Antigenic differences between the major glycoproteins of bovine herpesvirus type 1.1 and bovine encephalitis herpesvirus type 1.3

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Differences in the antigenic structure of the major glycoproteins, gI, gIII and gIV, of bovine herpesvirus type 1.1 (BHV1.1) and the neurovirulent BHV1.3 were demonstrated with a panel of monoclonal antibodies (MAbs) prepared against the BHV1.1 glycoproteins. Glycoprotein gIII of BHV1.3 was the most dissimilar, reacting with only four of 15 gIII-specific MAbs. Glycoproteins gI and gIV of BHV1.3 reacted with eight of 11 and eight of 12 specific MAbs, respectively. Monospecific bovine antisera to the two viruses supported findings from the MAb analysis in that gI and gIV glycoproteins were cross-recognized, but gIII was not. Virus-neutralizing MAbs reactive to each glycoprotein and which reacted with both viruses also neutralized both viruses. Previously undescribed glycoproteins which were antigenically related to the intact gIII glycoproteins, but of reduced sizes and lacking at least one gIII epitope, were found for both viruses. Tunicamycin inhibition experiments and immunoprecipitation data suggested that these proteins were intracellular degradation products. Comparisons of the peptide footprints of the glycoproteins from the two viruses using protease V8 digestion after immunoprecipitation with cross-reactive MAbs revealed distinctive footprint patterns for the respective glycoproteins.

Introduction

Bovine herpesvirus type 1 (BHV1) has been isolated from outbreaks of fatal encephalitis in Argentina (Carillo et al., 1983a, b), Australia (French, 1962), the United States (Eugster et al., 1974), and sporadically from other countries around the world (Bartha et al., 1969; Gough & Jones, 1975; Moretti et al., 1964). It has recently been recognized that the herpesviruses isolated from these outbreaks have genome characteristics (Brake & Studdert, 1985; Engels et al., 1987) and antigenic differences (Metzler et al., 1986; Friedli & Metzler, 1987) indicating that they represent a unique group of herpesviruses of cattle. Whereas genomic differences between BHV1 isolates have previously led to the segregation of viruses into respiratory or BHV1. Cooper-like, and genital or BHV1.K22-like variants based on restriction endonuclease digestion maps (Ludwig, 1983; Metzler et al., 1985; Misra et al., 1983; Miller et al., 1988), similar studies with the viruses recovered from the outbreaks of encephalitis have shown that they are more disparate (Brake & Studdert, 1985; Engels et al., 1987). Restriction endonuclease maps obtained using HindIII, BamH1 and BstEII of the DNA genomes from the prototype strains from Australia, N569, and from Argentina, A663, are very similar and yet altogether distinct from prototype respiratory and genital tract isolates, even though the representative BHV1 genomes are collinear in sequence (Brake & Studdert, 1985; Engels et al., 1987). Additionally, Engels found that the sequence identity between the encephalitis isolate and Cooper strain was approximately 85%, whereas the similarity between respiratory and genital isolates was greater than 95% (Engels et al., 1987). Comparison of sequence differences found by cross-hybridization among cloned BamH1 fragments between encephalitic and Cooper strains has demonstrated unique areas of sequence divergence (Bulach & Studdert, 1990).

Because of these unique strain differences among BHV1 isolates, several different designations have been given to the viruses. One proposed name is BHV1.3 (Engels et al., 1987; Metzler et al., 1986; Schudel et al., 1986), continuing the previously adopted BHV1.1 and BHV1.2 designations for respiratory and genital isolates, respectively (Ludwig, 1983; Misra et al., 1983). However, because of the unique genome characteristics and the possibility of a significantly different natural history of the virus, Studdert has suggested a new name, bovine encephalitis herpesvirus or BEHV (Studdert, 1989; Bulach & Studdert, 1990).
Although all BHV1 isolates are readily identified with polyclonal antisera directed against BHV1. Cooper-type strains (Metzler et al., 1986; Bratanich et al., 1991), significant antigenic differences between the encephalitis strain and other BHV1 subtypes have been found by crossed virus neutralization tests (Bagust, 1972; Metzler et al., 1986), neutralization kinetics (Bratanich et al., 1991), SDS–PAGE of radiolabelled polypeptides (Schudel et al., 1986; Metzler et al., 1985, 1986), and by monoclonal antibody (MAb) reactivity (Metzler et al., 1986; Friedli & Metzler, 1987).

