Exchange of the VP5 of Infectious Bursal Disease Virus in a Serotype I Strain with that of a Serotype II Strain Reduced the Viral Replication and Cytotoxicity

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Infectious bursal disease virus (IBDV), belonging to *Avibirnavirus* genus in the *Birnaviridae* family, consists of two segments of double-strand RNA. There are two distinct serotypes of IBDV, the pathogenic serotype I and the non-pathogenic serotype II. Comparison of the deduced amino acid sequences of a panel of VP5 genes retrieved from GenBank revealed a high identity among strains within the serotype I or serotype II group but a low identity between strains across two serotypes. In this study, we rescued two mosaic viruses, rGtGxVP5 and rGt2382VP5 by exchanging the VP5 gene of a cell culture-adapted serotype I Gt strain with its counterpart of the very virulent IBDV Gx strain, or a non-pathogenic 23/82 strain of the serotype II. In comparison to the parental strain rGt virus, the rGtGxVP5 showed the similar viral replication, cytotoxicity and the ability of inducing apoptosis; however, the other mosaic virus rGt2382VP5 had a lower titer and a reduced cytotoxicity. Although exchange of VP5 within serotype I group did not alter the viral replication and cytotoxicity of Gt strain, exchange of VP5 in the serotype I with that of a serotype II reduced the viral replication and cytotoxicity on chicken embryo fibroblast (CEF) cells. Therefore, the VP5 of serotype II may be one of the factors responsible for the distinct pathogenic features of two serotypes.

***Keywords*:** infectious bursal disease virus, VP5, mosaic virus, replication, cytotoxicity

Infectious bursal disease virus (IBDV) is the causative agent of a highly contagious disease in young chickens known as infectious bursal disease (IBD). IBD causes significant losses of the poultry industries due to its high mortality and immunodepression in young birds by the destruction of the developing B lymphocytes in the bursa of Fabricius (BF) (Cheville, 1967; Kibenge *et al.*, 1988). IBDV belongs to *Avibirnavirus* genus of the *Birnaviridae* family. Its genome consists of two segments of double-stranded RNA, segment A and segment B (Dobos *et al.*, 1979). Segment A contains two partially overlapping opening reading frames (ORFs). The smaller ORF encodes the nonstructural viral protein 5 (VP5, 17 kDa) (Mundt *et al.*, 1995) and the larger ORF encodes a precursor polypeptide chain (105 kDa), which is subsequently cleaved into VP2, VP4, and VP3. The segment B encodes VP1 protein, the viral RNA-dependent RNA polymerase (Von Einem *et al.*, 2004). There are two distinct serotypes of IBDV, designated as serotype I and II. Viruses of the serotype I group are pathogenic to chickens, whereas the serotype II, mostly isolated from turkey, do not replicate in the chicken bursal cells and are avirulent to chickens (Mcferran *et al.*, 1980). Serotype I viruses are sub-divided into the classical virus, the antigenic variant virus, and the very virulent virus (Müller *et al.*, 2003).

VP5 was first discovered only in the IBDV-infected cells but not packed into virion particles (Mundt *et al.*, 1995). It was not essential for the viral replication, but played a key role in the induction of apoptosis of the infected cells and pathogenesis of the disease (Mundt *et al.*, 1997; Yao *et al.*, 1998). By expressing VP5 in CEF cells, BSC-1 and Cos-1 cells, VP5 was shown to accumulate within the plasma membrane and induce cell lysis (Lombardo *et al.*, 2000). Recently, Liu reported that VP5 inhibited apoptosis at the early stage of the viral infection (Liu and Vakharia, 2006). In our previous study, we showed that although the deduced amino acid sequence for VP5 from a cell culture-adapted strain Gt was highly identical to that of its parental very virulent IBDV (vvIBDV) Gx strain (Wang *et al.*, 2007), the two viruses displayed remarkably different properties both *in vivo* and *in vitro*. Although VP2 was shown to be mainly responsible for the differences in the viral replication and pathogenicity between the attenuated IBDV and vvIBDV (Wang *et al.*, 2004), it was not clear whether VP5 also contribute to these different characteristics. Deduced amino acid sequences of VP5 of IBDV are highly conserved among viruses within the serotype I (Lombardo *et al.*, 2000) or serotype II groups, but shared a low identity among strains across the two serotype groups. Therefore, it is interesting to discuss the difference for VP5 in the pathogenic mechanisms of serotype I and II viruses.

