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(54) Title: PSEUDORABIES VIRUS VACCINE		
(57) Abstract Monoclonal antibodies to pseudorabies virus (PRV) glycoproteins useful for passive immunization of swine against PRV infection.		

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PSEUDORABIES VIRUS VACCINE

FIELD OF INVENTION

This invention relates to a passive vaccine for pseudorabies virus. More specifically, the invention relates to monoclonal
5 antibodies to pseudorabies virus glycoproteins, useful in alleviating pseudorabies virus infections in swine.

BACKGROUND OF THE INVENTION

Pseudorabies virus (PRV) is a disease which infects many species of animals worldwide. PRV infections are variously called infectious
10 Bulbar paralysis, Aujeszky's disease, and mad itch. Infections are known in important domestic animals such as swine, cattle, dogs, cats, sheep, rats and mink. The host range is very broad and includes most mammals and, experimentally at least, many kinds of birds (for a detailed list of hosts, see D.P. Gustafson, "Pseudo-
15 rabies", in Diseases of Swine, 5th ed., A.D. Leman et al., eds., (1981)). For most infected animals the disease is fatal. Adult swine and possibly rats, however, are not killed by the disease and are therefore carriers.

Populations of swine are particularly susceptible to PRV.
20 Although the adult swine rarely show symptoms or die from the disease, piglets become acutely ill when infected and death usually ensues in 24 to 48 hours often without specific clinical signs (T.C. Jones and R.D. Hunt, Veterinary Pathology, 5th ed., Lea & Febiger (1983)).

PRV vaccines have been produced by a variety of techniques and
25 vaccination in endemic areas of Europe has been practiced for more than 15 years. Losses have been reduced by vaccination, but vaccination has maintained the virus in the environment. No vaccine has been produced that will prevent infection. Vaccinated animals that
30 are exposed to virulent virus survive the infection and then shed more virulent virus. Vaccinated animals may therefore harbor a latent infection that can flare up again. (See, D.P. Gustafson, supra).

Live attenuated and inactivated vaccines for PRV are available
35 commercially in the United States and have been approved by the USDA (See, C.E. Aronson, ed., Veterinary Pharmaceuticals & Biologicals, (1983)).

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PRV is a herpesvirus. The herpesviruses generally are among the most complex of animal viruses. Their genomes encode at least 50 virus specific proteins and contain upwards of 150,000 nucleotides. Among the most immunologically reactive proteins of herpesviruses are the glycoproteins found, among other places, in virion membranes and the membranes of infected cells. The literature on PRV glycoproteins refers to at least four viral glycoproteins (T. Ben-Porat and A.S. Kaplan, *Virology*, 41, pp. 265-73 (1970); A.S. Kaplan and T. Ben-Porat, *Proc. Natl. Acad. Sci. USA*, 66, pp. 799-806 (1970)).

10 Based on research on herpes simplex virus type 1 (HSV-1) (B. Norrild, *Curr. Topics Microbiol. Immunol.*, 90, pp. 67-106 (1980)), the glycoproteins present on the surface of PRV infected cells and virions are believed to be the targets of humoral and cell-mediated immune responses in the infected host.

15 INFORMATION DISCLOSURE

The study of PRV glycoproteins with monoclonal antibodies has recently been described by M.W. Wathen and L.K. Wathen, *J. Virol.*, 51, pp. 57-62 (1984) who refer to a PRV containing a mutation in a viral glycoprotein (gp50), a method for selecting the mutant utilizing neutralizing monoclonal antibody directed against gp50, and a monoclonal antibody directed against gp50. Wathen and Wathen also indicate that their monoclonal antibody directed against gp50 is a strong neutralizer of PRV, with or without the aid of complement, and that polyvalent immune serum is highly reactive against gp50,

20 therefore concluding that gp50 may be one of the important PRV immunogens. On the other hand, it has been reported that monoclonal antibodies that react with the 98,000 MW envelope glycoprotein (gIII) neutralize PRV infectivity but that monoclonal antibodies directed against some of the other membrane glycoproteins (e.g., gII) have

25 very little neutralizing activity (H. Hampl, et al., *J. Virol.*, 52, pp. 583-90 (1984); and T. Ben-Porat and A.S. Kaplan, "Molecular Biology of Pseudorabies Virus", in B. Roizman ed., *The Herpesviruses*, 3, pp. 105-73 (1984)).

