JOURNAL OF VIROLOGY, Dec. 1987, p. 4030–4032
0022-538X/87/124030-03$02.00/0

Vol. 61, No. 12

Role of a Structural Glycoprotein of Pseudorabies in Virus Virulence

THOMAS C. METTENLEITER,[1] LASZLO ZSAK,[2] ALBERT S. KAPLAN,[1] TAMAR BEN-PORAT,[1]* AND BELA LOMNICZI[2]

Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232,[1] and Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, Hungary[2]

Received 17 July 1987/Accepted 10 September 1987

The virulence of deletion mutants of pseudorabies virus defective in the expression of glycoprotein gI, gp63, or both was tested in 1-day-old chickens and young pigs. In the absence of expression of gI, the virulence of a fully virulent laboratory strain, PrV(Ka), for 1-day-old chickens was reduced approximately fourfold. Inactivation of glycoprotein gp63 appeared also to affect the virulence of PrV(Ka) only slightly, as did inactivation of both gI and gp63. The level of reduction in virulence, however, was considerably more marked in Bartha 43/25aB4, a less virulent virus strain. Inactivation of the expression of gI in Bartha 43/25aB4 reduced virulence for chickens at least 100-fold. The results obtained when the virulence of the mutants for pigs was determined were compatible with those obtained for chickens. These results indicate that gI plays a role in virulence, but that it does so in conjunction with at least one other viral function (a function that is defective in Bartha 43/25aB4).

The genome of pseudorabies virus (PrV), a herpesvirus of pigs, is divided into two components, long (L) and short (S). The S component is composed of a unique sequence (U_S) bracketed by inverted repeats. PrV causes latent, as well as acute, often fatal infection of the nervous system in pigs and acute infection in other domestic and wild animals (3). In some vaccine strains of PrV, Bartha, for example, a segment of the S component of the genome is deleted, and in this strain the deleted segment of DNA plays a role in virulence (1, 2, 7, 8, 9). Restoration of an intact S component to Bartha does not, however, restore virulence; restoration of virulence is possible only by double-marker rescue with sequences that span the region of the S component of the genome that has been deleted, as well as sequences from the middle of the L component (which encode four genes involved in nucleocapsid assembly) (9). The doubly rescued Bartha, strain 43/25aB4, is virulent for both 1-day-old chickens and young pigs, but the dose of virus required for virulence is greater than that of wild-type virus (9).

The region in the S component that is deleted from the Bartha strain and that has to be restored before virulence is expressed encodes two structural glycoproteins, gI and gp63 (11, 13, 14); it may also encode several other viral functions, since several additional mRNAs transcribed from that region are present in the cytoplasm of cells infected with wild-type virus but not in the cytoplasm of cells infected with the Bartha strain (8). The experiments described in this paper were done to determine which of these genes are involved in virulence and to ascertain whether deletion of any one of them would suffice to affect the virulence of PrV. Our results show that glycoprotein gI plays a role in virulence of PrV and that it does so in conjunction with other viral genes.

Deletions that abolish the expression of either gI or gp63 or both were introduced into the wild-type PrV(Ka) genome or into the virulent doubly rescued Bartha vaccine genome (Bartha 43/25aB4). The following two types of mutants were used: (i) mutants in which a large part of the coding sequence of the gene was deleted (details concerning the construction of these mutants have been described [12]) and (ii) deletion/insertion mutants in which some sequences near the 5′ end of the coding sequence of the gene were removed by Bal 31 digestion and a sequence of M13 DNA was inserted (Fig. 1). Although each of these types of mutants has different

