VP5 to better understand the factors underl ing the pathogenicit of IBDV. In this stud , monoclonal cell lines e pressing the VP5 protein of IBDV ere constructed, and the function of VP5 protein inhibiting IBDV release from infected cells as con rmed.

Virus strain, cell lines, antibod and chickens

The NB strain of IBDV, a pathogenic serot pe I virus isolated in China, as adapted to gro th in chicken embr o broblasts (CEF) [27, 32]. Vero cells permissive for IBDV infection ere purchased from the Shanghai Institute of Biochemistr and Cell Biolog , Chinese Academ of Sciences, and ere maintained in MEM (Gibco BRL, USA) supplemented ith 10% ne born calf serum (NCS). Speci c-pathogen-free (SPF) Leghorn chickens and embr onated chicken eggs ere purchased from Beijing Merial Vital Laborator Animal Technolog Co. Ltd, Beijing, China. Primar CEF cells ere prepared from 10-da -old embr onated SPF chicken eggs and maintained in MEM supplemented ith 10% NCS. Both cell lines ere used for propagation of IBDV. The monoclonal antibod (mAb) to VP4 protein of infectious bursal disease virus as generated in our laborator [23].

Preparation of monoclonal antibod (mAb) against VP5 protein

To generate mAbs to VP5, recombinant VP5 protein e pressed in *E. coli* as prepared as an immunogen for mouse vaccination. Brie , the vector pCI-A containing the complete genomic A segment of IBDV (unpublished data) as used as a template for PCR. A 439-bp cDNA fragment encoding the complete VP5 protein as ampli ed b RT-PCR ith primers VP5-up (Table 1) containing an *Eco*RI site and VP5-do n (Table 1) containing a *Sal*I site, hich ere designed according to the cDNA sequence of segment A of IBDV strain NB (GenBank accession no. AY319786). RT-PCR as performed at 95 C for 5 min, 95 C for 1 min, 58 C for 1 min and 72 C for 2 min for 30 c cles, ith a nal elongation step for 5 min at 72 C. Then, PCR products ere inserted into the prokar otic e pression vector pET-28a (+) (Novagen, Madison, WI, USA). The resulting plasmid, pET-VP5, as con rmed b PCR, and its DNA sequence as veri ed. It as then used to transform BL21 (DE3) *E. coli* for e pression. His-tagged VP5 as optimall e pressed in *E. coli* as insoluble inclusions, induced b a nal concentration of 1 mM isoprop l-β-D-thiogalactoside (IPTG). The e pressed protein became soluble after treatment of *E. coli* cells ith l sis buffer containing urea, NaH_2PO_4 and Tris. SDS-PAGE anal sis sho ed that the His-VP5 fusion protein appeared to have a molecular eight of appro imatel 23 kDa (data not sho n). After puri cation ith an Ni-NTA af nit chromatograph column (Novagen, Madison, WI, USA), His-VP5 protein could be recogni ed b anti-His mAb in estern blot anal sis (data not sho n).

Five SPF BALB/c mice ere immuni ed subcutaneousl (S/C) ith rVP5 (50 μg rVP5 per mouse) emulsi ed in complete Freund's adjuvant (CFA, Sigma Aldrich, St. Louis, MO) and boosted t ice at 2- eek intervals ith rVP5 emulsi ed in incomplete Freund's adjuvant (Sigma) after the rst immuni ation. T o eeks after the third immuni ation, the vaccinated mice ere injected intraperitoneall ith 0.1 mg of rVP5. Three da s after the nal immuni ation, spleen cells ere fused ith SP2/0 cells as described b Kohler and Milstein [10]. The resulting h bridoma cells ere cultured in RPMI-1640 containing h po anthine-aminopterin-th midine (HAT) and h po-anthine th midine (HT) for 10 14 da s. Further selection and con rmation of mAb recognition of VP5 protein ere

Oligonucleotide primers used in PCR

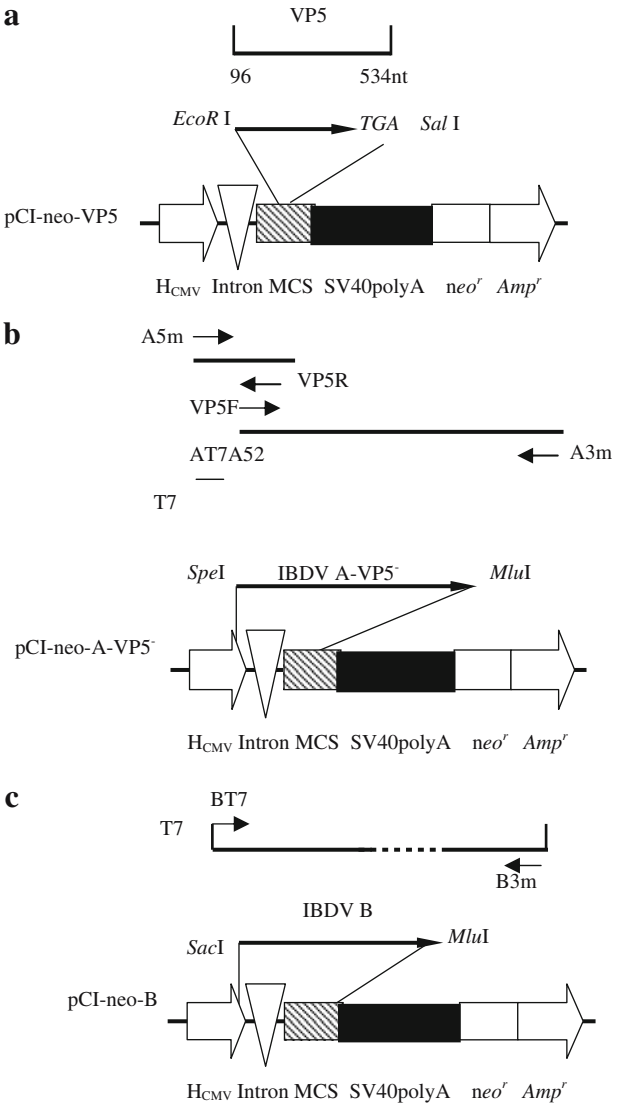
Name	Sequence	Orientation	Position (nt)
VP5-up	CC ATGGTCAGTAGAGATCAGACAAACG	+	96 121
VP5-do n	GATC TCACTCAGGCTTCCTTGGAAGGTC	−	511 531
AT7	TA TAATACGACTCACTATAGGGGATACGAT	+	1 9
A5 m	CTATAGGGGATACGATCGGTCTGA	+	1 17
VP5F	GCTATCATTG*ATT*GTCAGTCGA	+	97 108
VP5R	CTGAC*AAT*CAATGATAGCGTT	−	84 104
A3 m	CC GGGGACCCGCGAACGGAT	−	3,242 3,259
BT7	TA TAATACGACTCACTATAGGGGATACGAT	+	1 9
B3 m	CC GGGGGCCCCCGCAGGCGAA	−	2,809 2,827

