Chimeras in noncoding regions between serotypes I and II of segment A of infectious bursal disease virus are viable and show pathogenic phenotype in chickens

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Two serotypes, I and II, have been identified for infectious bursal disease virus (IBDV), a member of the family Birnaviridae. Here, the generation by reverse genetics of IBDV chimeras in segment A of the bisegmented genome is reported. The 5- and 3′-noncoding regions (NCRs) of a serotype II strain were exchanged with the NCRs of a full-length cDNA clone of segment A of a serotype I strain. Isolated chimeric viruses were characterized in cell culture and susceptible chickens. The results show that IBDV chimeras in segment A were able to replicate in cell culture and that VP1 encoded by a serotype I segment B is functionally active with serotype I NCRs as well as with serotype II NCRs. Chimeric viruses infected susceptible chickens and caused mild depletion of bursal cells. Thus, the noncoding regions of segment A are not responsible for the different pathotypes of IBDV serotypes I and II.

Introduction

Infectious bursal disease was first described in chickens by Cosgrove (1962). The causative agent of this highly contagious, immunosuppressive disease is infectious bursal disease virus (IBDV), specifically strains belonging to serotype I. A second serotype (serotype II), isolated from fowl, turkeys and ducks (McFerran et al., 1980), is apathogenic for chickens. The serotypes can be differentiated by cross-neutralization assays (McFerran et al., 1980). IBDV belongs to the genus Avibirnavirus of the family Birnaviridae (Dobos et al., 1995). The genome consists of two segments of double-stranded RNA, A and B, which are localized within a single-shelled icosahedral capsid of approximately 60 nm diameter. The complete genomic sequences of both segments of three serotype I and one serotype II strains of IBDV are known (Mundt & Müller, 1995). The larger segment, A, encodes a polyprotein of approximately 110 kDa, which is cleaved autoproteolytically (Hudson et al., 1986) to form the viral proteins VP2, VP3 and VP4. A second open reading frame (ORF) preceding and partially overlapping the polyprotein gene has been identified (Spies et al., 1989; Bayliss et al., 1990). A protein encoded by this ORF, designated VP5, is present in IBDV-infected chicken embryo cells (CEC) as well as bursal cells of IBDV-infected chickens (Mundt et al., 1995). Segment B encodes the 97 kDa protein VP1, which represents the putative viral RNA-dependent RNA polymerase (Spies et al., 1987).

Serotype I strains are pathogenic, since they cause lesions by lymphocytic depletion in the bursa of Fabricius (BF) of susceptible chickens. Serotype II strains lack the ability to induce lesions in the BF and are therefore not pathogenic for susceptible chickens (Ismail et al., 1988). The reasons for these different biological properties are unknown. Nieper & Müller (1996) showed binding of serotype I as well as serotype II virus particles to lymphoid cells such as bursal, thymic and spleen cells. This indicates that restriction of IBDV to lymphoid B cells might not be determined by the presence of specific receptors, but might be due to alternative regulatory elements, possibly involved in regulation of replication. The 5′-noncoding regions (NCRs) of segment A of strains of the two serotypes differ in 13 of 96 nucleotides and the 3′-NCRs differ in four of 93 nucleotides (Mundt & Müller, 1995). The differences in the 5′-NCRs led to a different calculated RNA secondary structure (Mundt & Müller, 1995), which might represent such a regulatory element involved in the regulation of replication. In members of the Picornaviridae, changes in the 5′-NCR can influence attenuation (Kawamura et al., 1989) and can also enhance cap-independent translation in vivo (Martinez-Salas et al., 1993). An influenza A virus with a chimeric neuraminidase...
gene containing the 5' - and 3'-NCRs of influenza B virus showed markedly reduced mortality in mice (Muster et al., 1991). The influence of the different NCRs of segment A of IBDV was investigated by the generation of chimeric viruses containing exchanged NCRs of segment A by using the reverse genetics system for IBDV (Mundt & Vakharia, 1996). Virus that was recovered was subsequently characterized in vitro and in vivo.

Methods

- **Cells.** CEC derived from embryonated, specific-pathogen-free (SPF) eggs (VALO, Lohmann, Cuxhaven, Germany), grown in Dulbecco’s minimal essential medium (DMEM) supplemented with 10% foetal calf serum (FCS), were used for growth kinetics and for immunofluorescence tests. Transfection experiments were performed on quail muscle cells (QM-7, ATCC, CRC, 1962) grown in medium 199 supplemented with 10% FCS. Buffalo green monkey (BGM, ECACC 90092601) cells were grown in MEM supplemented with 10% FCS and used for plaque assays. Vero cells grown in MEM supplemented with 10% FCS were used for propagation of recovered virus.

