Review Article

Infectious Bursal Disease Virus: A Review of Molecular Basis for Variations in Antigenicity and Virulence

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INTRODUCTION

Infectious bursal disease (IBD) is an acute contagious viral disease of young chickens (1,2). The etiological agent, IBD virus (IBDV), has a predilection for the cells of the bursa of Fabricius where the virus infects actively dividing and differentiating lymphocytes of the B-cell lineage (3). The chickens are susceptible to clinical disease at 3–6 wk of age. Clinical disease is characterized by inflammation of the bursa of Fabricius, hemorrhages in skeletal muscles and death. Economic losses in the poultry industry result from high mortality rates due to this acute form of the disease or from a subclinical infection in chickens below 3 wk old characterized by B-cell dependent immunodeficiency (1). The latter enhances the susceptibility of chickens to other infections and depresses the response of infected chickens to vaccines against other diseases such as Newcastle disease, Marek's disease and infectious bronchitis. Because vaccination is the principal method of viral disease control in commercial poultry worldwide (2), IBDV should be considered as one of the most important viral pathogens of the commercial poultry industry.

The virus belongs to the family Birnaviridae of the genus *Avibirnavirus* (4). Members of the family contain a double stranded (ds)RNA genome consisting of 2 segments, designated A and B, within a nonenveloped single-shelled icosahedral capsid of 60 nm diameter. These include infectious pancreatic necrosis virus (IPNV) of young salmonid fishes, tellina virus (TV), oyster virus (OV) and crab virus of bivalve molluscs in the genus *Aquabirnavirus* and Drosophila X virus (DXV) of *Culicoides* sp. in the genus *Entomobirnavirus* (4). Of the 2 recognized serotypes of IBDV, only serotype 1 strains are pathogenic and replicate in proliferating B-cells of the bursa of Fabricius. In the past few years, antigenic and pathotypic variant strains of IBDV, distinct from the standard or classical virulent serotype 1 strains were isolated from vaccinated flocks on the Delmarva peninsula in the United States (5). More recently in Europe, there were outbreaks of disease caused by very virulent (VV) strains of IBDV in the Netherlands (6), Great Britain (7), Belgium (8), Germany (9), and France (10). Since the latter part of 1990, similar cases of VV IBDV infections with more than 50% mortality in layer flocks were reported in Japan (11), Taiwan (12), Poland (13), Middle East and Northern and Southern Africa (2). Interestingly, the VV strains of IBDV, similar to the United States antigenic and pathotypic variants, caused disease even in the presence of protective maternal antibody against the classical vaccine strains (7). However, in contrast to the US variants, the VV strains produced bursal lesions and inflammation typical of classical serotype 1 strains and their enhanced virulence was not accompanied by any significant alterations in their antigenicity. The molecular basis for such virulence variations among IBDV strains has not been defined.

VIRAL GENOME STRUCTURE AND ORGANIZATION

Important clues to the virulence and antigenicity of IBDV have originated from the determination of the nucleotide sequences of the IBDV genomes of virulent and avirulent isolates and the antigenic variants of IBDV. The IBDV genome segment A (3254 bp) contains 2 open reading frames (ORF); a small ORF preceding and partially overlapping the larger ORF encodes VP5 (Fig. 1) (14–16). The function of VP5 is not known although the protein has been detected in IBDV infected cells (17). The larger ORF encodes a 109 kDa precursor polyprotein (N-VPX-VP4-VP3-C) (18,19) which is processed into 2 structural proteins VP2 (40–45 kDa) and VP3 (32–34 kDa) and the putative viral protease VP4 (28–30.5 kDa). VP2 contains the major antigenic site responsible for eliciting neutralizing antibodies (20) and VP3, the group-specific antigens (21) and a minor neutralizing site (22,23). The C-terminal region of VP3 has also been implicated in either packaging or stabilizing the RNA genome within the interior of the capsid (18). Deletion expression studies of cDNA fragments of segment A of 002-73-IBDV suggest VP4 to be the viral protease involved in the processing of the precursor polyprotein to VPX (the VP2 precursor, 47–48 kDa), VP3 and VP4 (19). Even though the active site of the viral protease has not been established, polyprotein residues H 546, D 589 and S 652 are suggested to form the catalytic triad of a serine protease (24). The dibasic residues at 453 and 723 or alternatively the repeats of the sequence A-X-A-A-S in the polyprotein are considered to be the likely protease cleavage sites (18). It is not known how VPX is processed to mature VP2. There are only minor molecular weight differences between the structural proteins of serotype 1 strains of classical and variant strains of IBDV (25).