T.C. Mettenleiter et al., *J. Virol.*, 53, pp. 52-57 (1985), also

35 refer to the use of monoclonal antibodies to map a gene encoding a PRV glycoprotein called gI. Monoclonal antibodies have also been used to partially characterize several PRV glycoproteins with respect to glycosylation and synthesis within infected cells (Hampl et al.,

supra.; and N. Lukacs et al., J. Virol., 53, pp. 166-73 (1985)(gI and gII)).

N.S. Balachandran, et al., Infect. Immun., 37, pp. 1132-37 (1982) and R.D. Dix, et al., Infect. Immun., 34, pp. 192-99 (1981) refer to protecting mice from homologous virus challenge using passive immunization with monoclonal antibodies reactive with herpes simplex virus glycoproteins. D.P. Gustafson et al., J. Am. Vet. Med. Assoc., 160, pp. 623-28 (1972) refer to passive administration of PRV hyperimmune serum and gamma globulin preparations and their effectiveness as a short-term prophylaxis against Aujeszky's disease in swine. R.A. Crandell et al., J. Am. Vet. Med. Assoc., 171, pp. 59-63 (1977) refers to suckling pigs given hyperimmune serum incurring a lower rate of mortality than control pigs after exposure to PRV.

The short-term prophylactic treatment of the instant invention is useful after exposure of a herd of pigs to PRV. Highly susceptible young pigs must be protected at that time in order to survive. Vaccination programs would then be started after initial protection is achieved to protect the pigs for a longer time.

The monoclonal antibodies of the instant invention are more advantageous than hyperimmune serum or gamma globulin in a passive immunization program for such protection because they are easier to mass produce and have greater specificity as well as less chance of passing on incidental infection.

SUMMARY OF THE INVENTION

The present invention relates to a method of treating PRV infection in swine by administering an anti-PRV effective amount of a composition comprising a monoclonal antibody against PRV gII, gIII, or gp50 to a swine in need of such treatment.

More particularly, the invention relates to a method of treating PRV infection in swine by administering an anti-PRV effective amount of a composition comprising a monoclonal antibody selected from the group consisting of 3A4, 3D1, and 3D11 to a swine in need of such treatment.

The present invention also relates to a monoclonal antibody selected from the group consisting of 3D11, 3D1, and 3A4.

The present invention also relates to a hybridoma selected from the group consisting of hybridomas 3A-4C, 3D-1C, and 3D-11C.

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The present invention also relates to a vaccine for passive immunization of swine against PRV infection comprising a monoclonal antibody against PRV gII, gIII, or gp50.

More particularly, the invention relates to a vaccine for
5 passive immunization of swine against PRV infection comprising a monoclonal antibody selected from the group consisting of 3A4, 3D1, and 3D11.

DETAILED DESCRIPTION OF THE INVENTION

To make the monoclonal antibodies of the instant invention one
10 can use any of the techniques well known to those skilled in the art, e.g., as set forth in R. Kennett, Monoclonal Antibodies, Plenum Press, (1980). We fused spleen cells from an immune mouse with Sp2/0-Ag14 tumor cells. Hybrid cell growth was visible in 145 of 720 microtiter wells initially seeded after cell fusion. The supernatant
15 from each growth-positive well was assayed for the presence of neutralizing antibody by a microneutralization assay (T.C. Holland et al., J. Virol., 45, pp. 672-82 (1983)). Neutralizing activity was detected in 10 of the initial 145 growth-positive wells. The hybridomas that were secreting neutralizing antibody were subsequent-
20 ly cloned by limiting dilution and their isotypes were determined by the Elisa method, supra.

The footpad model of peripheral inoculation of virus was used in the passive immunization studies in mice because the ascending neurological illness associated with this model resembles the
25 pathogenesis of PRV in swine. Footpad inoculation of mice with PRV results in pruritus within 3 to 7 days, followed by ascending myelitis, encephalitis, and death (H.J. Field and T.J. Hill, J. Gen.-Virol., 23, pp. 145-57 (1974)). Although it has been shown that the course of viral infection in mice by HSV can be abrogated by admini-
30 stration of protective antibody either prior to or soon after viral infection (Dix et al., supra. and Balachandran et al., supra.), this is the first report which describes the use of monoclonal antibodies to passively transfer immunity against PRV in swine (throughout the specification and claims, the terms swine and pig(s) are used
35 synonymously).

