Antigenic and genomic comparison between non-cytopathic and cytopathic bovine viral diarrhoea viruses isolated from cattle that had spontaneous mucosal disease

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Antigenic and genomic relationships between five pairs of non-cytopathic and cytopathic bovine viral diarrhoea (BVD) viruses isolated from cattle with mucosal disease were examined. Antigenic similarity was evaluated by studying the binding characteristics of 10 monoclonal antibodies (MAbs) directed against the gp53 BVD virus glycoprotein. The MAb binding match between members of the same virus pair ranged from 10/10 to 7/10. The genomic relationship was evaluated by studying the hybridization characteristics of two cDNA probes and six complementary 20 base oligomer probes with virus DNA, selected on the basis of conservation between published BVD virus sequences; cDNA probes were hybridized at two stringencies (60 °C and 45 °C). The match of hybridization results between members of the same virus pair ranged from 4/4 to 1/4 with the cDNA probes and from 6/6 to 3/6 with the oligomer probes. The results indicate that members of the same virus pair can differ at both the antigenic and genomic levels.

Bovine viral diarrhoea (BVD) virus, a positive-stranded RNA virus of the pestivirus group, is ubiquitous among cattle. Two biotypes, non-cytopathic and cytopathic, are differentiated by their effect on susceptible cell cultures (Lee & Gillespie, 1957; Gillespie et al., 1960, 1962). Mucosal disease (MD) is among the numerous disease processes induced by BVD viruses. This disease is induced by a unique mechanism that requires persistent infection with a non-cytopathic BVD virus followed by superinfection with an appropriate cytopathic BVD virus (Brownlie et al., 1984; Bolin et al., 1985a). Not every combination of non-cytopathic and cytopathic BVD virus results in fatal disease (Bolin et al., 1985b) and the interaction between the pairs of cytopathic and non-cytopathic BVD virus isolates that results in MD is unknown.

Antigenic similarity, based on virus neutralization, was reported between non-cytopathic and cytopathic pairs of viruses isolated from cattle that had MD (Howard et al., 1987; Corapi et al., 1988). From these data it was hypothesized that the cytopathic BVD virus isolated from cattle with MD arose by mutation of the persistent non-cytopathic BVD virus (Howard et al., 1987; Corapi et al., 1988). To date, the genomic similarity between cytopathic and non-cytopathic viruses involved in MD has not been examined. In this study we have compared MD virus pairs at both the antigenic and genomic levels. Similar to earlier studies comparing MD virus pairs, we evaluated similarity using a panel of monoclonal antibodies (MAbs) that had virus neutralizing activity and reacted with the viral polypeptide gp53. The genomic comparison was performed by hybridization using cloned BVD virus cDNA and BVD virus complementary oligomers as probes.

Pairs of non-cytopathic and cytopathic BVD viruses used in this study were isolated from cattle that had MD. In all cattle, MD occurred spontaneously and was not induced experimentally, nor had it occurred after vaccination. The non-cytopathic/cytopathic virus pairs NEB/2110, VM/190 and 7443/9657 were isolated from cattle at the National Animal Disease Center (NADC), Iowa, U.S.A. in 1987 and 1988. The non-cytopathic/cytopathic virus pairs Ill-NC/Ill-C and TGAN/TGAC were isolated from bovine spleens submitted to the NADC in 1981 and 1982, respectively. The non-cytopathic/cytopathic virus pairs Ill-NC/Ill-C and TGAN/TGAC were isolated from bovine spleens submitted to the NADC in 1981 and 1982, respectively. The cytopathic virus isolates were purified by three successive plaque pickings. The non-cytopathic virus isolates were purified by serial passage at limiting dilutions. Stock viruses were propagated in bovine turbinate (BT) cells, determined to be free of adventitious BVD viruses by direct fluorescence assay and radioimmunoprecipitation procedures, and harvested after one cycle of freezing and thawing.
Table 1. Binding of MAbs specific for gp53 to non-cytopathic and cytopathic BVD viruses isolated from cattle that had spontaneous MD

<table>
<thead>
<tr>
<th>MAb</th>
<th>Pair 1</th>
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<th>Pair 3</th>
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<td>2110-C</td>
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<td>CA-82</td>
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* c, Cytopathic.
† NC, Non-cytopathic.