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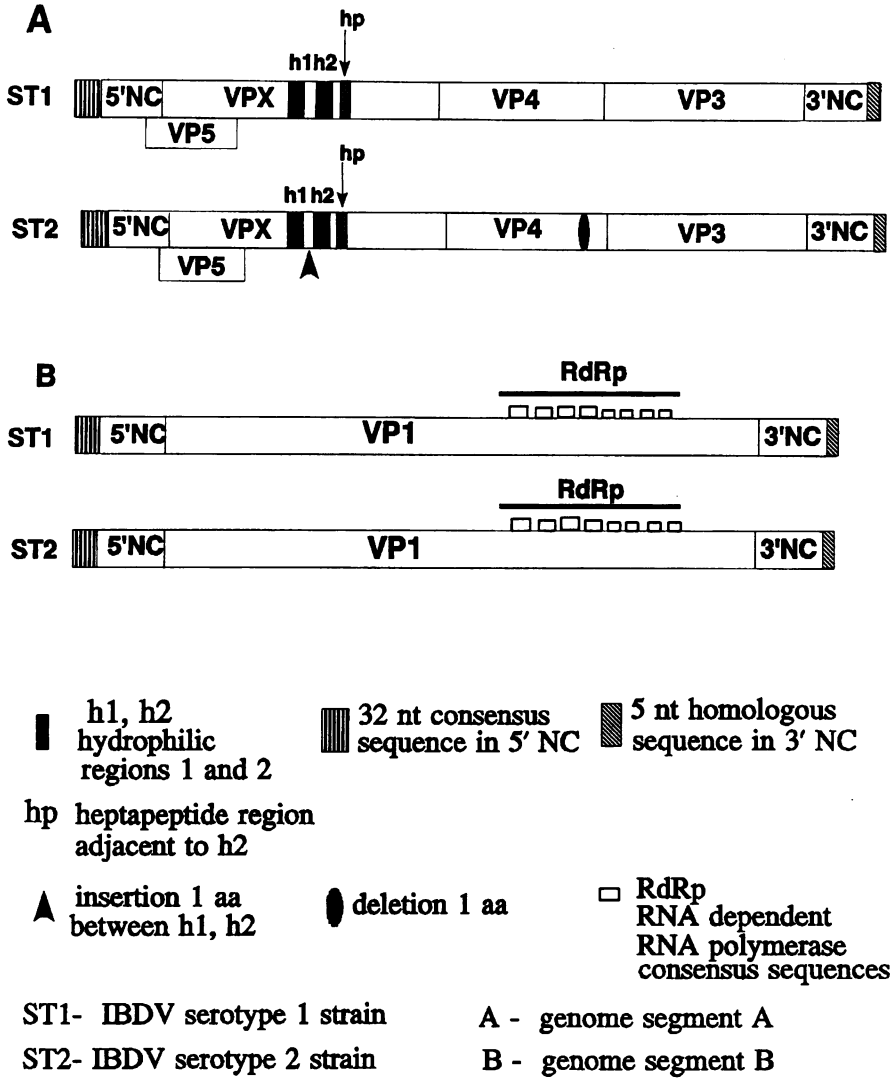

Figure 1. Genomic organization of avibirnaviruses.