In the present study, MAb analysis and protein footprinting techniques have been applied to the major glycoproteins of BHV1.1 and BHV1.3. These glycoproteins include gI, analogue of the herpes simplex virus (HSV) gB glycoprotein (Fitzpatrick et al., 1990; Misra et al., 1988), gIII, analogue to the HSV gC glycoprotein (Fitzpatrick et al., 1990) and gIV, analogue to the HSV gD glycoprotein (Tikoo et al., 1990). Major differences in epitope structure, peptide maps and in the bovine antibody response to the viral glycoproteins are described.

**Methods**

**Monoclonal antibodies.** MAbs were derived previously: D9, C6, C11, F2, G2, H2 (Collins et al., 1984); 4407, 4807, 5106, 5606, 1507, 1808, 2905, 3002, 1808, 1102, 1106, 3402, 4906, 5006 (Marshall et al., 1986); 1E11, 1F8, 1F10, 3C7, 3F3, 5G2, 5G11, 3H7, 3E3, 3F12, 1E2, 3G8, 1D6, 2A11, 1F11 (van Drunen Littel-van den Hurk et al., 1985); 136, 4C1, 10C2, 3C1, 3E7, 9D6 (Hughes et al., 1988); 3D9S (van Drunen Littel-van den Hurk & Babiuk, 1986).

**Virus strains.** The following viruses were used: a previously characterized strain (no. 10902) of BHV1.1 recovered from the respiratory tract of a case of classic rhinotracheitis (Collins et al., 1984, 1985) and a strain of BHV1.3 30326 recovered from the brain of an animal with encephalitis in an outbreak in Texas (Eugster et al., 1974).

**Bovine antiserum.** A Holstein calf, 5 months of age, was given 2 ml (10⁶ TCID₅₀/ml) of a field isolate of BHV1.1 (strain no. 10902) by intranasal aerosolization (Collins et al., 1985) and boosted 4 and 6 weeks later with two subcutaneous injections containing 10⁷ TCID₅₀/ml of virus. Monospecific viral antiserum (no. 561) to BHV1.1 Cooper strain (ATCC VR-864) and antiserum (no. 560) to BHV1.3 Texas strain (Eugster et al., 1974), were prepared by giving three intramuscular injections of the respective purified viruses.

**Virus neutralization by plaque reduction assay.** Twofold dilutions of MAb were mixed with a dilution of virus sufficient to give 30 to 40 plaques. This mixture was incubated for 18 h at 37 °C, then inoculated in duplicate (0.2 ml/well) in six-well tissue culture plates onto monolayers of primary bovine turbinate cells, grown as described previously (Collins et al., 1984). These plates were rockered on a platform at 37 °C for 90 min, washed, and the cells were overlaid with medium containing 0.6% agarose and 1% fetal bovine serum. After 3 days at 37 °C the overlays were removed, the monolayers were stained with 1% crystal violet in 20% ethanol, and the plaques were counted. The titer was recorded as the highest dilution of antibody that reduced plaques by at least 50% compared with the control using an unrelated antibody.

**Radioimmunoprecipitation.** Radioimmunoprecipitation (RIP) of virus-specific polypeptides was performed as described previously (Collins et al., 1984), using 1 to 3 μl of ascites fluid MAb. Secondary immunosorbers consisted of solid-phase Protein A (Protein A-Sepharose CL-4B, Sigma) or rabbit anti-mouse immunoglobulin (Immunobeads, Bio-Rad). Reduced proteins were boiled in sample buffer with 5% 2-mercaptoethanol; non-reduced proteins were boiled without this reducing agent.

**Protein electrophoresis.** Ten percent SDS-PAGE was performed as described previously (Collins et al., 1984). Mrs were determined using pre-stained markers (Amersham) and by averaging the Mr determinations from three PAGE experiments.

**Protein footprints obtained by protease V8 digestion of MAb-bound glycoproteins.** To perform protease digestion, RIP was first performed with a selected MAb. After allowing the MAb, infected cell lysate and immunosorbent to react, the MAb-antigen-immunosorbent complex was washed three times with lysing buffer (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 0.85% sodium chloride in 0.01 M-Tris-HCl pH 7.2 to 7.4) and the complex was suspended in water. This was then divided into 0.05 ml samples containing 0, 0.5, 2.5, 12.5 and 50 units of V8 protease (Sigma). After incubation of the complexed immunosorbents with protease for 18 h at room temperature (23 °C), they were again washed with lysing buffer, resuspended in sample buffer, boiled and analysed by SDS–PAGE. Fragment patterns observed using the same glycoprotein, but immunoprecipitated and therefore protected by different MAbs, were designated footprints characteristic for those conditions.