EXAMPLE 1

1. Cell Cultures, Media and Virus

Sp2/0-Ag14 cells (M. Shulman et al., *Nature*, 276, pp. 269-70 (1978); American Type Culture Collection, ATCC CRL 1581)) were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100
5 $\mu\text{g/ml}$) and 8-azaguanine (10 $\mu\text{g/ml}$). The Sp2/0-Ag14 cells were then maintained in the absence of 8-azaguanine for four to six days prior to fusion with spleen cells.

Selection medium used after fusion of spleen cells and Sp2/0-Ag14 cells comprised DMEM supplemented with 20% Zeta Serum (AMF
10 Biological and Diagnostic Products Co., Seguin, TX), hypoxanthine (13.6 $\mu\text{g/ml}$), aminopterin (0.18 $\mu\text{g/ml}$), and thymidine (3.9 $\mu\text{g/ml}$).

Hybrid cells shown to secrete neutralizing antibody were cloned in medium comprised of DMEM supplemented with 20% fetal bovine serum, hypoxanthine (13.6 $\mu\text{g/ml}$), and 20% conditioned medium obtained from
15 SP2/0-Ag14 cell cultures (cloning medium).

Vero (African Green Monkey Kidney) cells were grown in Eagle Minimum Essential Medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 $\mu\text{g/ml}$), and fungizone (0.25 $\mu\text{g/ml}$).

20 PRV (Rice strain) was propagated in primary rabbit kidney cells as described by Rea et al., *J. Virol.*, 54, pp. 21-29 (1985).

2. Mouse Immunization

A female Balb/c mouse (Charles River Breeding Laboratories, Inc., Wilmington, MA), 5-6 weeks old, was injected subcutaneously
25 with 0.2 ml of a soluble, inactivated PRV-infected cell extract (PR-VAC; Norden Laboratories, Lincoln, NE) on Day 0. The mouse was given booster injections subcutaneously with the same preparation on Day 14 and challenged with PRV (Rice Strain) intraperitoneally on Day 21 with a dose approximately 20 times the 50% lethal dose. A final
30 intraperitoneal injection of 1 ml of soluble PRV-infected cell extract was given on Day 43, three days prior to fusion (Day 46).

3. Fusion and Cloning

Spleen cells obtained from the immune mouse (above) were fused with Sp2/0-Ag14 cells essentially using the procedure described by
35 R. Kennett, *Monoclonal Antibodies*, Plenum Press, (1980). Spleen cells and Sp2/0-Ag14 cells were mixed at a ratio of 2:1 and washed twice with DMEM. After the final centrifugation, the cells were dispersed and the centrifuge tube containing the cell mixture was

placed in a heating block at 40°C. One ml of 40% (w/v) polyethylene glycol 1450 (J.T. Baker Chemical Co., Phillipsburg, NJ) was added at a rate of 0.25 ml/15 sec. The cell mixture was then incubated at 40°C for 1 min followed by addition of 1 ml of DMEM at a rate of 0.25 ml/15 sec. An additional 10 ml of DMEM was immediately added at a rate of 0.5 ml/15 sec. During all additions of polyethylene glycol and DMEM the cell mixture was periodically agitated to insure mixing.

The cell mixture was subsequently centrifuged for 7 min at 1000 RPM. The cells were then dispersed and DMEM supplemented with 20% Zeta Serum was added. Cells were transferred by pipette into 96-well tissue culture plates so that each well received the equivalent of 2×10^5 spleen cells and 1×10^5 Sp2/0-Ag14 cells.

The plates were incubated at 37°C in a humidified incubator with 5% CO₂. One drop of selection medium was placed in each well on each of three consecutive days after fusion. Subsequently, the wells were fed weekly by replacing 75% of the medium with fresh selection medium.

Supernatants from growth-positive wells were assayed by a micro-neutralization assay which is described below. Hybrid cell cultures secreting neutralizing antibody were subsequently transferred to cloning medium and cloned by limiting dilution. Ascites fluids were produced by intraperitoneal injection of 5×10^6 hybrid cells into each of several pristane-primed Balb/c mice.