The smaller genome segment B (2817 bp) encodes VP1 (90 kDa), the putative dsRNA dependent RNA polymerase (RdRp) (Fig. 1) (16,26). VP1 exists as a genome linked protein (VPg) circularizing segments A and B by tightly binding to their ends (27). In IPNV, VP1 is linked to the 5′ ends of both genome segments by a serine-5′-GMP phosphodiester bond (28). Since IBDV and IPNV behaved similarly during in vitro guanylylation reactions (29), VP1 of IBDV is also considered to be attached to a guanine residue at the 5′ terminus of the genome segments.

The nucleotide sequences of segment A of several serotype 1 strains (14,18,24,30,31), and serotype 2 IBDV strains, OH (15) and 23/82 (32), and segment B of serotype 1 strain 002-73 (33) and serotype 2 strains OH (16) and 23/82 (32) have been reported. Between the pathogenic serotype 1 and nonpathogenic serotype 2 strains that were sequenced for the coding region of segment B, there exist a high degree of nucleotide (89%) and amino acid (93–98%) sequence identities (24). The RdRp consensus sequence motifs 1-8 (26) are conserved in both IBDV serotypes. In segment A, lower nucleotide (83%–84%) and amino acid (90%) sequence identities are noted in coding regions between serotype 1 and 2 strains (15). This is mainly attributed to a hypervariable region corresponding to the serotype-specific epitope(s) in the structural protein VP2 (14,15,30).

Complete 5′ and 3′ terminal noncoding sequences of segments A and B of serotype 1 strains (SK140a, IN, P2, Cu-1, Cu-1M) and serotype 2 strains (OH, 23/82) of IBDV have been reported (16,34). The 5′ terminal sequences in both genome segments consist of a 32-nucleotide consensus sequence GGA U(A/G)C GAU (C/G)GG UCU GAA CC(C/U) C(G/U)G G(G/-)A GUC AC (Fig. 1). The 3′ terminal sequences of both segments of IBDV strains SK140a, IN and OH end with a conserved pentamer -GCGGU (16). Thus the termini of the IBDV genome segments resemble those of other segmented RNA viruses such as reovirus (35) and influenza virus (36) where both 5′ and 3′ termini are homologous between the genome segments. At the 5′ and 3′ ends in both genome segments of IBDV, there are direct terminal and inverted repeats that are likely to contain important signals for replication, transcription and packaging, and it is not known whether virulence variations are due to mutations in these regions.

The inverted adjacent repeats at the 3′ terminus on segment A and 5′ terminus on segment B have the potential to form stem and loop secondary structures (16). Stem and loop structures are involved in the processes of RNA replication, translation and encapsidation of other RNA viruses such as poliovirus (37). In segment A of IBDV, there are differences between serotype 1 and 2 strains in the predicted secondary structures formed by the 5′ noncoding region preceeding the VP5 gene; these structures may be involved in viral replication, in determining host cell-type specificity and possibly virulence (34).

Segments A and B of IBDV also contain serotype-specific nucleotide changes in the noncoding regions. When serotype 2 strains of IBDV (OH, 23/82) were compared to serotype 1 strains, there were 5 nucleotide changes in the 5′ noncoding region which were serotype-specific on segment A and 3 nucleotide changes on segment B (16). No serotype-specific changes were found in the 3′ terminal sequences of segment A whereas a single nucleotide change was identified in the 3′ terminal sequences of segment B (16).

VIRAL REPLICATION

A number of IBDV strains have been adapted to replicate and produce cytopathic effect (CPE) in primary cell cultures of chicken origin such as bursal lymphoid cells, chicken embryo kidney cells (CEK) and fibroblast (CEF) cells (38). IBDV specific polypeptides were identified in chicken bursal lymphoid cells as early as 90 min after infection and in the culture medium of such cells 6 h after infection (39). Prolonged multiplication cycle of more than 48 h was noted in mammalian cell lines such as Vero and BGM-70 cells (40,41). Moreover, of all the known birnaviruses, only IBDV is able to replicate in mammalian cells, a unique cross-species biological property (41). Incomplete IBDV particles with aberrant protein composition and which interfere with the replication of complete virus were formed by repeated passage of IBDV at high multiplicity of infection in CE-cells (42). By contrast, such interfering particles were rare when IBDV was propagated in the bursa of Fabricius in chickens (39). Both pathogenic serotype 1 and nonpathogenic serotype 2 strains replicate efficiently in CEF, but only serotype 1 strains replicate in bursal lymphoid cells. Both CEF and bursal lymphoid cells were reported to have common receptors of approximately 46 and 40 kDa for serotypes 1 and 2 strains (43). In addition, CEF had receptors specific for each serotype whereas lymphoid cells had receptors specific for serotype 2 strains only. However, the serotype 2 strains do not replicate in lymphoid cells (43).