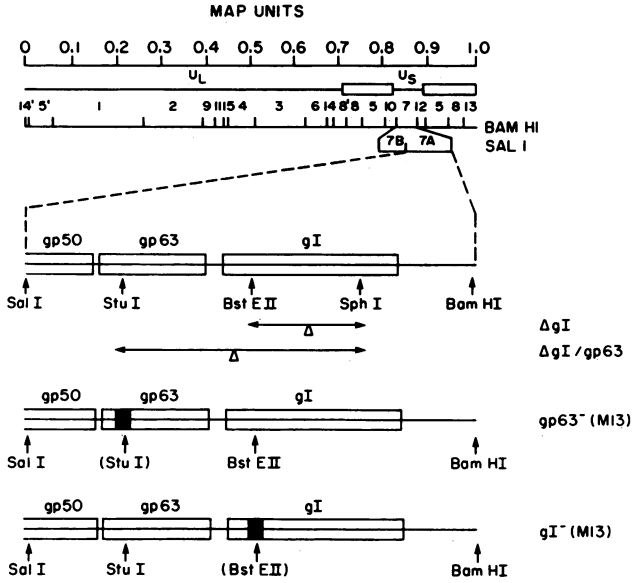

FIG. 1. Maps of PrV mutants with deletions or deletion/insertions in the genes encoding gI and gp63. The top of the figure shows the *Bam*HI restriction map of PrV DNA. The positions of the genes encoding gI, gp63, and part of gp50 are illustrated in line 4. The deletions which span sequences in gI (ΔgI) or gI and gp63 (ΔgI/gp63) are shown in lines 5 and 6. Details concerning the procedures used to obtain these mutants are described elsewhere (12). Lines 7 and 8 illustrate the structures of the region of interest in the deletion/M13 insertion mutants of gp63 and gI, respectively. In these mutants, approximately 200 base pairs flanking the *Stu*I or *Bst*EII sites were removed by digestion with Bal 31 nuclease, and a *Hae*III fragment of M13, approximately 200 base pairs in size, was inserted. The darkened areas indicate the M13 sequences that have been inserted into these genes. Details concerning the construction of these mutants will be reported elsewhere (T. C. Mettenleiter and T. Ben-Porat, manuscript in preparation).

* Corresponding author.

TABLE 1. Dose of gI⁻ and gp63⁻ virus mutants required to kill day-old chickens

Virus	Phenotype	LD_{50} (PFU)[a] for:		
		PrV(Ka)	Phylaxia	Bartha 43/25aB4
Wild type	gI⁺/gp63⁺	5.0×10^1	6.5×10^1	1.0×10^4
Deletion mutant	gI⁻/gp63⁺	2.0×10^2	2.5×10^2	$>10^6$
	gI⁻/gp63⁻	2.5×10^2	ND[b]	$>10^6$
Deletion/M13 insertion mutant	gI⁻(M13)	2.5×10^2	ND	ND
	gp63⁻(M13)	1.0×10^2	ND	ND

[a] Chickens (1 day old) were injected intracerebrally with 30 μl of 10-fold dilutions of the indicated virus stock (eight chickens per dilution). The LD_{50}s were calculated by the method of Reed and Muench.
[b] ND, Not determined.

experimental advantages, the salient point related to the experiments in the present study is that both types of mutants do not express glycoprotein gI or gp63, either alone or together.

The virulence of the gI⁻, gp63⁻, and gI⁻/gp63⁻ mutants for day-old chickens injected intracerebrally was determined (Table 1). The virulence of gI⁻ mutants of two different virulent strains, PrV(Ka) and Phylaxia, was only slightly reduced compared with that of wild-type virus; their 50% lethal doses (LD_{50}s) were increased approximately fourfold. Inactivation of gp63 alone in PrV(Ka) also had only a small (if any) effect on virulence.

While inactivation of gI in wild-type virus affected virulence only slightly, inactivation of gI in Bartha 43/25aB4 had a marked effect on virulence and increased the LD_{50} at least 100-fold. Bartha 43/25aB4 is only partially virulent; i.e., it is defective in some gene(s) that affects virulence. Because inactivation of gI had a more profound effect on the virulence of Bartha 43/25aB4 than on that of wild-type virus, one can conclude that gI affects virulence in conjunction with at least one other gene product.

Table 1 also shows that the doubly defective mutants, PrV(Ka)gI⁻/gp63⁻ and Bartha 43/25aB4gI⁻/gp63⁻, did not appear to be less virulent than the gI⁻ mutants of these viruses, indicating either that gp63 plays no role in virulence or that, if it does, its effect is not additive to that of gI.

Figure 2 illustrates the effect of infection with gI⁻ or gI⁻/gp63⁻ mutants on the weight of young pigs, as well as the appearance of signs of disease and death. Table 2 summarizes these results. Infection of 3-week-old pigs with wild-type PrV(Ka) under the conditions of infection used killed all five of the animals within 8 days of inoculation. Mutants defective in gI or gI and gp63 were significantly less virulent; in only 60% of the infected animals was a loss of weight, as well as signs of disease and death, observed.