- **Construction of IBDV chimeras of segment A of serotypes I and II.** For construction of a chimeric segment A, a full-length clone of strain D78 (pAD78/EK; Mundt et al., 1997) was used. Fragments containing the 5' - and 3'-NCRs of segment A of serotype II strain 23/82 were generated by PCR with plasmid pAD78/EK and two primer pairs (Table 1). For the 5'-NCR of segment A, an oligonucleotide (5'-STIIA; Table 1) specifying the complete NCR of serotype II strain 23/82 up to the start codon of VP5 (Mundt & Müller, 1995) and a second oligonucleotide (A15; Table 1) localized at the 5'-part of the polyprotein gene were used for PCR amplification. The resulting PCR fragment was cloned blunt-ended and sequenced. A plasmid containing an appropriate insert (p5'-STII-D78A) was used for a second PCR to place the T7 RNA polymerase promoter immediately upstream of the viral sequence. To this end, an oligonucleotide containing the promoter sequence and parts of the 5'-NCR of segment A (FKA5'; Table 1) was combined with oligonucleotide A15 for PCR amplification. The PCR fragment was cloned and sequenced and an appropriate plasmid (pT7–5'-STII-D78A) was selected. A unique restriction endonuclease cleavage site (RsrII) present in segment A and a unique EcoRI cleavage site in the oligonucleotide FKA5' as well as in pAD78/EK were used for insertion of the appropriate part of pT7–5'-STII-D78A into pAD78/EK, resulting in plasmid pFL5'-D78A. The 3'-NCR of segment A of serotype II strain 23/82 was generated by PCR with an oligonucleotide containing the serotype II 3'-NCR (STIIA-3'; Table 1) and a second oligonucleotide (A40; Table 1) localized at the 3'-part of the polyprotein gene. After cloning and sequencing of the PCR fragment (pD78A-STII-3'), an appropriate insert was ligated into BglII/BstGI-cleaved pAD78/EK (pFLD78A-3'). An appropriate fragment of pD78A-STII-3' was cloned into pFL5'-D78A to generate a plasmid containing the 5'- and 3'-NCRs of serotype II strain 23/82 and the coding region of serotype I strain D78 (pFL5'-D78A-3'). The plasmids are depicted in Fig. 1.

- **Virus recovery from cRNA.** For in vitro transcription of RNA, plasmids were linearized by cleavage with BglII (pAD78/EK, pFLD78A-3', pFL5'-D78A and pFL5'-D78A-3'; Fig. 1) or PstI (pBP2; Mundt & Vakharia, 1996). Further treatment of linearized DNA and transcription and transfection into QM-7 cells were carried out as described by Mundt & Vakharia (1996). Two days after transfection, cells were frozen and thawed and centrifuged at 700 g to remove cellular debris. The resulting supernatants were clarified further by filtration through 0.45 μm filters and stored at −70°C.

- **Propagation of chimeric virus.** Virus progeny recovered from QM-7 cells transfected with pAD78/EK/pBP2 (IBDV/EK), pFLD78A-3'/pBP2 (IBDV-3'), pFL5'-D78A/pBP2 (5'-IBDV) or pFL5'-D78A-3'/pBP2 (5'-IBDV-3') were passaged once on Vero cells and the presence of IBDV antigen was detected by immunofluorescence assay (IFA) by using rabbit anti-IBDV serum (Mundt et al., 1995). For propagation of the chimeric viruses, transfection supernatants were passaged once on Vero cells until a CPE was visible. Vero cells were frozen and thawed and scraped off the flask and the supernatants obtained after low-speed centrifugation were titrated on BGM cells as described previously (Hassan et al., 1996). Virus stocks were stored at −70°C.

- **Characterization of chimeric viruses in cell culture.** To assay the replication of chimeric viruses, growth kinetics were established.

| Table 1. Oligonucleotides used for amplification of the genomic 5'- and 3'-NCRs |

Sequences and locations of the oligonucleotide primers used for constructs of chimeric segments A. Restriction sites used are highlighted in bold. The underlined nucleotides are virus specific. The T7 promoter is shown in italics. The positions where the primers bind (nucleotide number) are in accordance with the published sequence of strain P2 (Mundt & Müller, 1995).

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Characterization of chimeric viruses in chickens.