Nucleotide positions correspond to the published sequence of IBDV strain NB p38 -TD-5GAT.5063 TDo1906 0 TD320]. 7-(prmrotr-speci cV)-47717(se

carried out b indirect en me-linked immunosorbent assa (ELISA), using cell l sates of *E. coli* BL21 (DE3) and rVP5 as antigens. H bridomas that secreted rVP5 antibodies ere subcloned three times b limiting dilution. The reaction of mAbs to native VP5 as assa ed using VP5 protein e pressed in IBDV-infected CEF and Vero cell monola ers as antigen as described previousl [28]. Brie , CEF and Vero cells inoculated ith IBDV ere cultured for 36 h. The cells ere ashed t ice ith PBS and ed ith 4% paraformaldeh de. IFA assa s ere performed as described belo . In addition, the mAbs against rVP5 ere identi ed b estern blot assa s using rVP5 as antigens. The IgG subt pe of each mAb as determined using SBA Clonot ping S stem/HRP (Southern Biotechnolog , Birmingham, AL, USA).

Construction of the eukar otic e pression vector, transfection and cloning

To subclone the VP5 gene into the eukar otic e pression vector pCI-neo (Promega, USA), a 439 bp-cDNA segment encoding the VP5 protein as obtained as described above. The resulting plasmid, designated pCI-neo-VP5 (Fig. 1a), as used to transform Top10 competent *E. coli* cells (Invitrogen, USA), and cloned fragments ere identi ed b PCR and sequencing. Finall , the plasmids ere isolated using a high-purit plasmid puri cation s stem (Marligen, USA) and lineari ed ith *Bam*HI. Vero cells ere gro n to 95% con uence and transfected ith lineari ed recombinant plasmid using Lipofectamine™ (Invitrogen) according to the manufacturer's instructions. Fort -eight hours post-transfection (h.p.t.), Vero cells transfected ith the pCI-neo-VP5 ere tr psini ed and persistentl selected in MEM containing neom cin G418 (500 µg/mL; B. M. Scienti c) for 2 3 eeks, until G418-resistant "cell islands" formed. Ever G418-resistant cell island as cloned and selected three times b limiting dilution in MEM containing G418 to obtain a single cell clone. The G418-resistant cell clones that gre ell ere selected for further anal sis. Non-transfected cells and cells that ere mock-transfected ith pCI-neo vectors ere anal ed in parallel as controls.



PCR and Southern blot assa s of genomic DNA

To detect chromosomal integration of the VP5 gene in the cloned, pCI-neo-VP5-transfected Vero cells, genomic DNA as isolated from each G418-resistant clone b spooling, as described previousl [17]. Genomic DNA as treated ith RNase and subjected to PCR as described above. Southern blot anal sis as performed using an alpha-^{32}P-labeled VP5 probe (300 bp) prepared ith a random prime DNA labeling kit Ver.2 (TaKaRa, Japan) as described previousl [17]. Genomic DNA from Vero cells transfected ith the parental pCI-neo vector as used as a negative control.

RT-PCR and northern blot anal sis of mRNA transcription

Transcription anal sis as carried out for each G418-resistant pCI-neo-VP5-transfected clone that gave a positive Southern blot result. Total cellular RNA as

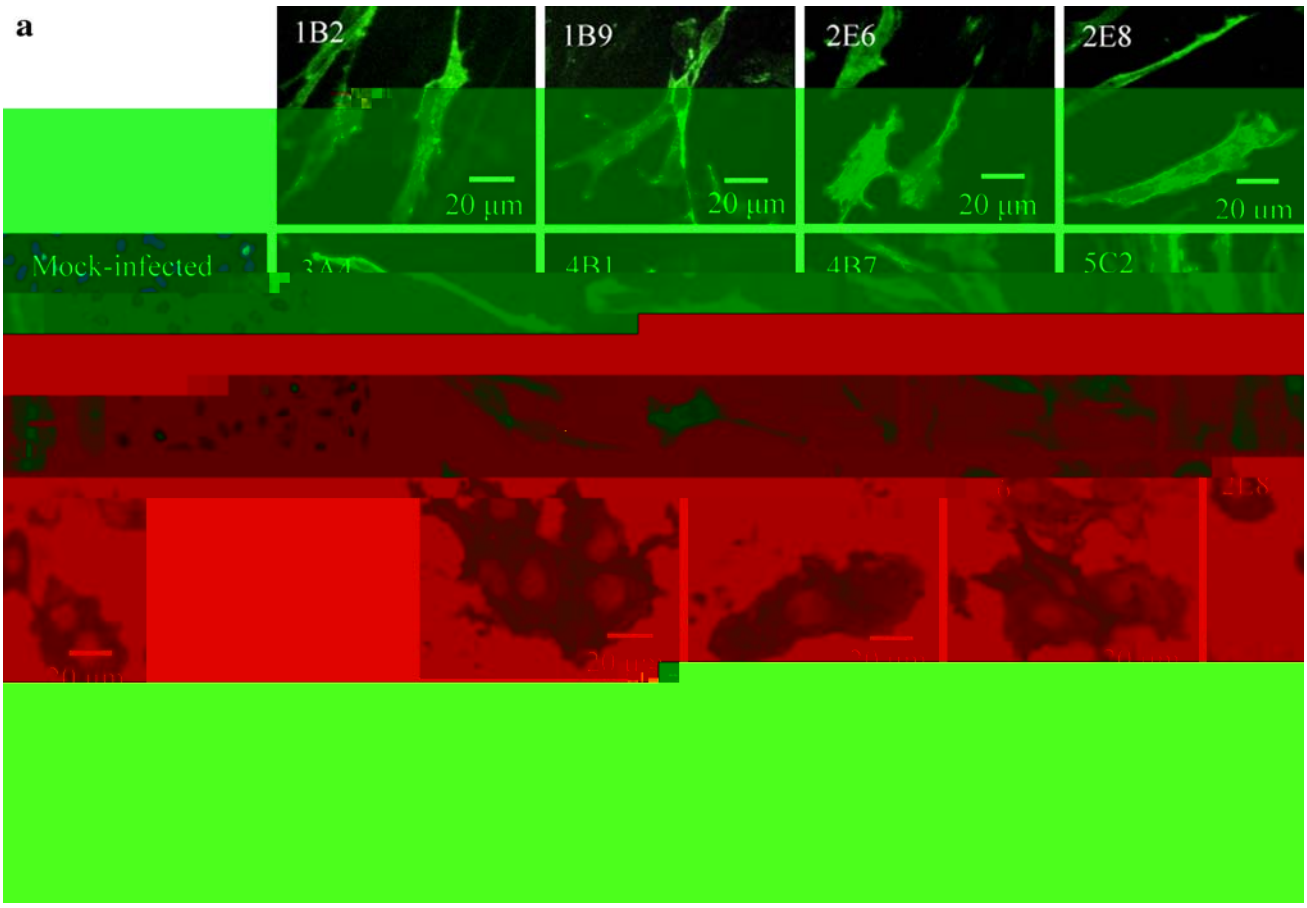
prepared ith Tri ol reagent (Invitrogen) according to the manufacturer's instructions. Anal sis of VP5 transcription as performed using the One Step RT-PCR Selective PCR Kit Ver.1.1 (TaKaRa), and northern blot anal sis ith an alpha-^{32}P-labeled VP5 probe, as described previousl [17].

