Induced Latency in Pseudorabies Vaccinated Pigs

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ABSTRACT
A latent pseudorabies virus infection was established in pigs despite vaccination with a modified-live pseudorabies virus vaccine. Although the vaccinated pigs developed high concentrations of antibody, virus was recovered from the tonsils and lungs of pigs treated with dexamethasone three months after inoculation with virulent virus. These results may explain why vaccination programs have failed to eliminate the persistence and spread of virulent pseudorabies virus in infected herds.

RÉSUMÉ
Les auteurs ont réussi à réaliser une pseudorabie latente, chez des porcs, en dépit de leur vaccination atténuée avec un virus atténué. Même si ces porcs dévéloppèrent des titres élevés d'anticorps, on recouvra le virus des amygdales et des poumons de ceux qui avaient reçu de la dexaméthasone, trois mois après une infection de défi. Ces résultats expliqueraient l'échec des programmes de vaccination destinés à enrayer la persistance et la dissémination du virus de la pseudorabie, dans les troupeaux infectés.

INTRODUCTION
The occurrence of a latent state after an initial infection is a known characteristic of the herpesvirus group. Pseudorabies (PR) is caused by a herpesvirus, and the pig is considered to be the natural host (10). Latent PR infections have been suspected in pigs by the transmission of the virus to cattle from clinically normal pigs, by the demonstration of neutralizing antibody in swine without clinical illness, and by the persistence of the virus in swine herds. More recently, latency was demonstrated experimentally in hydrocortisone-treated and untreated pigs (8). The PR virus has been shown to circulate in infected "closed" herds, despite the use of vaccines and presence of serum-neutralizing antibody (7). Vaccination in Central and Eastern Europe has failed to diminish the enzootic areas or to decrease the number of outbreaks of PR (11). Before a control program can be effective, it is imperative to determine whether latent infections with virulent viruses occur in vaccinated swine.

The purpose of the present study was to induce a latent infection in pigs immunized with a modified live virus vaccine and to cause recrudescence.

MATERIALS AND METHODS
EXPERIMENTAL DESIGN
The experimental groups and treatments are shown in Table I. Twenty-four pigs of mixed breed of both sexes, weighing between 12 and 14 kg, were divided into four groups of six and each group was housed in an isolation unit. All pigs were from a PR-free herd and were seronegative for PR antibody. Groups I and II were vaccinated twice, 14 days apart, with 1 mL of a commercially available modified live PR virus vaccine.¹

Three weeks after the second inoculation, one vaccinated group (group I) and one nonvaccinated group (group III) were challenged intranasally with a field isolate of PR virus (IL-1215). Each pig was given 1.0 mL (0.5 mL into each nostril) of the sixth cell culture passage containing 2 x 10⁷ median tissue culture infective dose (TCID₅₀)/0.1 mL.

TABLE I. Experimental Groups and Treatments

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pig No.</th>
<th>Vaccination</th>
<th>Virulent Virus Challenge Exposure</th>
<th>Dexamethasone Treatment</th>
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<tbody>
<tr>
<td>I</td>
<td>1-6</td>
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<td>+</td>
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<tr>
<td>II</td>
<td>7-12</td>
<td>+</td>
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<tr>
<td>III</td>
<td>13-18</td>
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<tr>
<td>IV</td>
<td>19-24</td>
<td>-</td>
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<td>+</td>
</tr>
</tbody>
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¹PR Vac, Norden Laboratories, Lincoln, Nebraska.

DEXAMETHASONE TREATMENT

Pigs in group I were treated with dexamethasone \(^9\) 90 days after exposure to the challenge virus. Pigs in groups II and IV were each treated with dexamethasone 90 days after group II pigs were given a second vaccine inoculation.

Two pigs from each group were treated with 100 mg of dexamethasone daily for four, five, or six days. The pigs were killed on the day after the last treatment.

CELL CULTURES

Pig kidney (PK-15) cells were grown in tubes and flasks with Eagle's minimal essential medium (MEM) with nonessential amino acids, glutamine, 0.5% lactalbumin hydrolysate plus 10% bovine fetal serum (BFS). Minimal essential medium with 2% BFS was used as maintenance medium. Primary bovine embryonic kidney (BEK) cells were obtained from a commercial source. All cell culture mediums contained 200 U of penicillin, 200 g of streptomycin, and 2.5 g of amphotericin B/mL.

