REVIEW ARTICLE

Biochemistry and Immunology of Infectious Bursal Disease Virus

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INTRODUCTION

The aetiological agent of infectious bursal disease (IBD), IBD virus (IBDV), belongs to a new group of viruses referred to as 'birnaviruses' (Dobos et al., 1979), which has been characterized only recently (Brown, 1986). There are excellent reviews dealing with the clinical, pathological, serological and epidemiological aspects of IBDV infection (Faragher, 1972; Becht, 1980; Okoye, 1984; Cummings et al., 1986). The molecular biology of birnaviruses has also been reviewed (Dobos & Roberts, 1983) but with an emphasis on infectious pancreatic necrosis virus (IPNV), the birnavirus genus prototype. The purpose of the present review is to compile information on structural and immunological aspects of IBDV. These are subjects of much recent interest, and have great relevance to the control of IBD in chickens.

IBD is a highly contagious viral disease of young chickens which is characterized by destruction of the lymphoid cells in the bursa of Fabricius; other lymphoid organs are also affected but to a lesser degree (Cheville, 1967). In a fully susceptible chicken flock (between 3 and 6 weeks of age), the clinical disease is responsible for losses due to impaired growth and death, and from excessive condemnation of carcasses because of skeletal muscle haemorrhages (Lukert & Hitchner, 1984). Susceptible chickens less than 3 weeks of age do not exhibit clinical signs (Hitchner, 1971), but have a subclinical infection characterized by microscopic lesions in the bursa of Fabricius (Winterfield et al., 1972) and immunosuppression (Allan et al., 1972). The greatest economic losses result from immunosuppression. This causes increased susceptibility to other diseases, and interferes with effective vaccination against Newcastle disease, Marek's disease and infectious bronchitis. Therefore, IBDV is one of the most important viral pathogens of commercial poultry.

The target cells for the virus are actively dividing B lymphocytes (Müller, 1986; Burkhardt & Müller, 1987). Studies in bursectomized and non-bursectomized chickens show that following oral inoculation, initial viral replication occurs in gut-associated lymphoid cells and that secondary replication in the bursa of Fabricius is responsible for high titres of virus and for mortality. Chickens are protected by bursectomy (Käufer & Weiss, 1980). Infection of B lymphocytes in the bursa of Fabricius is cytolytic and leads directly to immunosuppression. Mortality and clinical signs of IBD have also been associated with immune complexes (Ley et al., 1979; Skeeles et al., 1979b), and a depletion in circulating levels of haemolytic complement (Skeeles et al., 1979a) and clotting abnormalities (Skeeles et al., 1980).

IBDV is a very stable virus and can therefore persist in poultry houses after thorough cleaning and disinfection (Lukert & Hitchner, 1984). It is more resistant than reoviruses to heat, ultraviolet irradiation and photodynamic inactivation (Petek et al., 1973) and is resistant to ether and chloroform, it is inactivated at pH 12·0 but unaffected at pH 2·0 (Benton et al., 1967b),

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and is unaffected by exposure for 1 h at 30 °C to 0·5% phenol and 0·125% thiomersal (Benton et al., 1967b). Exposure to 1% phenol or 1% cresol for 1 h inactivates the virus (Cho & Edgar, 1969) and virus infectivity is markedly reduced by exposure to 0·5% formalin for 6 h (Benton et al., 1967b) or to 1% formalin for 1 h (Cho & Edgar, 1969). The stability of the virus and the economics of commercial poultry production, which require short time intervals between batches of chickens and in some cases the re-use of litter, dictate that adequate control of IBD is only possible by vaccination.

There are two known IBDV serotypes. Serotype I contains some viruses that are pathogenic to chickens whereas serotype II viruses infect chickens and turkeys but these infections are of unknown clinical significance (Cummings et al., 1986). The disease, recognized world-wide in all major poultry-producing areas (Okoye, 1984), is normally controlled by the combined use of live virus and inactivated oil emulsion vaccines containing serotype I IBDV. In recent years, infection with variant viruses which are not neutralized by antibodies against standard serotype I IBDV have been identified in field outbreaks of IBD, particularly in eastern U.S.A. (Saif, 1984; Rosenberger & Cloud, 1986).

**BIOCHEMISTRY**

**Structure of IBDV**

IBDV is a member of the birnavirus genus, family Birnaviridae (Brown, 1986). The generic name describes animal viruses with two segments of dsRNA (Dobos et al., 1979). It includes IPNV of fish, tellina virus (TV) and oyster virus (OV) of bivalve molluscs, and drosophila X virus (DXV) of the fruit fly (Drosophila melanogaster). These viruses are non-enveloped, have a single capsid structure of icosahedral symmetry and a diameter of 58 to 60 nm (Dobos et al., 1979).

IBDV has a sedimentation rate of 460S in sucrose gradients (Dobos et al., 1979; Todd & McNulty, 1979). The buoyant density of mature (complete) virions in caesium chloride (CsCl) gradients is 1·33 g/ml (Becht, 1981; Fahey et al., 1985a; Müller & Becht, 1982; Nick et al., 1976). A higher buoyant density of 1·34 g/ml has been reported (Hirai et al., 1979); lower densities of 1·31 g/ml (Pattison et al., 1975; Todd & McNulty, 1979) and 1·32 g/ml (Jackwood et al., 1982, 1984) have also been reported. Immature (incomplete) virus particles have buoyant densities lower than 1·33 g/ml in CsCl gradients.

As many as six virus-containing bands have been detected in CsCl gradients of a single virus preparation (Müller et al., 1986). Müller & Becht (1982) examined four of these using electron microscopy and SDS-PAGE: a prominent band seen at 1·33 g/ml contained particles with the typical appearance of IBDV. Another band at 1·32 g/ml contained particles with a relatively small amount of dsRNA (incomplete virus particles). A third band at 1·31 g/ml represented immature virus particles containing no RNA. These three virus populations have similar polypeptide patterns in SDS-PAGE. The virus contained in the fourth band ('top component') at a density of 1·29 g/ml differed from the incomplete virus particles in bands 2 and 3 by showing stain penetration, irregular shape and poor assembly (Müller et al., 1986), unusual amounts of dsRNA without being infectious, and a different polypeptide pattern consisting of a relative abundance of the 44K to 47K polypeptide and lack of the 90K, 33K and 28K polypeptides. This type of incomplete virus particle is commonly seen when IBDV is grown in chicken embryo fibroblast (CEF) cultures. By comparison, IBDV from the bursa of Fabricius forms very faint or no clearly discernible bands at the 1·29 g/ml density (Müller & Becht, 1982). The PAGE profile of this virus preparation shows the 28K polypeptide replacing the 32K protein (Lange et al., 1987). These differences in PAGE profiles of the incomplete virus particles formed in the bursa of Fabricius compared to CEF are in contrast to identical polypeptide patterns of mature (complete) IBDV particles (buoyant density 1·33 g/ml) formed in both cell types. Since B lymphocytes are probably the optimal host cells for IBDV replication, the low density (1·29 g/ml) particles formed in infected bursa cells might represent incompletely assembled premature particles whose PAGE pattern is different from that of the incomplete particles in
CEF (Müller et al., 1986). Incomplete IBDV particles purified from infected bursa cells are rare (Müller & Becht, 1982), and have both genome segments present in approximately equal amounts (Lange et al., 1987), whereas repeated high m.o.i. passage of IBDV in CEF results in stable small plaque mutants with interfering capacities and an abundance of incomplete particles (of buoyant density 1.29 g/ml in CsCl gradients) with an aberrant protein composition (Müller et al., 1986).