Indirect immuno uorescence assa

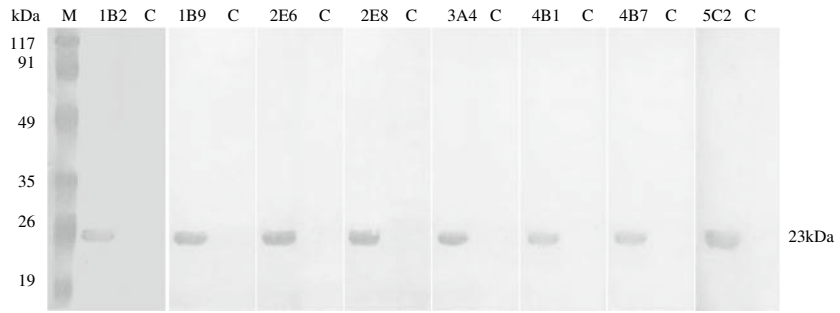
Indirect immuno uorescence assa s (IFAs) ere performed as described in ref. [28]. Brie , a plate of cells as ed ith 4% paraformaldeh de (in PBS, pH 7.4) for 30 min at room temperature and allo ed to air dr . The ed cells ere blocked ith PBS (717 mmol/L K_2HPO_4, 283 mmol/L KH_2PO_4, pH 7.2) containing 5% dr skimmed milk for 60 min at 37 C. The plate as ashed three times ith PBST (PBS ith 0.25% T een-20). Mouse anti-VP5 mAb or mouse anti-VP4 mAb (1:100, diluted ith PBS containing 5% dr skim milk) as added to the plate, hich as incubated for 60 min at 37 C. After three ashes in PBST, diluted FITC-conjugated goat anti-mouse IgG (KPL, USA) as added to the ells, and the plate as incubated for 60 min, follo ed b three PBST ashes. Additional nuclear staining ith 4′,6-diamidino-2-phen - lindole (Sigma) as performed as described previousl [28]. The cells ere then observed under an inverted uorescence research microscope (Ol mpus, Japan) for IFA anal sis. IBDV-infected cells ere used as positive control.

Construction of a VP5-deleted infectious IBDV clone

Vectors pCI-A and pCI-B, containing the complete genomic A and B segments, respectivel , of IBDV strain NB (unpublished data) ere used as templates. (The GenBank accession number of the cDNA sequence of segment B is AY654284.) To construct a mutant cDNA clone of segment A lacking the initiation codon of the VP5 gene, using a previousl reported method [15], e ampli ed t o DNA segments ith unique primer pairs (A5 m plus VP5R and VP5F plus A3 m; Table 1) and used the resultant segments as a template for fusion PCR ith AT7 and A3m primers. After replacing the VP5 gene initiation codon (ATG) of the A segment ith "ATT", an ampli ed fragment anked b *Spe*I and *Mlu*I sites as cloned into pCI-neo to ield the



. 2 Reactivit of VP5-speci c mAbs ith natural VP5 protein in IBDV-infected CEF and Vero cells b IFA. IBDV-infected CEF and Vero cells ere detected using mAbs 1B2, 1B9, 2E6, 2E8, 3A4, 4B1, 4B7 and 5C2, speci c for VP5. and sho that the anti-VP5 mAbs binds transientl to naturall e pressed VP5 protein in IBDV-infected CEF and Vero cells. *Bar* 20 μm (original magni cation ×40)



. Immunoactivit of mAbs to rVP5 protein b estern blot. Puri ed rVP5 separated b SDS-PAGE as used as antigen for estern blot anal sis ith mAbs 1B2, 1B9, 2E6, 2E8, 3A4, 4B1, 4B7 and 5C2, speci c for rVP5 protein. L sates of *E. coli* BL21 (DE3) containing pET-28a ere used as controls. *M* prestained protein molecular eight marker (MBI Fermentas, USA); *C E. coli* BL21 (DE3) containing pET-28a