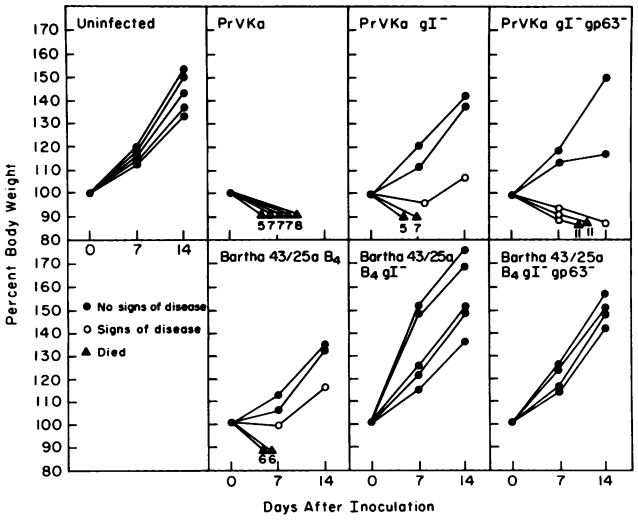

FIG. 2. Effects of infection with different PrV mutants on young pigs. See the legend to Table 2 for experimental details.

Infection of the pigs with Bartha 43/25aB4 (the vaccine strain to which partial virulence had been restored) induced a loss of weight, signs of disease, or death in three of five experimental animals. Thus, as expected, Bartha 43/25aB4 was virulent, but less so than wild-type PrV(Ka) virus. The gI⁻ and gI⁻/gp63⁻ mutants of Bartha 43/25aB4, however, were completely avirulent; pigs inoculated with these mutants gained weight at the same rate as did uninfected animals.

The LD_{50}s of the virus in pigs were not determined because of the expense involved. It is not possible, therefore, to state unequivocally that the inactivation of the gI gene in the Bartha 43/25aB4 genome caused a greater reduction in virulence for pigs than it did in the PrV(Ka) genome, as was the case for chickens (see Table 1). The results show clearly, however, that gI⁻ mutants are less virulent for pigs than are viruses that express gI and that the double gI⁻/gp63⁻ mutants do not appear to be less virulent than the gI⁻ mutant, i.e., that, as mentioned above, gp63 either plays no role in virulence for pigs or, if it does, its effect is not additive to that of gI.

Different regions of the genome of both herpes simplex virus and PrV that affect virulence have been identified. With the exception of thymidine kinase (4, 5, 8, 10, 16) and gC of herpes simplex virus (6), which have been shown to affect virulence, little is known about the functions that are responsible for virulence of the herpesviruses. The experiments in this study identify another viral function, the gI

TABLE 2. Virulence of gI⁻ and gI⁻/gp63⁻ mutants for young pigs[a]

Phenotype	Virulence (no. of animals affected/no. inoculated) of:			
	PrV(Ka)		Bartha 43/25aB4	
	Signs of disease	Death	Signs of disease	Death
gI⁺/gp63⁺	5/5	5/5	3/5	2/5
gI⁻/gp63⁺	3/5	2/5	0/5	0/5
gI⁻/gp63⁻	3/5	2/5	0/4	0/4

[a] Three-week-old pigs were inoculated both intranasally and intramuscularly with 10^7 PFU each of either wild-type (gI⁺/gp63⁺) PrV(Ka) virus, deletion mutants that do not express gI (gI⁻/gp63⁺), or mutants that do not express gI and gp63 (gI⁻/gp63⁻). The animals were observed for 14 days for signs of disease (loss of appetite, respiratory distress, fever, and loss of weight) and death.

glycoprotein of PrV, as one that plays a role in the virulence of this virus. Recently, also, Quint et al. (15) reported that deletions in the S component of the viral genome (which includes the region that is known to encode gI) affect virulence of the virus for pigs and mice.

Inactivation of gI alone (and possibly also of gp63 alone) decreased the level of virulence of the virus only slightly. However, inactivation of gI in conjunction with another viral function affected virulence much more profoundly. This conclusion was reached from the following considerations. Inactivation of gI in Bartha 43/25aB4 affected virulence for chickens much more severely than its inactivation in the PrV(Ka) or Phylaxia strain did. Bartha 43/25aB4 is a variant of the Bartha vaccine strain to which partial virulence has been restored by marker rescue with two regions of the viral genome. It was, however, 100-fold less virulent than wild-type virus; i.e., it appears to be defective in at least one other gene that is necessary for the full expression of virulence. Because inactivation of gI in Bartha 43/25aB4 affected virulence to a greater extent than it did in fully virulent virus strains, it appears that the effect of inactivation of gI on virulence depends on the genetic background of the virus strain. We conclude that gI affects virulence in conjunction with another gene(s).

