The different interactions of a gIII mutant of pseudorabies virus with several different cell types

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Glycoprotein gIII of pseudorabies virus (PrV) is multifunctional. It plays a role in the stable adsorption of the virus to its host cells by interacting with a cellular heparin-like substance. It also affects both release of mature virus from infected cell types and virulence. Thus, although non-essential for growth in vitro, gIII plays a central role in the biology of the virus. The primary attachment of a mutant, PrV2, which has an in-frame internal deletion and expresses a shortened version of gIII, and of wild-type (wt) virus, to MDBK cells has been shown to occur similarly. To ascertain whether different domains of gIII control the expression of the different biological functions of the gIII protein, we have compared several aspects of virus-host cell interactions of PrV2, of a gIII-null virus, and of wt virus. Our results showed that the deletion of the internal segment of the gIII glycoprotein affects adsorption and virus release differently, i.e. that these two functions of gIII appear to be independent of each other. Furthermore, we observed that although the primary adsorption of PrV2 and wt virus to MDBK cells is similar, PrV2 behaved like a gIII-null mutant with respect to virulence. The apparent contradiction between these two findings was resolved when it was found that although PrV2 binds as well as does wt to some cell types, it binds poorly to other cell types. The functional importance of different domains of gIII in virus adsorption thus differs, depending on the cell type with which the virus interacts.

Introduction

Eight genes encoding glycoproteins of pseudorabies virus (PrV) have been mapped and sequenced (Mettenleiter et al., 1985; Petrovskis et al., 1986a, b; Robbins et al., 1986a, 1987; Rea et al., 1985; Klupp & Mettenleiter, 1991; Wathen & Wathen, 1984, 1986). Four have been shown to be nonessential for growth in cell culture (Ben-Porat et al., 1986; Keeler et al., 1986; Petrovskis et al., 1986a, c; Robbins et al., 1986b; Wathen & Wathen, 1986; Mettenleiter et al., 1985; Whealy et al., 1988). We have been interested in ascertaining the functions of these non-essential glycoproteins and have focused on gIII, one of the major glycoproteins of PrV. Glycoprotein gIII is a homologue of glycoprotein gC of herpes simplex virus (HSV) (Robbins et al., 1986a) and is one of the more abundant membrane proteins of PrV.

Glycoprotein gIII is multifunctional. It plays a pivotal role in the stable adsorption of the virus to its host cells (Schreurs et al., 1988) by interacting with a cellular heparin-like substance (Mettenleiter et al., 1989). An alternative, less efficient, mode of adsorption, however, also exists which is independent of either the viral gIII glycoprotein or the heparin-like cellular receptor, and consequently gIII− mutants are viable. A similar situation also prevails for the gC glycoprotein of HSV (Herold et al., 1991; Campadelli-Fiume et al., 1990).

In addition to being involved in adsorption, gIII of PrV also plays a role in the release of mature virus from the infected cells (Whealy et al., 1988; Zsak et al., 1989), an effect that is detectable in some cell types only in conjunction with a defect in glycoprotein gI (Schreurs et al., 1988). Thus, gIII-null mutants are released poorly from chick embryo fibroblasts (CEFs) but are released as efficiently as wild-type (wt) virus from primary rabbit kidney (RK) cells. Mutants defective in both gI and gIII, however, are released poorly from RK cells (Zsak et al., 1989; Schreurs et al., 1988). Furthermore mutants defective in both gI and gIII also have a significantly reduced level of virulence for mice, chickens as well as swine, whereas mutants defective only in gI or gIII retain...
virulence (Mettenleiter et al., 1988a). Thus, gIII, although non-essential for growth in vitro, plays a central role in the biology of the virus.

