

Effect of a Subunit Vaccine Prepared from Virus-Infected Cell Membrane on Aujeszky's Disease in Pigs

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Aujeszky's disease (AD) is caused by Suid herpesvirus 1 which infects various animal species. Natural infections with AD virus, however, occur most often in pigs. The disease has been distributed in many countries of the world and caused a great economic loss for porcine production. Susceptibility of pigs to AD virus is apparently dependent on their ages. Morbidity and mortality approach frequently 100% in suckling piglets less than 10 days old. Mortality in pigs over 6 weeks old is usually low, although morbidity is very high. Only mild clinical signs are developed or subclinical infection may occur in adult pigs, and they usually survive the infection. Latent infections are frequently established in pigs surviving the disease, and the virus may be reactivated and excreted by following certain stress.¹⁾

Both live and inactivated vaccines to AD have been developed and put into practical use in some countries. They can protect clinical disease in vaccinated pigs following exposure to virulent AD virus. It has been reported, however, that both vaccines failed to prevent infection, replication and shedding of the virus when vaccinated pigs were exposed to virulent AD virus.^{3,9,11)} Although occurrence of the clinical disease can be protected, latent infections are established in vaccinated pigs after subsequent infection with the virulent virus, and they cause the virus to spread to susceptible pigs.^{1,5)}

Recently Roger et al.⁸⁾ reported that a subunit

vaccine prepared from virus-infected cells induced high virus neutralizing (VN) antibody and the virus was not excreted from pigs vaccinated previously and challenged the virulent AD virus.

The purpose of this paper is to evaluate the effect of the subunit vaccine prepared from infected cell membrane on AD in pigs in comparison with an inactivated virus vaccine.

Materials and methods

1) Virus

The Y-81 strain²⁾ of AD virus which had been passaged 3 times in the continuous porcine kidney cell line (PK-15 cell) cultures was used for production of vaccines, a serum neutralization test and challenge inoculation.

2) Preparation of Vaccines

(1) Subunit vaccine

Confluent monolayers of PK-15 cells grown in Roux bottles were inoculated with the Y-81 strain of AD virus at multiplicity of infection 1, and the virus was allowed to be adsorbed by the cells for 1 hr at 37°C. The cultures were refed with Eagle's minimum essential medium supplemented with 0.15% NaHCO₃, and incubated for 16 hr at 37°C. Cells and culture fluids were harvested separately and used for production of the subunit and the inactivated virus vaccines, respectively. Infected cells were washed twice with phosphate buffered saline (pH 7.2) and suspended with 0.2% nonionic detergent Nonidet P-40 in RSB (0.01M NaCl, 0.0015M MgCl₂, 0.01M Tris-HCl, pH 7.4) at concentration of

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3×10^7 cells/ml. The cell suspension was stirred gently for 15 min at 0°C to solubilize infected cell membrane, and centrifuged for 20 min at 6,000 rpm. Supernatants were collected and used as the subunit vaccine. Absence of infectious virus in the vaccine was verified by inoculation into PK-15 cell cultures.

(2) Inactivated virus vaccine

AD virus was concentrated and purified from infected cell culture fluids by combination of differential and sucrose density gradient centrifugations. Viral infectivity of final material was $10^{9.5}$ median tissue culture infective doses (TCID₅₀)/ml, and inactivated by adding an equal volume of 0.2% beta-propiolactone solution (in 0.85% NaCl and 1.68% Na₂CO₃ aqueous solution).

3) Pigs and vaccination

A total of 9 pigs weighing about 10 kg, which had been negative for AD virus neutralizing antibody, were used in this experiment. They were divided into 3 groups which included 3 pigs each. Two groups of pigs were inoculated subcutaneously twice at 3 weeks interval with 1 ml of the subunit and the inactivated virus vaccines, respectively. DEAE-dextran (50 mg/ml) was supplemented to both vaccines as an adjuvant. The other 3 pigs served as non-vaccinated controls. Serum samples were periodically collected and tested for VN antibody.

4) Challenge inoculation

All pigs were exposed intranasally to 10^7 TCID₅₀/ml of the Y-81 strain of AD virus 10 days after the second vaccination, and clinical symptoms were observed for 2 weeks. Nasal secretions were obtained from all pigs 1, 3, 5, 7, 10 and 14 days after challenge exposure by inserting tampons into nasal cavities. The nasal secretions in the tampons were extracted with 2 ml of the tissue culture medium and tested for viral contents.

5) Serum neutralization test

VN antibody titers of sera were determined by the standard microplate method. CPK cell cultures (continuous porcine kidney cell line) were used in the test. Incubation of virus-serum mixture was carried out for 1 hr at 37°C .

6) Virus isolation

AD virus excreted in nasal secretions was titrated by the microplate method, using CPK cell cultures.

Results

1) Antibody responses

The subunit vaccine induced high antibody responses in inoculated pigs (Table 1). VN antibody was first detected 7 days and reached 8 in titer 14 days after vaccination in pigs inoculated with the subunit vaccine. After the second vaccination, VN antibody showed a remarkable increase in titer and reached 128 after 10 days. Antibody responses in pigs inoculated with the inactivated virus vaccine were poor. They produced only a low level of VN antibody even after the second vaccination was given. However, they produced high titer VN antibody after challenge exposed to the virulent virus. Non-vaccinated control pigs produced low titer VN antibody after challenge exposure.