Density equilibrium banding of the virus on CsCl is a common method of purifying IBDV. However, the Australian IBDV strain 002/73 is unstable in CsCl gradients (Azad et al., 1985). We have experienced a similar phenomenon with IBDV strain IM (Winterfield, 1969) propagated in the bursa of Fabricius (F. S. B. Kibenge, R. G. Russell & A. S. Dhillon, unpublished data). The IM virus was pelleted through the CsCl gradients whereas other IBDV strains (grown in cell culture) could be banded by repeated centrifugations in CsCl. These observations indicate that differences in the propagation and purification steps used for different isolates of IBDV can be a source of variation in the structure of the virus observed.

**Genome of IBDV**

The genome of IBDV consists of two pieces of high M, dsRNA that sediment as 14S components in sucrose gradients (Müller et al., 1979; Todd & McNulty, 1979). IBDV dsRNA is resistant to RNase degradation at high molarities of salts (Spies et al., 1987). Other properties of IBDV RNA consistent with a double-stranded structure include a buoyant density of 1.62 g/ml in caesium sulphate gradients, a melting point of 95-5 °C in the presence of RNase, a base composition reflecting the pairing of adenine and uracil as well as guanine and cytosine (Müller et al., 1979), precipitation from 4 M but not 2 M-LiCl and green staining with acridine orange (Azad et al., 1985).

The bisegmented dsRNA genome is characteristic of the birnavirus genus (Dobos et al., 1979). Linear dose–response curves of IPNV and DXV infectivity show that the divided genome resides in a single virus particle (Macdonald et al., 1977; Nagy & Dobos, 1984b). However, the two dsRNA segments are different as demonstrated by oligonucleotide maps (Macdonald et al., 1977), nuclease digestion patterns (Nagy & Dobos, 1984b), in vitro translation of individual dsRNA segments (Mertens & Dobos, 1982; Nagy & Dobos, 1984a; Azad et al., 1985) and by production, through genetic reassortment, of virus recombinants (Macdonald & Dobos, 1981).

The migration rate of the two segments of IBDV dsRNA in polyacrylamide gels is influenced by the presence of urea. In gels containing urea, the segments migrate more slowly (Nick et al., 1976) than in gels without urea (Müller et al., 1979). In the latter, IBDV RNA segments migrate faster than the reovirus L segment (the 10 reovirus RNA segments are commonly used as M, standards) (Becht, 1980). In polyacrylamide gels without urea differences are observed between the migration patterns of the RNA segments of the two serotypes of IBDV. The migration rate of the heavier RNA segments of the serotype II MO strain and serotype I SAL strain are similar but that of the OH strain of serotype II is different (Jackwood et al., 1984). Segment A of the genomic RNA of strain 23/82 of serotype II migrates considerably faster than the corresponding genomic segment of strain Cu-1 of serotype I. Segment B in the serotype II strains also migrates faster than its counterpart in serotype I strains but this is evident only after long electrophoretic separation (Jackwood et al., 1984; Becht et al., 1988). A wider range of mobilities of the RNA segments is found between viruses in the different serotypes of IPNV (Macdonald & Gower, 1981; Hedrick & Okamoto, 1982; Hedrick et al., 1983).

A comparison of IBDV RNA with the dsRNA segments of bovine rotavirus under non-denaturing conditions shows that the individual lengths of the two segments of dsRNA from strain 002/73 of serotype I are about 3400 (M, 2.06 x 10^6) and 2900 (M, 1.76 x 10^6) base pairs long (Azad et al., 1985). Similar values are obtained when IBDV dsRNA is electrophoresed after complete denaturation (Azad et al., 1985). The M, values of the two RNA segments of strain Cu-I are similar to those of strain 002/73 cited above (Müller & Nitschke, 1987a). Segments A and B of genomic RNA of strain 23/82 (serotype II) are smaller than those of strain Cu-I by approximately 70 and 20 base pairs, respectively (Becht et al., 1988).
IBDV replication

General
The biochemical steps involved in the replication of IBDV or other birnaviruses have not been characterized. Dobos (1977) listed a number of factors that have restricted studies of IPNV replication. One of the major factors is that, even at high m.o.i., only a limited number of cells infected in vitro support virus replication (Hirai & Calnek, 1979; Müller et al., 1986). Another difficulty encountered in studying IBDV replication is the sensitivity of this virus towards actinomycin D (Petek et al., 1973; Müller & Becht, 1982) which is normally used selectively to inhibit cellular RNA synthesis.

In vitro IBDV replication
IBDV has been adapted to replicate and produce c.p.e. in primary cell cultures including chicken bursal lymphoid cells, chicken embryo kidney (CEK) and CEF cells (Lukert & Davis, 1974; McNulty et al., 1979). Cell culture-adapted IBDV also grows in several mammalian continuous cell lines such as RK-13, Vero, BGM-70 and M4-104 cells (Petek et al., 1973; Rinaldi et al., 1972; Jackwood et al., 1987; Leonard, 1974; Lukert et al., 1975). Synthesis of IBDV-specific polypeptides in chicken bursal lymphoid cells as early as 90 min after infection in vitro has been reported, and mature viral polypeptides have been demonstrated in the culture medium of such cells 6 h after infection (Müller & Becht, 1982). Serotype I IBDV attaches to the CEK cell monolayers maximally after 75 min incubation at 37 °C (Lukert & Davis, 1974). The multiplication cycle in CEF and CEK cultures is 10 to 16 h (Nick et al., 1976; Lukert & Davis, 1974). The latent period of IBDV in CEF cultures is 4 to 6 h (Becht, 1981; Jackwood et al., 1984). In Vero cells, both serotypes I and II of IBDV showed maximum attachment 70 min post-inoculation (p.i.) at 37 °C. The viruses studied had latent periods of 12 to 16 h in Vero cells and multiplication cycles of more than 48 h (Kibenge et al., 1988a). This prolonged replicative cycle of IBDV in Vero cells was initially observed by Lukert et al. (1975) and has since been reported for IBDV grown in BGM-70 cells (Jackwood et al., 1987). It might be characteristic of IBDV replication in mammalian cell lines.