The adsorption (i.e. association of virus with cells persisting after extensive washing) of PrV2, a mutant with an internal deletion of codons 157 to 292 of gIII expressing a shortened version of gIII (Whealy et al., 1988), to MDBK cells occurs as efficiently as that of wt virus (Zsak et al., 1991). To ascertain whether different domains of gIII control the expression of the different biological functions of the gIII protein, we have compared several aspects of the virus-host cell interactions of the PrV2 mutant, a gIII-null mutant and wt virus. Our results show that the deletion of the internal segment from the gIII glycoprotein of PrV2 affects adsorption and virus release differently, i.e. that these two functions of gIII appear to be independent of each other. Furthermore, although the attachment of PrV2 to MDBK cells and its release from RK cells appeared to be like those of a gIII+ virus, this mutant behaved like a gIII− virus with respect to virulence. To clarify this apparent contradiction, the interactions of PrV2 with four different cell lines were compared. We found that although the gIII of PrV2 functions as well in adsorption as does the gIII of wt virus when it interacts with some cell types, it is defective in its adsorption to other cell types. The functional importance of different domains (or configurations) of the gIII glycoprotein thus differs, depending on the cell type with which the virus interacts.

### Methods

**Media and solutions.** The following were used: Eagle's synthetic medium plus 5% dialysed bovine serum (EDS), Tris buffered saline containing 1% crystalline bovine albumin (TBSA) and Tris buffered saline containing 2% SDS (TBS-SDS).

**Radiochemicals and chemicals.** [3H]Thymidine (specific activity, 45 Ci/mmol) was purchased from New England Nuclear Corporation. Polylysine was purchased from Sigma.

**Cells and virus mutants.** MDBK (Madin-Darby bovine kidney), pig kidney (PK), RK cells and CEFs were cultivated in EDS. Virus was titrated by plaque assay.

Mutants of two PrV strains were used. One set of mutants was derived from the Becker strain of PrV, PrV(Be). Some of these mutants have been isolated by Lynn Enquist and his group at DuPont and have been characterized (Whealy et al., 1988). The mutants PrV(Ka)gIII− and PrV10 both have a deletion of the gIII promoter, as well as of most of the gIII coding sequence. Cells infected with these mutants do not synthesize gIII (Robbins et al., 1986b; Schreurs et al., 1988). Thus, PrV2 and PrV(Ka)2 virions possess a smaller version of gIII, PrV(Ka)gIII− and PrV10 are gIII-null. PrV4 contains a stop codon at codon 157 of the gIII gene. It produces a truncated gIII protein which is excreted from the infected cells and is not integrated into the viral membrane (Whealy et al., 1988).

Deletions to abolish the expression of gI were produced as described previously (Mettenleiter et al., 1987a, b). Marker rescue of PrV2gI− was performed as follows. PrV2gI− DNA was cotransfected either with the BamHI/SalI fragment 7A of PrV(Ka), which includes the gI gene (Mettenleiter et al., 1987a), or with the 4.3 kbp PstI fragment of PrV(Ka), which includes the gIII gene (Robbins et al., 1986a; Schreurs et al., 1988). The progeny were plaque-assayed and screened by the black plaque technique using monoclonal antibodies against gI or against gIII (Holland et al., 1983; Smith et al., 1981). A description of the mutants used and their nomenclature are summarized in Table 1.

The mutants were analyzed by Southern blotting (Southern, 1975) to ensure that they had the expected deletion in the gI genes (data not shown). Protein extracts of cells infected with the mutants were immunoprecipitated with anti-gI as well as with anti-gIII monoclonal antibodies (Hampel et al., 1984; Ben-Porat et al., 1986) to ensure that the mutants had the desired phenotype.

**Determination of LD₅₀.** Tenfold dilutions of virus stocks were injected intramuscularly into the hind leg or intracerebrally in BALB/c mice and intracerebrally into l-day-old chickens (six animals per dilution). The number of animals that died each day up to 2 weeks after inoculation was determined and the LD₅₀ obtained each day was calculated by the method of Reed & Muench (1938). The LD₅₀ was calculated at daily intervals, as well as at the end of the experiment, to illustrate differences in the number of animals that died, as well as differences in the time of death of animals inoculated with the different mutants.

