Generation of VP5 deficient mutant of infectious bursal disease virus strain HZ2

LI Long¹,², WEI Yongwei¹, HUANG Yaowei¹* & YU Lian¹

¹. Institute of Preventive Veterinary Medicine, Zhejiang University, Hangzhou 310029, China; ². Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, China

Correspondence should be addressed to Yu Lian (email: yulian@zju.edu.cn)

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Abstract Infectious bursal disease virus (IBDV) is a bi-segmented, dsRNA virus of the Birnaviridae family. The nonstructural protein VP5 has been reported to be associated with virus-induced cell apoptosis and pathogenicity, but its role in viral replication has not been unequivocally identified. Based on a PCR introduced mutagenesis strategy, the 33 bp of 96–129 bp located between ORF A1 and ORF A2 of genomic segment A of IBDV strain HZ2 were deleted, and an NheI (GcTaGc) site was inserted at 96–102 bp simultaneously. The mutated segment A was ligated into pCI, resulting in pCI-ANhe3. A chimeric and deficient IBDV strain, named strain ANhe3, was recovered from chicken embryo fibroblast (CEF) cells by co-transfection with pCI-ANhe3 and pCI-mB, derived from the genomic segment B strain HZ2. The indirect fluorescent assay identified that strain ANhe3 could replicate on CEF cells without expression of VP5. Further examination showed that the pathogenesis of strain ANhe3 replicating on SPF chicken embryos was attenuated compared to strain HZ2. This paper provides a new rapid rescue strategy for gene-deleted virus. This strategy lays a basis for gene-deleted vaccine of IBDV.

Keywords: infectious bursal disease virus, IBDV, infectious clones, VP5, gene-deleted.

Infectious bursal disease virus (IBDV) is one of the most significant viral pathogens in chickens¹¹. It causes high mortality in young chickens and establishes an immunosuppression state by destroying the precursors of B lymphocytes in the bursa of Fabricius and the post-bursal B lymphocytes, leading to vaccination failure against other pathogens. IBDV is a member of the Birnaviridae family and has two double-stranded RNA segments, A and B. Segment B (~2.9 kb) encodes the viral RNA dependent RNA polymerase VP1²,³. The larger segment A (~3.3 kb) contains two partially overlapping open reading frames (ORFs). The larger ORF encodes a 110 kD precursor polyprotein (VP2/4/3) that can be post-translationally cleaved into three mature proteins: VP2 (40 kD), VP4 (28 kD) and VP3 (32 kD), and the cleavage sites of the polyprotein have been determined. The proteins VP2 and VP3 form the viral capsid, while VP4 is the viral protease. VP2 is the major protective immunogen of the virus and contains the antigenic determinants responsible for induction of neutralizing antibodies. VP3 contains the group-specific antigens⁴,⁵. The smaller ORF in the upstream of segment A encodes the nonstructural protein VP5 (17 kD). Few reports are available on the function of this protein⁶–⁹.

The VP5 of IBDV is highly basic, cysteine-rich and conserved among all serotype I strains of IBDV. It has been shown that VP5 is expressed in IBDV-infected cells but it is not incorporated into the virion. VP5 protein was shown to accumulate within the cell plasma membrane and induce cell apoptosis and lysis⁷–⁹. VP5-knockout IBDV mutant showed that VP5 is dispensable for virus replication, but related with virus pathogenicity⁸,⁹, suggesting that VP5 plays a key role in IBDV pathogenesis and dissemination. VP5 has attracted attention as a potential target for the development of new control strategies and vaccines of IBDV. In this paper, VP5 deficient IBDV strains with the 5′ terminus deleted was recovered in the chicken embryo fibroblast (CEF) cells after co-transfection with mutated cDNA of segment A and B, by a plasmid-based infectious cDNA system¹⁰.

Plasmids pTAK/HZ2, pCI-AKHZ2 and pCI-mB (from cell attenuated IBDV strain HZ2) have been described previously¹¹. Plasmid pTAK/HZ2 was first digested with NdeI into two bands and the longer fragment (about 3.7 kb) was purified for self-ligation, resulting in a new plasmid pGEM-T-AN which contains EcoRI/NdeI fragment of the segment A. The PCR-introduced deletion of VP5 gene on pGEM-T-AN was performed with MutanBEST Kit (TaKaRa) using...
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Fig. 1. Construction of full-length cDNA clones for IBDV recovery. The map of the genomic organization of IBDV segments A and B is shown. The coding sequences are marked by open boxes. Restriction enzymes and their cleavage sites used for cloning are indicated. The mutated sites of VP5 and names of the resulting plasmids are shown. Full-length cDNAs were constructed under the control of the cytomegalovirus (CMV) immediate-early enhancer/promoter in plasmid pCI. All infectious clones generated viable virus as marked by “+”.

The linearized PCR products were re-circled (pGEM-T-The3). After sequencing for confirmation, the mutated EcoR I/Nde I fragments of the above plasmids were further released and ligated with Nde I/Kpn I fragments (about 2.6 kb) of segment A from pCI-AKHZ2. The resulting EcoR I/Kpn I fragments (about 3.3 kb) were inserted into pCI digested with EcoR I/Kpn I, to give infectious clone pCI-ANhe3 (Fig. 1).

The CEF cells were isolated from 11-d SPF chicken embryos and cultured overnight to 80%–90% confluence in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS). After being washed with 0.01 mol/L phosphate buffered saline (PBS, pH 7.4), the cells were co-transfected respectively with different plasmid (High-purity plasmid miniprep system, Invitrogen) combinations (pCI-AKHZ2/pCI-mB and pCI-ANhe3/pCI-mB) accompanied with Lipofectamine reagent 2000 (Invitrogen). The transformed cells were further cultured and the cell pathogenic effect (CPE) appeared on all plasmids and Lipofectamine transformed cells 24 h after transfection. All cells were harvested, frozen, thawed and centrifuged at 72 h post-transfection. After filtration through a 0.22-mm-pore-size filter, the supernatants were used to infect fresh cells. This process was repeated five times to increase virus titer. It was found that the CPE appeared on all transformed cells and lasted throughout the infection process (Fig. 2(a)). However, the CPE disappeared in Lipofectamine transfected control in the infection process (Fig. 2(b)). The IBDV-like virions were also observed in the thin section of all plasmid transfected cells by electronic microscopy (Fig. 3).

Indirect fluorescence assay (IFA) was used to detect the viral proteins expression. At 36 h post-infection, cell monolayers were washed twice in PBS and fixed.

Fig. 2. Cell pathogenic effect on chicken embryo fibroblast (CEF) cells infected with recovered viruses (a). (b) Negative control. 100×.