Reverse genetics offers a convenient and indispensable tool for the study of viral life cycles and the associated

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Table 1. Primers used for fusion PCR and RT-PCR

Primer	Sequence	Orientation	Position
GtA5U	AGCGATCGATtgttaagcgtctgatgag	+	-58~-41
GtA5L	CAATGATAGCGTTGTAGAAGGAGGAGTC	-	96~69
GtA3U	ACTGACAGATGTTAGCTACAATGGGTTG	+	535~562
GtA3L	ATTAGGTACCcgccctcccttagccatc	-	+88~+71
GxVP5U	CTCCTCCTTCTACAACGCTATCATTG *ATGGTCAGTAGAGATCAGACAAAC*	+	71~120
GxVP5L	CCCATTGTAGCTAACATCTGTC *AGTTCACTCAGGCTTCCTTGGAAGGTCA*	-	559~510
2382VP5U	CTCCTCCTTCTACAACGCTATCATTG *ATGGTGAGTAGAGATCAGACAAAC*	+	71~120
2382VP5L	CCCATTGTAGCTAACATCTGTC *AGTTCACTCAGGCTTCCGTGGAAGGTCA*	-	559~510
39U	ACAGGCCGTCAAGGTCTTGT	+	39~58
545L	ATCAACCCATTGTAGCTAAC	-	564~545

The virus Gt strain specific sequences are underlined. Ribozyme sequences are in lower-case characters. Restriction sites used are framed. The sequence of VP5 gene of Gx strain and 23/82 strain are in italics. Orientations of the virus-specific sequences of the primers are shown as sense (+) or antisense (-). The "+" or "-" symbols in front of the positions of nucleotides mean that the nucleotides positions are in the upstream or downstream of the genome, respectively. The positions where the primers bind (nucleotide number) are in accordance with the published sequence of strain Gt (**DQ403248**) (Qi *et al.*, 2007).

pathogenic mechanisms, which has been applied in studies of IBDV (Mundt and Vakharia, 1996). To gain a deeper insight into the role of the VP5 for the viral replication and pathogenicity, recombinant viruses (rGt) were previously generated using RNA polymerase II system (Qi *et al.*, 2007). In this follow-up study, we described the rescue and characterization of two new mosaic Gt viruses with VP5 gene exchanged by that of the Gx strain (very virulent virus) or the 23/82 strain (the non-pathogenic serotype II virus), respectively.

Materials and Methods

Viruses, plasmids, cells, and antibodies

The attenuated Gt strain of serotype I IBDV was derived from the vvIBDV Gx strain (Wang *et al.*, 2004, 2007). The recombinant virus of this strain rGt was successfully rescued in our earlier study (Qi *et al.*, 2007). The eukaryotic expression vector pCAGGS (Niwa *et al.*, 1991) was kindly provided by Dr. J. Miyazaki (University of Tokyo, Japan). The recombinant plasmid, pUC18GxA, carrying segment A of the Gx strain , as well as two infectious clones each carrying A or B segment of the Gt strain, pCAGGGtAHRT and pCAGGGtBHRT (shortened as pCGtA and pCGtB), respectively, were constructed from this laboratory as previously described (Qi *et al.*, 2007). VP5 gene of the serotype II virus 23/82 strain inserted in pUC57 plasmid, named as pUC2382VP5, was synthesized by Invitrogen (China). DFI cells used for transfection and Vero E6 cells for indirect immunofluorescence assay (IFA) were cultured in Dulbecco modified Eagle medium (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified 5% CO_2 incubator. Primary CEF cells were prepared from 10-day-old SPF chicken embryos. Secondary CEF cells were used for replication kinetics, apoptosis assay and cell viability assay. The VP2-specific monoclonal antibodies (McAb) 8G and VP5-specific McAb 4B4 (reactive to both serotype viruses) were prepared in our laboratory using

standard procedures. All primers used were synthesized by Invitrogen, and their sequences were listed in Table 1.