It is of paramount importance to understand the mechanism of viral replication since the replicative ability of the virus has an influence on its virulence. RNA-dependent RNA polymerase activity is associated with IBDV particles grown in CE-cells (44). In vitro single-stranded (ss)RNA synthesis studies show that the RNA polymerase synthesizes viral ssRNA by a semi-conservative strand displacement mechanism, whereby the nascent strand displaces one of the parental strands (44,45). Two genome-length 24S mRNA hybridizing to the 2 segments were detected both in vivo (46) and in vitro (45). In both cases, birnaviruses were transcriptionally active without the need for uncoating or degradation of the capsid (44). The 24S ssRNA and 14S dsRNA are synthesized in vitro (44–46); 24S ssRNA component is believed to be the viral RNA, serving as the template for the synthesis of complementary strands to form dsRNA (46). More recent experiments indicate that virion-associated VP1 catalyzes a guanylylation reaction which serves to prime viral RNA synthesis; apparently only the plus strands of the two genome segments are synthesized in vitro which remain base-paired to their templates (47). The initiation of viral RNA synthesis is suggested to involve either 2 VP1 molecules, one serving as a primer and the other as polymerase for chain elongation or just a single VP1 molecule in both functions (47).

Regulated expression of viral genes may be essential for the multiplication of IBDV. In IBDV infected cells, 5 mature viral proteins VP1, VP2, VP3, VP4 (48) and VP5 (17) are synthesized. A precursor-product relationship has been demonstrated in the biosynthesis of VP2, VP3 and VP4 polypeptides. A two-step cleavage has been described in which a polypeptide of 50K could be chased to form 49K (VPX), the precursor of VP2 (40K) (39). Since VP2 does not accumulate intracellularly, post-translational modification of the 50K polypetide into the 40K (VP2) may occur during virus maturation and assembly (39). Similarly, a 55K to 60K polypeptide is suggested to be the precursor for VP3 and VP4 (18,19).

VIRAL IMMUNOSUPPRESSION

IBDV seems to have a predilection for actively proliferating cells such as precursor B-cells of the bursa of Fabricius than for mature B-cells (49,50), causing severe necrosis, lymphoid depletion, and subsequent immunosuppression (51). Other mechanisms of immunosuppression such as the development of suppressor cells in the spleen of infected chicks causing in vitro mitogenic hyporesponsiveness and impairment of helper T-cell function have been suggested (52,53). In vitro studies using IBDV infected chicken peripheral lymphocytes showed features typical of apoptosis (54) suggesting that IBDV, in addition to causing necrosis, can induce apoptosis in avian lymphocytes. Indeed, there was depletion of cortical thymocytes due to apoptosis following infection with a highly virulent strain of IBDV (HPS-2) (55). Chickens infected with some of the VV IBDV strains from Japan developed not only bursal lesions but also thymic and bone marrow lesions (11). High virus titres were detected not only in the bursa of Fabricius, but also in the thymus, spleen and bone marrow suggesting that these organs may also be involved in efficient replication of VV IBDV in susceptible chickens (56).