It is not known which of the two capsid proteins (VP2 and VP3) of IBDV recognizes cell receptors. Monoclonal antibodies (MAbs) 17/82 (Azad et al., 1987) and 1/A6 (Becht et al., 1988) directed against VP2 of IBDV show virus-neutralizing activity while MAb 27/B1 (Becht et al., 1988) directed against VP3 is non-neutralizing, indicating that VP2 may be important in virus adsorption.

RNA synthesis
The mechanism of synthesis of both virus-specific ssRNA and dsRNA during infection with IBDV has not been clearly determined, partly for reasons discussed above. Initial steps of viral replication of dsRNA viruses have been extensively studied with reovirus (Shatkin, 1969) which utilizes a virion-associated RNA polymerase for transcription of one strand of the viral RNA. Similar results have been published with other dsRNA viruses such as bluetongue virus (Martin & Zweerink, 1972; Verwoerd et al., 1972) and cytoplasmic polyhedrosis virus (Lewandowski et al., 1969). Similarly, within the birnavirus genus, an RNA-dependent RNA polymerase has been demonstrated in IPNV (Cohen, 1975; Mertens et al., 1982), DXV (Bernard & Petitjean, 1978) and IBDV (Spies et al., 1987). However, the method of transcription in birnaviruses is not known (Mertens et al., 1982). Genome-linked proteins have been demonstrated in the three birnaviruses (Revet & Delain, 1982; Persson & Macdonald, 1982; Spies et al., 1987; Müller & Nitschke, 1987) indicating that they replicate their nucleic acid by a strand displacement (semi-conservative) mechanism (Bernard, 1980; Mertens et al., 1982; Spies et al., 1987). This is in contrast to reovirus and cytoplasmic polyhedrosis virus which produce ssRNA conservatively (Joklik, 1974; Smith & Furuichi, 1980).

The viral RNA polymerase of IBDV is thought to be VP1 (Spies et al., 1987). Its activity requires removal of Ca\(^{2+}\) ions from the reaction mixture and is optimum at 40 °C and pH 8.5. It requires the presence of Mg\(^{2+}\) but no special treatment of the virus particles. Monovalent cations (Na\(^{+}\) and K\(^{+}\)) significantly increase the enzyme activity. The RNA polymerase of IPNV
is also considered to be VP1 (Huang et al., 1986; Nagy et al., 1987). It is insensitive to actinomycin D and rifampicin, and also requires Mg$^{2+}$ (Cohen, 1975; Mertens et al., 1982), and not Ca$^{2+}$, but unlike the IBDV RNA polymerase, Na$^+$ or K$^+$ diminishes its activity (Mertens et al., 1982). Optimum activity is at 30 °C, pH 8-05 (Mertens et al., 1982). Pretreatment of the virions is not necessary, in fact pretreatment with chymotrypsin or with Triton X-100 decreases the enzyme activity (Cohen, 1975). The ability to demonstrate RNA polymerase activity without any pretreatment of the virions (Cohen, 1975; Mertens et al., 1982; Bernard, 1980; Spies et al., 1987) indicates that transcription and replication can be initiated when the virus has penetrated into the host cell without the need for uncoating (Spies et al., 1987) such as occurs with reovirus (Skehel & Joklik, 1969).

IPNV RNA synthesis has been studied in CHSE-214 cells (Somogyi & Dobos, 1980). The rate of IPNV-specific RNA synthesis is maximal at 8 to 10 h after infection and is completed by 12 to 14 h. A single cycle of replication of IPNV in various fish cell lines takes 16 to 20 h at 24 °C (Malsberger & Cerini, 1965), which is comparable to the time span for IBDV replication in CEF at 37 °C (Nick et al., 1976).

Three forms of intracellular, virus-specific RNAs have been described in IPNV by Somogyi & Dobos (1980). First, there is a putative transcription intermediate (probably a complex of template RNAs and their products) which barely enters 2% acrylamide gels. It is partially RNase-sensitive and is the most abundant RNA form early in the infection cycle. Second, a 24S genome length ssRNA exists which is thought to be the viral mRNA. It can be resolved into two bands by PAGE, is RNase-sensitive and is associated with polysomes. Third, a 14S dsRNA has been found to be indistinguishable from virion RNA by gradient centrifugation and gel electrophoresis. The 24S virus-specific ssRNA in IPNV-infected cells was initially reported by Alayse et al. (1975). It is considered to be of genome length since it also forms when the 14S viral dsRNA genome is denatured (Dobos, 1976).

The slowly migrating RNA fraction (transcription intermediate), 24S ssRNA (viral mRNA), and 14S dsRNA (virion RNA) have also been demonstrated in vitro as reaction products of the RNA-dependent RNA polymerase of DXV (Bernard, 1980) and IBDV (Spies et al., 1987). The slowly migrating RNA fraction disappeared and only virion dsRNA could be demonstrated in IBDV reaction mixtures incubated for more than 2 to 3 h (Spies et al., 1987). These authors demonstrated the different degrees of hybridization of the reaction products to IBDV dsRNA and the different susceptibilities of the reaction products to RNase activity. In a rabbit reticulocyte lysate translation system the 24S RNA yields products corresponding to IBDV polypeptides (Spies et al., 1987).

**Protein synthesis**

The patterns of newly synthesized proteins do not differ between uninfected and IBDV-infected cells analysed by SDS-PAGE indicating that IBDV does not shut off the synthesis of cellular proteins (Becht, 1981). The low level of viral protein synthesis observed in CEF cultures could also be due to the limited fraction of cells replicating IBDV. Viral protein bands from lymphoid cells from the bursa of Fabricius are visible in polyacrylamide gels against the background of newly synthesized cellular proteins (Müller, 1986). The ability of the TLT-1 cell line (avian B cell-derived lymphoblastoid cell line) to yield high titres of IBDV (Hirai & Calnek, 1979), if accompanied by a relatively low cellular protein background would warrant further examination of its use in studying IBDV replication.