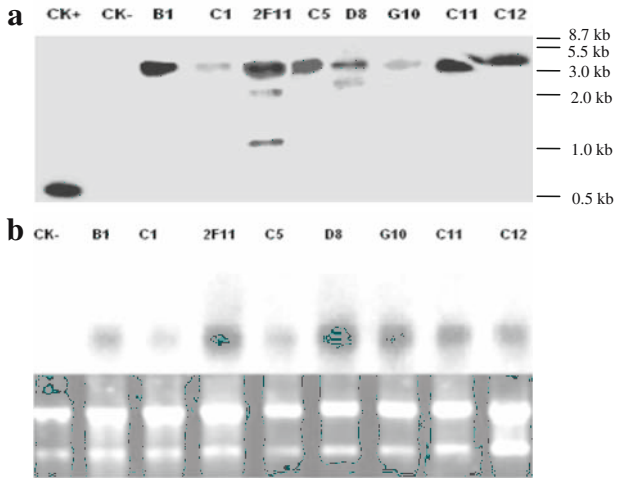
plasmid pCI-neo-A-VP5^{-} (Fig. 1b). We also ampli ed the hole segment B, anked b *Sac*I and *Mlu*I (BT7 and B3 m; Table 1), and cloned the resultant segment into pCI-neo to obtain plasmid pCI-neo-B (Fig. 1c). Plasmids pCI-neo-A-VP5^{-} and pCI-neo-B ere digested ith *Mlu*I. After puri cation ith TE phenol (pH 8.0), in vitro transcription as performed using the lineari ed plasmids as template, according the protocol of the T7 RiboMAX^{TM} E press RNAi S stem (Promega). Vero cells ere co-transfected ith transcripts of mutant segment A and of segment B, using Lipofectamine^{TM} 2000 (Invitrogen). The resulting progen ere designated recombinant VP5^{-}/IBDV.

The role of VP5 protein in replication and c topathogenicit of IBDV

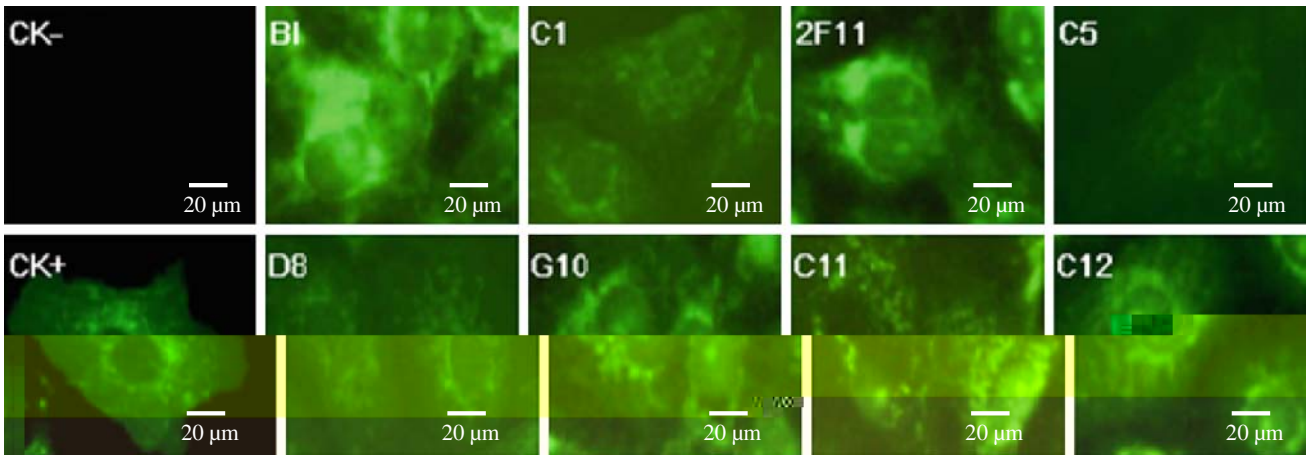
To determine the role of VP5 protein in IBDV infection, a CEF monola er as inoculated ith the parental IBDV or VP5^{-}/IBDV at 500 $TCID_{50}$. The cell supernatant, cellular monola er (CM), and cell supernatant plus cellular monola er (CSC) ere collected at various time points post-infection (p.i.) for virus titration. All virus samples ere free e-tha ed three times, follo ed b centrifugation at 2,000×*g* for 10 min. Finall , the virus titer as determined b end-point dilution (tenfold serial dilution) on CEF monola ers cultured in 96- ell plates. The c topathogenic effect (CPE) as observed ever da until 7 da s p.i., and the 50%-tissue culture infectious dose ($TCID_{50}$ ml^{-1}) as determined according to Karber [8].

Detection of VP5 protein b o c tometr (FCM)

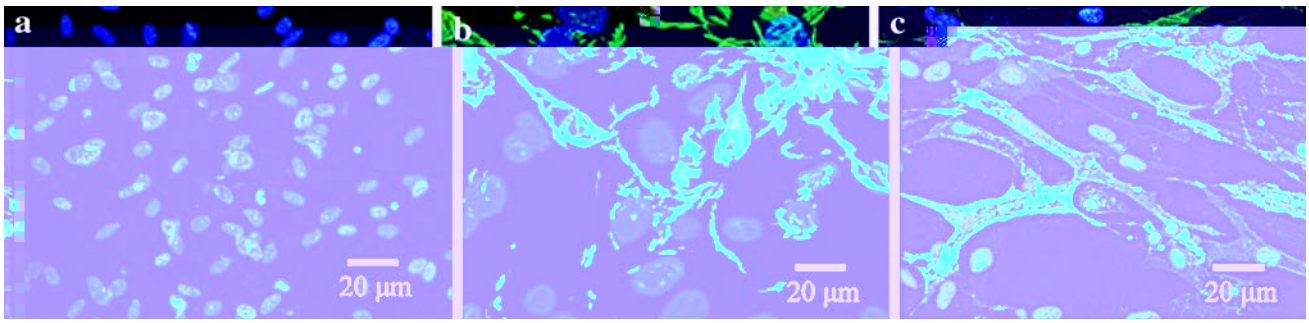
VP5 protein on the surface of bursal cells of chicken infected ith virulent IBDV, a VP5^{-}/IBDV-infected Vero monola er and VP5-e pressing Vero cells as anal ed b FCM. Brie , t elve 30-da -old SPF Leghorn chickens ere housed in negative-pressure isolator cages ith HEPA- ltered air and inoculated intranasall ith 50 BLD_{50} IBDV NB strain in a volume of 0.1 mL. Single-cell suspensions ere produced from bursa of Fabricus (BF) tissues collected from infected chickens at 12, 24, 48 and 72 h p.i. as described previousl [31]. Single-cell suspensions of Vero cell monola ers ere prepared b digestion ith 0.25% tr psin for 3 min, follo ed b three ashes ith PBS. Using the previousl reported method [21], cells (1×10^6 cells) ere rst blocked in PBS containing 5% normal rabbit serum for 30 min at 4 C and then incubated ith VP5 mAb and a secondar PE-labeled goat anti-mouse-IgG (SBA) on ice. Cells ere anal ed using a