Analysis of VP5 sequences

A panel of VP5 sequences were retrieved from GenBank under the following accession numbers: **X92760** (UK661), **AY444876** (Gx), **AF240686** (D6948), **AF092943** (HK46), **D49706** (OKYM), **AF194428** (CEF94), **AF499929** (D78), **X84034** (P2), **X16107** (Cu-1), **DQ403248** (Gt), **D00499** (STC), **D00869** (52/70), **M97346** (GLS), **AF133904** (Variant E), **U30818** (OH), and **AF362773** (23/82). The deduced amino acid sequences from these DNA sequences were aligned using Meglign programme of DNAStar 5.01 software and a phylogenetic tree was constructed by the neighbor-joining method with 1000 times bootstrapping replicates using MEGA version 4.1 software.

Construction of the VP5 mosaic full-length segment A cDNA clones

Fusion PCR was used to construct the recombinant mosaic full-length segment A cDNA clones. Briefly, two primer pairs GtA5U and GtA5L, GtA3U and GtA3L (Table 1), respectively, were used to amplify two DNA fragments from the template pCGtA, one containing the HamRz sequence and the 5'NCRs (-68 bp~96 bp) (F1), the other containing the partial sequence of the Gt strain in the segment A (535 bp~+3260 bp) and the HdvRz sequence (F2) (Fig. 1). Another two primer pairs GxVP5U and GxVP5L, 2382VP5U and 2382VP5L were synthesized to amplify VP5 gene of the Gx strain (F3) and the 23/82 strain (F4), respectively, with pUC18GxA or pUC2382VP5, respectively, as templates. The products F3 and F4 were partly overlapped with F1 or F2 (Fig. 1). To obtain the mosaic full-length segment A cDNA products, mixtures of three overlapping templates, one containing F1, F3, and F2, the other containing F1, F4, and F2, were used for fusion PCR with primers GtA5U and GtA3L. The fusion PCR was carried out for first 8 cycles of denaturation at 95°C for 30 sec, annealing at 50°C for
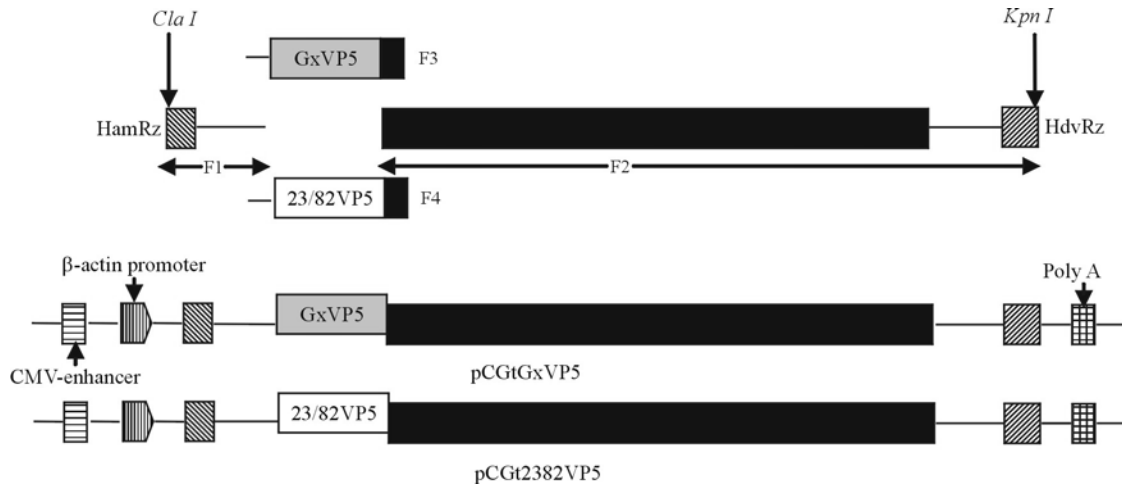
Fig. 1. Construction of mosaic cDNA clones of segment A of IBDV strain Gt. Boxes filled with black represent Gt strain sequences as indicated, and grey-filled boxes represent Gx strain sequences and blank boxes represent 23/82 strain sequences, respectively. Fragments amplified by PCR and used in the fusion PCR were marked as F1, F2, F3 and F4. 5'NCR and 3'NCR of the viral genome were fused with HamRz (▨) or HdvRz (▨), respectively. Full-length mosaic cDNAs were inserted into pCAGGs plasmid under the control of the CMV enhancer (▤), β-actin promoter (▨) and Poly A terminator (▥).