**Competition ELISA.** Competition ELISA tests were performed between peroxidase-conjugated MAbs and ascites containing different MAbs as described previously using microtitre plates coated with purified BHV1.1 (Ayers et al., 1989).

**Results**

**Monoclonal antibody reactivity**

Panels of MAbs were selected from three sources and those reactive to distinct epitopes on one of the three major glycoproteins of BHV1.1, gI, gIII or gIV, were screened for reactivity to BHV1.3 by RIP assay (Table 1). All of the MAbs immunoprecipitated the respective glycoproteins of BHV1.1, using either Protein A or rabbit anti-mouse Ig solid-phase immunosorbers. All but three of 11 gI-specific MAbs reacted with BHV1.3 and eight of 12 gIV-specific MAbs were reactive; however, only four of 15 gIII-specific MAbs were reactive. The three non-reacting gI-specific MAbs (3F3, 5G2 and 5G11) represented three different, previously defined epitopes (van Drunen Littel-van den Hurk et al., 1985). The non-reactive gIV MAbs represented three
Table 1. Radioimmunoprecipitation of BHV1.3 with gI, gIII and glV-specific MAbs against BHV1.1

<table>
<thead>
<tr>
<th>Glycoprotein gI</th>
<th>Glycoprotein gIII</th>
<th>Glycoprotein glV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ep* MAb RIP</td>
<td>Ep MAb RIP</td>
<td>Ep MAb RIP</td>
</tr>
<tr>
<td>D9† +</td>
<td>C11† +</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>F2 —</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>G2 +</td>
<td>—</td>
</tr>
<tr>
<td>I‡ 5106 +</td>
<td>II‡ 1507 —</td>
<td>III‡ 5006 +</td>
</tr>
<tr>
<td>II 4807 +</td>
<td>III 2905 —</td>
<td>II 1106 +</td>
</tr>
<tr>
<td>III 5606 +</td>
<td>IV 3002 —</td>
<td>III 4906 +</td>
</tr>
<tr>
<td>IV 4407 +</td>
<td>V 1808 +</td>
<td>IV 1102 +</td>
</tr>
<tr>
<td></td>
<td>V 3402 —</td>
<td>—</td>
</tr>
<tr>
<td>III§ 3F3 —</td>
<td>Ia§ 3H7 —</td>
<td>—</td>
</tr>
<tr>
<td>III 1E11 +</td>
<td>II 3E3 —</td>
<td>1b 9D6 —</td>
</tr>
<tr>
<td>IVa 1F8 +</td>
<td>III 3F12 —</td>
<td>2 3E7 +</td>
</tr>
<tr>
<td>IVb 5G2 +</td>
<td>IV 1E2 —</td>
<td>3a 10C2 +</td>
</tr>
<tr>
<td>IVc 5G11 —</td>
<td>V 3G8 +</td>
<td>3b 4C1 +</td>
</tr>
<tr>
<td>V 1F10 +</td>
<td>VI 1D6 +</td>
<td>3d 3C1 +</td>
</tr>
<tr>
<td></td>
<td>VII 2A11 +</td>
<td>3D9S¶ —</td>
</tr>
<tr>
<td>VIII 1F11 —</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Epitope designation.
† All monoclonal antibodies immunoprecipitated the respective glycoproteins from BHV1.1. gI and gIII MAbs were from Collins et al. (1984).
‡ gI, gIII and glV MAbs were from Marshall et al. (1986).
§ gI and glIII MAbs were from van Drunen Littel-van den Hurk & Babiuk (1985).
¶ glV MAbs were from Hughes et al. (1988).