. Southern and northern blot anal sis of monoclonal IBDV-VP5-e pressing Vero cell lines. Genomic DNA from monoclonal Vero cells e pressing VP5 protein as anal ed b Southern blot using α-^{32}P-labelled VP5 probe. *Lane CK+* contains a PCR-ampli ed IBDV-VP5 gene; *Lane CK-*contains genomic DNA from Vero cells transfected ith the parental pCI-neo vector. *Lanes B1*, *C1*, *2F11*, *C5*, *D8*, *G10*, *C11* and *C12* contain genomic DNA from monoclonal IBDV-VP5-e pressing Vero cell lines. Genomic DNA from monoclonal Vero cells e pressing VP5 protein as anal ed b northern blot using alpha-^{32}P-labelled VP5 probe. Lane CK- contains mRNA from Vero cells transfected ith the parental pCI-neo vectors, *lanes B1*, *C1*, *2F11*, *C5*, *D8*, *G10*, *C11* and *C12* contain mRNA from monoclonal cells e pressing IBDV-VP5



. E pression of IBDV-VP5 protein in monoclonal cells, visuali ed b IFA. Using anti-VP5 mAb, the e pressed VP5 protein as observed in the monoclonal IBDV-VP5-e pressing Vero cells under a uorescence microscope. *CK-*, Vero cells transfected ith pCI-neo vector; CK+ , Vero cells infected ith IBDV; *B1*, *C1*, *2F11*, *C5*, *D8*, *G10*, *C11*, *C12* represent the eight strains of monoclonal IBDV-VP5-e pressing Vero cells. *Bar* 20 μm (original magni cation 40×)



. CEF cells infected b the VP5¯/IBDV chimeric virus e press VP4, but not VP5. Immuno uorescence staining as used to detect protein e pression. CEFs infected ith VP5¯/IBDV ere stained ith mouse anti-VP5 mAb. CEFs infected ith VP5¯/IBDV ere stained ith mouse anti-VP4 mAb. Parental IBDV-infected CEF cells ere stained ith mouse anti-VP5 mAb. The nucleus as stained ith 4',6-diamidino-2-phen lindole (*blue*). *Bar* 20 μm (original magni cation ×40)

FACSCalibur™ o c tometer using Cellquest™ softare (BD, Pharmingen). Uninfected cells incubated ith VP5 mAb and PE-conjugated mouse IgG1 (SBA) ere used as parallel controls.

Generation, characteri ation and reactivit of mAbs against VP5 protein of IBDV

BALB/c mice ere immuni ed ith the puri ed rVP5 protein e pressed in *E. coli*. After cell fusion and screening, using the puri ed rVP5 protein as ELISA-coating antigen, eight h bridomas secreting mAbs to rVP5 ere established and designated 1B2, 1B9, 2E6, 2E8, 3A4, 4B1, 4B7 and 5C2. As sho n in Fig. 2, IFA anal sis indicated that all eight mAbs recogni ed the VP5 protein ithin IBDV-infected CEF (Fig. 2a) and Vero cells (Fig. 2b). Moreover, mock-infected CEF and Vero cells ere not recogni ed b these mAbs. These anal ses con rmed that the rVP5-derived mAbs could recogni e the native VP5 protein of IBDV. Further estern blot anal sis demonstrated that all of the eight mAbs speci call recogni ed the puri ed rVP5 protein (Fig. 3), but none of these antibodies sho ed an reaction against cell l sate of *E. coli* BL21 (DE3) ith pET-28a. Immunoglobulin subt pe anal sis indicated that the heav and light chains of all mAbs ere IgG1 and κ, respectivel .

Generation of monoclonal Vero cells e pressing VP5 protein of IBDV

After transfection ith the pCI-neo-VP5 plasmid, the majorit of Vero cells died b 7 8 da s after inoculation in MEM containing 500 μg/mL G418. Surviving cells