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### Table 1. Genotype and nomenclature of mutants used

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Strain PrV(Be)</th>
<th>Strain PrV(Ka)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrV(Be); Be</td>
<td>PrV(Ka); Ka</td>
<td>Wt</td>
<td>Deletion of codon 157 to 292 of the gIII gene</td>
</tr>
<tr>
<td>PrV2; B2*</td>
<td>PrV(Ka)2+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PrV10; B10*</td>
<td>PrV(Ka)gIII++</td>
<td>Deletion of most of the gIII gene</td>
<td></td>
</tr>
<tr>
<td>PrV4; B4*</td>
<td>−</td>
<td>Stop codon at codon 157 of gIII gene</td>
<td></td>
</tr>
<tr>
<td>PrV(Be)gI−; BegI+</td>
<td>PrV(Ka)gI+</td>
<td>Deletion of gI gene from Wt</td>
<td></td>
</tr>
<tr>
<td>PrV2gI−; B2gI+</td>
<td>PrV(Ka)2gI+</td>
<td>Deletion of gI gene from PrV2</td>
<td></td>
</tr>
<tr>
<td>PrV4gI−; B2gI+</td>
<td>−</td>
<td>Deletion of gI gene from PrV4</td>
<td></td>
</tr>
<tr>
<td>B2/10+</td>
<td>PrV2gI+ marker rescued by gI gene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2/90+</td>
<td>PrV2gI+ marker rescued by gIII gene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mutants isolated in the laboratory of L. Enquist. † Mutants isolated by L. Zsak.
Preparation and adsorption of [3H]thymidine-labelled virus. Purified [3H]thymidine-labelled virus was prepared as described previously (Ben-Porat & Kaplan, 1976). The purified [3H]thymidine-labelled virus in TBSA was added to monolayers that had been preincubated in TBSA for 15 min (to minimize unspecific binding) and the monolayers were further incubated for 1 h at 37 °C, when they were washed extensively to remove non-adsorbed virus. The cells were scraped into TBS-SDS and the amount of radioactivity associated with the cell monolayers was determined.

Results

PrV2 behaves like wt virus with respect to release from infected cells

Mutants that are deficient in gIII adsorb poorly to MDBK cells and are released much less readily from CEFs than is wt virus (Schreurs et al., 1988; Zsak et al., 1989). PrV2 (with an internal in-frame deletion of a DNA segment encoding 134 amino acids) adsorbs as well as does wt virus to MDBK cells (Zsak et al., 1991). To ascertain whether the shortened version of the gIII glycoprotein present in PrV2 would also function as does the wt gIII glycoprotein in virus release from CEFs, we compared the release of PrV2 with that of a gIII-null mutant and of wt virus. Fig. 1(a) shows that the gIII-null mutants (PrV10 and PrV4), as expected, were released less efficiently from CEFs than was wt virus but that PrV2 behaved like wt virus.

Although mutants deficient in gIII were not released as efficiently as wt virus from CEFs, no difference in their release from RK cells was observed. However mutants deficient in both gI and gIII were released poorly from these cells (Schreurs et al., 1988). To ascertain whether PrV2 behaves like wt virus or like a gIII-null mutant in this respect, i.e. whether PrV2gI- is released efficiently from RK cells, we deleted the gI gene from PrV2 as well as from wt PrV(Be) and from the gIII-null mutant PrV4, and determined how these mutants behaved with respect to release from RK cells. No difference in the release of PrV2gI- and PrV2 from RK cells was detected (Fig. 1b), but, as expected, PrV4gI- was not released. The absence of the middle domain of gIII, either alone or in conjunction with a defect in gI, thus did not appear to affect the ability of the glycoprotein to promote virus release; PrV2 behaved as wt with respect to its release from both CEFs and RK cells. This was found to be true for a similar mutant isolated from the PrV(Ka) strain, PrV(Ka)2.