Table 2. Comparison of virus neutralization titres of selected cross-reactive MAbs

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>MAb</th>
<th>BHV1.1</th>
<th>BHV1.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>gI</td>
<td>5106</td>
<td>1:1600</td>
<td>&lt;1:100</td>
</tr>
<tr>
<td></td>
<td>1E11</td>
<td>1:800</td>
<td>1:6400</td>
</tr>
<tr>
<td></td>
<td>1F8</td>
<td>1:3200</td>
<td>1:1600</td>
</tr>
<tr>
<td></td>
<td>1F10</td>
<td>1:1600</td>
<td>&lt;1:100</td>
</tr>
<tr>
<td>gIII</td>
<td>G2</td>
<td>1:25600</td>
<td>1:12800</td>
</tr>
<tr>
<td></td>
<td>5006</td>
<td>1:102400</td>
<td>1:6400</td>
</tr>
<tr>
<td></td>
<td>1106</td>
<td>1:6400</td>
<td>1:3200</td>
</tr>
<tr>
<td></td>
<td>3E7</td>
<td>1:102400</td>
<td>1:102400</td>
</tr>
<tr>
<td></td>
<td>10C2</td>
<td>1:409600</td>
<td>1:31200</td>
</tr>
<tr>
<td>gIV</td>
<td>4C1</td>
<td>1:102400</td>
<td>1:6400</td>
</tr>
<tr>
<td></td>
<td>3C1</td>
<td>1:102400</td>
<td>1:12800</td>
</tr>
</tbody>
</table>

* Endpoint 50% plaque-reduction titres determined in the absence of complement. Those MAbs cross-reactive by RIP, and previously shown to neutralize virus at ≥1:100 titres were compared.

Identification of the gI, gIII and glV glycoproteins of BHV1.3

Several of the MAbs had been shown previously to neutralize BHV1.1, and were therefore tested for complement-independent neutralization of BHV1.3. Most MAbs that had strong neutralizing activity against BHV1.1 and that reacted with BHV1.3 also neutralized this virus (Table 2). These included the gI-specific MAbs 1E11 and 1F8, the gIII-specific MAb G2 and the gIV-specific MAbs 5006, 1106, 3E7, 4C1, 3C1 and 10C2.

Using cross-reactive MAbs to gI, gIV and p100, a major non-glycosylated protein of BHV1.1, and two MAbs to gIII, the corresponding proteins of BHV1.3 were identified by RIP-SDS-PAGE analysis (Fig. 1). Fig. 1 (lanes 2, 4) shows the gI heterodimer glycoproteins that were immunoprecipitated from the two viruses. Lanes 1 and 3 show gIII, lanes 5 and 7 show gIV, and lanes 6 and 8 show a major non-glycosylated protein of BHV1.1 and
The immunoprecipitation of reduced and non-reduced glycoprotein gI was also compared (Fig. 2). Slightly higher $M_r$s of the major non-reduced gI glycoprotein, the gI precursor and the reduced heterodimer subunits (glb, glc) were apparent for BHV1.3. An additional non-reduced higher $M_r$ band (Fig. 2) was also precipitated from BHV1.3, and glc appeared as a distinct doublet. A lower apparent $M_r$ for gIV was observed but p100 was similar in size for both viruses (Fig. 1).

Immunoprecipitation of BHV1.3 gIII by MAb 1D6 (Fig. 1, lane 3) demonstrated bands in addition to those found for BHV1.1 gIII using MAb F2 (Fig. 1, lane 1). Because of this, the two viral glycoproteins were both analysed further using the cross-reactive MAb, 1D6, and using tunicamycin inhibition of glycosylation (Fig. 3, lanes 1 to 4). The additional bands seen after RIP using MAb 1D6 and BHV1.3 (Fig. 1, lane 3) were also seen upon immunoprecipitation of BHV1.1 with MAb 1D6 (Fig. 3, lane 1) and with an additional MAb, 1507 (lane 5), which was non-cross-reactive. The apparent $M_r$s of gIII and gIII-related proteins of BHV1.3 were correspondingly lower than those of BHV1.1 (87K versus 91K, 64K versus 72K, and 48K versus 51K). Upon analysis of the proteins found after tunicamycin treatment, it was observed that the principal gIII glycoprotein bands (BHV1.1, 91K; BHV1.3, 87K) shifted to corresponding lower $M_r$s (72K and 64K). This suggested a precursor–product relationship between the 91K and 72K proteins for BHV1.1 (Fig. 3, lanes 1, 2), and between the 87K and 64K proteins for BHV1.3 (lanes 3, 4). However, the 51K and 48K gIII-related proteins immunoprecipitated by 1D6 (from both viruses, lanes 1 and 3) and by 1507 (from BHV1.1 only, lane 5) showed altered mobility (lower $M_r$s) after tunicamycin treatment (lanes 2, 4, 6), suggesting that these were not non-glycosylated polypeptides, but were more likely to be antigenically related degradation products. The 51K gIII-related glycoprotein from BHV1.1 was not precipitated by MAb F2 (lane 7).