Seventy-five 2-week-old SPF chickens (Intervet) were divided randomly into five groups. Each group was maintained in negative-pressure, filtered-air isolators. Chickens were infected via eye drop with 10° TCID₅₀ virus of IBDV serotype or 5°-IBDV-3’ at an m.o.i. of 1. After incubation at room temperature for 1 h, the inoculum was removed and the cells were rinsed twice with PBS and overlaid with 1 ml DMEM. The supernatant was removed immediately from one well and stored at −70 °C [0 h post-infection (p.i.)]. The remaining wells were incubated and supernatants were removed at 8, 12, 24, 36 and 48 h p.i. and stored at −70 °C. Supernatants were centrifuged and tiritated on BGM cells.

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Histopathology of bursae. Formalin-fixed samples of BF were embedded in paraffin (Paraplast), sectioned and stained with haematoxylin and eosin (H&E). Microscopic bursal lesion score (BLS) was determined to be the reciprocal of the highest dilution, expressed in log₁₀, in which there was no visible CPE. Positive and negative controls, as well as internal serum standards with different concentrations of antibodies against IBDV, were run at all times.

Analysis of NCRs of segment A of virus reisolated after passage in chickens. For investigation of the NCRs of segment A after passage in chickens, virus-containing supernatants of CEC showing CPE during passage of the bursa homogenate were analysed. For this purpose, groupwise-pooled aliquots of bursa homogenates were used for the presence of virus in the BF. To this end, QB-7 cells were infected with the supernatant obtained and assayed for the presence of IBDV antigen 24 h p.i. by using indirect IFA (Mundt et al., 1995). Supernatants that tested positive were titrated on BGM cells. Titres were calculated per gram bursal tissue.

Confluent secondary CEC grown in a 24 well tissue-culture dish were infected with IBDV/EK, IBDV-3’, 5°-IBDV or 5°-IBDV-3’ at an m.o.i. of 1. After incubation at room temperature for 1 h, the inoculum was removed and the cells were rinsed twice with PBS and overlaid with 1 ml DMEM. The supernatant was removed immediately from one well and stored at −70 °C [0 h post-infection (p.i.)]. The remaining wells were incubated and supernatants were removed at 8, 12, 24, 36 and 48 h p.i. and stored at −70 °C. Supernatants were centrifuged and tiritated on BGM cells.

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Analysis of chicken serum. Chicken sera were analysed by Western blot. For Western blot, virus particles of IBDV serotype I strain P2 (Schobries et al., 1977) were used that had been purified by ultracentrifugation as described previously (Müller et al., 1986). Two µl purified IBDV particles was separated by SDS–PAGE and blotted onto nitrocellulose membrane (Schleicher & Schüll). Groupwise-pooled chicken sera were used as the first antibody in different dilutions. After incubation with the second antibody (alkaline phosphatase-conjugated anti-species serum, Sigma), the reaction was visualized by using BCIP/NBT (Roth).

To test the ability of the chimeric viruses to induce neutralizing antibodies, a virus-neutralizing (VN) test was performed. The VN test was based on the tests described by Skeeles et al. (1979) and Lütticken & Cornelissen (1981) with modifications. Briefly, serum was serially diluted twofold in a constant amount (750 TCID₅₀/100 µl) of virus (strain D78, Intervet). To this end, 100 µl of the diluted virus solution, containing 750 TCID₅₀/100 µl, was placed in each well of a microtitre plate with the exception of the first well in each row, which was left empty. Next, 100 µl serum was added to the empty first well of each row. One hundred µl of a virus suspension containing 1500 TCID₅₀/100 µl was then added to the serum-containing wells in the first well of each row. After mixing the virus and serum, resulting in a virus concentration of 750 TCID₅₀/100 µl in the first well of each row, serial dilutions were made by transferring 100 µl per well to the next well of each row. After incubation for approximately 1–5 h, 100 µl per well of a CEC suspension (0.5–1.0 × 10⁶ cells/ml) was added and the microtitre plates were covered and incubated at 37 °C for 5–7 days. After incubation, wells were scored for the presence of a CPE. The end-point of the VN test for a serum sample was determined to be the reciprocal of the highest dilution, expressed in log₁₀, in which there was no visible CPE. Positive and negative controls, as well as internal serum standards with different concentrations of antibodies against IBDV, were run at all times.