30 sec and extension at 72°C for 3 min without the primers, then primers were added, the reaction was processed for another 27 cycles of 95°C for 30 sec, 56°C for 30 sec and 72°C for 3 min 30 sec with DNA Polymerase (TaKaRa, China). The full-length mosaic segment A products were named as GtAGxVP5HRT and GtA2382VP5HRT, respectively. They were then purified, digested with *Cla*I/*Kpn*I and ligated into pCAGGS vector digested by the same restriction enzymes. Two recombinant plasmids were obtained and named pCAGGGtAGxVP5HRT (pCGtGxVP5) and pCAG GGtA2382VP5HRT (pCGt2382VP5) (Fig. 1).

Rescue and identification of the mosaic viruses
Extraction of recombinant plasmids and transfection were performed as described in details previously (Qi *et al.*, 2007). Purified recombinant plasmids, pCGtGxVP5 or pCGt2382VP5, were co-tranfected with pCGtB into DF I cells with Lipofectamin™2000 (Invitrogen, USA). Three days post-transfection, the cells were lysed by freezing and thaw, and cell lysates were centrifuged. The supernatants containing the viral particles were transferred onto fresh CEF cells. After three passages of the infected CEF cells, viruses were harvested from the culture supernatants, titered and stored at -70°C.

To determine the rescue of recombinant viruses, VeroE6 cells were infected with rescued viruses and indirect IFA was performed with VP2 specific McAb (8G) and VP5 specific McAb (4B4) to detect the presence of VP2 and VP5 proteins, respectively, following the method described previously (Qi *et al.*, 2007). Mock infected VeroE6 cells were processed similarly as negative controls for the indirect IFA.

To verify the presence of mosaic VP5 gene, total RNA was isolated from infected cells and RT-PCR was performed with the specific primers 39U and 545L (Table 1). PCR products were directly sequenced.

Replication kinetics of mosaic viruses
To assay the viral replication kinetics, confluent secondary CEF cells were seeded in a 12-well tissue culture plate and infected with rescued viruses at an MOI of 0.1. After incubation for 1 h, inoculum was removed and cells were overlaid with 1 ml DMEM (1% FBS), following rinse with Hank's buffer. The first well was collected immediately and stored at -70°C, marked as 0 h post-infection (p.i.). Portions of the remained wells were collected at 12, 24, 48, 72, 96, and 120 h p.i, and stored, and then the viral titers of lysates were measured by the $TCID_{50}$ values on CEF cells. The average titers were calculated from three independent experiments by the method of Reed-Muench.

Assay for viability of infected cells
MTT [3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, USA] colormetric assay was carried out to analyze relative viability of cells infected with the rescued viruses (Yao *et al.*, 1998). Briefly, secondary CEF cells were seeded in 96-well plates and infected with rescued virus at an MOI of 0.1. MTT was dissolved in PBS (5 mg/ml) and filtered with 0.22 μm filter. At daily intervals, 10 μl of MTT solution was added to each well and the plate was incubated at 37°C for another 4 h. At that time, the medium was replaced by 100 μl of dimethysulfoxide. The plate was then read for the optical density at 570 nm (OD_{570}) in a microplate reader. The data were averaged from values obtained in three independent experiments. The OD_{570} of the control mock infected cells was defined as 100% viability, which was used to normalize all the values from infected cells to relative cell viabilities.

Assay for apoptosis of infected cells
Apoptosis was analyzed by Cell Death Detection ELISA[plus] kit (Roche, Germany) following the manufacturer's protocol. Secondary CEF cells grown in 96-well microplate to 80% confluence were infected with rescued viruses at an MOI of
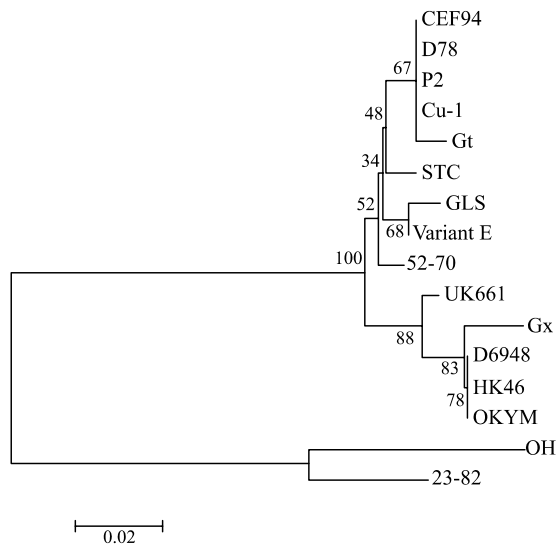
Fig. 2. Phylogenetic tree of the deduced VP5 amino acid sequences from different IBDV strains. Serotype I and serotype II strains compose two different branches. The vvIBDV strains and attenuated strains of serotype I belong to two subgroups.