The apparent $M_r$s of the major glycoproteins of the two viruses are summarized in Table 3. A precursor–
Table 3. Ms of the major glycoproteins of BHV1.1 and BHV1.3

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>BHV1.1</th>
<th>BHV1.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>gI non-reduced</td>
<td>150000</td>
<td>157000</td>
</tr>
<tr>
<td>gIa (reduced)</td>
<td>132000</td>
<td>135000</td>
</tr>
<tr>
<td></td>
<td>122000</td>
<td>123000</td>
</tr>
<tr>
<td>gII</td>
<td>68000</td>
<td>70000</td>
</tr>
<tr>
<td>gIII</td>
<td>122000</td>
<td>123000</td>
</tr>
<tr>
<td>glb</td>
<td>53000</td>
<td>56000</td>
</tr>
<tr>
<td>gII precursor</td>
<td>68000</td>
<td>70000</td>
</tr>
<tr>
<td>gIV</td>
<td>51000</td>
<td>48000</td>
</tr>
<tr>
<td>gII-related</td>
<td>51000</td>
<td>48000</td>
</tr>
<tr>
<td>gIII precursor</td>
<td>72000</td>
<td>64000</td>
</tr>
<tr>
<td>glV</td>
<td>91000</td>
<td>87000</td>
</tr>
<tr>
<td>glII precursor</td>
<td>71000</td>
<td>67000</td>
</tr>
<tr>
<td>glV precursor</td>
<td>63000</td>
<td>55000</td>
</tr>
</tbody>
</table>

product relationship was also suggested for the multiple gII glycoproteins through the use of tunicamycin inhibition experiments (data summarized in Table 3).

**Protein footprints obtained by protease V8 digestion of MAb-bound glycoproteins**

To compare the glycoproteins of the two viruses further and to obtain additional information on differences or similarities in protein structure, immunoprecipitated (MAb-protected) glycoproteins were digested with protease V8, and the cleaved fragments or footprint patterns were then compared by SDS–PAGE. The digestion of proteins while MAb-protected has been shown to create characteristic protein footprints, dependent on the glycoprotein, the MAb and the protease (Sheshberadaran & Payne, 1988; Eisenberg et al., 1982).

Using a cross-reactive MAb, D9, to immunoprecipitate gI glycoproteins, protease V8 digestion clearly distinguished the two viral glycoproteins (Fig. 4). After reduction followed by SDS–PAGE, the two heterodimer subunits were shown to be cleaved into different, discrete patterns. After digestion, both of the gI subunits of BHV1.3 appeared as doublet bands, in contrast to those of BHV1.1, for which the subunits appeared as one band each.

Protein footprints of gIII from the two viruses were analysed after immunoprecipitation of the glycoproteins by MAbs F2, 1507 and ID6 for BHV1.1, and with ID6 for BHV1.3. The three MAbs used for immunoprecipitation of gIII from BHV1.1 further elucidated the different immunoprecipitation patterns observed. These three MAbs, from three different laboratories, each recognized different epitopes on gIII as revealed by reciprocal competition ELISA (Table 4). The patterns of gIII footprints were also quite different with the three MAbs (Fig. 5). MAb F2 protected gIII from digestion but with MAbs 1507 and ID6, distinct footprints were obtained. The gIII precursor and the additional smaller-sized bands immunoprecipitated by MAbs 1507 and ID6 from BHV1.1 were found to remain after protease digestion, suggesting that these were resistant to further digestion, and that they may represent intracellular digestion fragments bearing these epitopes.

Using the cross-reactive MAb ID6, the footprints obtained from BHV1.1 and BHV1.3 gIII glycoproteins were clearly distinct (Fig. 5). Although several similarly sized protease-resistant digestion fragments were observed, virus-specific fragments were also identified. Both gIII glycoproteins were relatively resistant to V8 digestion, since intact gIII was still present after 18 h of digestion.
Fig. 5. Footprints of gIII glycoproteins obtained from BHV1.1 (R) and BHV1.3 (E). The gIII glycoproteins were immunoprecipitated with the indicated MAbs then digested with no V8 protease (lanes C) or with 0.5, 2.5, 12.5 or 50 units of protease (lanes 1, 2, 3 and 4, respectively) and analysed by SDS-PAGE.