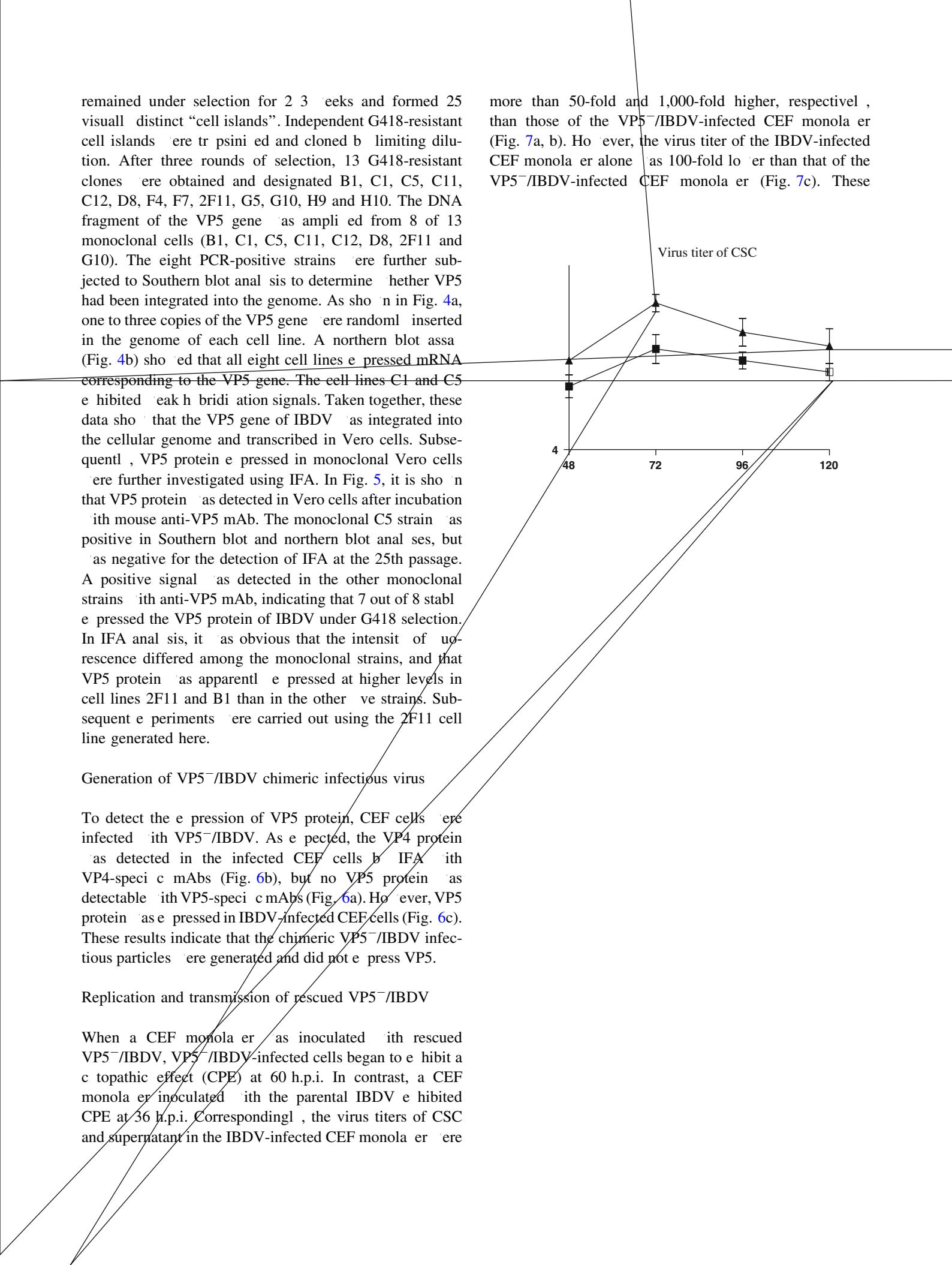
remained under selection for 2 3 eeks and formed 25 visuall distinct “cell islands”. Independent G418-resistant cell islands ere tr psini ed and cloned b limiting dilution. After three rounds of selection, 13 G418-resistant clones ere obtained and designated B1, C1, C5, C11, C12, D8, F4, F7, 2F11, G5, G10, H9 and H10. The DNA fragment of the VP5 gene as ampli ed from 8 of 13 monoclonal cells (B1, C1, C5, C11, C12, D8, 2F11 and G10). The eight PCR-positive strains ere further subjected to Southern blot anal sis to determine hether VP5 had been integrated into the genome. As sho n in Fig. 4a, one to three copies of the VP5 gene ere randoml inserted in the genome of each cell line. A northern blot assa (Fig. 4b) sho ed that all eight cell lines e pressed mRNA corresponding to the VP5 gene. The cell lines C1 and C5 e hibited eak h bridi ation signals. Taken together, these data sho that the VP5 gene of IBDV as integrated into the cellular genome and transcribed in Vero cells. Subsequentl , VP5 protein e pressed in monoclonal Vero cells ere further investigated using IFA. In Fig. 5, it is sho n that VP5 protein as detected in Vero cells after incubation ith mouse anti-VP5 mAb. The monoclonal C5 strain as positive in Southern blot and northern blot anal ses, but as negative for the detection of IFA at the 25th passage. A positive signal as detected in the other monoclonal strains ith anti-VP5 mAb, indicating that 7 out of 8 stabl e pressed the VP5 protein of IBDV under G418 selection. In IFA anal sis, it as obvious that the intensit of uorescence differed among the monoclonal strains, and that VP5 protein as apparentl e pressed at higher levels in cell lines 2F11 and B1 than in the other ve strains. Subsequent e periments ere carried out using the 2F11 cell line generated here.

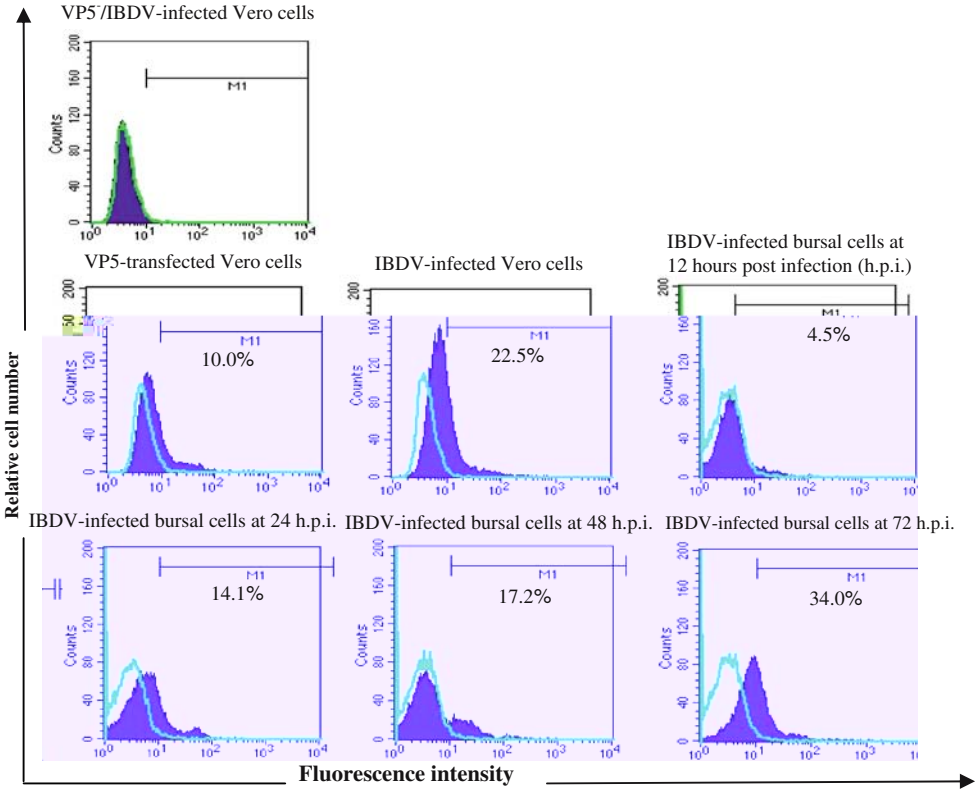
Generation of VP5⁻/IBDV chimeric infectious virus