1.0. At 24 h p.i., CEF cells were lyzed directly in the well and 20 μl supernatants were transferred to ELISA microplate. Each well was added with 80 μl of the immunoreagent (anti-histone-biotin and anti-DNA-POD) and incubated under gently shaking (300 rpm) for 2 h at room temperature. After rinsing, each well was pipetted into 100 μl ABST solution and incubated for 10 min. Then 100 μl ABST stop solution was added to stop reaction. The microplate was read for the optical density at 405 nm (OD_{405}) in a microplate reader. Each sample was analyzed triplicate wells.

Analysis for expression of viral protein in infected cells
To analyze expression of viral protein, VeroE6 cells (in a

six-well plate) were infected with rescued virus at an MOI of 2.0. At 24 h p.i., cells were harvested and lysed. For immnoblotting, the proteins were electrophoretically separated in a 12% sodium dodecyl sulfate-polyacrylamide gel. The primary antibody was VP2 specific McAb (8G) diluted 1:500. β actin McAb (Sigma, USA) was used as the control.

Results

**Phylogenetic analysis and homology analysis of VP5**
A panel of VP5 genes from different IBDV strains was retrieved from GenBank database. As shown in Fig. 2, their deduced amino acid sequences can be divided into two groups: serotype I group which includes vvIBDV strains, classical strains, attenuated strains, variant strains and the serotype II group (OH and 23/82 strains). In the serotype I group, although vvIBDV strains and attenuated strains shared a high identity, they belonged to two different subgroups. Homology analysis showed that VP5 amino acid sequences of IBDV were highly conserved among strains of serotype I (93.8%~99.3%) or serotype II (91.8%) groups; however, the identity between strains across two serotypes was less than 81.5% (data not shown).

Production of recombinant viruses and identification of the rescued viruses
To construct the recombinant viruses, two full length DNA fragments of the segment A from the Gt strain were obtained as GtAGxVP5HRT and GtA2382VP5HRT (Fig. 1). And these two plasmids were used to construct two mosaic viruses rGtGxVP5 and rGt2382VP5 by co-transfection along with another plasmid pCGtB in DF I cells. The supernantants derived from the third passage of rescued viruses were used to infect VeroE6 cells and indirect IFA with VP2 specific McAb detected fluorescent signals in all cells infected with rGt (Fig. 3A), rGtGxVP5 (Fig. 3B), and rGt2382VP5 (Fig. 3C). Indirect IFA also showed that all the infected cells reacted with VP5 specific McAb (Fig. 3E, F, and G). No flu-
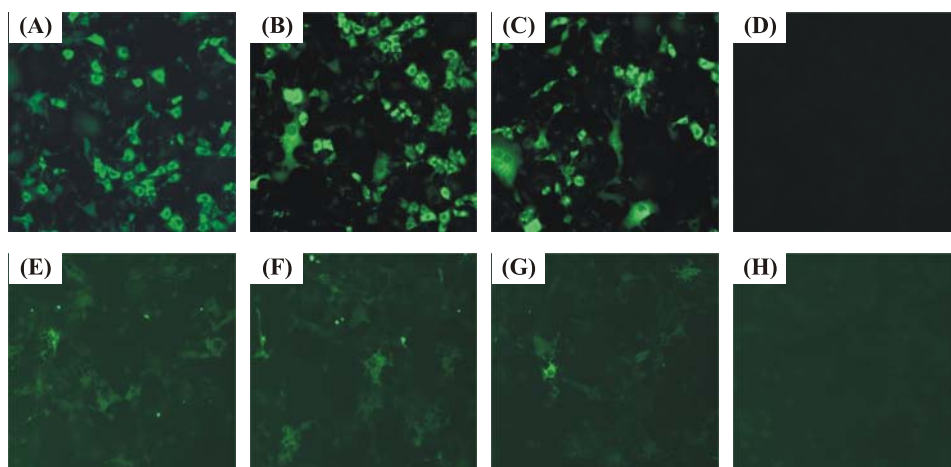