Virulence of PrV2gI- mutants for chickens and mice

Inactivation of either the gI or of the gIII gene alone affects the virulence of PrV for chickens and mice only slightly, whereas inactivation of both drastically reduces its virulence (Mettenleiter et al., 1988a). As PrV2 behaves as wt with respect to adsorption to MDBK cells as well as to release from CEFs and RK cells, it was of interest to ascertain whether it also would behave as wt with respect to virulence, i.e. whether the PrV2gI- mutant would be virulent.

The results (Fig. 2 and 3) show that wt PrV(Be), PrV2 and PrV4 exhibited approximately the same level of virulence. In animals inoculated with PrV(Be)gI-, the time of death was somewhat delayed but the final LD50 was approximately the same as that of wt virus. On the other hand, the virus LD50 for PrV4gI- and PrV2gI- was considerably higher. Similarly, PrV(Ka)gIII- and PrV(Ka)2 were as virulent as wt virus and both PrV(Ka)gIII-/gI- and PrV(Ka)2gI- were avirulent (data not shown). Thus, even though PrV2 behaved as does wt virus with respect to adsorption to MDBK cells as well as to release from RK cells and CEFs, it behaved like a gIII-null mutant with respect to virulence; like gIII- mutants, PrV2 [and PrV(Ka)2] are as virulent for chickens and mice as is wt virus and, like the gIII-/gI- mutants, PrV2gI- and PrV(Ka)2gI- have lost most of their virulence.
To ensure that the reduced virulence of PrV2gII− was related to the defect in the gIII and gI genes, the mutant was marker-rescued with either the gIII or the gI gene of PrV(Ka) (see Methods). The PrV2gII− mutant, to which either an intact gI gene or intact gIII gene had been restored (B2/10 or B2/90, respectively), were as virulent as wt virus or the PrV(Be)gII− virus, respectively (Fig. 3b). Thus, the reduced virulence of PrV2gII− can be ascribed to the fact that the gIII protein was modified and that the gI gene had been deleted from this mutant.

We conclude that, although the deletion of the middle section of gIII had no measurable effect on the parameters of viral growth that we measured in cell culture, it affects virulence significantly.

The defect in the gIII glycoprotein of PrV2 affects virus–cell interactions in a cell type-specific manner

In the experiments described above, to ascertain whether PrV2 behaved like a gIII-null mutant or like wt virus with respect to adsorption and release, we used cell types in which the effects of gIII on virus–cell interactions were most marked: MDBK cells for adsorption and CEFs or RK cells for release. However, because in conjunction with a deletion of the gI gene the defect in the gIII gene of PrV2 reduces its virulence significantly, we surmised that either aspects of virus growth not yet studied are affected in PrV2 or that PrV2 is defective in its interaction with some cell types with which it interacts in vivo. To test this possibility, we ascertained whether the defect in PrV2 might affect virus–cell interaction in a cell type-specific manner.

Table 2 shows the titre of the virus mutants when plaque-assayed on four different cell lines. As previously reported, gIII-null mutant stocks (such as PrV10), in general, have a low titre of infectious virus but plaque with a somewhat higher efficiency on CEFs and RK cells than on MDBK or PK cells (Mettenleiter et al., 1990). Wild-type PrV(Be) gave approximately the same titre when plaqued on MDBK and RK cells but gave a somewhat lower titre on PK cells and CEFs. PrV2, however, produced a considerably larger number of plaques on MDBK cells and CEFs than on RK or PK cells.

The difference between the number of plaques that developed when PrV2 was assayed on PK or RK cells and on MDBK cells can be attributed to differences in the efficiency of adsorption of PrV2 to these cells (Table 3). Wild-type PrV(Be) gave approximately the same titre when plaqued on MDBK and RK cells but gave a somewhat lower titre on PK cells and CEFs. PrV2, however, produced a considerably larger number of plaques on MDBK cells and CEFs than on RK or PK cells.