Glycoprotein gI plays a role (also in conjunction with other viral functions) in virus release, a role that appears to be cell type specific (12). It is interesting that both virus release from certain cells (12) and virulence (this study) are affected more profoundly by inactivation of gI in Bartha 43/25aB4 than in PrV(Ka). The possibility that the effect of inactivation of gI on virus release and on virulence are related is appealing.

This investigation was supported by Public Health Service grant AI-10947 from the National Institutes of Health. Thomas Mettenleiter was supported by a fellowship (Me 854/1-2) from the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. **Berns, A., A. van den Ouweland, W. Quint, J. van Oirschot, and A. Gielkens.** 1985. Presence of markers for virulence in the unique short region or repeat region or both of pseudorabies hybrid viruses. J. Virol. **53**:89–93.
2. **Gielkens, A. L. J., J. T. van Oirschot, and A. J. M. Berns.** 1985. Genome differences among field isolates and vaccine strains of pseudorabies virus. J. Gen. Virol. **66**:69–82.
3. **Gustafson, D. P.** 1975. Pseudorabies, p. 391–410. *In* H. W. Dunne and A. P. Leman (ed.), Diseases of swine, 4th ed. Iowa State University Press, Ames.
4. **Kit, S., M. Kit, and E. C. Pirtle.** 1985. Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. Am. J. Vet. Res. **46**:1359–1367.
5. **Klein, R. J.** 1982. The pathogenesis of acute, latent, and recurrent herpes simplex virus infections. Arch. Virol. **72**:143–168.
6. **Kümel, G., H. C. Kaerner, M. Levine, C. H. Schröder, and J. C. Glorioso.** 1985. Passive immune protection by herpes simplex virus-specific monoclonal antibodies and monoclonal antibody-resistant mutants altered in pathogenicity. J. Virol. **56**:930–937.
7. **Lomniczi, B., M. L. Blankenship, and T. Ben-Porat.** 1984. Deletions in the genomes of pseudorabies virus vaccine strains and existence of four isomers of the genomes. J. Virol. **49**:970–979.
8. **Lomniczi, B., S. Watanabe, T. Ben-Porat, and A. S. Kaplan.** 1984. Genetic basis of the neurovirulence of pseudorabies virus. J. Virol. **52**:198–205.
9. **Lomniczi, B., S. Watanabe, T. Ben-Porat, and A. S. Kaplan.** 1987. Genome location and identification of functions defective in the Bartha vaccine strain of pseudorabies virus. J. Virol. **61**:796–801.
10. **McGregor, S., B. C. Easterday, A. S. Kaplan, and T. Ben-Porat.** 1985. Vaccination of swine with thymidine kinase-deficient mutants of pseudorabies virus. Am. J. Vet. Res. **46**:1494–1497.
11. **Mettenleiter, T. C., N. Lukàcs, and H.-J. Rziha.** 1985. Pseudorabies virus avirulent strains fail to express a major glycoprotein. J. Virol. **56**:307–311.
12. **Mettenleiter, T. C., C. Schreurs, F. Zuckermann, and T. Ben-Porat.** 1987. Role of pseudorabies virus glycoprotein gI in virus release from infected cells. J. Virol. **61**:2764–2769.
13. **Petrovskis, E. A., J. G. Timmins, T. M. Gierman, and L. E. Post.** 1986. Deletions in vaccine strains of pseudorabies virus and their effect on synthesis of glycoprotein gp63. J. Virol. **60**:1166–1169.
14. **Petrovskis, E. A., J. G. Timmins, and L. E. Post.** 1986. Use of λgt11 to isolate genes for two pseudorabies virus glycoproteins with homology to herpes simplex virus and varicella-zoster virus glycoproteins. J. Virol. **60**:185–193.
15. **Quint, W., A. Gielkens, J. van Oirschot, A. Berns, and H. T. Cuypers.** 1987. Construction and characterization of deletion mutants of pseudorabies virus: a new generation of "live" vaccines. J. Gen. Virol. **68**:523–534.
16. **Tenser, R. B., S. J. Ressel, F. A. Fralish, and J. C. Jones.** 1983. The role of pseudorabies virus thymidine kinase expression in trigeminal ganglion infection. J. Gen. Virol. **64**:1369–1373.