Fig. 3. Indirect immunofluorescence assay of cells infected with the rescued viruses. VeroE6 cells were infected with rGt (A, E), rGtGxVP5 (B, F), and rGt2382VP5 (C, G) showed positive green fluorescence signals with VP2 specific McAb 8G or VP5 specific McAb 4B4. No fluorescence was detected in the mock infected cells (D, H).
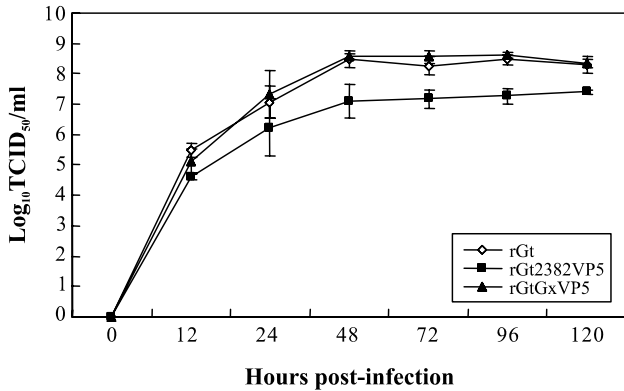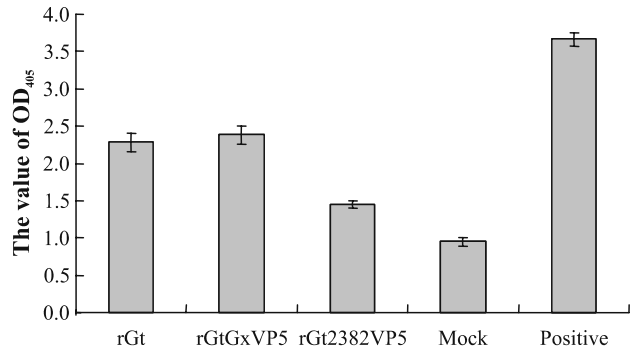
Fig. 4. Replication kinetics of rescued viruses on CEF cells. Secondary CEF cells were infected with the rGt, rGtGxVP5, and rGt2382VP5 at the MOI of 0.1, respectively. The viral titers of supernatants from infected cells harvested at different time points were determined by median tissue culture infective experiment and expressed in $\log_{10}TCID_{50}/ml$. Average titers and standard deviation (error bars) from three independent experiments are indicated.

orescence was detected in the mock infected cells (Fig. 3D and H). The results of indirect IFA indicated that recombinant viruses were rescued and VP5 was expressed in cells infected with rescued viruses.

The full-length ORF of the two mosaic VP5 genes were detected by RT-PCR in RNA isolated from CEF cells infected with rGtGxVP5 and rGt2382VP5. The sequence analysis confirmed nucleotide sequences containing ORF of VP5 gene of Gx strain or 23/82 strain fused to partial sequence
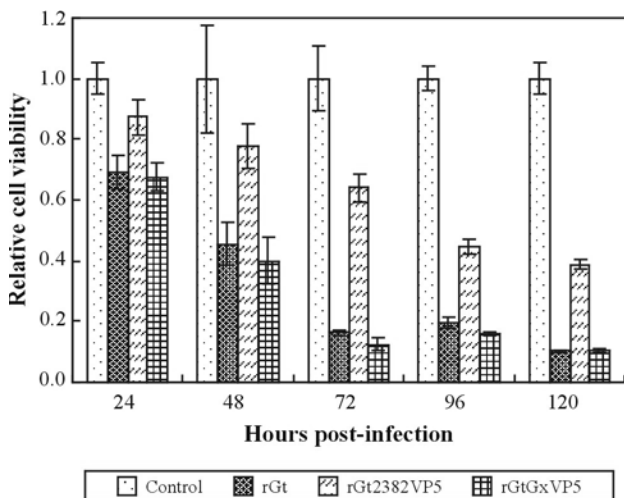
Fig. 5. Relative viabilities of cells infected with the rescued viruses. Secondary CEF cells infected with the rGt, rGtGxVP5, and rGt2382VP5 at an MOI of 0.1 were analyzed for viabilities at different time points by MTT colormetric assay. The OD_{570} of the mock infected cells was defined as 100% viability as the control. All the data represented are cell viabilities relative to the control. Average titers and the standard deviations (error bars) from three independent experiments are indicated.

of Gt strain (data not shown). These results indicated that mosaic viruses were successfully rescued from mosaic full-length infectious clones.