Effects of polylysine on adsorption to RK and MDBK cells of mutants defective in gIII

Polylysine, a polycationic compound, is effective in inhibiting adsorption of HSV-1 (Langeland et al., 1988; WuDunn & Spear, 1989) but its effect on the adsorption of wt PrV is only slight (Zsak et al., 1990). However, it greatly stimulates the adsorption of gIII− mutants of
Table 2. Plaque formation by PrV(Be)gIII defective mutants on different cell types*

<table>
<thead>
<tr>
<th>Virus mutant</th>
<th>Expt.*</th>
<th>MDBK</th>
<th>PK</th>
<th>RK</th>
<th>CEF</th>
<th>PK/MDBK</th>
<th>RK/MDBK</th>
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<tbody>
<tr>
<td>PrV(Be) (wt)</td>
<td>1</td>
<td>100t</td>
<td>36†</td>
<td>92†</td>
<td>0-36</td>
<td>0.36</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>220</td>
<td>70</td>
<td>250</td>
<td>87†</td>
<td>0.32</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>130</td>
<td>47</td>
<td>120</td>
<td>0.36</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>120</td>
<td>42</td>
<td>110</td>
<td>0.35</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>PrV2</td>
<td>1</td>
<td>39</td>
<td>5</td>
<td>5</td>
<td>0.13</td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>120</td>
<td>9</td>
<td>12</td>
<td>0.07</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>85</td>
<td>4</td>
<td>6</td>
<td>0.05</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>150</td>
<td>11</td>
<td>15</td>
<td>0.07</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>PrV10</td>
<td>2</td>
<td>9</td>
<td>5</td>
<td>23</td>
<td>0.56</td>
<td>2.60</td>
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<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>1.00</td>
<td>2.20</td>
<td></td>
</tr>
</tbody>
</table>

* Stocks of the mutants were serially diluted (10-fold) and each was plated in triplicates on monolayers of either MDBK, PK, CEF or RK cells. After a 1 h adsorption period, the monolayers were washed, overlaid with agarose and plaques counted 4 d later. [The ratio of p.f.u. (on MDBK cells) and physical particles has been found to be approximately 1:20 for wt virus and PrV2, and approximately 1:200 for PrV10.]
† P.f.u./ml × 10⁻⁶.

Table 3. Comparison of the adsorption of PrV(Be) mutants defective in gIII*

<table>
<thead>
<tr>
<th>Virus mutant</th>
<th>MDBK</th>
<th>PK</th>
<th>RK</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrV(Be)</td>
<td>9200†</td>
<td>14000†</td>
<td>8250†</td>
</tr>
<tr>
<td>PrV2</td>
<td>8100</td>
<td>1410</td>
<td>1200</td>
</tr>
<tr>
<td>PrV10</td>
<td>520</td>
<td>375</td>
<td>1270</td>
</tr>
</tbody>
</table>

* Approximately 10⁵ c.p.m. of [³H]thymidine-labelled virus was added in triplicate to monolayers of either MDBK, PK or RK cells. After a 1 h adsorption period, the monolayers were washed, the cells scraped into TBS-SDS and the number of counts associated with the cells was ascertained.
† C.p.m./monolayer.

Table 4. Effect of polylysine on plaque formation by PrV(Be)gIII mutants*

<table>
<thead>
<tr>
<th>Virus mutant</th>
<th>MDBK cells</th>
<th>RK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polysine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt</td>
<td>900†</td>
<td>280†</td>
</tr>
<tr>
<td>PrV2</td>
<td>430</td>
<td>320</td>
</tr>
<tr>
<td>PrV10</td>
<td>45</td>
<td>460</td>
</tr>
</tbody>
</table>

* MDBK or RK cell monolayers were incubated for 15 min in either TBSA (-) or TBSA containing polylysine (50 μg/ml) (+). Aliquots (0.1 ml) of 10-fold dilutions of the virus stocks in TBSA were then added to the monolayers, which were washed 1 h later and overlaid with agarose. Plaques were counted 4 days later.
† P.f.u. × 10⁻⁶.

