

The Effects of Challenge on the Humoral and Cellular Immune Responses in Pseudorabies Vaccinated Swine

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ABSTRACT

The effects of challenge exposure on the humoral and cellular immune responses in pseudorabies vaccinated swine were studied in 84 barrows. The pigs were divided into seven groups and challenge exposed to a virulent strain of pseudorabies virus on months 1, 3, 5, 8, 10, 12 and 14 after vaccination. The pigs were vaccinated with commercial attenuated and inactivated pseudorabies virus vaccines. The protection conferred by vaccination was equally effective with both types of vaccines. The levels of cellular and humoral immunity after challenge exposure in pigs vaccinated with either type of vaccine were similar. The cell-mediated immune response can be effectively used for the early detection of pigs exposed to pseudorabies virus. Virus isolation attempts from the brain and spleen in most of the vaccinated pigs were unsuccessful.

Key words: Pseudorabies immunity, cell-mediated immune response, humoral immune response, pseudorabies, vaccines and virus isolation.

RÉSUMÉ

Cette expérience portait sur 84 porcs castrés et vaccinés con-

tre la pseudo-rage; elle consistait à déterminer les effets d'une infection de défi, avec le virus correspondant, sur leur immunité humorale et cellulaire. Les auteurs répartirent leurs sujets d'expérience en sept groupes et les soumirent à l'infection de défi, avec une souche virulente du virus de la pseudo-rage, au bout d'un, trois, cinq, huit, 10, 12 et 14 mois après leur vaccination. Ils utilisèrent des vaccins commerciaux atténués et inactivés. La protection conférée par la vaccination et la qualité de l'immunité cellulaire ou humorale se révélèrent comparables, indépendamment du vaccin employé. L'immunité cellulaire peut servir efficacement à l'identification précoce des porcs exposés au virus de la pseudo-rage. Les tentatives visant à isoler le virus, du cerveau et de la rate de la plupart des sujets infectés, s'avèrent infructueuses.

Mots clés: immunité contre la pseudo-rage, immunité cellulaire, immunité humorale, vaccins contre la pseudo-rage, isolation du virus.

INTRODUCTION

The cell-mediated immune response has been detected in swine infected with pseudorabies virus (PRV) by using the lympho-

cyte transformation test *in vitro* (1, 2, 3).

Lymphocytes from nonvaccinated pigs on day 6 after exposure to a virulent strain of PRV (4) were shown to have a proliferative response when exposed to a Shope strain of PRV, while lymphocytes from pigs exposed to a modified live PRV vaccine were shown to have a proliferative response between days 7 and 14 after vaccination (1). Humoral antibodies were detected in nonvaccinated pigs on days 7 and 8 after challenge exposure to PRV (2, 3).

The objectives of this study were: 1) to determine the degree of protection conferred by the cellular and humoral immune response in pigs vaccinated with two commercially available PRV vaccines and challenge exposed later to an Iowa strain of PRV and 2) to compare the virus isolation, fluorescent antibody and virus coculture tests among vaccinated and nonvaccinated challenge exposed pigs.

MATERIALS AND METHODS

Eighty-four seven week old barrows which were negative for PRV antibody titers at the start of the study were utilized. Twenty-eight pigs received a modified live PRV (MLPRV) vaccine (Norden Laboratories, Inc, Lincoln, Nebraska, U.S.A.), 28 received an inactivated PRV (IPRV) vaccine (Salsbury Laboratories, Charles City, Iowa,

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U.S.A.) and 28 received a placebo (PL) solution of sterile distilled water. The MLPRV vaccine and PL solution were administered IM once (2 mL) while the IPRV vaccine was given subcutaneously twice (2 mL) fourteen days apart. The pigs were vaccinated at nine weeks of age and kept in separate pens according to vaccine category until month 3 after vaccination. Seven groups were formed (I, II, III, IV, V, VI, VII) and exposed to the PRV on months 1, 3, 5, 8, 10, 12 and 14 after vaccination respectively. Each group consisted of 12 pigs (four vaccinated with the MLPRV vaccine, four vaccinated with the IPRV vaccine and four injected with the PL solution).

The cell-mediated immune response was determined with the lymphocyte transformation test *in vitro*. The test was done in all the pigs in each group before challenge exposure and on day 6 after challenge exposure, in addition the test was done in half of the pigs in each group on days 11, 36 and 60 after challenge exposure. The level of antibodies in serum was determined with the microtitration neutralization test (5) in half of the pigs in each group on day 7 after challenge exposure and on day 60 in the other half. Furthermore, the microtitration neutralization test was done in all the pigs in each group after vaccination but before challenge exposure at monthly intervals to evaluate how long after vaccination the serum antibodies could be detected. The test was considered positive at the 1:2 dilution or greater.