4. Microneutralization Assay

A microneutralization assay, as described by T.C. Holland et al., J. Virol., 45, pp. 672-82 (1983), was used to screen hybrid cell supernatants for neutralizing activity and to determine the neutralization titers of supernatants and ascites fluids. 50 µl of each supernatant or serially diluted ascites fluid was added to 50 µl of virus (100 times the 50% tissue culture infectious dose) and 20% rabbit complement. The microtiter plates were incubated for 3 hrs at 37°C and 3×10^4 Vero cells per well were added. The microtiter plates were then incubated for 48 hrs at 37°C.

Cells were stained with 0.025% crystal violet and the antibody titer was expressed as the reciprocal of the highest dilution of supernatant or ascites which protects greater than 50% of the cells from cytopathic effects. Supernatants obtained from initial growth-positive hybrid cultures were determined to have neutralizing

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activity if greater than 50% of the cells were protected from cytopathic effects.

5. Immunoprecipitation

Immunoprecipitation of radiolabeled viral proteins with monoclonal antibodies was according to the method of T.J. Rea, et al. J.Virol., 54, 21-29 (1985).

6. Isotype Determination

The isotype of monoclonal antibody secreted by each hybrid cell line was determined by an enzyme-linked immunosorbent assay using a commercially available isotyping kit (Hyclone Laboratories, Logan, UT). This assay was conducted according to the manufacturer's specifications. Of the four finally selected hybridomas, two secreted IgG_{2a}, one secreted IgG_{2b}, and one secreted IgA (Table 1). Monoclonal antibodies 3A4, 3D1, 3D11, and 2A2 neutralized PRV only in the presence of complement.

Immunoprecipitations using either supernatants or ascites fluids of hybrid cell lines demonstrated that each monoclonal antibody was specific for gII, gIII (gp82), or gp50 (the nomenclature is that of Hampl et al., supra, and Wathen and Wathen, supra.). Immunoprecipitation patterns of monoclonal antibodies 3D11, 3A4, 3D1, and 2A2 were also determined by immunoprecipitation of ¹⁴C-glucosamine-labeled--infected-cell polypeptides using the monoclonal antibodies. The specificities are set forth in Table 1.

Table 1

		Monoclonal Antibodies			
		Neutralization ^a		Specificity ^b	
Monoclonal					
Antibody	Isotype	+C	-C	Wathen	Hampl
30	3A4	IgG _{2a}	+	-	gp50
	3D1	IgG _{2b}	+	-	gII
	3D11	IgG _{2a}	+	-	gp82 gIII
	2A2	IgA	+	+	gp50

^aSupernatants obtained from hybrid cultures were determined to have neutralizing activity (in the presence or absence of complement) if greater than 50% of the cells were protected from cytopathic effects.

^bSpecificity was determined by immunoprecipitation.

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Hybridomas 3A-4C (UC HB-7), 3D-1C (UC HB-13), and 3D-11C (UC HB-14) which produce monoclonal antibodies 3A4, 3D1, and 3D11 respectively and exemplify the invention, were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 on December 18, 1985, and have been assigned accession numbers HB8980, HB8981, and HB 8982 respectively (Note: UC is a registered trademark of The Upjohn Co.).

EXAMPLE 2

In this example we demonstrate that administration of the monoclonal antibodies of Example 1 protected mice from footpad challenge with PRV.

1. Monoclonal Antibodies

The production of hybridomas and the generation of ascites fluids is described in Example 1.

15 2. Microneutralization Assay

The microneutralization assay used to determine the neutralization titers of ascites fluids is described in Example 1.

3. Passive Transfer Studies

The administration of ascites fluids and subsequent challenge of mice with PRV was conducted as described by N.S. Balachandran et al., supra, with several modifications. Female CF-1 mice, 6-7 weeks old, were each administered 1 ml of the respective antibody or control preparations by the intraperitoneal route. Three hours after administration of the respective preparations mice were challenged by the footpad route with a volume of 0.05 ml containing PRV at 20 times the 50% lethal dose (LD_{50}). Mice were observed for mortality for a period of 21 days.