CONTROL OF IBDV

In the past, a combination of live and inactivated vaccines used in the parent breeder flocks was sufficient to induce the production of high levels of maternal antibody in the broiler progeny which prevented early infections and therefore immunosuppression. However, most intermediate vaccines are presently inadequate in providing protection against VV IBDV. Some of the less attenuated ('hot') vaccine strains with acceptable reduction of mortality are being evaluated by determining the optimum age for vaccination using a formula which predicts the decline in maternal antibody (57). With the increase in knowledge on the molecular structure and immunology of IBDV, better attenuated and genetically engineered vaccines are continually being developed. Structural protein genes of IBDV have been expressed in fowlpox and baculovirus-vector systems. VP2 from a virulent IBDV strain 52/70 expressed as a β-galactosidase fusion protein in a recombinant fowlpox virus, fpIBD 1, provided protection against mortality, but not against damage to the bursa of Fabricius (58). Recombinant FPV-VP2 containing the VP2 coding region under the control of the fowlpox early/late promoter inserted immediately downstream of the thymidine kinase gene provided considerable level of protection when challenged

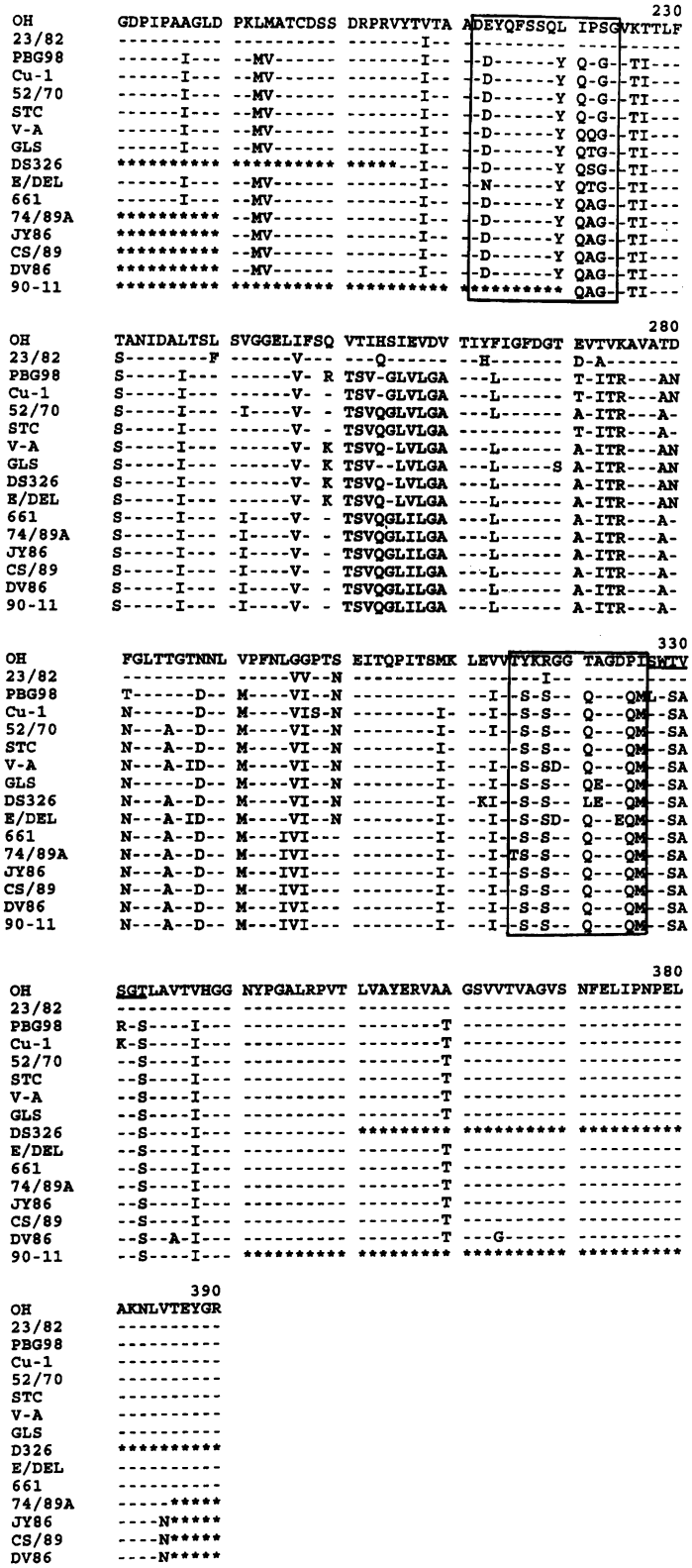

Figure 2. Deduced amino acid sequence of VP2, from amino acid position 181-390 (numbering from the sequence of segment A of serotype 2, strain OH of IBDV (15), is indicated above the amino acid sequence). Sequence of another serotype 2 strain 23/82 (32) is indicated in the upper 2nd line and they are compared to the following serotype 1 strains: STC (14), 52/70, PBG98, Cu-1 (30), V-A (68), GLS, DS326, E/DEL (31), 90-11 (11), 661, 74/89A, JY86, CS/89, DV86 (64). Hyphens denote sequences identical to IBDV strain OH, gaps represent deletion, and * represents unavailable sequence. The 2 hydrophilic regions are boxed and the heptapeptide region adjacent to the 2nd hydrophilic region is underlined.

with IBDV strain 002-73, although the level was lower than the protection provided by an oil adjuvanted inactivated whole IBDV vaccine (59). Baculovirus-expressed IBDV antigens conferred 79% protection against subclinical infection. A chimeric cDNA clone of the large segment A of an antigenic variant strain GLS, encoding VP3, VP4 and the VP2 with the B69 epitope was expressed in a recombinant baculovirus (60). When used as a vaccine in SPF chicks, it conferred protection against virulent challenge with the classical IM and STC strains and the antigenic variant strains E/DEL and GLS (61). A novel complex IBDV vaccine containing a mixture of IBDV with viral antibodies (bursal disease antibody; BDA) has been evaluated for safety and protection of chicks following subcutaneous administration (62). However, the efficacy of such vaccines in providing protection against challenge with the naturally occurring VV variants needs to be established.

