signal molecule for the processes that occur after virus attachment, such as penetration, uncoating and replication. Obviously, argument exists as to if SIgM acts as IBDV receptor. We believe that molecular biotechnology is the one of the best methods to resolve the matter in the latter confirmed experiments.

IBDV OF VIRULENT STRAINS AND CEF CELL-ADAPTED STRAINS MAY RECOGNIZE DIFFERENT MOLECULES FOR THE VIRUS INFECTION

Generally, field isolates (virulent strains) of IBDV can directly replicate in chicken lymphocytes but not in CEF cells (Lukert et al., 1974). With successive blind passages in CEF (Yamaguchi et al., 1996) or Vero cells (Kwon and Kim, 2004), the virus becomes progressively adapted to growth in both cells. Adaptation of wild-type IBDV, however, always seems to correlate with virulence attenuation (Yehuda et al., 1999). With binding studies Nieper and Mülle (1996) found that strains of both IBDV serotypes 1 and 2 can all bind to lymphoid B cells. With the saturation binding studies and competition experiments, they also found that CEF had receptors common to both serotypes as well as specific sites for each strain. Receptor sites common to both serotypes were also present on lymphoid cells but an additional serotype-specific site was only demonstrated for the apathogenic serotype 2 strains. According to the results we speculated that the presence of specific binding sites could not completely determine the restriction of certain IBDV replication to a certain target cells. And except the binding receptors, certain molecules existing on various cells may be helpful factor in the interaction between the receptors and virus for the determination of cell tropism.

Virus overlay protein blotting assay (VOPBA) is a useful method to study the interaction between virus and receptor *in vitro* (Kim et al., 2006). Using the method Nieper et al. (1996) and Setiyono et al. (2001) detected the molecules showing properties of binding to IBDV particles , which were expressed on CEF cells and LSCC-BK3, respectively. Nieper found that proteins with molecular mass of 40 and 46 kDa expressed in both CEF cells and chicken B lymphocytes could bind to the viral particles propagated in CEF cells. While, Setiyono used a highly virulent strain of IBDV in VOPBA assay and found that the viral particles bind to proteins of 70, 80 and 110 KDa expressed in LSCC-BK3 cells. The genes encoding the protein of 40, 46, 70, 80 and 110 kDa have not so far been cloned. To study the receptors on DF-1 cells, Lin et al. (2007) developed the monoclonal antibody against recombinant VP2. With the monoclonal antibody and purified recombinant VP2, by affinity chromatography approach and mass spectrometry analysis, chicken heat shock protein (cHsp90α) was identified as a functional part of the receptor complex for IBDV infection in DF-1 cells. The cHsp90α interacting with IBDV was also confirmed by VOPBA assays *in vitro*. This is the first molecule identified as the putative cellular receptor com-

plex of IBDV.

IBDV RECEPTORS ON VERO CELLS

Adapted strains of IBDV also infect Vero cells so there must be viral binding sites on the surface of these cells. Yip et al. (2007) studied the binding properties of different VP2 to Vero cells. For that study, both VP2s of an attenuated strain (D78) and a very virulent strain (HK46) of IBDV were expressed in AD293 cells (a mammalian cell line), generating RAVP2 and RVVP2, respectively. In flow cytometry assy, both RAVP2 and RVVP2 are demonstrated to bind with Vero cells while these bindings are blocked by D78 viral particles, implying both vvIBDVs and attenuated IBDVs bind to Vero cells through the same receptor(s). Since vvIBDVs cannot be propagated directly in tissue cultures, the specific binding between RVVP2 and Vero cells confirmed the speculation that except the binding receptors some molecules or co-receptors lacking on the surface of Vero cells may be another factor to determine vvIBDV cell infectivity.

THE RECEPTOR IN LSCC-BK3 CELLS WAS IDENTIFIED TO BE AN N-GLYCOSYLATED PROTEIN

Viruses are able to utilize a wide variety of cell-surface molecules as their receptors, including proteins, carbohydrates, glycolipids and so on. For further characterization of the IBDV receptor, Ogawa et al. (1998) treated target cells with a variety of enzymes and reagents to modify individual components of the plasma membrane. Treatment of cells with proteases reduces the binding of IBDV to the target cells, whereas, treatment with phospholipase did not noticeably affect the binding. These results suggested that the IBDV attachment molecule is at least partially composed of protein. Incubation of cells with two N-glycosylation inhibitors, tunicamycin and swainsonine, reduced the percentage of the cells that bound the virus. Tunicamycin also reduced the number of virus-infected cells. Tunicamycin inhibits the first step in N-glycosylation, but it simultaneously affects protein synthesis and glycosylation of glycolipid. On the other hand, swainsonine, an N-glycosylation processing inhibitor, has no effect on protein synthesis or glycosylation of glycolipid. Therefore, this confirmed that inhibition of N-glycosylation directly reduced the binding of IBDV. The results strongly suggest that the cellular binding site for IBVD is composed of an N-glycosylated protein.