SPECIMENS

Tonsillar swabs collected once before treatment and daily after dexamethasone treatment were placed in 2.0 mL of culture medium and stored at -70°C until tested. Specimens of tonsil, lung, liver, brain, adrenal gland and trigeminal ganglia collected at necropsy were stored at -70°C until tested. All tissues were prepared as a 10% suspension as previously described (1). Blood samples for neutralization test were collected before vaccination and periodically throughout the experiment. The serum was harvested and stored at -20°C until tested.

NEUTRALIZATION TEST

Microtests were performed on PK-15 cells as recommended by the Pseudorabies Diagnostic Standardization Committee (2). Serum-neutralizing (SN) titers were determined against 100 to 300 TCID\(_{50}\) doses of the field isolate of PR virus (IL-1215). The SN titers were expressed as the reciprocal of the greatest dilution of serum that inhibited cytopathic effect (CPE).

VIRUS ISOLATION AND IDENTIFICATION

The BEK cell cultures were each inoculated with 0.2 mL of tonsil swab or tissue specimen and incubated at 36°C; negative cultures (tubes without CPE) were passed one more time (on fresh cells) before they were reported as negative. Isolates were identified as PR virus by serum-neutralization and fluorescent antibody staining (1).

TEST FOR VIRULENCE

The PR isolate (IL-9133) recovered from lung tissue from pig 3 (group I) after dexamethasone treatment was inoculated into five pigs, weighing 12 to 14 kg. Each pig was given (intramuscularly into right thigh) 1 mL of third cell culture passage of the virus containing 10\(^6\) TCID\(_{50}/0.1\) mL. One pig served as a contact control.

RESULTS

RESPONSE TO VACCINATION

Pigs in groups I and II showed no clinical signs or adverse effects after vaccination. All vaccinated pigs developed specific SN titers against PR virus. The SN titers ranged from four to 16 with a geometric mean titer (GMT) of 7.4 at 14 days after the first vaccination. Fourteen days after the second vaccination, the SN titers ranged from eight to 32, with a GMT of 19.7. Virus was not isolated from tonsillar swabs collected after vaccination.

RESPONSE TO CHALLENGE EXPOSURE

A mild clinical illness characterized by anorexia and depression was observed in all vaccinated pigs (group I) after challenge exposure. By postchallenge exposure day (PCD) 7, all pigs seemed normal and were eating. The SN titers in pigs of group I ranged from 512 to 2,048 with a GMT of 1,290 on PCD 14. On PCD 90, the day dexamethasone treatment began, the SN titers ranged from 64 to 256 with a GMT of 128.

The results of virus isolation from the tonsillar swabs from vaccinated (group I) and nonvaccinated (group III) after challenge exposure are shown in Table II. Virus was recovered from pigs 1, 2, 4, 5, and 6 on PCD 1 through 3 and from pig 6 on PCD 5. Virus was recovered from pig 3 only on PCD 3. Virus was not recovered after PCD 5.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>0</th>
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<td>Group I (vaccinated)</td>
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<td>18</td>
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<td>+</td>
<td>NT</td>
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<td>-</td>
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</tr>
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</table>

- = Negative  
+ = Positive  
NT = Not tested  
* Died

\(^9\)Azium, Schering Corporation, Kenilworth, New Jersey.
All of the nonvaccinated pigs in group III developed clinical signs of illness by PCD 2. The pigs were depressed, refused to eat, and showed signs of central nervous system involvement. They circled the isolation unit, rubbing their snouts and heads on the floor and walls. Three pigs died on PCD 3, two died on PCD 4, and one died on PCD 5. Antibody to PR virus was not detected in any of the six pigs.

Virus was recovered from tonsillar swabs on each PCD tested until death (Table II). The PR virus was isolated from the brain, trigeminal ganglia and tonsil of all six pigs, the lung of five pigs, the adrenal glands of three pigs and the liver of one pig.

**RESPONSE TO DEXAMETHASONE TREATMENT**

On the third day after dexamethasone treatment, all pigs had loose feces which persisted until the animals were killed. Pigs 3 and 4 (group I) held their head in a titled position after five days of treatment. No other signs were observed in the three groups.

The PR virus was recovered from five of the six pigs in group I (Table III). The virus was recovered from tonsillar swabs from pig 1 on the third day of dexamethasone treatment and from pigs 1, 2 and 3 on the fourth day of treatment. At necropsy, virus was isolated from the tonsils of pigs 3, 4 and 5 and from the lungs of pig 3. Virus was not recovered from any of the other tissues tested. All viruses were detected on primary inoculation of cell cultures. Cytopathic changes were observed in the monolayers between two and four days after inoculation. Blind passage of cells and fluid of each negative specimen did not reveal additional isolates.