ANTIGENIC AND VIRULENCE VARIATIONS

Since 1985, antigenic drift in field IBDV populations has been recognized in the United States with the isolation of several serotype 1 strains from the bursa of birds properly vaccinated with mild, live IBD vaccine (63). These virus isolates were designated variant viruses since they infected broiler chickens with relatively high levels of maternal antibodies (6). They were antigenically different from the classical strains isolated before 1985 and were highly immunosuppressive, causing rapid bursal atrophy without symptoms of clinical disease (6). Variant strains such as E/DEL (63), GLS and DS326 isolated on the Delmarva peninsula (64) were therefore both antigenic and pathotypic variants of classical virulent strains. Vaccination with variant strains of IBDV protected against the variant strains as well as the classical virulent type 1 strains (63). Since 1987, a new class of pathotypic variants has emerged in different parts of Europe and Asia constituting the very virulent strains of IBDV which cause severe damage to the bursa of

Fabricius, thymus, spleen and bone marrow and high mortality (2). Such VV IBDV strains do not show any differences in antigenicity from the classical virulent strains (64), and the underlying molecular mechanisms for their virulence variations needs further clarification.

Nevertheless, since all known serotype 2 strains of IBDV are naturally avirulent for chickens (65), considerable knowledge on the molecular basis for antigenicity and virulence has accumulated from identifying sequence differences between the naturally avirulent serotype 2 strains and the virulent serotype 1 strains (Fig. 2) (15). Previously, the majority of efforts were focused on the VP2 coding region responsible for inducing virus neutralizing (VN) antibodies (20). The greatest amount of amino acid sequence variations in VP2 among the various strains of IBDV are between amino acid residues 206 and 350 (*Acc*I-*Spe*I fragment) (Fig. 2) (14,30). This hypervariable region, of 151–152 amino acid residues long encodes the conformational epitope recognized by VN mAb 17/82 (19). Two symmetrically spaced hydrophilic regions (amino acid residues 212–224 and 314–324) are recognized in this hypervariable region (Fig. 2) (66). These hydrophilic regions and the internal sequences of VP2 are not conserved between the pathogenic serotype 1 and nonpathogenic serotype 2 strains (Fig. 2). The first hydrophilic region has been speculated to be responsible for stabilizing the conformation epitope and the 2nd hydrophilic region for recognition by VN mAbs (67).