Half of the pigs in each group (two of each vaccine category) were killed on day 7 and the other half on day 60 after exposure. Virus isolation from the brain and spleen and fluorescent antibody test on the lungs, tonsils and trigeminal ganglia (5) was attempted in all the pigs while virus coculture from the trigeminal ganglia were attempted only in the pigs killed on day 60 after challenge exposure (4).

The pigs in groups I and II were challenge exposed intranasally with 4 mL of a field isolate of an

Iowa strain of PRV at the 10th tissue culture passage containing 1.6×10^7 plaque forming units (PFU)/mL, while the pigs in groups III, IV, V, VI and VII received 4 mL of the same strain of PRV at the 11th tissue culture passage containing 5×10^6 PFU/mL in the same manner.

For the lymphocyte transformation test *in vitro*, blood was collected from the anterior vena cava into siliconized tubes containing ten units of heparin/mL. Mononuclear cells were separated and a dilution containing 4.7×10^6 cells/mL prepared. One hundred and fifty μ L of cells were added to each well of 96 well U bottom microtiter plate. The inactivated PRV antigen used to sensitize the lymphocytes was prepared by using the technique described by Gutekunst and Pirtle (6). The antigen was diluted 1 to 80 with RPMI 1640 and 50 μ L of the diluted antigen added to each well. Control wells received 50 μ L of RPMI 1640 media while positive control wells received 10 μ g of phytohemagglutinin P; 3 H thymidine was added and the cells harvested onto glass fiber filter paper. Each sample was set up in six wells. Samples were counted in a liquid scintillation counter (Packard Tricarb Model 2425 Liquid Scintillation Spectrometer System, Downer's Grove, Illinois, U.S.A.). Results were expressed in counts per minute (CPM) and stimulation indices (SI) which were the ratios of the CPM of the cells containing the PRV antigen by the CPM of the cells containing the RPMI 1640 media. Only the stimulation indices above three were considered positive. Stimulation indices were compared by Student's *t* test (7). The neutralization test, virus isolation, fluorescent antibody and virus coculture tests were evaluated as positive or negative.

RESULTS

All the pigs from groups I, III, IV, V, VI and VII had clinical signs of illness on about day 3 after challenge exposure. The clinical

signs consisted of increased rectal temperature, anorexia, prostration, reluctance to move, sneezing and purulent exudate flowing from the nostrils. Most of the pigs in group II however failed to develop clinical illness. The pigs which received the PL injections in groups I, III and IV had a more severe clinical illness than the pigs which received the MLPRV and the IPRV vaccines in the same groups. The protection conferred by vaccination in the pigs of these groups was equally effective for both types of vaccines. All the pigs in groups V, VI and VII had an equal degree of clinical illness regardless of the type of vaccine received (PL, IPRV OR MLPRV). Only two pigs in group II had evidence of clinical illness (one pig vaccinated with the MLPRV vaccine and one with the PL solution).

The results obtained with the lymphocyte transformation test were as follows:

Group I (pigs challenge exposed one month after vaccination). The stimulation indices (SI) before challenge and on day 6 after challenge exposure were negative in all the vaccinated and nonvaccinated pigs. Only the pigs vaccinated with the IPRV vaccine had positive SI of 3 to 1 on day 11. The mean SI of the PL injected and MLPRV vaccinated pigs were 3.6 to 1 and 3.4 to 1 respectively on day 36, while the IPRV vaccinated pigs had negative indices (Table I).

Group II (pigs challenge exposed three months after vaccination). The mean SI of the MLPRV vaccinated pigs before challenge exposure was 3.2 to 1 but was negative in the PL injected and IPRV vaccinated pigs. On day 6, the mean SI of the pigs injected with the PL and IPRV vaccine were 4.8 to 1 and 4.2 to 1 respectively, while the pigs vaccinated with the MLPRV vaccine had negative indices. All the vaccinated and nonvaccinated pigs had negative indices on days 11, 36 and 60 (Table I).