The results of these passive transfer studies are shown in Table 2. The most protective monoclonal antibodies were those specific for PRV glycoproteins gp50 or gIII. Monoclonal antibody 3A4, reactive with gp50, was especially protective in both studies 1 and 2 (90% and 100% survival, respectively). Mice that were administered saline or ascites fluids with no antibody (saline, Sp2/0, and hybridoma control groups) were not protected from PRV infection. In addition, monoclonal antibody 3D1, which is specific for PRV glycoprotein gII, was not protective despite those antibodies having a significant neutralization titer (Tables 2 and 3).

In Studies 1 and 2 monoclonal antibody 3A4 was the most protective, even though monoclonal antibody 3D11 had higher neutralization titers. Monoclonal antibody 3D11 neutralized in the absence of complement (neutralization titer = 40) at a very low level. Neutralization by the other monoclonal antibodies was dependent on the presence of complement.

A challenge dose of 20 LD₅₀ was used in each of the two passive transfer studies. The mean day of death (MDD) observed for control mice that were administered Sp2/0 and hybridoma control ascites fluids in Study 1 were each 4.0 days, whereas in Study 2 the MDD for mice that were administered hybridoma control ascites fluid and saline were 5.8 and 7.1 days, respectively. Since the LD₅₀ determination is only an approximation, the actual challenge doses in Studies 1 and 2 were probably different, therefore accounting for the slight variation in MDD for control mice between studies. This explanation may also account for the differences in the survival of mice given monoclonal antibodies 3A4 and 3D11 when comparing Studies 1 and 2. These passive transfer studies indicated that monoclonal antibodies directed against gIII and gp50 are protective, whereas the monoclonal antibodies directed against gII do not protect mice against PRV challenge.

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Table 2
Mortality of Mice^a

	Preparation	Dead/ Total	MDD ^b
5			
	Study 1		
	3A4	1/10	5.0
	3D1	10/10	4.0
10	3D11	5/10	7.4
	Conv. Pig Serum ^c	0/10	---
	Sp 2/0 Control ^d	10/10	4.0
	Hybridoma Control ^e	10/10	4.0
	Study 2		
15	3A4	0/10	---
	3D11	1/10	8.0
	2A2	4/10	8.3
	Conv. Pig Serum	1/10	10.0
	Hybridoma Control	9/10	5.8
20	Saline Control	9/10	7.1

^aMice were challenged by the footpad route with PRV at a dose of 20 LD50 3 hrs after administration of the antibody or control preparations.

25 ^bMDD: mean day of death.

^cConvalescent pig serum: serum from a recovered PRV-infected pig.

^dSp 2/0 control: ascites fluid from mice injected with Sp 2/0 cells.

30 ^eHybridoma control: ascites fluid from mice injected with a hybrid oma cell line not secreting antibody.

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Table 3

Properties of Preparations Passively Administered to Mice

				Neutralization		
				Titer ^a		
	Preparation	Isotype	Specificity	+C	-C	% Survival
	Study 1					
10	3A4	IgG2 _a	gp50	327,680	<20	90
	3D1	IgG2 _b	gII	10,240	<20	0
	3D11	IgG2 _a	gIII	655,360	40	50
	Conv. Pig					
	Serum	---	---	20,480	640	100
15	Sp 2/0					
	Control	---	---	<20	<20	0
	Hybrid					
	Control	---	---	<20	<20	0
20	Study 2					
	3A4	IgG2 _a	gp50	327,680	<20	100
	3D11	IgG2 _a	gIII	655,360	40	90
	2A2	IgA	gp50	2,560	2,560	60
	Conv. Pig					
25	Serum	---	---	20,480	640	90
	Hybrid					
	Control	---	---	<20	<20	10
	Saline					
	Control	---	---	<20	<20	10
30						

^aThe neutralization titer (+ or - complement) expressed as the reciprocal of the highest dilution of each preparation that protected greater than 50% of the cells from cytopathic effects.

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EXAMPLE 3

In this example we demonstrate that passive immunization of swine with the monoclonal antibodies of Example 1 protects swine from PRV infection.

5 1. Animals

Study 1: Thirty-six crossbred pigs, 5-6 weeks old and of mixed sex, randomly allotted into six groups (six pigs/group), were used. Sixteen pigs of the same age were also used for the determination of the 50% lethal dose. Swine were given unmedicated feed and water ad
10 libitum.