In variant viruses such as variant E/DEL, the amino acid substitutions in the 2nd hydrophilic region appeared to enable variant viruses to escape VN by antibodies induced by vaccination with a classical type 1 vaccine (67). Six amino acid changes were identified within the variable domain of VP2 of variant A virus when compared to the consensus sequence of 5 other IBDV isolates (68). In variants DS326, E/DEL and GLS only 1 or 2 amino acid exchanges were noted in each of the 2 hydrophilic regions (67–69) and all these strains had a Gln → Lys substitution at position 249 (Fig. 2). Interestingly, studies with escape mutants (mAb-resistant mutants) of a mildly pathogenic strain of IBDV (Cu-1) also supported amino acid exchanges within the hydrophilic regions of *Acc*I-*Spe*I fragment for antigenic variation (9).

The antigenic variation of IBDV strains has also been elucidated with a select panel of mAbs raised against various isolates of IBDV and the mAbs B69 and R63 recognized 2 distinct neutralizing epitopes on VP2 (70). The US variants lacked the VN B69 epitope found in all classical serotype 1 strains except vaccine strain PBG98 (31). It is speculated that Glyn 249 might be involved in binding with mAb B69 (31). Closely related GLS and DS326 variants lacked R63 epitope and shared a common mAb 57 epitope that differentiated them from variant strain E/DEL. Another neutralizing epitope in close proximity, possibly flanked by B69 and R63 binding sites on VP2 which is recognized by mAbs 9214 and 771 could also be attributed to serotype 1 specificity similar to the B69 binding site (71).

Comparison in the VP2 region of serotype 1 strain of a classical virulent isolate 52/70 (30%–50% mortality) and an attenuated vaccine strain PBG98 from the same geographic region revealed 5 amino acid changes that might be associated with virulence (Fig. 2) (30). Another region of interest in terms of virulence is a heptapeptide region (residues 326 to 332) adjacent to the 2nd hydrophilic region of VP2 (Fig. 2). Various VV IBDV strains isolated in Japan (11) and the antigenic virulent variants isolated in the United States contain a conserved serine rich heptapeptide S-W-S-A-S-G-S in this region (Figs. 1 & 2). The less virulent strains have fewer serine residues (31,67). Avirulent serotype 2 strain OH has 3 substitutions in this region. The serotype 2 strains also have an insertion of an amino acid residue at position 249 (serine) in the VP2 coding region (Fig. 2). Serotype 2 strain OH also has a deletion of residue at position 680 in the VP4 region (15). These changes might contribute to the loss of pathogenicity of the serotype 2 virus (31). Another region adjacent to the 1st hydrophilic area representing a 14 amino acid segment (residues 249–263) with 10 amino acid mismatches between the avirulent serotype 2 strain OH and the virulent serotype 1 strains STC, 52/70, Cu-1 is also considered to be associated with virulence (Fig. 2) (72).

By comparing the nucleotides in the variable region of VP2, the UK VV isolates (661, 74/89A, JY86, CS/89) were found to be closely related to each other (Fig. 2) (64). Among themselves, they differed by no more than 2 nucleotides whereas from the classical virulent strains such as STC and 52/70 by at least 29 nucleotides and 4 amino acids. The Dutch VV isolate DV86 was also very closely related to the UK VV isolates differing by only 3 nucleotides in this region. Interestingly, all the European isolates resembled the Japanese (VV) isolate 90-11 with no amino acid changes in the hypervariable region of VP2 but differing from it only by 1 or 2 nucleotides (64). Moreover, serines in the serine-rich heptapeptide region adjacent to the 2nd hydrophilic region were conserved in all the European VV isolates similar to the classical virulent strains suggesting that a virulent phenotype might impose a restraint to conserve more serine residues in this region (Fig. 2). All the amino acid changes except 1, observed in the VV isolates, were between the 2 hydrophilic regions of the VP2 gene. However, 1 amino acid change occurred in the 1st hydrophilic region (P to A at 222) (Fig. 2) similar to that of antigenic variants A, E/DEL and GLS. Mutations in the 2nd rather than the 1st hydrophilic region are believed to be responsible for escape from antibody and for failure of neutralizing mAbs to recognize this site (66–68). Hence, no significant antigenic marker characterizing the VV isolates has yet been identified.