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using oligo(dGTP) tailing for the 5'–NCR and poly(A) tailing followed by RT–PCR for the 3'–NCR (Mundt & Müller, 1995), using oligonucleotides shown in Table 1. The resulting PCR fragments were cloned blunt-ended and sequenced. Sequences were analysed by using the Wisconsin package, version 8 (Genetics Computer Group, Madison, WI, USA).

Results

Recovery of chimeric IBDV

For transfection experiments, a full-length cDNA clone of segment A of serotype 1 strain D78 (pAD78/EK) or chimeric segments A (pFLD78A-3', pFL5'-D78A, pFL5'-D78A-3') was transcribed into plus-strand cRNA and co-transfected with segment B (pBP2) full-length plus-strand cRNA into QM-7 cells. To validate that replicating virus was generated, secondary CEC were infected with transfection supernatant. At 24 h p.i., cells were fixed with acetone and incubated with rabbit anti-IBDV serum and fluorescein-conjugated secondary antibody. IBDV antigen was detected in the cytoplasm of cells infected with supernatants of all cRNA-transfected cells. Cells infected with supernatants of mock-transfected cells showed no specific fluorescence (data not shown). The resulting virus progeny IBDV/EK, IBDV-3', 5'-IBDV and 5'-IBDV-3' were passaged once on Vero cells and titrated. Titres were

Replication of chimeric IBDV in CEC

In order to compare replication of the different chimeric IBDV in more detail, growth kinetics were analysed (Fig. 2). Supernatants of confluent secondary CEC infected with IBDV/EK, IBDV-3', 5'-IBDV or 5'-IBDV-3' were removed at 0, 8, 12, 24, 36 and 48 h p.i. and titrated on BGM cells. Titres produced by IBDV containing the 5'-NCR of serotype I (IBDV/EK, IBDV-3') were about 0.5 log₁₀ higher than those of the IBDV containing the 5'-NCR of serotype II (5'-IBDV, 5'-IBDV-3') at 12 h p.i. From 12 h p.i. onwards, no significant differences could be observed between the four chimeric IBDV analysed.

Replication of chimeric IBDV in chickens

In order to investigate the properties of chimeric IBDV in chickens, animal experiments were performed. Neither clinical signs nor macroscopic abnormalities of the BF during necropsy could be observed. H&E-stained bursal sections were also analysed for lesions to determine a bursal lesion score (BLS). Bursae of chickens infected with the different IBDV showed depletion of bursal cells in bursal follicles. In affected bursal follicles, necrosis of lymphocytes and infiltration by heterophils was detected at 3 and 7 days p.i. A transition from acute into chronic lesions in the affected bursal follicles, characterized by activity of macrophages and early fibrosis, was observed from 7 to 13 days p.i. Furthermore, a loss of distinction between the bursal cortex and medulla was detected. All chimeric viruses induced mild bursal lesions by day 7 p.i. (Fig. 3A–D). IBDV/EK and 5'-IBDV-3' caused bursal lesions in chickens at 3 days p.i. Bursae of chickens infected with IBDV-3' or 5'-IBDV showed no depletion at day 3 p.i. Lesions were observed...
in bursae of chickens infected with IBDV/EK, IBDV-3’, 5′-IBDV and 5′-IBDV-3’ at day 7 p.i. At this point, most bursae examined showed a BLS of 1. At 13 days p.i., bursae with lymphoid depletion were detected in all groups of infected chickens. Neither depletion of bursal cells nor signs of inflammation were observed in negative controls (data not shown). The BLS of each chicken is shown in Table 2.

The presence of chimeric IBDV in supernatants of homogenized bursal samples was assayed by indirect IFA using QM-7 cells. Cell cultures infected with supernatants of bursal samples of infected chickens necropsied on days 3 and 7 p.i. showed specific fluorescence in IFA. Bursal samples of infected chickens necropsied at day 13 p.i. and non-infected controls (data not shown) caused no specific fluorescence in QM-7 cells. To estimate the titre in p.f.u./g bursal tissue, infectivity assays of bursal homogenates of each positive chicken were performed by plaque assay. As summarized in Table 2, the highest titres in homogenized bursal tissue were detected on day 3 p.i. Titres decreased until day 7 p.i. and no virus was detectable on day 13 p.i. The titres were not significantly different, since considerable variations were observed within each group and day p.i.