Study 2: Thirty crossbred pigs, 5-6 weeks old and of mixed sex, randomly allotted into five groups, were used. Sixteen pigs of the same age were also used to determine the LD50.

2. Production of Antibody Preparations

15 Study 1: Ascites fluids were prepared by injecting 5×10^6 cells of each hybridoma cell line into separate pristane-primed mice. After the appearance of an ascites tumor the mice were tapped for ascites fluids on alternate days until death.

Study 2: The monoclonal antibodies were prepared by culturing
20 hybridoma cell lines in 16-liter-suspension culture vessels operated as 10 liters. Each 10-liter harvest was concentrated to 1 liter with a Pellicon Ultrafiltration unit using 10,000 MW cut-off membranes. The concentrate was brought to 4°C and 1 liter of saturated ammonium sulfate at 4°C was added slowly under vigorous stirring, allowed to
25 stand for 1 hour, and centrifuged at 3000 xg. The precipitate was placed in Spectra/Por 6 dialysis tubing (2.7 mm dia.) and dialyzed for 24 hours at a 1:100 dialysate:buffer volume ratio. The buffer was replaced once, giving a total of 48 hours for dialysis and a 10^4 reduction of salts. Dialysis buffer was 0.15 M NaCl in Milli-Q
30 quality water. Each dialysate was then sterile filtered through 0.45 μ filters and later divided into 6 doses in 50 ml sterile serum bottles.

3. Determination of 50% Lethal Dose (LD50)

Sixteen pigs randomly allotted into four groups (four pigs/-
35 group), were used to determine the LD50 of the Rice strain of PRV. The four groups received 10^4 , 10^3 , 10^2 , and 10^1 plaque forming units (pfu)/pig, respectively, by intranasal inoculation. The LD50 was

determined by the method of L. J. Reed and H. Muench, Am. J. Hygiene, 27, pp. 493-97 (1938).

4. Virus Isolation from Nasal Swabs

Nasal swabs collected from pigs were each placed in one ml of
5 Eagles Basal Medium (BME; M.A. Bioproducts) supplemented with 3% fetal bovine serum (FBS) and antibiotics. Swabs were stored at -70° until they were assayed for the presence of virus. For the virus isolation assay, the nasal swabs in BME were thawed and the individual swabs were discarded. Samples (0.1 ml) were inoculated in
10 duplicate onto porcine kidney-15 (PK-15; ATCC CCL33) cell monolayers and incubated for 1 hr at 37° to allow virus adsorption. An overlay of medium-199 (Flow Laboratories) supplemented with 4% FBS, antibiotics, and 1% agar was placed on the cell cultures. After 3 days the cell monolayers were stained with neutral red and the plaques
15 were enumerated.

5. Passive Transfer

Study 1: Pigs were treated as outlined in Table 5. Pigs in groups 1 through 3 were administered 5 ml ascites fluid containing monoclonal antibodies (3A4, 3D11, or 3D1) by the intraperitoneal (IP)
20 route. The amount of immunoglobulin was not quantitated in this study. Pigs in group 4 served as positive controls and received 5 ml serum obtained from a pig recovered from PRV infection (convalescent pig serum). Pigs in group 5 served as negative controls and received 5 ml ascites fluid containing no monoclonal antibody.

25 All pigs were challenged intranasally with 20 times the 50% lethal dose ($20 \text{ LD}_{50} = 3.8 \times 10^4$ plaque forming units/pig) of PRV (Rice strain) 4 hrs after administration of the antibody or control preparations on Day 0. Nasal swabs were obtained from each pig daily beginning on Day 2. The study was terminated 21 days after challenge
30 (Day 21).

Study 2: Pigs were administered about 150 mg of the respective antibody preparations by the intraperitoneal route (Table 4). Control pigs were given either fetal calf serum proteins prepared in a manner similar to the monoclonal antibody preparations or convalescent pig serum. Four hours after administration of the respective
35 preparations swine were challenged intranasally with a volume of four ml (two ml/nostril) containing PRV (Rice strain) at 20 times the 50% lethal dose ($20 \text{ LD}_{50} = 5.0 \times 10^4$ plaque forming units/pig). Swine were

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observed for mortality for a period of 14 days and nasal swabs were obtained daily from each pig. In addition, swine were weighed prior to challenge (day 0) and at termination (day 14) or death.