At necropsy, virus was not isolated from tonsillar swabs or tissues from pigs in groups II and IV.

**TEST FOR VIRULENCE**

The five pigs inoculated intraocularly with the isolate (IL-9133) from pig 3 and the contact control all showed signs of PR infection and some died. Clinical signs consisted of anorexia, depression and varying degrees of paralysis. Paralysis occurred first in the rear legs and progressed cranially until the animal was recumbent. One pig died on PCD 6, and the others were killed between PCD 6 and 17. The contact control died 13 days after its pen mates were inoculated with the virus.

Virus was recovered from tonsillar swabs as early as PCD 3 and as late as PCD 14. Virus was first isolated from the contact control pig seven days after the pen mates were inoculated.

**DISCUSSION**

The present study demonstrates that vaccination with a modified live virus vaccine fails to prevent infection and the subsequent establishes a PR carrier state in pigs challenged exposed with virulent virus.

Corticosteroid treatment has been shown to induce reactivation of virus in latent infections in vaccinated animals with herpesvirus (5, 9). Latent infections with infectious bovine rhinotracheitis (IBR) virus was induced in calves immunized with an activated IBR vaccine. The IBR virus was recovered from calves two to three months after virus challenge by administering large doses of corticosteroids and ACTH (9). A feline viral rhinotracheitis (FVR) virus carrier state was established in both FVR-vaccinated and non-vaccinated cats (5). A combination of dexamethasone trimethylacetae and prednisolone administered intramuscularly induced virus shedding.

The induction of a latent infection in vaccinated pigs with virulent PR virus was shown by the reactivation and recovery of virus after dexamethasone treatment. Virus was recovered without the use of tissue explant or cocultivation techniques from five of six vaccinated-challenge exposed pigs, but not from vaccinated, non-challenged exposed pigs. Virulence of the PR virus isolate from pig 3 after dexamethasone treatment was shown by its pathogenicity in susceptible pigs. Paralysis and death in the inoculated pigs and the illness in the contact control pig are evidence that the isolate is not vaccine virus (13).

The pigs immunized with modified live PR virus vaccine developed serum-neutralization antibodies and withstood virulent PR virus challenge exposure. The challenge virus induced an anamnestic response in each vaccinated pig to PR virus. Similar findings have been reported in pigs vaccinated with the BUK strain (3, 12, 13) and with the Bartha (K) strain (4).

The present data support earlier observations that vaccination does not prevent reinfecion of pigs with virulent virus (3, 4, 6, 13). In one study, virus was recovered from vaccinated pigs for 20 days after challenge exposure. Viral persistence was considered to be an important factor in the dissemination of the virus in swine herds (6). Virus has been shown to persist in the nasopharyngeal region of both vaccinated and nonvaccinated animals for varying periods (3, 4, 6, 13).

The results further show that in pigs with latent infections the PR virus can be reactivated in the presence of high titers of neutralizing antibody. The fact that immunized pigs with neutralizing antibody can be reinfected and become latent carriers of virulent virus raises the question of the merit for

**TABLE III. Isolation of Pseudorabies Virus from Tonsils of Vaccinated Pigs Exposed to Challenge Virus and Later Treated with Dexamethasone**

<table>
<thead>
<tr>
<th>Pig No.</th>
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<td>6</td>
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<td>-</td>
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<td>N</td>
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</tbody>
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- = Negative  
+ = Positive  
N = At necropsy  
*Virus also isolated from lung
continued use of vaccines in the control of PR. Vaccines have been reported to decrease the severity of illnesses and reduce mortality of PR virus infections.

It appears that vaccination reduces virus dissemination by limiting the time virus is present in the nasopharyngeal sites (3, 4, 6, 13). This would tend to decrease the spread of the virus but does not ensure that latent infections cannot develop within vaccinated animals. Although it has been stated that induced permanent infections in vaccinated stock have not been substantiated (14), epidemiological evidence indicates that latent carriers do exist in vaccinated herds. On a farm where vaccination was effective in stopping an outbreak, the virulent virus was believed to persist because sick pigs appeared sporadically (4). In another study, the uninterrupted circulation of virulent virus was demonstrated in vaccinated pigs (7). Although no attempt was made in this study to demonstrate contact transmission between the induced latent carrier and susceptible pigs, the fact that vaccinated pigs were shown to be carriers identifies them as potential shedders of virulent virus.

ACKNOWLEDGMENTS

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REFERENCES