The presence of polylysine allows the virus to bypass efficiently the interactions between viral glycoprotein gIII and the cellular heparin-like receptor, i.e. to bypass the normal pathway of adsorption used by the majority of wt virions (Zsak et al., 1990).

To study further the differences in the ability of the modified gIII present in PrV2 to adsorb to different cell types, we have compared the effects of polylysine on plaque formation of wt virus, of PrV2 and of a gIII-null mutant. The results of these experiments (Table 4) showed that the presence of polylysine during the adsorption period reduced somewhat the number of plaques formed by wt virus, particularly on MDBK cells. As expected, the titre of the gIII-null mutants (PrV10) was considerably lower than that of wt virus when assayed on either cell type, but the presence of polylysine significantly enhanced its titre on both cell types. The presence of polylysine considerably enhanced plaque formation of PrV2 on RK cells but somewhat reduced the titre of PrV2 on MDBK cells. Mutant PrV(Ka)2 behaved in a manner indistinguishable from that of PrV2 (data not shown).

The results of an experiment in which adsorption to MDBK and RK cells of radiolabelled virus preparations was ascertained yielded similar results (Table 5). However, polylysine did not appear to affect adsorption of wt PrV(Be) to either MDBK cells or RK cells. [Its effect on plaque formation of wt virus (see Fig. 3) is probably due to interference with a step subsequent to adsorption (WuDunn & Spear, 1989; Zsak et al., 1990).] Polylysine considerably enhanced adsorption of PrV10 to both cell types. Adsorption of PrV2 to PK cells but not to MDBK cells was also greatly stimulated by polylysine. Thus, the response of PrV2 to the presence of polylysine was similar to that of a wt virus when it interacted with
possesses a shortened version of glII, i.e. from which 134 internal amino acids have been deleted. Our purpose in performing these experiments was to identify the regions of the gill protein that function in adsorption and in release can be uncoupled, (ii) although deletion of the middle domain of gIII does not affect its ability to bind to some cell types, it strongly affects its binding to other cell types and (iii) in conjunction with a deletion in gl, which in itself does not greatly affect virulence, PrV2 mutants are avirulent.

We had previously concluded that although gIII plays a role in both virus adsorption and virus release, these two functions of gIII are probably not linked (Schreurs et al., 1988). Thus it was observed that gIII-null mutants were released as well as wt virus from RK cells, but they adsorbed very poorly to these cells. The results described here corroborate these findings. Whereas PrV2 adsorbs poorly to RK cells and behaves in this respect like a gIII-null mutant, the PrV2gI- mutant, unlike the gIII- mutant, is released efficiently from RK cells. Thus, the domain (or the configuration) of the gIII glycoprotein that is necessary for optimum adsorption to RK cells is not necessary for optimum virus release from these cells.

Our results showed that PrV2 behaved like wt virus both with respect to attachment to MDBK cells and with respect to release from CEFs and RK cells. However, it behaved like a gIII- mutant with respect to its virulence for chickens and mice; in conjunction with a defect in gl- (which in itself does not greatly affect virulence of wt virus), the defect in gIII of PrV2 resulted in a loss of virulence. This finding alerted us to the possibility that the modification in the gIII protein of PrV2 (and PrV2-gI-) may affect the interaction of the virus with different host cells differently, i.e. that the virus might behave as wt virus when interacting with some cell types and as a gIII-mutant when interacting with other cell types. When this possibility was investigated, we found that although the attachment of PrV2 and wt virus to MDBK cells is similar, PrV2 attaches poorly to RK and PK cells and appears to behave like a gIII-null mutant with respect to adsorption to these cells.

The reasons for the differential behaviour of PrV2 with respect to its interaction with different cell lines is at present unclear. It is possible that the PrV2 gIII glycoprotein has a low binding affinity for the heparin-like cellular receptor, so that upon interacting with a cell that has relatively few such receptors its adsorption may be detectably impaired compared to that of wt virus. However, upon interacting with a cell type rich in this receptor, differences in adsorption rate between wt and PrV2 virions might not be detectable. We had attempted to test this possibility by ascertaining the binding affinities of the wt virus and PrV2 gIII to heparin-Sepharose beads and have been unable to detect differences between the two (Zsak et al., 1991). As differences in the binding of wt virus and PrV2 gIII to heparin, were not detected, the possibility that different cell types may differ with respect to the structure of their heparin-like receptors must be considered.