Table 4

5 Composition of Preparations Used in Study 2

					Fetal Bovine Serum
10	Monoclonal Ab	3A4	3D1	3D11	
	No. of Doses	6	6	6	6
	Volume (ml)/Dose	25.6	26.2	33.0	32.0
	mg/ml IgG	5.86 ± 0.54 ^a	5.73 ± 0.48 ^a	4.55 ± 0.68 ^a	0
15	mg/ml Total				
	Protein	33.8 ± 0.2	42.8 ± 1.5	34.3 ± 1.3	35.3
	mg IgG/Dose	150 ± 14 ^b	150 ± 13 ^b	150 ± 23 ^b	0
	mg Total				
	Protein/Dose	865 ± 2	1120 ± 20	1130 ± 20	1130
20	(IgG/Total				
	Protein)x100	17.3 ± 1.6	13.4 ± 1.2	13.2 ± 2.0	0
	Total IgG				
	Produced (mg)	936 ± 69	1000 ± 80	1260 ± 100	--
	Percent Recovered	103 ± 12	134 ± 18	78 ± 13	--

25

Total protein determinations were conducted using the Bradford assay (M. Bradford, Anal. Biochem., 72, p. 248 (1976)), with bovine serum albumin as the standard.

30 ^aRelative standard deviation is a combination of relative standard deviations from two or more independent fractions that were mixed to produce the formulated dose.

^bNote that the calculated concentrations of IgG have large standard deviations. The IgG concentrations were determined several times and in most instances were calculated to be within two standard deviations of 150 mg.

35

6. Criteria for Efficacy Evaluation

Efficacy of each monoclonal antibody preparation was evaluated on the basis of protection against PRV-induced mortality.

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The results of the study are set forth in Table 5.

Table 5

5	Group ^a			Glycoprotein	Neutraliza-	Survivors/
	No.	Preparation ^b	Isotypes	Specificity	tion Titer ^c	Total
	STUDY 1					
	1	mAb 3A4	IgG _{2a}	gp50	40,960	2/6
10	2	mAb 3D11	IgG _{2a}	gIII	327,680	5/6
	3	mAb 3A4, 3D11, 3D1	IgG _{2a} , IgG _{2a} , IgG _{2b}	gp50, gIII, gII	655,360	3/6
	4	mAb 2A2	IgA	gp50	320	0/6
15	5	Convalescent Pig Serum	--	--	163,840	4/6
	6	Ascites Control	--	--	<20	0/6
	STUDY 2					
20	1	mAb 3A4	IgG _{2a}	gp50	81,920	3/5
	2	mAb 3D11	IgG _{2a}	gIII	81,920	6/6
	3	mAb 3D1	IgG _{2b}	gII	5,120	3/5
	4	Convalescent Pig Serum	--	--	81,920	5/6
25	5	FBS Control ^d	--	--	<20	1/6

^aFive or six pigs per group.

^bPigs were treated as set forth in the text. mAb = monoclonal
30 antibody; FBS = fetal bovine serum.

^cThe neutralization titer is expressed as the reciprocal of the
highest dilution of serum that protected greater than 50% of the
cells from cpe.

^dFBS control was used as a negative control for protection and
35 contained no PRV-specific antibody.

Monitoring of virus shedding from nasal secretions demonstrated
that virus was shed by pigs administered any monoclonal antibody or

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control preparation. No difference in viral shedding was apparent between pigs administered specific monoclonal antibody preparations.

Pigs that were given monoclonal antibody preparations did not gain weight as readily as pigs that were given convalescent pig serum. This may have been due to either the effect of convalescent pig serum antibodies in decreasing the onset of morbidity or the adverse effect of contaminants such as ammonium sulfate, phenol red, and bovine serum proteins present in monoclonal antibody preparations.

Monoclonal antibody 2A2 did not protect in this study at the levels tested. While not wishing to bound by theory, this is possibly because it is an IgA immunoglobulin and not IgG as were those that protected. Also, as set forth supra, we did not quantitate the amount of immunoglobulin in Study 1 and therefore only a small amount of 2A2 may have been administered in the experiment.