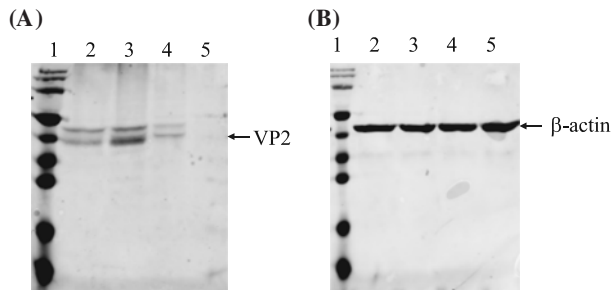**Replication kinetics of rescued viruses**
Based on titers of collections at different time points from infected CEF cells, the replication kinetic of rGtGxVP5 was identical to that of rGt (Fig. 4). This indicated that VP5 of vvIBDV Gx strain did not affect the replication of attenuated virus Gt strain *in vitro*. However, rGt2382VP5 replicated slower than the two rescued viruses, rGt and rGtGxVP5 (Fig. 4). At 48 h p.i., rGt2382VP5 reached the highest titer of $10^{7.09}$ TCID50/ml, approximately 20-fold lower than those of rGt ($10^{8.32}$ TCID50/ml) and rGtGxVP5 ($10^{8.56}$ TCID50/ml). The results showed that VP5 of 23/82 strain (serotype II) decreased the replication efficiency of Gt strain *in vitro*.

Viability of infected cells
As shown in Fig. 5, the relative cell viabilities of all infected cells were lower than the mock infected cells. At 48 h p.i., relative viabilities of infected cells decreased to 40% (rGt) and 45% (rGtGxVP5) which was significantly more dramatic when compared with rGt2382VP5 (to about 78%). After 72 h p.i., the relative viabilities of cells infected with both rGt and rGtGxVP5 decreased to about 20%, however cells infected with rGt2382VP5 decreased to a 38.8%. These results showed that rGt2382VP5 had lower cytopathogenicity for CEF cells than rGt, which indicated that replacement of VP5 gene of 23/82 strain decreased the cytopathogenicity of Gt strain in CEF cells.

Apoptosis of infected cells
Secondary CEF cells were infected with each of the rescued viruses at an MOI of 1.0 and the cell apoptosis was analyzed by Cell Death Detection ELISA^{plus} kit at 24 h p.i. As shown in Fig. 6, the OD_{405} of infection with rGt or rGxVP5 was more than twice as high as that of mock infection control. The value of infection with rGt2382VP5, which was more than that of mock infection control, was lower

Fig. 6. Assay of apoptosis for cells infected with the rescued viruses. Secondary CEF cells infected with the rGt, rGtGxVP5, and rGt2382VP5 at an MOI of 1.0 were analyzed for apoptosis by Cell Death Detection ELISA^{plus} kit. The OD_{405} of samples represented the apoptosis level of infected cells. Average value of triplicates and the standard deviations (error bars) were indicated.

(A)

(B)

Fig. 7. Analysis of expression of VP2 in cells infected with the rescued viruses. VeroE6 cells were infected with rGt (lane 2), rGtGxVP5 (lane 3), rGt2382VP5 (lane 4) or left uninfected (lane 5). Cells were harvested and lysed for Western blot assay with VP2 specific McAb (8G) (A) and β-actin McAb (Sigma, USA) as the control (B).

than that of infection with rGt or rGtGxVP5. The results indicated that the exchange of VP5 gene with the counterpart of the 23/82 strain might decrease the cytopathogenicity of rGt2382VP5 strain.

Expression of viral protein

To analyze the expression of viral protein in detail, VeroE6 cells were infected with rescued viruses and harvested at 24 h p.i. The lysates were analyzed by Western blot using VP2 specific McAb (8G) and β actin McAb. The results showed that no significant difference in the levels of VP2 between infections with rGt and rGtGxVP5 (Fig. 7A, lanes 2~3). However, the expression of VP2 in cells infected with rGt2382VP5 (Fig. 7A, lane 4) was lower than that infected with rGt or rGtGxVP5. The expressions of β actin protein reference were equal in all groups (Fig. 7B).