CONCLUSIONS

IBDV is an aechaeo-virus which emerged in 1957, and was formally documented in 1962. Progress has been made mainly in characterizing the binding properties of viral particles to the target cell surface molecules. Compared with other virus, for example, human immunodeficiency virus, avian influenza virus, the knowledge of virus receptor(s) or coreceptor(s) in IBDV is very poor. Encouragingly, cHsp90α has been identified as a component of the putative cellular receptor complex of IBDV in DF-1 cell, but the other components of the receptor complex are still unknown. And the molecules allowing competent infection in B lymphocytes, chicken macrophage, CEF and other permissive cell lines are still needed for further investigation. It is certificated that CEF, B lymphocytes and the other permissive cell have the same binding site for both vvIBDV and attenuated IBDV, while certain IBDV can only replicate in a specific kind of cells. Apparently, more than one molecule is involved in the process of virus attachment. But conclusive evidence is lacking to elucidate the mechanism. It is important to note that except the binding sites the other molecules should be attached great importance for antiviral substance design. The study of all the molecules is complicated. We believe that the method of developing the monoclonal antibodies that can inhibit virus infection immunized with the whole target cells is validate for the investigation of the molecules involved in the cause of virus entry.

ACKNOWLEDGEMENT

This study was supported by grants from the Chinese National Science Foundation Grant (No.30571374 and No. 30771603).

REFERENCES

Balamurugan V, Kataria JM (2006). Economically important no-oncogenic immunosuppressive viral diseases of chicken--current status. Vet. Res. Commun. 30(5): 541-566.

Birghan C, Mundt E, Gorbalenya AE (2000). Anon-canonical lon proteinase lacking the ATPase domain employs the ser-Lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. EMBO J. 19: 114-123.

Cummings TS, Broussard CT, Page RK, Thayer SG, Lukert PD (1986). Infectious bursal disease virus in turkeys. Vet. Bull. 56: 757-762.

Coulibaly F, Chevalier C, Gutsche I, Pous J, Navaza J, Bressanelli S, Delmas B, Rey FA (2005). The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell. 120: 761-772.

Den Berg TP, Morales D, Eterradossi N, Rivallan G, Toquin D, Raue R, Zierenberg K, Zhang MF, Zhu YP, Wang CQ, Zheng HJ, Wang X (2004). Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. Avian Pathol. 33: 470-476.

Fahey KJ, Erny K, Crooks J (1989). A conformational immunogen on VP-2 of infectious bursal disease virus that induces virusneutralizing antibodies that passively protect chickens. J. Gen. Virol. 70: 1473-1481.

Giambrone JJ, Ewert DL, Eidson CS (1977). Effect of infectious bursal disease virus on the immunological responsiveness of the chicken. Poult. Sci. 56: 1591-1594.

Hirai K, Calnek BW (1979). In vitro replication of infectious bursal disease virus in established lymphoid cell lines and chicken B lymphocytes. Infect. Immunol. 25(3): 964-970.