Four mature viral proteins designated VP1, VP2, VP3 and VP4 are synthesized in IBDV-infected cells (Becht, 1981; Dobos, 1979; Dobos et al., 1979; Nick et al., 1976). This is also characteristic of other members of the birnavirus genus (Dobos et al., 1979). As many as nine viral proteins have been reported for IBDV (Müller & Becht, 1982), suggesting precursor–product relationships in the biosynthesis of these polypeptides. Müller & Becht (1982) used cultured bursal lymphoid cells to analyse $^{35}$S methionine pulse-labelled viral polypeptides by SDS-PAGE. As described for IPNV (Dobos, 1977; Dobos & Rowe, 1977; Macdonald & Dobos, 1981), Müller & Becht (1982) found a two-step cleavage processing of medium-size polypeptides for maturation of the virus. A polypeptide of 50K could be chased to form 49K (VPX), the
precursor of VP2 (40K). VP2 could not be detected in cell extracts in which 50K was demonstrable, suggesting that VP2 does not accumulate intracellularly as the other viral proteins do. This indicates that post-translational modification of the 50K polypeptide probably goes on during virus maturation or assembly (Müller & Becht, 1982). A two-step cleavage process has also been described for DXV (Nagy & Dobos, 1984b).

Additional information on the synthesis of structural proteins has been obtained by determination of genome coding assignments of birnaviruses (see below). It has been established that VP2, VP3 and VP4, which are the products of genomic segment A, arise by cotranslational proteolytic cleavage of a precursor polypeptide. VP4 is involved in the processing of the precursor polyprotein, in cleaving between VPX and VP4 (Duncan et al., 1987), and between VP4 and VP3 (Azad et al., 1987; Jagadish et al., 1988).

**IBDV genome coding assignments**

The coding assignments of the individual genomic segments of IPNV, DXV and IBDV have been determined by analyses of virus recombinants obtained through genetic reassortment (Macdonald & Dobos, 1981), and of protein products of *in vitro* translation of denatured genomic segments (Mertens & Dobos, 1982; Nagy & Dobos, 1984a; Azad et al., 1985; Nagy et al., 1987).

Virus recombinants have been successfully used to identify proteins encoded by each genomic segment of IPNV (Macdonald & Dobos, 1981). This has been possible because some of the serotypes differ significantly in the apparent *M*<sub>o</sub> of their RNAs and proteins (Macdonald & Gower, 1981). Since the virus has only two segments of RNA, virus recombinants produced by genetic reassortment provide unambiguous coding assignments for each RNA segment. However, this technique has not been used on IBDV, probably because there are no major differences in the RNAs of the two known serotypes (Jackwood et al., 1984; Becht et al., 1988).

The technique of *in vitro* translation of dsRNA genome segments requires denaturation of the RNA segments prior to translation in a cell-free protein-synthesizing system. The IBDV dsRNA can be translated *in vitro* only after extensive denaturation (Azad et al., 1985; Spies et al., 1987). This is in contrast to DXV in which undenatured (native) dsRNA can also be translated *in vitro* with high fidelity (Nagy & Dobos, 1984a). The latter is unexpected since dsRNAs, by virtue of their secondary structure, are unsuitable messengers.

The genomic organization of IBDV and IPNV is illustrated in Fig. 1. The terminology of Macdonald & Dobos (1981) is used to refer to the individual genomic segments. The smaller genomic segment (segment B) of IBDV codes for a single 90K polypeptide (VP1) and the larger genomic segment (segment A) encodes five polypeptides of *M*<sub>o</sub> 52K (VPX), 41K (VP2), 32K (VP3), 28K (VP4) and 16K (Azad et al., 1985). The function of the 16K polypeptide is unknown, and will not be discussed further since the existence of this polypeptide has not yet been corroborated. Genomic coding assignments for IPNV and DXV dsRNAs, determined by *in vitro* translation (Mertens & Dobos, 1982; Nagy & Dobos, 1984a), and by using temperature-sensitive recombinant viruses (Macdonald & Dobos, 1981), are similar to those for IBDV.

The finding that the total *M*<sub>o</sub> of the translated products of genomic segment A of IBDV strain 002/73 is 169K yet it encodes proteins of total *M*<sub>o</sub> 125K, suggests a precursor–product relationship between some of the translation products (Azad et al., 1985). Peptide mapping has shown that VPX and VP2 of IBDV strain Cu-1 have very similar amino acid sequences. The sequences of the other proteins are completely different (Müller & Becht, 1982; Dobos, 1979). In addition, both VPX and VP2 react with the same MAb on Western blots (Fahey et al., 1985b; Becht et al., 1988). These observations suggest that VP2 is a specific cleavage product of a VPX precursor. Similarly, a 55K to 60K polypeptide (Fig. 1) may be the precursor protein for VP3 and VP4 (Hudson et al., 1986). This 55K to 60K polypeptide was found on Western blots of solubilized viral proteins of IBDV strain 002/73 developed with chicken immune sera raised against fusion proteins from recombinant *Escherichia coli* colonies containing VP3-encoding cDNA inserts (Azad et al., 1986).

We have retained the NS and pVP2 terminology used by Nagy et al. (1987) in the text and Fig. 1 of this review for reasons of consistency only. The term NS is used by Nagy et al. (1987) to refer to VP4 in IPNV. They speculated that VP4 was a non-structural polypeptide. However, VP4
IBDV genome Segment A (approx. 3400 bp) 

mRNA A 5'→ 3' 3033 bp 3' 5'→ mRNA B 5'→ Translation (108K) 3' Translation

Proteins VPX VP2 VP4 VP3

IBDV genome Segment B (approx. 2900 bp) 

mRNA A 5'→ 3' 3' 5'→ mRNA B 5'→ Translation VP1 Protein 3' Translation

Proteins VP1 Protein

IPNV genome Segment A (approx. 3097 bp) 

mRNA A 5'→ 2916 bp 3' 5'→ mRNA B 3' Translation

Proteins PreVP2 NS VP3 (106K–108K)

IPNV genome Segment B (approx. 2900 bp) 

mRNA A 5'→ 3' 3' 5'→ mRNA B 5'→ Translation 3' Translation

Fig. 1. Genomic organization of IBDV and IPNV based on the work of Macdonald & Dobos (1981), Azad et al. (1985, 1987), Hudson et al. (1986), Duncan & Dobos (1986), Duncan et al. (1987) and Nagy et al. (1987). The open reading frame in mRNA A is indicated by double lined bars and proteolytic processing of the primary translation product is also illustrated. The viral polypeptides, designated by their names (or M+), are indicated by single lines. Upper panel, IBDV genome. Lower panel, IPNV genome.

appears in purified virus indicating that it is a normal structural viral protein. Nagy et al. (1987) also use the term pVP2 for the VP2 precursor polypeptide generally referred to as VPX.