Monoclonal Antibody as Therapy

1. Composition of vehicle(s) - e.g., water, saline, buffer suspensions of purified and/or processed monoclonal antibody or unaltered ascites fluid.
2. Route of administration - intraperitoneal, intravenous, intramuscular or subcutaneous, oral or intranasal.
3. Dosage regimen - at first signs of pseudorabies in the herd, treat all susceptible (non-vaccinated) pigs. One dose is generally adequate but a second dose may be required in the event of further pseudorabies infections. No more than two doses should be administered due to the possible adverse immune reactions to mouse protein.
4. Duration of protection - for the first dose, up to about 10 days; for the second dose, no more than about one week.
5. Vaccination with commercial vaccines during treatment - administration of vaccines currently available commercially (such as the attenuated virus or inactivated virus vaccines) is contraindicated since the antibody of the monoclonal therapeutic agent will react with the vaccine antigen and cause rapid clearance of antigen and monoclonal antibody. Commercial vaccine may be administered at about seven days post therapy as long as a second dose of monoclonal antibody therapy is not contemplated.
6. Amount of monoclonal antibody - About 20 mg to about 300 mg of monoclonal antibody may be administered per pig. The preferable

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amount is from about 50 to about 150 mg per pig. Preferably about 1 ml to about 10 ml of a composition comprising the monoclonal antibody will be administered. The maximum administered will be about 50 ml. The amount of material administered will be route dependent and animal dependent (e.g., based on age, size, and general health). Less volume can be administered into the muscle or intravenously than can be administered intraperitoneally or subcutaneously.

We claim:

1. A method of treating PRV infection in swine by administering an anti-PRV effective amount of a composition comprising a monoclonal antibody against PRV gII, gIII, or gp50 to a swine in need of such treatment.
2. A method according to claim 1 wherein the monoclonal antibody is selected from the group consisting of 3A4, 3D1, and 3D11.
3. A monoclonal antibody selected from the group consisting of 3D11, 3D1, and 3A4.
4. A hybridoma selected from the group consisting of hybridomas 3A-4C, 3D-1C, and 3D-11C.
5. A vaccine for passive immunization of swine against PRV infection comprising a monoclonal antibody against PRV gII, gIII, or gp50.
6. A vaccine according to claim 5 wherein the monoclonal antibody is selected from the group consisting of 3A4, 3D1, and 3D11.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02809

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC ⁴ : A 61 K 39/42; C 12 P 21/00; C 07 K 15/00; C 12 N 5/00 // C 12 N 15/00; (C 12 P 21/00; C 12 R 1:91		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁴	A 61 K; C 12 P; C 12 N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Chemical Abstracts, volume 104, no. 5, 3 February 1986, (Columbus, Ohio, US), L.M.K. Wathen et al.: "Production and characterization of monoclonal anti- bodies directed against pseudorabies virus", see page 443, abstract 32830c, & Virus Res. 1985, 4(1), 19-29 --	3-6
A	Biological Abstracts, volume 78, no. 9, 1984, (Philadelphia, PA., US), M.W. Wathen et al.: "Isolation characterization and physical mapping of a pseudorabies virus mutant containing antigenically altered gp50", see page 8142, abstract 72347, & J. Virol. 51(1): 57-62, 1984 cited in the application --	3-6
A	Chemical Abstracts, volume 101, no. 23, 3 December 1984, (Columbus, Ohio, US), H. Hampl et al.: "Characterization of the envelope proteins of pseudorabies virus", see page 330, abstract 207336p, ./.	3-6
¹⁰ Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
8th April 1987		15 MAY 1987
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		 L. ROSSI

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

J. Virol. 1984, 52(2), 583-90
cited in the application

P,X

Biological Abstracts / Reports, Reviews
and Meetings, volume 31, 1986,
(Philadelphia, PA., US),
C.C. Marchioli et al.: "Protection of
mice and swine from pseudorabies virus-
induced mortality with monoclonal anti-
bodies directed against pseudorabies
virus glycoproteins", see abstract no.
7477, & Abstracts of the Annual Meeting
of the American Society for Microbio-

3-6

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 1, 2... because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv):

Methods for treatment of the human or animal body by means of
surgery or therapy, as well as diagnostic methods

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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	logy (US), 1986, vol. 86, no. 0, p. 315	