Hirai K, Funakoshi T, Nakai T, Shimakura S (1981). Sequential changes

in the number of surface immunoglobulin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. Avian Dis. 25(2): 484-96.
Ismail NM, Saif YM, Moorhead PD (1988). Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. Avian Dis. 32: 757-759.
Jackwood DJ, Saif YM, Moorhead PD (1985). Immunogenicity and antigenicity of infectious bursal disease virus serotypes I and II in chickens. Avian Dis. 29: 1184-1194.
Kim JK, Fahad AM, Shanmukhappa K, Kapil S (2006). Defining the cellular target(s) of porcine reproductive and respiratory syndrome virus blocking monoclonal antibody 7G10. J. virol. 80 (2): 689-696.
Khatri M, Palmquist JM, Cha RM, Sharma JM (2005). Infection and activation of bursal macrophages by virulent infectious bursal disease virus. Virus Res. 113(1): 44-50.
Kim IJ, Karaca K, Pertile TL, Erickson SA, Sharma JM (1998). Enhanced expression of cytokine genes in spleen macrophages during acute infection with infectious bursal disease virus in chickens. Vet. Immunol. Immunopathol. 27,61(2-4): 331-41.
Kwon HM, Kim SJ (2004). Sequence analysis of the variable VP2 gene of infectious bursal disease viruses passaged in Vero cells. Virus Genes. 28: 285-291.
Khatri M, Sharma JM (2008). IFN-γ upregulation and protection by macrophage-adapted infectious bursal disease virus. Vaccine. 26: 4740-4746.
Lukert PD, Davis RB (1974). Infectious bursal disease virus: growth and characterization in cell cultures. Avian Dis. 18: 243-250.
Lukert PD, Saif YM (1997). "Infectious bursal disease virus." In Diseases of Poultry, pp. 721-738. Edited by Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM, Ames: Iowa State University Press.
Lin TW, Lo CW, Lai SY, Fan RJ, Lo CJ, Chou YM, Thiruvengadam R, Wang AH, Wang MY (2007). Chicken heat shock protein 90 is a component of the putative cellular receptor complex of infectious bursal disease virus. J. Virol. 81(16): 8730-41.
Muller H, Lange H, Becht H (1986). Formation, characterization and interfering capacity of a small plaque mutant and of incomplete viral particles of infectious bursal disease virus. Virus Res. 4: 297-309.
Ogawa M, Yamaguchi T, Setiyono A, Ho T, Matsuda H, Furusawa S, Fukushi H, Hirai K (1998). Some characteristics of a cellular receptor for virulent infectious bursal disease virus by using flow cytometry. Arch Virol. 143: 2327-2341.
Nieper H, Müller H (1996). Susceptibility of chicken lymphoid cells to infectious bursal disease virus does not correlate with the presence of specific binding sites. J. Gen. Virol. 77(1): 229-1 237.
Oladele OA, Adene DF, Obi TU, Nottidge HO (2008). Comparative susceptibility of chickens, turkeys and ducks to infectious bursal disease virus using immunohistochemistry. Vet. Res. Commun. DOI 10.1007/s11259-008-9078-2.
Palmquist JM, Khatri M, Cha RM, Goddeeris BM, Walcheck B, Sharma JM (2006). In vivo activation of chickenmacrophages by infectious bursal disease virus. Viral Immunol. 19(2): 305-15.
Rodenberg J, Sharma JM, Belzer SW, Nordgren RM, Naqi S (1994).Flow cytometric analysis of B cell and T cell subpopulations in specific-pathogen-free chickens infected with infectious bursal disease virus. Avian Dis. 38(1): 16-21.
Setiyono A, Hayashi T, Yamaguchi T, Fukushi H, Hirai K (2001). Detection of cell membrane proteins that interact with virulent infectious bursal disease virus. J. Vet. Med. Sci. 63: 219-21.
Terasaki K, Hirayama H, Kasanga CJ, Maw MT, Ohya K, Yamaguchi T, Fukushi H (2008). Chicken B Lymphoma DT40 Cells as a Useful Tool for *in vitro* Analysis of Pathogenic Infectious Bursal Disease Virus. J. Vet. Med. Sci. 70(4): 407-410.
Thierry P, Van Den Berg (2000). Acute infectious bursal disease in poultry: a review. Avian Pathol. 29: 175-194.
Yip CW, Yeung YS, Ma CM, Lam PY, Hon CC, Zeng F, Leung FC (2007). Demonstration of receptor binding properties of VP2 of very virulent strain infectious bursal disease virus on Vero cells. Virus Res. 123(1): 50-6.
Yamaguchi T, Kondo T, Inoshima Y, OgawaM, Miyoshi M, Yanai T, Masegi T, Fukushi H, Hirai K (1996). In vitro attenuation of highly virulent infectious bursal disease virus: Some characteristics of attenuated strains. Avian Dis. 40: 501-509.
Yehuda H, Pitcovski J, Michael A, Gutter B, Goldway M (1999). Viral protein 1 sequence analysis of three infectious bursal disease virus strains: a very virulent virus, its attenuated form, and an attenuated vaccine. Avian Dis. 43(1): 55-64.