To characterize the genomic coding assignments of IPNV, Nagy et al. (1987) separated and identified the complementary ‘plus’ and ‘minus’ RNAs making up the IPNV dsRNA. Complementary strands of each genomic segment of heat-denatured dsRNA migrate at different rates in low pH, 1.5% agarose gels containing urea (Nagy et al., 1987). Like simian rotavirus (Patton & Stacy-Phipps, 1986) and cytoplasmic polyhedrosis virus, but unlike human reovirus (Smith et al., 1981), IPNV plus strand RNA migrates faster than its complementary minus strand on agarose-urea gels. The basis for the differences in migration rates is not known. In the case of DNA, complementary strand separation by agarose gel electrophoresis is presumed to result from conformational differences between the separated strands arising from differences in base composition and/or nucleotide sequence since the separation increases as a function of electrophoresis time (Johnson & Grossman, 1977). It is possible that a common structural feature exists for all plus or minus strand RNAs (Smith et al., 1981).

IPNV genomic segment A consists of bands 1 (A-) and 3 (A+). Segment B has bands 2 (B-) and 4 (B+). Using hybrid-arrested cell-free translation (Paterson et al., 1977), VP2, VP3 and VP4 (NS) have been precisely mapped on the A+ (messenger) RNA strand (Nagy et al., 1987). The 5' end of the A+ RNA strand can be hybridized to cDNA to allow synthesis of NS and VP3 only, not pVP2. Use of longer 5' co-terminal cDNA allows synthesis of VP3 only. These results demonstrate directly that the order of the three major polypeptides on A+ RNA is 5'-pVP2–NS–VP3–3' and that initiation of protein synthesis can take place at some of the internal in-phase AUG codons on A+ RNA (Nagy et al., 1987). Huang et al. (1986) used selective deletions of viral cDNA to illustrate this linear gene arrangement along genomic segment A of IPNV. A similar VP2–VP4–VP3 coding assignment of genomic segment A of IBDV has been confirmed by peptide sequence analysis (Hudson et al., 1986). The same gene order was previously reported for genomic segment A of DXV (Nagy & Dobos, 1984a), indicating that this arrangement may
be common to all birnaviruses. This form of gene expression resembles that of the picornaviruses in that three of the four gene products of birnaviruses may be produced by polyprotein processing. In the case of birnaviruses, however, the structural genes are not clustered near the 5' end of the genome as in picornaviruses, but are located at opposite ends of the RNA A genomic segment (Nagy et al., 1987). The putative RNA polymerase of birnaviruses is encoded by the second genomic segment. This manner of gene expression has not been described for any other viruses and may be unique to members of the Birnaviridae.

RNA sequence studies

It was initially thought that the large RNA segment (A) of birnaviruses which encodes VP2, VP3 and VP4 was polycistronic (Dobos & Mertens, 1982; Mertens & Dobos, 1982; Azad et al., 1985). This was because no polyprotein precursor(s) could be detected in virus-infected cells and the frequency of synthesis of the three viral proteins was inversely related to their Mr, suggesting that they may have been the products of different cistrons (Dobos, 1977). Moreover, only two pieces of genome length 24S viral mRNA which hybridized to the two genomic segments, and no subgenomic mRNAs, had been detected either in vivo (Somogyi & Dobos, 1980) or in vitro (Mertens et al., 1982). Current evidence suggests that genomic segment A in IBDV and IPNV is expressed as a monocistronic template (Hudson et al., 1986; Duncan & Dobos, 1986). A single continuous open reading frame (ORF) capable of coding for a previously undetected 106K to 108K polypeptide can be translated from the nucleotide sequence obtained (Hudson et al., 1986; Duncan & Dobos, 1986). The primary translation product (polyprotein precursor) is subsequently processed into the mature viral proteins VP2, VP3 and VP4. The fact that such a polypeptide precursor has never been detected in infected cells suggests rapid co-translational processing as shown recently with some of the flaviviruses (Rice et al., 1985). A 101K precursor polyprotein has been detected recently in an in vitro translation system with IPNV (Duncan et al., 1987). The peptide map of the IPNV polypeptide is compatible with the combined peptide maps of pVP2, NS and VP3 (Duncan et al., 1987). The synthesis and processing of the polyprotein precursor also vary when different reticulocyte translation systems are used (Nagy et al., 1987). This variability may explain, in part, why such a polyprotein was not detected earlier (Mertens & Dobos, 1982).

Kozak (1981) has proposed that for efficient initiation of translation an AUG initiator codon must exist in a favourable sequence context, the most favourable consensus sequence for eukaryotic initiation sites being 5'XXAUGG (where X is any base). The initiator codon of the ORF of genomic segment A in both IBDV (Hudson et al., 1986) and IPNV (Duncan & Dobos, 1986) does not obey this consensus. Therefore initiation of protein synthesis at internal in-phase AUG codons is not abolished (Kozak, 1986). Consequently, both polyprotein processing and initiation from downstream sites may operate for genomic segment A of birnaviruses as has been shown during in vitro translation of poliovirus RNA (Celma & Ehrenfeld, 1975; Phillips & Emmert, 1986).

Proteins of IBDV

It is generally agreed that IBDV has four viral proteins (Becht, 1981; Dobos, 1979; Dobos et al., 1979; Todd & McNulty, 1979; Nick et al., 1976): VP1 to VP4 (Dobos, 1979). Of these, VP2 is the most abundant, making up 51% of serotype I IBDV proteins. VP3 is another major protein (40%) and VP4 (6%) and VP1 (3%) are minor proteins (Dobos et al., 1979).