Group III (pigs challenge exposed five months after vaccination). The SI before challenge exposure were negative in all the vaccinated and nonvaccinated

TABLE I. Mean Stimulation Indices of Procine Peripheral Blood Lymphocytes Cultures

Day of Test	Vaccine Category	GROUPS						
		I	II	III	IV	V	VI	VII
Before Challenge Exposure	PL	—	—	—	—	—	—	—
	IPRV	—	—	—	—	—	—	—
	MLPRV	—	3.2	—	—	—	—	—
6 Days	PL	—	4.8	—	—	—	7.0	24.4
	IPRV	—	4.2	—	3.3	6.3	46.5	12.1
	MLPRV	—	—	3.3	5.8	26.6	70.6	15.6
11 Days	PL	—	—	—	—	—	8.4	14.0
	IPRV	3.0	—	—	8.6	3.1	8.2	3.2
	MLPRV	—	—	—	15.7	6.5	26.8	12.4
36 Days	PL	3.6	—	—	—	7.2	12.2	—
	IPRV	—	—	4.1	—	4.0	16.8	—
	MLPRV	3.4	—	—	3.6	4.2	16.1	3.6
60 Days	PL	5.8	—	—	—	—	—	10.1
	IPRV	—	—	—	—	—	7.4	3.1
	MLPRV	4.2	—	—	11.9	5.1	—	4.4

Positive stimulation indices are those with a 3 or higher number. The — sign indicates stimulation indices below 3

pigs. Only the pigs vaccinated with the MLPRV vaccine had mean SI of 3.3 to 1 on day 6, while the PL and IPRV vaccinated pigs had negative indices. All vaccinated and nonvaccinated pigs had negative SI on day 11. On day 36 only the IPRV vaccinated pigs had positive mean indices of 4.1 to 1, while on day 60 all the vaccinated and nonvaccinated pigs had negative indices (Table I).

Group IV (pigs challenge exposed eight months after vaccination). The SI before challenge exposure were negative for all the vaccinated and nonvaccinated pigs. The SI of the pigs vaccinated with the MLPRV vaccine were positive on days 6, 11, 36 and 60 and were greater than the SI for the PL injected and the IPRV vaccinates. The pigs vaccinated with the IPRV vaccine had positive indices on days 6 and 11 while the PL injected pigs had negative indices on days 6, 11, 36 and 60 (Table I).

Group V (pigs challenge exposed ten months after vaccination). The SI before challenge exposure were negative for all the vaccinated and nonvaccinated pigs. The SI of the MLPRV vaccinated pigs on days 6, 11, 36 and 60 were positive and greater than the SI of the IPRV vaccinated pigs. Only on day 36 were the SI of the PL injected pigs positive and greater than the indices of the vaccinated pigs.

Group VI (pigs challenge exposed 12 months after vaccination). The SI before challenge exposure were negative for all the vaccinated and nonvaccinated pigs. The MLPRV vaccinated pigs had positive and greater SI than the IPRV vaccinated pigs on days 6 and 11, while the IPRV vaccinates had positive and greater SI than the MLPRV vaccinates on days 36 and 60. The PL injected pigs had positive SI on days 6, 11 and 36. The PL injected pigs had higher SI than the IPRV vaccinated pigs on day 11 (Table I).

Group VII (pigs challenge exposed 14 months after vaccination). The SI before challenge exposure were negative in all the vaccinated and nonvaccinated pigs. The SI of the pigs vaccinated with the MLPRV vaccine were positive and greater than the indices of the IPRV vaccinates on days 6, 11, 36 and 60. Only on day 36 were the SI of the IPRV vaccinates

negative. The SI of the PL injected pigs on days 6, 11 and 60 were positive and greater than the SI of the IPRV and MLPRV vaccinates respectively (Table I).

When the SI of the pigs in all the groups were combined by vaccine category, all vaccinated and nonvaccinated pigs had negative SI when tested before challenge exposure and positive SI when tested on days 6, 11, 36, and 60 after challenge exposure (Table II). The MLPRV vaccinates had a higher overall SI than the IPRV vaccinates on days 6, 11, 36 and 60; however, the difference was statistically significant ($P < 0.05$) on day 11. The MLPRV vaccinates had higher overall SI than the PL injected pigs on days 6, 11, 36 and 60; however, this difference was statistically significant ($P < 0.05$) only on days 6 and 11. The IPRV vaccinated pigs had higher SI than the PL injected pigs on day 6 and 36 but the difference was statistically significant ($P < 0.05$) on day 6. The pigs injected with the PL solution had higher overall SI than the pigs vaccinated with the IPRV vaccine on days 11 and 60; however, the difference was not statistically significant (Table II). The counts per minute in the cells used as positive controls (cells exposed to the mitogen phytohemagglutinin P) were about 27 times higher than the counts per minute of the cells exposed to the RPMI 1640 media and about seven times higher than the counts per minute of the cells exposed to the PRV antigen after challenge exposure.