To detect the e pression of VP5 protein, CEF cells ere infected ith VP5⁻/IBDV. As e pected, the VP4 protein as detected in the infected CEF cells b IFA ith VP4-speci c mAbs (Fig. 6b), but no VP5 protein as detectable ith VP5-speci c mAbs (Fig. 6a). Ho ever, VP5 protein as e pressed in IBDV-infected CEF cells (Fig. 6c). These results indicate that the chimeric VP5⁻/IBDV infectious particles ere generated and did not e press VP5.

Replication and transmission of rescued VP5⁻/IBDV

When a CEF monola er as inoculated ith rescued VP5⁻/IBDV, VP5⁻/IBDV-infected cells began to e hibit a c topathic effect (CPE) at 60 h.p.i. In contrast, a CEF monola er inoculated ith the parental IBDV e hibited CPE at 36 h.p.i. Correspondingl , the virus titers of CSC and supernatant in the IBDV-infected CEF monola er ere more than 50-fold and 1,000-fold higher, respectivel , than those of the VP5⁻/IBDV-infected CEF monola er (Fig. 7a, b). Ho ever, the virus titer of the IBDV-infected CEF monola er alone as 100-fold lo er than that of the VP5⁻/IBDV-infected CEF monola er (Fig. 7c). These





. Flo c tometr anal sis demonstrates that VP5 protein is e pressed in IBDV-infected cells. VP5 e pression in VP5⁻/IBDV- and IBDV-infected Vero cells, VP5-transfected monoclonal Vero cells and bursal cells of IBDV-infected chickens as assa ed b single-color o c tometric anal sis. Cells ere stained as described ith anti-VP5 mAb follo ed b PE-conjugated goat anti-mouse IgG. Mock-infected Vero cells served as negative controls (*green line*). The data sho n are from a single representative e periment of three independent e periments)

results demonstrated that the absence of VP5 protein e pression postpones and minimi es the cellular replication of IBDV and speci call inhibits the release of IBDV virions from infected cells.

Flo c tometric anal sis of VP5 protein in infected cells

The e pression of VP5 protein in VP5⁻/IBDV- and IBDV-infected Vero cells, VP5-transfected monoclonal Vero cells and bursal cells of IBDV-infected chickens as assessed b the FCM technique. As sho n in Fig. 8, VP5⁻/IBDV- and mock-infected Vero cells had no VP5 e pressing ($VP5^+$) populations. Ho ever, $VP5^+$ cell populations ere identi ed in VP5-transfected and parental IBDV-infected Vero cells. This con rmed that VP5 as detectable on the surface of IBDV-infected Vero cells. To further investigate hether VP5 protein molecules ere present on the surface of bursal cells in IBDV-infected chickens, bursal single-cell suspensions ere prepared at several different time points p.i. The data sho n in Fig. 8 illustrate that the percentage of $VP5^+$ cells as 4.5 0.5% at 12 h p.i. and reached a peak (34.0 1.6%) at 72 h p.i. These data indicate that the number of $VP5^+$ bursal cells increased graduall in IBDV-infected chickens during the rst three da s of IBDV infection.

In the present stud , e produced mAbs to the VP5 protein of IBDV using recombinant VP5 protein e pressed in *E. coli.* These mAbs recogni ed native VP5 molecules e pressed in IBDV-infected cells, con rming that the are capable of binding naturall e pressed VP5 epitopes, and the mAbs provide an important tool for FCM of IBDV-infected cells.

Cell lines that e press viral proteins are an important tool for understanding the protein's activit . Here, e obtained eight monoclonal Vero cell lines ith one to three copies of the full-length VP5 gene inserted into the cellular genome, as sho n b Southern blot (Fig. 4a). Subsequent IFA revealed that one of these monoclonal cell lines as not recogni ed b the anti-VP5 mAb, leading to the h pothesis that transcription of the VP5 gene is follo ed b post-transcriptional gene silencing (PTGS) in transfected cells. PTGS acts to degrade e isting RNA and is a common defense mechanism against invasive nucleic acids in a broad range of organisms. It is also used to regulate controlled e pression of endogenous genes [24]. We have previousl demonstrated PTGS in transgenic potatoes [29, 30].

It is essential to understand cellular pathogenesis after virus infection in order to develop effective viral disease

control. The formation of cellular lesions results from a series of interactions bet een viral components and virus-sensitive cells, and virus-induced cell death pla s an important role in the pathogenesis of virus infection. A nonstructural protein of IBDV, VP5, is a highl conserved, basic, c steine-rich class II membrane protein [11, 16] that has been sho n to induce cell death or apoptosis hen transientl overe pressed in BSC40, RP9 or CEF cells [11, 26]. In this stud , ho ever, seven monoclonal cell lines stabl e pressing VP5 under G418 selection did not undergo cell death or morphological changes, suggesting that the VP5 protein is not responsible for the induction of these processes. Moreover, in our e periments, VP5-protein-de cient IBDV as successfull recovered and produced a dela of c topathic effect (CPE) in the infected cells, demonstrating that VP5 is not essential for viral replication but is relevant to the c topathogenicit of IBDV. Instead, e observed that the virus titer is decreased in the supernatant of VP5⁻/IBDV-infected cells (Fig. 7b), indicating that the VP5 protein promotes the release of mature IBDV virions from infected cells, as suggested b Yao and Vakharia [26]. Besides, b rescuing the infectious clones of ild-t pe and VP5-de cient IBDV, Yao et al. [26] suggested that the VP5 protein of IBDV is involved in apoptosis of infected cells, but this as dif cult to con rm in our monoclonal Vero cell lines stabl e pressing VP5 protein of IBDV, impl ing that the c topathogenicit of IBDV-infected cells did not involve the VP5 protein of IBDV.