Fig. 6. Footprints of gIV glycoproteins obtained from BHV1.1 (R) and BHV1.3 (E). The gIV glycoproteins were immunoprecipitated with the MAbs indicated then digested with no V8 protease (lanes C), or with 2.5 or 12.5 units of protease (lanes 1, 2, 3, respectively). Despite this resistance to digestion, the two glycoproteins were readily distinguished.

Footprints obtained from the respective gIV glycoproteins after immunoprecipitation with two cross-reactive MAbs are shown in Fig. 6. Even though the two gIV glycoproteins shared many epitopes as shown by RIP analysis, the two glycoproteins were markedly different in the footprints that were generated. Digestion of the gIV glycoprotein of BHV1.1 revealed an intermediate digestion pattern of 10 bands which were further digested to a major V8-resistant band of about 30K. This pattern was observed with both MAbs 1102 and 1004, each of which recognizes the same epitope on gIV of BHV1.1 (Marshall et al., 1986). In contrast, glycoprotein gIV of BHV1.3 was digested to three bands and showed major V8-resistant end-products of either a single or doublet band at 37K to 40K, depending on the MAb. Minor differences in the final digestion patterns found with these two MAbs were observed for both BHV1.1 and BHV1.3 gIV glycoproteins. The different
bands observed by digestion of the same gIIV but with different MAb protection may represent transient and/or intermediate digestion fragments possibly present during each digestion but at different times.

Cross-reactivity of bovine antibodies to gI, gIII and gIV glycoproteins of BHV1.1 and 1.3

To examine cross-reactivity of bovine antibodies to the two viruses, monospecific immune sera were prepared to each virus and used to immunoprecipitate the viral proteins (Fig. 7). Differences in gIII antigens were demonstrated using bovine antisera. Two bovine antisera to BHV1.1 precipitated glycoprotein gIII of BHV1.1 as expected (lanes 8 and 9), but did so only weakly with gIII of BHV1.3 (pointer a, lane 11). The bovine antiserum to BHV1.3 failed to precipitate gIII from BHV1.1 (pointer b, lane 10), but did so from homologous BHV1.3 (lane 12). In contrast, glycoproteins gI and gIV were cross-precipitated by bovine antisera to the two viruses. The relative amounts of these glycoprotein bands were approximately equivalent for each virus and were not dependent on the antiserum used. Variation was also found in the precipitation of the major non-glycosylated protein p100 (lane 6), in that it was precipitated only weakly by BHV1.3-specific antiserum from both BHV1.1 and 1.3 (lanes 10, 12).

Discussion

Antigenic differences between the corresponding major glycoproteins of BHV1.1 and BHV1.3 were demonstrated by comparison of MAb reactivities to the two viruses. Of the three glycoproteins, gI, gIII and gIV, gIII showed major antigenic differences between the two viruses, with gIII of BHV1.3 reacting with only four of 15 gIII-specific MAbs. At least three of these four gIII MAbs represented unique, previously mapped epitopes (van Drunen Littel-van den Hurk et al., 1985); the fourth, G2, is related to one of them, ID6, as ascites fluid from ID6 competes in competitive binding assays with this MAb (V. K. Ayers and J. K. Collins, unpublished). Glycoproteins gI and gIV of the two viruses were more closely related antigenically. Analysis of the reactivity of the viruses with monospecific bovine antisera supported the MAb analysis findings. Finally, the application of footprinting to the comparison of the corresponding viral glycoproteins provided further evidence of their differences. Each of the corresponding gI, gIII and gIV glycoproteins from the two viruses showed distinctive footprint patterns.

To date, the isolate of BHV1.3 analysed in this study is the only isolate from the U.S.A. obtained from an outbreak of bovine encephalitis, which occurred in the state of Texas (Eugster et al., 1974). However, isolates of BHV1.3 may go unrecognized since diagnostic reagents that recognize BHV1.1 react with BHV1.3 equally well (J. K. Collins, unpublished). One additional isolate of BHV1.3 has been made in the U.S.A., from the brain of a moribund feedlot calf in Texas in 1989 (J. d'Offay, personal communication); this virus has an identical polypeptide profile to the isolate examined here (data not shown).