NEUTRALIZATION TEST

None of the PL injected pigs were seropositive before challenge exposure. The highest percentage

TABLE II. Overall Stimulation Ratios of Porcine Peripheral Blood Lymphocyte Cultures

Number of Pigs	Vaccine Category	Before Challenge Exposure	Days After Challenge Exposure			
			6	11	36	60
28-14*	PL	1.2	7.3 ^a	4.7 ^a	4.0	4.5
28-14	IPRV	1.3	10.6 ^b	4.5 ^a	4.7	2.9
38-14	MLPRV	1.6	17.4 ^b	11.5 ^b	5.9	5.9

*The vaccine categories before challenge exposure and on day 6 contained 28 pigs each while on days 11, 36 and 60 they consisted of 14 pigs each

^{a,b}Same letter = No significant difference; Different letter = Significant difference at $P < 0.05$

TABLE III. Percent of Seropositive Pigs After Vaccination and Before Challenge

Vaccine Category	Months After Vaccination															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IPRV	89	45	57	47	20	23	31	10	0	0	9	0	0	0	0	0
MLPRV	90	100	92	96	93	76	71	50	0	22	36	10	33	0	0	0

^a1:2 Dilution or higher

of seropositive pigs before challenge exposure were 89% (25 of 28) in the IPRV vaccine category on month 1 after vaccination and 100% (24 of 24) on month 2 after vaccination in the MLPRV vaccine category. In both cases the percent of seropositive pigs began to decline gradually two months after vaccination (Table III). The serum neutralization titers obtained after challenge exposure were as follows (Table IV):

Group I. All the vaccinated and nonvaccinated pigs were seropositive on days 7 and 60.

Group II. The pigs vaccinated with the IPRV and MLPRV vaccine were all seropositive on day 7 but they were negative on day 60. None of the PL injected pigs seroconverted by days 7 or 60.

Groups III, IV, V and VII. All the vaccinated pigs were seropositive on days 7 and 60 while the PL injected pigs were negative on day 7 and positive on day 60.

Group VI. All the vaccinated pigs were seropositive on day 7 and 60 while 1 and 2 of the PL injected pigs were seropositive on days 7 and 60 respectively.

The MLPRV vaccinated pigs had higher antibody titers than the IPRV vaccinated pigs on day 7 (in groups I, III and IV) and on day 60 in the pigs of group V.

The IPRV vaccinated pigs had higher antibody titers than the MLPRV vaccinated pigs on day 7 (in groups II, V, VI and VII) and on day 60 in groups I, VI and VII.

The pigs injected with the PL solution that seroconverted after challenge exposure had lower antibody titers when compared to the vaccinated pigs. Only 21% (3 of 14) of the PL injected pigs seroconverted by day 7 while 100% (14 of 14) of the vaccinated pigs did so by the same day. The percent of seropositive pigs on day 60 was 86% (12 of 14) in each vaccine category.

VIRUS ISOLATION TEST

In the PL injected pigs the virus was isolated in six of the 14 pigs killed on day 7 (two pigs from group II, two pigs from group VII, one pig from group IV and one pig from group V) and in one of the 14 pigs killed on day 60 (group V).

In the pigs immunized with the IPRV vaccine the virus was isolated in only one of 14 pigs killed on day 7 (group VI) while no isolations occurred from the pigs killed on day 60.

In the pigs immunized with the MLPRV vaccine, the virus was isolated in only one of 14 pigs killed on day 7 (group II) and in one of the 14 pigs killed on day 60 (group V).

FLUORESCENT ANTIBODY TEST

In the pigs injected with the PL solution, the fluorescent antibody test was positive in the tonsils of seven and in the lungs of two of the 14 pigs killed on day 7 (in the tonsils of one pig from group II, one from group III, two from group V, two from group VI, one from group VII and in the lungs of one pig from group III and one from group IV). All the pigs examined on day 60 had negative results.

In the pigs vaccinated with the MLPRV vaccine, the test was positive in the tonsils of six of the 14

TABLE IV. Number of Pigs That Seroconverted by Days 7 and 60 After Challenge Exposure