The lack of virulence of gl-/gIII- mutants has been shown to result from the inability of these mutants to spread. Virus spread can occur by direct cell-to-cell transmission of the virus; this mode of spread is impaired in gl- virus (Zsak et al., 1992). Virus spread can also occur by readsoption of released virus to uninfected cells; gIII- virus is impaired in adsorption (Schreurs et al., 1988). Virus with defects in one of these genes can still use the alternative mode of spread and therefore be virulent; virus with defects in both of these genes will spread poorly and therefore lose its virulence (Zsak et al., 1992). Since PrV2 behaves like a gIII mutant with respect to virulence for chickens and mice, i.e. upon inactivation of its gl gene it loses its virulence, it is likely that PrV2 is defective in its ability to adsorb to its target tissues in vivo.

Our results indicate that the requirement for adsorption of a virus mutant to different cell types (to which wt virus adsorbs efficiently) may differ significantly. Therefore, results obtained from studies on the interactions of a mutant with one cell type may not necessarily be applicable to its interactions with other cell lines.

This investigation was supported by Public Health Service Grant AI-10947 from the National Institutes of Health.

**Table 5. Effect of polylysine on virus adsorption***

<table>
<thead>
<tr>
<th>Virus mutant</th>
<th>MDBK cells</th>
<th>RK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Wt</td>
<td>5 x 10^4</td>
<td>3300†</td>
</tr>
<tr>
<td>PrV2</td>
<td>7 x 10^4</td>
<td>2600</td>
</tr>
<tr>
<td>PrV10</td>
<td>9 x 10^4</td>
<td>190</td>
</tr>
</tbody>
</table>

*One ml of [3H]thymidine-labelled virus in TBSA was added to monolayers of MDBK or RK cells. After a 1 h adsorption period, the monolayers were washed, the cells scraped into TBS-SDS and the number of counts associated with the cells was ascertained.
† C.p.m.

MDBK cells, but was similar to that of a gIII-null mutant when it interacted with RK cells.

**Discussion**

The experiments presented in this paper compare the properties of wt PrV with those of a mutant, PrV2, which possesses a shortened version of glIII, i.e. from which 134 internal amino acids have been deleted. Our purpose in performing these experiments was to identify the domains of gIII that may control the different facets of its functions. The results obtained show that (i) the regions of the gIII protein that function in adsorption and in release can be uncoupled, (ii) although deletion of the middle domain of gIII does not affect its ability to bind to some cell types, it strongly affects its binding to other cell types and (iii) in conjunction with a deletion in gl, which in itself does not greatly affect virulence, PrV2 mutants are avirulent.

The lack of virulence of gl-/gIII- mutants has been shown to result from the inability of these mutants to spread. Virus spread can occur by direct cell-to-cell transmission of the virus; this mode of spread is impaired in gl- virus (Zsak et al., 1992). Virus spread can also occur by readsoption of released virus to uninfected cells; gIII- virus is impaired in adsorption (Schreurs et al., 1988). Virus with defects in one of these genes can still use the alternative mode of spread and therefore be virulent; virus with defects in both of these genes will spread poorly and therefore lose its virulence (Zsak et al., 1992). Since PrV2 behaves like a gIII mutant with respect to virulence for chickens and mice, i.e. upon inactivation of its gl gene it loses its virulence, it is likely that PrV2 is defective in its ability to adsorb to its target tissues in vivo.

Our results indicate that the requirement for adsorption of a virus mutant to different cell types (to which wt virus adsorbs efficiently) may differ significantly. Therefore, results obtained from studies on the interactions of a mutant with one cell type may not necessarily be applicable to its interactions with other cell lines.

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References


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