Generall , the lack of VP5 e pression does not block intracellular virus production but does signi cantl hinder the release of infectious virus from infected cells. Using monoclonal Vero cell lines e pressing the VP5 protein, it as con rmed that the VP5 protein is not responsible for the induction of c topathogenicit . In addition, anti-IBDV VP5-speci c mAbs have been generated as reagents for FCM anal sis to nel map target cells.

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1. A ad AA, Barrett SA, Fahe KJ (1985) The characteri ation and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. Virolog 143:35 44
2. Bottcher B, Kiselev NA, Stel'Mashchuk VY, Perevo chikova NA, Borisov AV, Cro ther RA (1997) Three-dimensional structure of infectious bursal disease virus determined b electron cr omicroscop . J Virol 71:325 330
3. Dobos P, Hill BJ, Hallett R, Kells DT, Becht H, Teninges D (1979) Bioph sical and biochemical characteri ation of ve animal viruses ith bisegmented double-stranded RNA genomes. J Virol 32:593 605
4. Hudson PJ, McKern NM, Po er BE, A ad AA (1986) Genomic structure of the large RNA segment of infectious bursal disease virus. Nucleic Acids Res 14:5001 5012
5. Ismail NM, Saif YM, Moorhead PD (1988) Lack of pathogenicit of ve serot pe 2 infectious bursal disease viruses in chickens. Avian Dis 32:757 759
6. Ismail NM, Saif YM (1991) Immunogenicit of infectious bursal disease viruses in chickens. Avian Dis 35:460 469
7. Jack ood DH, Saif YM (1987) Antigenic diversit of infectious bursal disease viruses. Avian Dis 31:766 770
8. Kaerber G (1931) Beitrag ur kollektiven Behandlung pharmakologischer Reihenversuche. Arch E p Pathol Pharmakol 162:480 483
9. Kibenge FS, Dhillon AS, Russell RG (1988) Biochemistr and immunolog of infectious bursal disease virus. J Gen Virol 69(Pt 8):1757 1775
10. Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibod of prede ned speci cit . Nature 256:495 497
11. Lombardo E, Maraver A, Espinosa I, Fernande -Arias A, Rodrigue JF (2000) VP5, the nonstructural pol peptide of infectious bursal disease virus, accumulates ithin the host plasma membrane and induces cell l sis. Virolog 277:345 357
12. McFerran JB, McNult MS, McKillop ER, Connor TJ, McCracken RM, Collins DS, Allan GM (1980) Isolation and serological studies ith infectious bursal disease viruses from fo l, turke s and ducks: demonstration of a second serot pe. Avian Pathol 9:395 404
13. Muller H, Scholtissek C, Becht H (1979) The genome of infectious bursal disease virus consists of t o segments of double-stranded RNA. J Virol 31:584 589
14. Muller H, Becht H (1982) Bios nthesis of virus-speci c proteins in cells infected ith infectious bursal disease virus and their signi cance as structural elements for infectious virus and incomplete particles. J Virol 44:384 392
15. Mundt E, Vakharia VN (1996) S nthetic transcripts of double-stranded Birnavirus genome are infectious. Proc Natl Acad Sci USA 93:11131 11136
16. Mundt E, Kollner B, Kret schmar D (1997) VP5 of infectious bursal disease virus is not essential for viral replication in cell culture. J Virol 71:5647 5651
17. Sambrook J, Russell DW (2001) Southern blotting and northern h bridi ation. Cold Spring Harbor Laborator Press, Ne York
18. Schroder A, van Loon AA, Goovaerts D, Teifke JP, Mundt E (2001) VP5 and the N terminus of VP2 are not responsible for the different pathot pe of serot pe I and II infectious bursal disease virus. J Gen Virol 82:159 169
19. Shai Y (1995) Molecular recognition bet een membrane-spanning pol peptides. Trends Biochem Sci 20:460 464
20. Tacken MG, Van Den Beuken PA, Peeters BP, Thomas AA, Rottier PJ, Boot HJ S0(Hom)-3308.7(J,)-3in-49as PJ,-251.657

discriminating pathogenic and nonpathogenic IBDV infection. Mol Immunol 46:1964 1969
24. Wassenegger M (2002) Gene silencing. Int Rev C tol 219:61 113
25. Yao K, Good in MA, Vakharia VN (1998) Generation of a mutant infectious bursal disease virus that does not cause bursal lesions. J Virol 72:2647 2654
26. Yao K, Vakharia VN (2001) Induction of apoptosis in vitro b the 17-kDa nonstructural protein of infectious bursal disease virus: possible role in viral pathogenesis. Virolog 285:50 58
27. Zheng X, Hong L, Li Y, Guo J, Zhang G, Zhou J (2006) In vitro e pression and monoclonal antibod of RNA-dependent RNA pol merase for infectious bursal disease virus. DNA Cell Biol 25:646 653
28. Zheng X, Hong L, Shi L, Guo J, Sun Z, Zhou J (2008) Proteomics anal sis of host cells infected ith infectious bursal disease virus. Mol Cell Proteomics 7:612 625
29. Zhou JY, Wu JX, Cheng LQ, Zheng XJ, Gong H, Shang SB, Zhou EM (2003) E pression of immunogenic S1 gl coprotein of infectious bronchitis virus in transgenic potatoes. J Virol 77:9090 9093
30. Zhou JY, Cheng LQ, Zheng XJ, Wu JX, Shang SB, Wang JY, Chen JG (2004) Generation of the transgenic potato e pressing full-length spike protein of infectious bronchitis virus. J Biotechnol 111:121 130
31. Zhou JY, Wang JY, Chen JG, Wu JX, Gong H, Teng QY, Guo JQ, Shen HG (2005) Cloning, in vitro e pression and bioactivit of duck interleukin-2. Mol Immunol 42:589 598
32. Zhou JY, Ye JX, Ye WC, Chen QX, Zheng XJ, Guo JQ (2005) Antigenic and molecular characteri ation of infectious bursal disease virus in China. Prog BiochemBioph s 32:37 45