The Mr of specific viral proteins appears variable (Table 1) depending on a number of factors such as differences in methodology for virus purification and protein detection in the gel, percentage of polyacrylamide gel used, amount of sample loaded, source and range of Mr markers, degree of dehydration of the gel, e.g. during destaining following Coomassie Brilliant Blue staining. These may account for the differences in estimates of the Mr of viral proteins of a particular strain of IBDV obtained by different laboratories, for example the Cu-1 strain (Table 1). Variations in Mr of viral proteins may also be due either to differences in the cleavage of specific precursor proteins such as VPX (Becht, 1981) or to different levels of viral mRNA translation (Duncan et al., 1987), especially if different host systems are used (Müller & Becht,
Table 1. Polypeptides (M_r values × 10^-3) of IBDV serotype I and II isolates

<table>
<thead>
<tr>
<th>Polypeptide field</th>
<th>Cu-I (a)</th>
<th>GBF-I (c) virus (d)</th>
<th>Cu-I (e)</th>
<th>Cu-I (f)</th>
<th>002/73 (g)</th>
<th>SAL (h)</th>
<th>MO (h)</th>
<th>OH (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP-1</td>
<td>86</td>
<td>90</td>
<td>90</td>
<td>91.5</td>
<td>90</td>
<td>95</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>VP-X</td>
<td>53</td>
<td>51</td>
<td>50</td>
<td>49</td>
<td>48</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>VP-2</td>
<td>45</td>
<td>40</td>
<td>41</td>
<td>40</td>
<td>41.5</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP-3</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>35.5</td>
<td>35.5</td>
<td>35.5</td>
</tr>
<tr>
<td>VP-4</td>
<td>30</td>
<td>32</td>
<td>32</td>
<td>33</td>
<td>32</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* References: (a) Dobos (1979); (b) Todd & McNulty (1979); (c) Hirai et al. (1979); (d) Nick et al. (1976); (e) Becht (1980); (f) Müller & Becht (1982); (g) Fahey et al. (1985a); (h) Jackwood et al. (1985).

† 55K to 60K polypeptide, considered to represent a precursor molecule, was detected in Western blots with chicken immune sera raised against fusion proteins from recombinant *E. coli* colonies containing cDNA inserts which encode the 32K polypeptide (Azad et al., 1986).

1982; Lange et al., 1987). However, variations in the M_r of viral proteins of some IBDV serotype I isolates may represent genuine differences between virus strains, for example the Australian isolate 002/73 of IBDV and the European IBDV isolates (Fahey et al., 1985a).

Variations in the M_r of viral proteins are seen between the two serotypes of IBDV. Although Jackwood et al. (1984) reported that serotype II viruses lack VP2, our observations demonstrate VP2 in the same two serotype II strains (MO and OH) propagated in Vero and BGM-70 cells. Differences in virus propagation and purification between the two laboratories may have facilitated detection of VP2 in our preparations (Kibenge et al., 1988a). The VP2 in these serotype II viruses is larger than in the SAL strain of serotype I (Kibenge et al., 1988b). We also found the M_r of VPX, VP3 and VP4 in serotype II viruses to be higher than in SAL virus. VP2 and VPX in IBDV serotype II strain 23/82 are also considerably larger than in serotype I strain Cu-1 although VP3 is almost identical in size in both strains (Becht et al., 1988). The viral proteins of different serotypes of IPNV also vary in M_r (Hedrick & Okamoto, 1982; Hedrick et al., 1983).

Due to the prominence of VP2 and VP3, Becht (1980) speculated that one or both make up the viral capsid. Among the different strains of IBDV, VP2 shows the greatest variation in size (F. S. B. Kibenge, R. G. Russell & A. S. Dhillon, unpublished data). This is also true of the different strains of IPNV (Hedrick & Okamoto, 1982). Fahey et al. (1985a) thought that there may be a relationship between the smaller size of VP2 and the relatively low pathogenicity of the Australian 002/73 isolate of IBDV. It has been recently demonstrated that VP2 contains the antigenic region responsible for the production of neutralizing antibodies and that this is highly
conformation-dependent and is a major host protective immunogen of IBDV (Azad et al., 1987; Becht et al., 1988). Neutralizing monoclonal antibodies directed against VP2 differentiate between the two serotypes of IBDV (Becht et al., 1988).

VP3 is considered to be a group-specific antigen because it is recognized by monoclonal antibodies directed against VP3 in strains from both serotype I and serotype II (Becht et al., 1988). It has also been suggested that the basic C-terminal region of this protein may be involved in either packaging or stabilizing the RNA genome within the interior of the viral capsid (Hudson et al., 1986).

VP1 is a minor internal component of the virion. It is presumed to be the viral RNA polymerase (Hudson et al., 1986; Nagy et al., 1987; Spies et al., 1987). This polypeptide is synthesized in small quantities in vivo and incorporated into virions without apparent changes in Mr, (Dobos & Mertens, 1982). In IBDV, this polypeptide is tightly bound to both ends of the two genomic segments which are circularized (Müller & Nitschke, 1987b).

The conclusion that VP4 is a viral protease in IBDV (Hudson et al., 1986) and IPNV (Nagy et al., 1987) is speculative. Although deletion expression studies of segment A of viral cDNA suggest that VP4 contributes to the processing of a precursor polyprotein of VP2, VP3 and VP4 (Azad et al., 1987; Duncan et al., 1987; Jagadish et al., 1988), the exact way in which this occurs in unclear.

**IMMUNOLOGY**

**Antigens of IBDV**

IBDVs share common group antigens which may be detected by agar gel precipitation, the fluorescent antibody test, and ELISA (Allan et al., 1984; Jackwood et al., 1982; Hirai et al., 1972, 1974; Chettle et al., 1985; Cullen & Wyeth, 1975; Wood et al., 1979; Marquardt et al., 1980). Both capsid proteins (VP2 and VP3) contain epitopes that are responsible for the IBDV group antigenicity (Becht et al., 1988).

In addition to the common group antigens, each IBDV contains an antigen or antigens responsible for serotype specificity. Serotypes are identified by neutralization tests with serotype-specific antisera (McFerran et al., 1980). It was initially thought that VP3 carried the antigenic determinant(s) of serotype specificity of IBDV (Fahey et al., 1985a, b). However, current evidence indicates that VP2 carries the serotype-specific antigens responsible for the induction of neutralizing protective antibodies (Azad et al., 1987; Becht et al., 1988). There are at least two virus-neutralizing epitopes on VP2, one of which is strictly serotype-specific (Becht et al., 1988). The existence of additional virus-neutralizing epitopes on viral proteins of IBDV cannot be ruled out.

Two serotypes of IBDV (I and II) are recognized in the U.S.A. (Barnes et al., 1982; Jackwood et al., 1982; Jackwood & Saif, 1983) and Europe (McFerran et al., 1980). The possible existence of a third serotype has been suggested (Lee, 1983; Lukert & Hitchner, 1984) but current evidence indicates that the establishment of a third serotype is not justified. Lee (1983) examined 15 chicken and turkey IBDV isolates by cross-neutralization tests and identified three serotypes with prototype strains BVM (serotype 1), MO (serotype 2) and NC (serotype 3). Jackwood & Saif (1987) used similar methods to examine 15 chicken and turkey IBDV strains including the three prototype strains of Lee (1983), and confirmed the existence of only two serotypes. The serotype 1 and 3 prototype strains (BVM and NC, respectively) (Lee, 1983) were identified as subtypes of serotype I (Jackwood & Saif, 1987). Six subtypes were distinguished among the 13 serotype 1 strains tested (Jackwood & Saif, 1987). By comparison, as many as 10 serotypes have been reported for aquatic birnaviruses (Hill & Way, 1983) indicating a high degree of variation in the serotype-specific proteins of these viruses.