**Analysis of NCRs**

In order to confirm the identity of the chimeric virus in tissue culture supernatants, reisolated viruses were analysed by RT–PCR. Sequence analysis of the amplified fragments revealed that clones specific for both ends differed in size. Therefore, sequencing of cDNA clones was performed until at least four clones with the longest nucleotide sequence had been analysed. Sequence analysis showed that nearly all IBDV sequences were 100% identical to the parental plasmids (Fig. 4). No nucleotide substitutions was detected in the 5′-NCRs of IBDV/EK or IBDV-3’. Single clones contained single nucleotide substitutions, e.g. in the 3′-NCR of IBDV-3’, one of 10 clones showed a C^3226^A substitution. One mutation detected in the in the 3′-NCRs of IBDV/EK and 5′-IBDV (C^3227^T) represented a mutation from C (serotype I strain D78) to T (serotype II strain 23/82). However, no substitution was a revertant mutation to the serotype I sequence. Since plus-sense RNAs transcribed from plasmids containing segment A have four additional nucleotides at the 3′-end, no nucleotide substitutions were detected in the 5′-NCRs of IBDV/EK and 5′-IBDV. Since plus-sense RNAs transcribed from plasmids containing segment A have four additional nucleotides at the 3′-endfo linearization with BsrGI, it was interesting to analyse whether the vector-derived nucleotides were retained. Sequence data for the 3′-ends of all four genomic segments A showed that the vector-derived nucleotides were not part of the viral genomic RNA.

**Immunological reaction of chickens**

To test the immunological reaction of chickens against the chimeric viruses, groupwise-pooled sera of chickens necropsied at days 7 and 13 p.i. were tested for IBDV-specific antibodies by Western blot assays using purified IBDV particles. Specific

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* BLS of BF of each chicken investigated.
† IBDV antigen was detected by indirect IFA using rabbit anti-IBDV serum (Mundt et al., 1995). Number of positives/total examined is shown.
‡ Titres were determined by plaque-overlay assay after homogenization of samples of BF.
reactions of the sera of chickens infected with the chimeric IBDV against IBDV proteins were detected for both days investigated. No reactivity was detectable in the control group. The log₂ titres of the neutralizing antibodies were tested on day 13 p.i. Sera of chickens infected with IBDV against IBDV proteins were detected for both days investigated. No reactivity was detectable in the control group. The log₂ titres of the neutralizing antibodies were tested on day 13 p.i.