Antigenic differences between BHV1.3 and other bovine herpesviruses have been found previously using MAbs directed against BHV1.1 and BHV1.3 (Friedli & Metzler, 1987). In this previous study, comparisons were made of the N569 neurotropic strain of BHV1.3 to representative strains of BHV1.1, BHV1.2, and caprine herpesvirus. Major antigenic differences were found between the glycoprotein gII (proteins 3/12) of BHV1.3 and BHV1.1 and BHV1.2, whereas minor differences were found between the gI glycoproteins (proteins 7/7A/17/23). Using four MAbs that reacted against BHV1.1, BHV1.2, BHV1.3 and caprine herpesvirus, but which recognized different proteins, Friedli & Metzler (1987) also found differences in the sizes of the corresponding viral proteins, including differences in the sizes of gIII and the heterodimer subunits of gI, similar to the differences described here. They also demonstrated that gl of BHV1.3 was a target for neutralizing MAbs, and this can be extended to include gIII and gIV from the present results.

Further RIP analysis of the viral gIII glycoproteins with various MAbs revealed proteins antigenically related to gIII of approximately 55K (BHV1.1) and 48K (BHV1.3). These have not been described previously. Based on the reduced Mr of these proteins and the finding that they reacted with some MAbs (ID6, 1507) and not others (F2), they probably represent degradation products of gIII. Tunicamycin inhibition experiments supported this possibility since the 55K and 48K proteins were found to be fully glycosylated. The Mr changes between tunicamycin-treated and untreated proteins were similar to the changes found for the respective intact gIII glycoproteins. From the relative labelling pattern of the gIII-related proteins with [35S]methionine, further differences could be observed between the viruses. With BHV1.1, the 55K protein was only faintly evident compared with the intact 91K protein, whereas with BHV1.3 the 48K protein was quite prominent, and was as abundant as the intact 87K protein. The meagre amount of the 55K protein observed with BHV1.1 may explain its lack of detection previously.

Despite major antigenic differences between gIII glycoproteins, major protein footprint differences were not found. This most likely reflected the resistance of gIII to protease digestion, as neither viral gIII glycoprotein...
could be completely digested, even with high levels of the protease. Similar results have been found with other proteolytic enzymes (data not shown). The gII-related 55K and 48K proteins were also resistant to protease digestion. This may help to explain why these proteins were observed upon RIP with the appropriate MAb, in that the proteins may accumulate inside infected cells owing to resistance to intracellular protease.

The differences observed in the viral glycoproteins involve several biological functions. Glycoprotein gIII has a predominant role in attachment of BHV1 to cells (Okazaki et al., 1991; Liang et al., 1991; Fitzpatrick et al., 1990); both gl and gIV may participate in attachment and penetration (Liang et al., 1991). Some of the functionally important epitopes on these BHV1.1 glycoproteins are absent from those of BHV1.3. On glycoprotein gl, one epitope involved in neutralization of BHV1.1 (5G2) (van Drunen Littel-van den Hurk et al., 1985) is missing from BHV1.3. From the virus neutralization data in this paper, three or four gl epitopes (5106, 1E11, 1F8, 1F10) are involved in attachment and/or penetration; two of these, 5106 and 1F10, may not function in attachment and/or penetration of BHV1.3 since the MBs lack virus-neutralizing activity. Glycoprotein gIII is lacking at least one epitope involved in attachment, F2 (Fitzpatrick et al., 1990; Collins et al., 1984); gIV lacks two important epitopes, 136 and 9D6, both involved in penetration (Hughes et al., 1988). Differences in the glycoproteins may possibly relate to in vivo viral tropism differences.

The antigenic differences observed have important implications for immunity and cross-protection between strains. Cross-protection has been demonstrated in challenge studies of calves immunized with killed BHV1.1 and challenged with BHV1.3 (strain A663) (Bratanich et al., 1991). This may explain why outbreaks with the neurotropic virus have occurred only in unvaccinated calves of an age at which colostral immunity would be expected to have waned (French, 1962; Eugster et al., 1974; Carillo et al., 1983a, b). However, it is not clear to what extent BHV1.1-vaccinated cattle are protected against this virus, the duration of immunity and its effectiveness in field conditions.

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References


Glycoprotein antigens of BHV1.3

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