All stock viruses, with the exception of TGAN and TGAC, were at cell culture passage six or less; the TGAN virus isolate was at cell culture passage eight and the TGAC virus isolate was at cell culture passage 12. Cell culture passages were limited to the number of passages necessary to separate biotypes. Virus was stored at -90 °C. BT cells were grown in F15 Eagle’s medium (Gibco) supplemented with 10% foetal bovine serum, tested at the NADC and found to be free of adventitious BVD virus and antibodies to BVD virus.

The panel of MAbs used to evaluate antigenic similarity consisted of 10 BVD viral glycoprotein gp53-specific antibodies. MAbs were prepared and characterized as described by Bolin et al. (1988). The immunoperoxidase staining procedure of Afshar et al. (1989) was used with the following modifications. Cells were seeded 24 h before the addition of virus, fixation was performed for 10 min with 35% acetone in phosphate-buffered saline pH 7.6, fixed cells were dried for 30 min at 37 °C and peroxidase-conjugated Protein G (Zymed) was used rather than horseradish peroxidase-labelled anti-IgG. The results of binding MAbs to non-cytopathic and cytopathic BVD virus pairs are shown in Table 1. Non-cytopathic and cytopathic viruses within three virus pairs could not be differentiated by MAb binding. These results are in agreement with the proposal that virus pairs involved in MD are antigenically similar. The remaining two virus pairs, TGAN/TGAC and NEB/2110-C, differed in their binding of three of 10 and two of 10 MAbs, respectively.

The probes used for the evaluation of genomic similarity were selected on the basis of conservation between published BVD virus sequences (Fig. 1). Two plasmids containing cloned cDNA sequences of the NADL isolate of BVD virus were obtained from M. Collett (Molecular Vaccines). Plasmid pBV-18 contained an insert encompassing nucleotides 24 to 1308 and plasmid pBV4-p80 contained an insert encompassing nucleotides 5644 to 7949 (Fig. 1). Although MAbs against gp53 were used to evaluate antigenic similarity, the portion of the genome encoding gp53 was not used as a cDNA probe. This decision was made because amino acid sequence identity analysis between NADL BVD virus and Osloss BVD virus (Collett et al., 1989) indicated relatively low identity between those two isolates in the region coding for gp53. Radiolabelled cDNA plasmid probes were prepared by nick translation following the method of Koch et al. (1986) as modified by Sambrook et al. (1989). Spin columns (5 Prime-3 Prime) were used to remove unincorporated label.

Complementary oligomers (20 bases) were synthesized to six of 29 homologous sites identified between the NADL and Osloss BVD virus sequences (Fig. 1). The oligomer probes were used for hybridization because preliminary studies in our laboratory had indicated that they were useful for discrimination between BVD viruses. Oligomers were synthesized an an Applied Biosystems Model 381A DNA synthesizer using phosphoramidite chemistry (Beaucage & Caruthers, 1981) and end-labelled using the T4 polynucleotide kinase reaction. The end-labelling reaction mix was 67 mM-Tris–HCl pH 8.0, 10 mM-MgCl₂, 10 mM-DTT, 400 ng oligomer and 100 μCi [γ-³²P]ATP. Unincorporated label was removed by anion exchange chromatography using DE-52 cellulose.

To generate RNA blots, confluent monolayers of BT cells grown in 25 cm flasks were inoculated with BVD virus isolates at an approximate m.o.i. of 10. Cytopathic viruses were harvested when 90% of the cells exhibited a c.p.e. (24 to 30 h after inoculation); non-cytopathic viruses were harvested at 48 h post-inoculation. The cultures, including cells and culture media (approximate-
ly 5 ml), were harvested by two cycles of freezing and thawing at \(-20^\circ\text{C}\). A 500 \mu l aliquot was removed at this point to determine the virus titre. The remainder of the harvested culture material was stored at \(-20^\circ\text{C}\). Harvested material that had an infectious virus titre of \(10^6/\text{ml}\) or greater was used for blotting. RNA was prepared for blotting by adding 5 ml lysis buffer (0.2 M-Tris–HCl pH 8.0, 25 mM-EDTA, 0.3 M-NaCl, 2% SDS and 200 \mu g/ml proteinase K) to 5 ml virus–cell lysate and incubating at 37 \(^\circ\text{C}\) for 30 min. The resulting mixture was extracted once with an equal volume of a 1:1 mixture of phenol and chloroform and once with an equal volume of chloroform. After extraction, 10 ml formaldehyde [6-15 \text{m M in 20} \times \text{SSC (3 m NaCl, 0.34 m sodium citrate pH 7-0)}] was added, the mixture was
incubated at 65 °C for 15 min and 1 ml solution was blotted onto nitrocellulose. Nucleic acids from cells infected with the NADL BVD virus isolate and uninfected cells were blotted as positive and negative controls respectively. After blotting, nitrocellulose membranes were rinsed in 2 × SSC and baked for 2 h at 80 °C.