Recent work comparing the amino acid sequences of parental highly virulent strains OKYM and TKSM in the VP2 region with their attenuated progeny revealed 2 amino acid substitutions at residues 279 and 284 from Asp → Asn and Ala → Thr, respectively (73). But the Asp residue at position 279 is well conserved in avirulent serotype 2 strains OH and 23/82 which were not included in the comparisons in that study. However, the Ala residue at position 284 between

the 2 hydrophilic regions may have a role in virulence determination. It is interesting to note that Thr is present at this position in GLS and Cu-1 strains similar to the avirulent serotype 2 strains (Fig. 2). Thus, the virulence determinants are not clearly established in spite of the molecular investigations of the VP2 gene and further studies on other regions of IBDV genome such as VP3, VP4, VP1 and the noncoding regions of both genome segments are warranted.

Previous studies of reassortants containing segment A from the pathogenic serotype 1 strain Cu-1 and segment B from the nonpathogenic serotype 2 strain 23/82 were found not to be lethal, causing only slight bursal lesions similar to some vaccine strains in chickens, suggesting that both segments have a role for establishing virulence (74). Sequence determination and analysis of segment A and segment B of a recent European VV isolate (UK661) have revealed some interesting aspects of the virulence of IBDV (24). The coding sequence of segment B of serotype 1 (VV) UK661 isolate is more closely related to those of 2 nonpathogenic serotype 2 strains (OH and 23/82) (24). In addition, the VP3 and VP4 coding sequences of segment A of (VV) UK661 are different from those of other pathogenic serotype 1 strains (24). Some amino acid substitutions identified in the (VV) UK661 strain in the VP4 viral protease and near the VP2-VP4 cleavage site and in the antigenic sites of VP2 and VP3 were considered to affect its phenotype, possibly including the virulence (24). Particularly, the substitutions in the polyprotein at 651 (N-S) adjacent to the predicted active site of the VP4 serine protease, another one at 452 (I-L) prior to the postulated dibasic residue protease cleavage site between VP2 and VP4 and a substitution (H-D 752) just upstream of the alternative VP4-VP3 cleavage site A-X-A-A-S could modify the protease thereby affecting the polyprotein processing and the virus replication rate (24). Further, the presence of higher relative homology of VP1 coding sequences of (VV) UK661 with those of serotype 2 strains 23/82 and OH indicated possible reassortment between segment A of virulent and segment B of avirulent strains (24). The significance of these observations cannot as yet be conclusively evaluated as more virulent strains have to be characterized and more IBDV strains have to be sequenced on genome segment B.

CONCLUDING REMARKS

It is worthy to note that cell-culture adaptation and serial passage of two IBDV variant strains IN and E/DEL resulted in loss of pathogenicity for SPF chickens without loss of antigenicity as indicated by the 2 in vitro tests (IIF test and VN) (75). By contrast, the newly evolved VV isolates of IBDV in Europe and Japan show increased virulence without any changes in their antigenicity. Understanding the mechanisms for such attenuation in the former and increased virulence in the latter in terms of nucleotide changes in the coding as well as the noncoding regions of the IBDV genome could provide clues to the likely sites involved in viral virulence. In reviewing the investigations so far addressing the molecular basis for antigenic and virulence variations of IBDV, it becomes obvious that amino acid residues within the "central" variable region of VP2 are responsible for antigenic variation in IBDV, and nucleotide changes in other areas of the genome such as VP4 and VP1 possibly contribute to gene constellation necessary for the evolution of virulent strains of IBDV. Since the complete nucleotide sequences of avirulent and virulent strains are known and studied in some detail, the next clear challenge is to map the principal determinants of virus virulence. Precise measures would then be possible for prevention of IBD by construction of improved vaccine strains.

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