### Discussion

IBDV strains of two serotypes have been isolated. These serotypes are distinguishable by serological methods but the two serotypes also show a marked biological difference in chickens. Serotype I strains are able to replicate in proliferating B lymphocytes (Müller et al., 1986) and affect the BF, resulting in depletion of bursal follicles (Winterfield et al., 1972; Fadly & Nazerian, 1983; Mazariagos et al., 1990), whereas serotype II strains do not (Jackwood et al., 1985; Ismail et al., 1991). Thus, serotype I strains are pathogenic and serotype II strains are apathogenic in chickens. Nieper & Müller (1996) showed that IBDV particles of serotype I as well as serotype II bound to lymphoid cells. Therefore, the restriction of IBDV to lymphoid B cells might not be determined by the presence of a specific receptor. cis-acting elements located in the NCRs of IBDV might play a role in the restriction of serotype I strains to replication in B lymphocytes. Such cis-acting elements involved in regulation of replication have been shown for members of the Picornaviridae (Kawamura et al., 1989; Martinez-Salas et al., 1993), the Togaviridae (Chen & Frey, 1999) and Dianthovirus (Turner & Buck, 1999). The re-assortment of segments of serotype I and II strains in vivo and in vitro has not been described so far. The availability of a reverse genetics system for IBDV (Mundt & Vakharia, 1996) has made it possible to investigate the molecular basis of the different phenotypes of the two serotypes. One salient result was that replication of both serotypes was not restricted by the different NCRs. Thus, VP1 of a serotype I strain can act with serotype II NCRs to generate viable virus containing chimeric segment A. The observed differences in replication in tissue culture were not significant. Furthermore, exchange of the NCRs did not affect pathogenicity in chickens. All chimeric IBDV induced seroconversion and resulted in mild bursal lesions characteristic of a mildly virulent IBDV, which is a general feature of tissue culture-adapted serotype I strains (Tsai & Saif, 1992). Completely different results were described for the Orthomyxoviridae (Muster et al., 1991). Chimeric influenza A virus carrying the NCRs of influenza B virus on the neuraminidase gene showed a different phenotype in vitro and in vivo compared with the wild-type influenza A virus (Muster et al., 1991). In this case, the NCRs of influenza B virus affected replication in tissue culture and attenuated the chimeric virus, whereas in the current study the NCRs of the apathogenic serotype II strain of IBDV did not. Although the importance of the NCRs has been shown for the phenotype of other viruses, this is not the case for IBDV. The effects of regulatory elements on virulence would be better analysed by using a virulent IBDV strain. However, pathogenic field strains are not easily adapted to cell culture, a process which requires either several passages in cell culture (Hassan et al., 1996) or extensive passaging on the chorioallantoic membrane as well as in the yolk sac of embryonated eggs. Thereafter, strains show reduced pathogenicity in vivo (Yamaguchi et al., 1996;
Hassan et al., 1996). Additionally, several field isolates failed to become adapted to cell culture (McFerran et al., 1980). All four chimeric viruses described in this study are pathogenic, since they caused damage in the BF. Serotype I strains in general cause histological changes in the BF. The alterations in the BF that we observed were like those induced by naturally occurring mild serotype I strains. In conclusion, the NCRs of segment A are not responsible for the restriction of serotype I strains to replication in B lymphocytes. It is possible that binding to B lymphocytes occurs (Nieper & Müller, 1996) and that the next step necessary for a productive infection, such as penetration or uncoating of the virus, resulting in intracellular replication is inhibited. The expression of VP5 of serotype I seems to be an important factor for pathogenicity in chickens, since a VP5− mutant of a serotype I strain was completely attenuated (Yao et al., 1998), but the mutant was able to infect and replicate in B lymphocytes. It is possible that VP5 or another viral protein of serotype II strains is a factor responsible for the inhibition of replication in B lymphocytes. The determination of the 5′- and 3′-NCR sequences of the four genomic segments A showed that the substitutions detected were point mutations present in the minority of the plasmids analysed. Single mutations in the NCRs are expected for RNA viruses. No mutations from serotype II-specific to serotype I-specific nucleotides were detected. One mutation for RNA viruses. No mutations from serotype II-specific to detected were point mutations present in the minority of the four genomic segments A showed that the substitutions in the natural host will be identified. responsible for the different pathotypes of the two serotypes has no significant influence on virus replication in cell culture. & Vakharia, 1998). described recently for infectious pancreatic necrosis virus (Yao trimmed exactly before being packaged into the virion, as virus replication is not clear. The 3′ mutation was also present in the minority of the plasmids present in the sequence of the plasmids analysed. But this since a VP5− mutant of a serotype I strain was completely replication is inhibited. The expression of VP5 of serotype I penetration or uncoating of the virus, resulting in intracellular that the next step necessary for a productive infection, such as cause histological changes in the BF. The alterations in the BF that we observed were like those induced by naturally occurring mild serotype I strains. In conclusion, the NCRs of segment A are not responsible for the restriction of serotype I strains to replication in B lymphocytes. It is possible that binding to B lymphocytes occurs (Nieper & Müller, 1996) and that the next step necessary for a productive infection, such as penetration or uncoating of the virus, resulting in intracellular replication is inhibited. The expression of VP5 of serotype I seems to be an important factor for pathogenicity in chickens, since a VP5− mutant of a serotype I strain was completely attenuated (Yao et al., 1998), but the mutant was able to infect and replicate in B lymphocytes. It is possible that VP5 or another viral protein of serotype II strains is a factor responsible for the inhibition of replication in B lymphocytes. The determination of the 5′- and 3′-NCR sequences of the four genomic segments A showed that the substitutions detected were point mutations present in the minority of the plasmids analysed. Single mutations in the NCRs are expected for RNA viruses. No mutations from serotype II-specific to serotype I-specific nucleotides were detected. One mutation detected in the 3′-NCR (C<sup>2227</sup>T) of IBDV/EK and 5′-IBDV was present in the sequence of the plasmids analysed. But this mutation was also present in the minority of the plasmids analysed and therefore the importance of this mutation for the virus replication is not clear. The 3′ end of segment A was trimmed exactly before being packaged into the virion, as described recently for infectious pancreatic necrosis virus (Yao & Vakharia, 1998).

In summary, exchange of the NCRs of segment A of IBDV has no significant influence on virus replication in cell culture. The restriction of replication of IBDV in lymphoid cells of BF is not determined by the NCRs of segment A. The binding of the virus to cells and the ability to replicate within these cells seem to be two different processes. By using other engineered chimeric viruses, it is hoped that the viral genomic element responsible for the different pathotypes of the two serotypes in the natural host will be identified.

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References


bursal disease virus is not essential for viral replication in cell culture. 


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