Discussion

Since the two serotypes of IBDV display completely different pathogenic properties (Ismail *et al.*, 1988), the underlying mechanism has been always a research focus of the field. Boot and co-worker found that VP3 might be a key factor responsible for such different pathogenic properties because exchange of VP3 C-terminal sequence of a serotype I virus with the serotype II counterpart reduced the virulence in SPF chickens with a slight effect on the viral replication (Boot *et al.*, 2002).

To date, the deduced amino acid sequences of VP5 are highly conserved in all studied IBDV strains within the serotype I or serotype II group with greater than 93.8% and 91% identity, respectively. However, they only share a lower than 81% identity among strains across two serotypes (data not shown). The other viral proteins have higher identities between two serotypes (VP1-99%, VP2-88%, VP3-95%, and VP4-92%) (Boot *et al.*, 2001; Yuwen *et al.*, 2008). Furthermore, VP5 was reported to play an important role in viral replication, release and pathogenicity (Mundt *et al.*, 1997; Yao *et al.*, 1998; Lombardo *et al.*, 2000), but whether it exerts different functions in the viral replication and pathogenicity in two serotype groups have not been well studied.

Although, both vvIBDV and attenuated viruses belong to the serotype I, they show distinct characteristics including cell tropism and pathogenicity. Researchers considered VP2 as the protein mainly responsible for the differences between the two pathotypes, but recent study showed that it was not the sole determinant of very virulent phenotype (Boot *et al.*, 2000).

Although VP5 of vvIBDV and attenuated virus have a high identity of above 93.8%, they belong to two different subgroups (Fig. 2). Comparison of the viral replication, cytotoxicity and induction of apoptosis for rGt and rGtGxVP5 showed that two rescued viruses had similar characteristics, indicating that exchange of VP5 gene of vvIBDV did not affect the replication and increase pathogenicity of rGtGxVP5 virus. This is consistent with our speculation that VP5 of vvIBDV was not responsible for cell tropism and pathogenicity in cell cultures.

Studies of the replication kinetics on CEF cells showed a delay of rGt2382VP5 in viral replication, and lower titers than the rGt virus. VP5 of 23/82 strain IBDV decreased the titers of the mosaic virus, which is similar to the results of Yao's, which showed that mutant virus lacking the expression of VP5 replicated slowly and had the final titer 10 fold lower than that of the parental vaccine strain (D78) (Yao *et al.*, 1998). They considered that lacking expression of VP5, which might affect viral release (Lombardo *et al.*, 2000), was the reason for the slow replication and the lower titers. Here our rescued virus expressing VP5 of 23/82 strain still replicated slowly and showed lower titers than the rescued virus (rGt). Although the mechanism was unknown, this was also in agreement with SchrÖder who showed that mosaic viruses replacing the NCR, VP5, and N terminus of VP2 of serotype I with their counterparts in serotype II strain affected the rival replication in cell cultures (SchrÖder *et al.*, 2001).

Yao and co-workers also found that VP5 played a role in viral pathogenesis because viruses lacking VP5 attenuated the virulence of the parental virus and VP5 could induce apoptosis (Yao *et al.*, 1998; Yao and Vakharia, 2001). Our study showed that VP5 of the 23/82 strain decreased the cytotoxicity of mosaic virus rGt2382VP5, by the results of MTT proliferation and apoptosis assays carried on CEF cells. Also, we found that expression of VP2 in rGt2382VP5 infected cells was lower than that in cells infected with rGt or rGtGxVP5. Probably, the reduced cytotoxicity of rGt2382VP5 is due to its poor replication efficiency.

In conclusion, we obtained two mosaic viruses with VP5 gene replaced by that of the vvIBDV or the serotype II 23/82 strain. The mosaic virus rGtGxVP5 is viable and shows characteristics similar to its parent strain rGt in the viral replication and the cytotoxicity *in vitro*. So VP5 is not responsible for replication and pathogenicity between attenuated virus and vvIBDV. In additional, it is not be responsible for cell tropism of vvIBDV. However, the other mosaic virus rGt2382VP5 containing VP5 gene of 23/82 strain (serotype II) showed decreased replication and cytotoxicity to CEF cells, indicating that it contribute to the distinct pathogenesis of serotype I and serotype II strains *in vitro*. Together, all these results suggest that VP5 is involved in the efficiency of viral replication and modulates the patho-

genicity of IBDV *in vitro*. Whether it plays the same role *in vivo* need to be further studied.

Acknowledgements

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