**Immune response**

The paradox of the immune response to IBDV infection is the simultaneous immunosuppression against many antigens (Allan et al., 1972; Faragher et al., 1974) but stimulation of very high antibody levels to the virus itself (Skeelers et al., 1979b). Indeed Käufer & Weiss (1980) described
an immune response to IBDV in surgically bursectomized chickens indicating that immunity to IBDV, at least in part, develops in the spleen and other lymphoid organs. Immunosuppression results directly from damage to the bursa. The immunosuppressive effects of IBDV infection in chickens and turkeys have been thoroughly reviewed (Okoye, 1984; Cummings et al., 1986) and will only be briefly referred to here.

**Antibody response**

Infection with IBDV is accompanied by formation of antibodies to the group antigens and the serotype-specific antigens (Jackwood et al., 1985). After field exposure or vaccination with IBDV, virus neutralization (VN) titres greater than 1:1000 are common (Lukert & Hitchner, 1984). However, weak and delayed antibody responses (detected by ELISA and VN assay) are obtained in adult chickens immunized with the purified viral polypeptides of IBDV (Fahey et al., 1985b). Two explanations have been suggested: first, that a relatively small antigenic mass is injected into the chickens, and second, that there is a reduced immunogenicity of purified polypeptides, perhaps as a result of destruction or alteration of important conformational epitopes by SDS treatment of the virus. The former is highly unlikely. VP3 fused to β-galactosidase, from recombinant *E. coli* colonies containing VP3-encoding cDNA inserts, is highly immunogenic (Azad et al., 1986). However, the antibodies produced generally have low VN titres suggesting that the fusion protein does not have the conformation needed to elicit VN antibodies. Additional evidence that virus protein conformation is important in immunity is shown by studies in which the native conformation of VP2 induces VN antibodies (Azad et al., 1987; Becht et al., 1988) which do not occur in response to SDS-denatured VP2 (Fahey et al., 1985a; Becht et al., 1988).

Humoral immunity is the primary mechanism of the protective immune response (Baxendale, 1976; Hitchner, 1971; Lucio & Hitchner, 1979, 1980; Wyeth & Cullen, 1976, 1978; Wyeth, 1980; Wood et al., 1981). During a study of the protective immune response to IBDV following vaccination with standard serotype I vaccines, Rosenberger et al. (1985) isolated several IBDVs. These isolates produced bursal lesions in vaccinated specific pathogen-free (SPF) birds. Others have also isolated IBDV from vaccinated broiler stock in farms throughout the U.S.A. (Lukert, 1986; Saif, 1984; Saif et al., 1987). Some of these viruses are presently classified as subtypes of serotype I (Jackwood & Saif, 1987). They are called ‘variant’ viruses but their origin is not clearly known.

The VN test using standard IBDV serotype I strains does not differentiate between antibodies prepared against some standard and variant strains of serotype I IBDV. When the Variant-A virus strain is used as antigen in the VN test, it is poorly neutralized by antibodies against standard serotype I IBDV (Kibenge & Dhillon, 1987). We have also observed an anamnestic response when birds inoculated with standard serotype I virus or Variant-A are challenged with the heterologous strain 2 weeks later. This indicates a one-way antigenic relationship between some variant viruses and the standard serotype I IBDV (Kibenge & Dhillon, 1987). The variant viruses protect chickens against challenge with pathogenic standard serotype I IBDV (Rosenberger & Cloud, 1987). They might be ideal for priming prior to immunization with inactivated serotype I IBDV vaccines.

The variant IBDVs are highly cytopathic and cause rapid bursal atrophy (within 72 h p.i.) with minimal inflammatory response. They incite little or no clinical IBD in susceptible 3- to 4-week-old SPF leghorns and appear to persist for longer periods in the thymus (Rosenberger & Cloud, 1986; Rosenberger et al., 1987). When inoculated into embryos, the variants produce little if any embryo mortality but typically cause marked liver necrosis and splenomegaly (Rosenberger et al., 1987). Variant IBDVs cause immunosuppression (subclinical IBD) in infected chickens which have maternal antibodies to serotype I vaccine viruses (Saif, 1984; Rosenberger & Cloud, 1986).

Neutralizing antibodies to a serotype II (OH) strain do not protect chicks from challenge with a virulent serotype I (ST-C) isolate. The reverse situation can not be tested because the available serotype II viruses are avirulent. Since the serotypes are less than 10% related (McFerran et al., 1980), and chickens inoculated with serotype I viruses do not produce neutralizing antibodies to
serotype II viruses (Jackwood et al., 1985), no cross-protection would be expected between the two serotypes.

It has been demonstrated with other viruses, for example, bluetongue virus of sheep (Jeggo et al., 1983), that several inoculations of homologous or heterologous viruses broaden the spectrum of the antibody response. This has not been investigated with IBDV but may provide a method for broadening the spectrum of IBDV antibodies to protect against variant viruses. A broad antibody response to IBDV might also reduce selection pressure on wild-type virus thereby hindering the emergence of antigenic variants.

Cell-mediated immune response

There is a lack of information on cell-mediated immune (CMI) responses to IBDV antigens and the role of a CMI response in protective immunity. Reports on the effect of IBDV on the CMI response have not been in total agreement (Lukert & Hitchner, 1984). This is probably because of limitations and variations in the tests available for measurement of CMI.

Cross-reactions with other birnaviruses

There are no serological cross-reactions between IBDV and other birnaviruses (Dobos et al., 1979). This is in contrast to aquatic birnaviruses (IPNV, TV, OV), which cross-react to some extent (Macdonald & Gower, 1981), although significant antigenic differences occur among the numerous isolates (Hill & Way, 1983; Caswell-Reno et al., 1986). A high level of cross-neutralization has been reported between OV and TV (Dobos et al., 1979), and between the AB strain of IPNV and the European eel virus (Hedrick & Okamoto, 1982), and IPNV and TV cross-react in immunofluorescence tests (Underwood et al., 1977).

Vaccines

Present vaccines

Because of the stability of IBDV in the environment, control through sanitation and isolation is not practical for commercial poultry production (Benton et al., 1967a; Cosgrove, 1962; Parkhurst, 1964). The principal method of control is therefore by vaccination. Of the two serotypes of IBDV recognized in the U.S.A. and Europe, only serotype I and its variants have been known to cause naturally occurring disease. Current vaccines are therefore made from these.