Group	Month of Challenge Exposure After Vaccination	Vaccine Category	Number of Pigs Day 7	Positive Pigs Day 7	SN Titers ^a Day 7	Number of Pigs Day 60	Positive Pigs Day 60	SN Titers Day 60
I	1	PL	2	2	(4-4)	2	2	(64-64)
I	1	IPRV	2	2	(256-64)	2	2	(256-64)
I	1	MLPRV	2	2	(256-256)	2	2	(256-32)
II	3	PL	2	0	(negative)	2	0	(negative)
II	3	IPRV	2	2	(256-2)	2	0	(negative)
II	3	MLPRV	2	2	(4-2)	2	0	(negative)
III	5	PL	2	0	(negative)	2	0	(64-128)
III	5	IPRV	2	0	(64-64)	2	2	(128-128)
III	5	MLPRV	2	2	(256-16)	2	2	(128-128)
IV	8	PL	2	0	(negative)	2	2	(128-32)
IV	8	IPRV	2	2	(32-32)	2	2	(256-34)
IV	8	MLPRV	2	2	(128-128)	2	2	(64-128)
V	10	PL	2	0	(negative)	2	2	(16-64)
V	10	IPRV	2	2	(64-16)	2	2	(32-128)
V	10	MLPRV	2	2	(8-16)	2	2	(64-128)
VI	12	PL	2	1	(neg-4)	2	2	(16-16)
VI	12	IPRV	2	2	(16-32)	2	2	(32-16)
VI	12	MLPRV	2	2	(4-4)	2	2	(16-16)
VII	14	PL	2	0	(negative)	2	2	(16-16)
VII	14	IPRV	2	2	(32-32)	2	2	(64-32)
VII	14	MLPRV	2	2	(8-32)	2	2	(16-64)

^aThe values in parentheses are the serum neutralization titers (SN) of the two pigs in each vaccine category

pigs killed on day 7 (one pig from group II, one from group III, one from group IV, two from group V and one from group VI).

All of the IPRV vaccinated pigs had negative results on day 7 and 60 after challenge exposure.

VIRUS COCULTURE TEST

All attempts to isolate the virus by tissue coculture from the trigeminal ganglia were unsuccessful.

DISCUSSION

The development of the cellular immune response was not detected after vaccination before challenge exposure in the great majority of the pigs. The IPRV vaccine induced a detectable humoral immune response for a period of 11 months after vaccination, while the MLPRV vaccine induced the same type of immunity for 13 months. The pigs vaccinated with the MLPRV vaccine had a higher and longer lasting humoral immune response than the pigs vaccinated with the IPRV vaccine before challenge exposure; however, the response declined sharply in titers and in percent of reactors on month 7 after vaccination. Overall the levels of cellular immunity detected after challenge exposure were higher in the pigs vaccinated with the MLPRV vaccine, however, this difference was not statistically significant.

The vaccinated pigs in groups I, III and IV had higher levels of humoral immunity and lower levels of cellular immunity when compared to the vaccinated pigs in groups V, VI and VII and the clinical signs of the vaccinated pigs in groups I, III and IV after challenge exposure were considerably less severe than the clinical signs in the vaccinated pigs in groups V, VI and VII. Therefore, it seems that the protection against chal-

lenge exposure seen in the vaccinated pigs was chiefly due to the high level of humoral immunity developed after exposure to the virus.

The reduced number of pigs with clinical illness and detectable levels of cellular and humoral immunity in group II could not be explained; however, it was noted that the virus used to inject these pigs was frozen and thawed twice and this treatment may have reduced the virus titers in the inoculum and therefore diminished the severity of clinical signs and failed to induce a detectable humoral and cellular immune response.

The transmission of vaccine virus to nonvaccinated pen mates as reported previously (8) was not observed in this experiment. None of the PL injected pigs developed serum neutralizing antibodies in spite of being penned together with the vaccinated pigs for several months beginning on month 3 after vaccination.

The evaluation of the cell-mediated immunity by the lymphocyte transformation test *in vitro* is useful in the early detection of pigs exposed to PRV especially in nonvaccinated individuals. Most of these pigs are not seropositive by day 7 after challenge exposure to PRV.

The variation in percent of seropositive pigs from month 9 to 13 after vaccination could not be explained; however, it is to be noted that those samples were tested separately at monthly intervals and this may have caused some variation.

The greater percentage of virus isolations was from the pigs which received the PL injections and which had the lowest levels of humoral and cellular immunity. The lowest percentage of virus isolations was from the vaccinated pigs which had the highest levels of humoral and cellular immunity. This indicates that virus isolation

attempts from vaccinated animals may be unsuccessful. This may be attributed to a possible reduction or neutralization of viral particles by the high levels of humoral and cellular immunity.

The failure to isolate the virus by tissue coculture technique from the trigeminal ganglia may have been due to: a) the high levels of cellular and humoral immunity which persisted for longer periods of time after challenge exposure, b) to the absence or low number of viral particles in the tissue at that time, or c) to a lack of sensitivity of this test to detect small numbers of viral particles.

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