Blots probed with cDNA plasmids were prehybridized for at least 6 h, at either 60 °C or 45 °C, in 6 × SSC, 2 × Denhardt’s reagent, 0-1 % SDS and 100 µg/ml denatured salmon sperm DNA. Blots were hybridized at the same temperature in the same buffer with the addition of 0-1 µg denatured cDNA probe (sp. act. at least 2 × 10⁸ c.p.m./µg). Hybridized filters were washed for 20 min at room temperature in 1 × SSC, 0-1 % SDS, followed by two washes of 1 h each at the hybridization temperature in 0-2 × SSC, 0-1 % SDS (Sambrook et al., 1989).

Blots probed with oligomers were prehybridized at 65 °C for 4 to 5 h in 6 × NET (0-15 M-NaCl, 15 mM-Tris–HCl pH 8-3, 1 mM-EDTA), 0-1 % SDS, 5 × Denhardt’s solution and 100 µg/ml denatured salmon sperm DNA. Hybridization reactions were carried out at 50 °C for 2 h in 6 × NET, 0-1 % SDS, 5 × Denhardt’s solution and 1-5 × 10⁷ to 2-0 × 10⁷ c.p.m. end-labelled oligonucleotide (5 to 10 ng/ml of probe). After hybridization, blots were washed three times in 6 × SSC, 0-1 % SDS at room temperature for 5 min each, twice in 3 × SSC, 0-1 % SDS at 50 °C for 30 min each and twice in 1 × SSC, 0-1 % SDS at 50 °C for 20 min each. After washing, blots were exposed to X-Omat AR or XAR-5 film (Eastman Kodak) for 48 h.

Hybridization reactions are summarized in Table 2 and an example of the hybridization results is shown in Fig. 2. The RNA from all virus isolates hybridized to the pBV-18 probe at 45 °C and to oligomer A, indicating that sufficient RNA was blotted for detection. One virus pair (NEB/2110-C) was not differentiated by its hybridization results. Genomic heterogeneity was detected among the remaining four virus pairs. Two virus pairs (VM/190 and 7443/9657) were differentiated by hybridization with both cDNA and oligomer probes. Viruses within the remaining two pairs were only differentiated by hybridization with cDNA probes (II1-NC/II1-C) or by hybridization with oligomer probes (TGAN/TGAC). Genomic heterogeneity detected by hybridization with cDNA probes is of greater significance than that detected by hybridization with oligomers because a larger portion of the genome is represented.

Our findings indicate that the virus pairs involved in the production of MD may be homologous or heterologous at the antigenic and/or genomic level. Antigenic similarity between viruses did not appear to correlate with genomic identity. Our data are not sufficient to determine the origin of the cytopathic viruses or the exact genetic relationship of virus pairs. Others have speculated that cytopathic BVD viruses arise from non-cytopathic viruses within the persistently infected cow (Howard et al., 1987; Corapi et al., 1988). Some of the differences observed between virus pairs may be due to mutations arising in cell passage. We have been successful in producing MD in animals using the virus pair, TGAC/TGAN, which had the highest passage number. The cytopathic TGAC virus was at the same passage number used in this study whereas the non-cytopathic TGAN virus, in addition to cell culture passage, had been passaged in animals three times. This suggests that any change that may have occurred in cell culture passage, and which may have contributed to the differences observed between paired viruses, did not affect the ability of the virus pairs to cause MD.

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References


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