The main emphasis for control of IBD is by vaccination of the dam in order to obtain chickens which have passive immunity for the first 4 to 5 weeks of life. Egg yolk antibodies protect progeny against early subclinical infection. Because maturing birds are refractory to natural infection with IBDV, highly antigenic parenteral immunizing products such as inactivated oil-adjuvant vaccines are used to boost immunity in breeding stocks previously sensitized by exposure to the live virus.

Because the level of passive immunity is variable and unpredictable, a common commercial practice is to vaccinate all chicks against IBD with a live vaccine during the first 3 weeks of life (Winterfield et al., 1980). Inactivated vaccines are ineffective in inducing active immunity in chicks with maternal antibody. Current attenuated IBDV vaccines vary in their safety and efficacy (Thornton & Pattison, 1975; Winterfield et al., 1980; Winterfield & Thacker, 1978; Naqi et al., 1980; Lukert & Hitchner, 1984; Giambrone & Clay, 1986). They are generally classified as 'mild' highly attenuated vaccines and 'intermediate' vaccines of moderate virulence. The latter vaccines are now more frequently used, particularly in the U.S.A. (Giambrone & Clay, 1986), since fully attenuated IBDV strains do not induce immunity in chickens in the presence of maternal antibodies (Baxendale, 1976; Hitchner, 1971; Lucio & Hitchner, 1979; Skeeles et al., 1979b; Winterfield et al., 1980; Winterfield & Thacker, 1978; Wood et al., 1981).

Because birds inoculated with serotype I vaccines are not protected against infection with the variant viruses (Rosenberger & Cloud, 1986), there has been a resurgence of interest in the
U.S.A. in IBDV vaccines. One result of this has been work on attenuation of the variant viruses by passage in cell culture (Rosenberger et al., 1987). An attenuated Variant-E strain of IBDV was safe for young chicks, stimulating a protective immune response against both variant and standard serotype I IBDV field isolates (Rosenberger & Cloud, 1987).

**Future vaccines**

The recent advances in knowledge of the molecular structure and immunology of IBDV provide a basis for the development of better attenuated or bio-engineered vaccines. Subunit vaccines can be prepared directly from the virus by recombinant DNA technology. This allows large scale production of the specific protective polypeptides. Synthetic peptides comprising the antigenic determinants of the protective immunogens of IBDV could be produced. However, before subunit vaccines can be used, ways will have to be found to increase the magnitude and duration of the immune response. Also these vaccines are expensive to produce and in labour costs to administer. This might be prohibitive in the poultry industry which prefers mass vaccination methods.

Although previous work (Azad et al., 1987; Becht et al., 1988) has shown that VP2 is the major host-protective antigen in IBDV, work on subunit vaccines should be influenced by the realization that serotype I variant viruses are not neutralized by standard serotype I antiserum. A single IBDV immunogen may not protect against all virulent field strains of IBDV, particularly in the U.S.A. Another possible disadvantage of subunit vaccines is that the specificity of the immune response may exert a selective pressure on wild virus, resulting in even more antigenic variants.

Another method of producing bio-engineered vaccines in the future may be by inserting immunogen genes of IBDV into live virus vectors such as fowlpox virus (Boyle & Coupar, 1986; Binns, 1987). These recombinant vaccines may be economical and effective. This is analogous to the use of vaccinia virus as a vaccine vector in mammals (Mackett & Smith, 1986; Smith et al., 1983; Paoletti et al., 1984, 1985; Panicali et al., 1983; Kienny et al., 1984; Mackett et al., 1985; Yilma et al., 1985).

Because vaccinia virus replication has been demonstrated in animals (Mackett et al., 1985; Perkus et al., 1985; Smith et al., 1985) and man (Mackett & Smith, 1986; Jones et al., 1986) previously infected or vaccinated with vaccinia virus (Paoletti et al., 1985), recombinant vaccines could be useful to stimulate active immunity in chickens that already have passive immunity. This may help overcome the problem of maternal antibody interference with IBD immunization.

Recombinant fowlpox vaccines could also be expected to produce good primary immunization against multiple poultry pathogens. This could be achieved by a variety of techniques such as those shown by Paoletti et al. (1985) with vaccinia virus vector vaccines. Since the expression level of the foreign gene(s) in the fowlpox virus recombinant could be manipulated, no interference with fowlpox vaccination programmes (Tripathy & Cunningham, 1984) would be expected as a result of immunity to the fowlpox virus vector itself. If interference were to occur, then alternatives such as using serologically unrelated or altered members of the poxvirus family could be investigated.

Recombinant vector vaccines also have the advantage over subunit vaccines of considerable flexibility. It may be possible to insert several IBDV genes (e.g. from subtypes of serotype I) to broaden the immunogenic spectrum. These vaccines could also be administered by mass vaccination methods or in combination with other poultry vaccines such as the turkey Marek's herpesvirus vaccine (Siccardi, 1975).

Other approaches are being used to develop better live attenuated virus vaccines. This has become possible as a better understanding of the molecular basis of attenuation is obtained (Chanock, 1982). Techniques used in recombinant DNA technology now allow specific insertions or deletions in viral genomes, thus facilitating the construction of attenuated viruses with defined mutations (Kit et al., 1985). With regard to IBDV, it is necessary to identify the part of the virion responsible for virulence to create live modified viruses that are immunogenic as well as safe to use.
CONCLUDING REMARKS

Knowledge of the molecular structure and immunology of IBDV is accumulating rapidly. The viral protein responsible for protective immunity against IBD has been identified and the viral genomic segment encoding it has been sequenced. This information may lead to development of better diagnostic tools and more effective vaccines to control IBD in chickens.

Because serotype I IBDV does not protect against the presently recognized variant viruses and the possibility that new variants will emerge in the future, it is essential to understand the antigenic relationships among present isolates of IBDV, to elucidate the mechanism causing antigenic variation, and to develop effective immunization strategies. Future immunization strategies will need to develop protection against all pathogenic field strains of IBDV and avoid setting up selection pressures that might cause new variants to emerge. In this regard, recombinant live vector vaccines offer the greatest potential because they give the greatest flexibility, they could be engineered to induce a broad immune response with less risk of inducing antigenic variants, and they would be economical for commercial poultry production.

The authors are grateful to Mrs Rosie Hueneka for secretarial help. We thank Susan R. Weiss, Graham H. Purchase, Phil D. Lukert, Roger G. Breeze and Graham E. Wilcox for critical reading of the manuscript.

REFERENCES