Table 1. Antibody responses of experimental pigs to AD virus

Vaccine inoculated	Day of experiment						
	0	7	14	21	31	41	51
Subunit	<1*	0.8	8	8	128	81	64
Inactivated virus	<1	<1	<1	0.8	1	32	81
Control	<1	<1	<1	<1	<1	1	3

* VN antibody titer. All data are expressed as geometric mean (3 pigs each). Pigs were vaccinated on day 0 and 21, and challenge exposed on day 31 of the experiment, respectively.

2) Clinical responses after challenge exposure

Pigs of both groups, inoculated previously with the inactivated virus vaccine and non-vaccinated, developed typical signs of AD such as depression, respiratory disorders and discharge of nasal secretions after challenge exposure. Their rectal temperatures rose over 40°C 2 days after challenge and persisted for 5 to 10 days. On the contrary, pigs inoculated previously with the subunit vaccine showed no considerable signs of the disease,

although rectal temperatures rose somewhat in 2 of them only 4 days after challenge.

3) Isolation of AD virus from nasal secretions

In non-vaccinated control pigs, AD virus was first detected in nasal secretions 1 day after challenge, and maximum titer of $10^{4.5}$ TCID₅₀/ml was obtained 2 days later (Table 2). AD virus was continually shed in their nasal secretions for 10 days. Similarly, AD virus was continually found in nasal secretions collected from pigs inoculated previously with the inactivated virus vaccine between 1 and 7 days after challenge exposed. AD virus was not detected in nasal secretions collected from pigs inoculated with the subunit vaccine and exposed subsequently to the virulent virus throughout the experiment.

Table 2. Detection of AD virus in nasal secretions after challenge exposure

Vaccine inoculated	Day of experiment						
	0	1	3	5	7	10	14
Subunit	0*	0	0	0	0	0	0
Inactivated virus	0	3.9	5.4	4.0	0.9	0	0
Control	0	2.8	4.5	4.3	2.8	1.0	0

* Viral infectivity (log TCID₅₀/ml), 0 indicates negative for virus isolation in undiluted materials. All data are expressed as geometric mean (3 pigs each).

Discussion

This study confirmed the results reported previously by Roger and Schultz.⁸⁾ The subunit vaccine used in this study induced high VN antibody, especially after the second vaccination, and protected the occurrence of clinical disease after challenge exposure to the virulent virus. Furthermore, AD virus could not be detected in nasal secretions of pigs inoculated with the subunit vaccine and subsequently challenged with the virulent virus. These results indicate that the subunit vaccine prepared from virus-infected cell membrane are more immunogenic than the inactivated virus vaccine. This study could not clarify, however,

whether the subunit vaccine prevented primary infection and virus shedding, because the virus might be neutralized by VN antibody possibly present in nasal secretions, even if the infection was established in vaccinated pigs following challenge exposure. Experiments on induction of recurrence in pigs immunized with the subunit vaccine and challenged subsequently with the virulent virus will be necessary to clarify this point.

High immunogenicity of the subunit vaccine seems to be due to glycoprotein(s) mass present in the vaccinal material, as it has been reported in other enveloped viruses.⁶⁾ Recently importance of glycoprotein(s) in induction of immunity to AD virus has been suggested by several workers.^{4,7,10)} Identification and purification of immunogenic glycoprotein(s) appear to produce useful information for understanding of immunity and development of more effective vaccine to AD virus. The conclusions obtained in this study are that the subunit vaccine prepared from virus-infected cell membrane is highly immunogenic and may be one of the promising candidates for effective vaccine to AD.

Acknowledgment

The author thanks the Central Laboratory, Kyoritsu Shoji Co. Ltd. for the cooperation in performing this study.

References

- 1) Crandell, R. A., Mock, R. E. & Mesfin, G. M.: Latency in pseudorabies vaccinated pigs. *Proc. U. S. Anim. Health Assoc.*, **83**, 444-447 (1979).
- 2) Fukusho, A. et al.: The first outbreak of Aujeszky's disease in swine in Japan. II. Virus isolation. *Bull. Nat. Inst. Anim. Health*, **82**, 5-11 (1981) [In Japanese].
- 3) Gustafson, D. P.: Pseudorabies. *In Diseases of swine*, V. ed. Leman, A. D. et al., The Iowa State University Press, Ames, Iowa (1980).
- 4) Hampl, H. et al.: Characterization of the envelope proteins of pseudorabies virus mutant containing antigenically altered gp 50. *J. Virol.*, **52**, 583-590 (1984).
- 5) Mock, R. E., Crandell, R. A. & Mesfin, G. M.: Induced latency in pseudorabies vaccinated pigs. *Can. J.*

- Comp. Med.*, **45**, 56-59 (1981).
- 6) Morein, B. et al.: Effective subunit vaccines against an enveloped animal virus. *Nature*, **276**, 715-718 (1978).
 - 7) Platt, K. B.: The porcine humoral response to detergent extracted Aujeszky's disease (pseudorabies) virus antigen. *Vet. Microbiol.*, **7**, 515-534 (1982).
 - 8) Roger, K. M. & Schutz, J. C.: Evaluation in swine of a subunit vaccine against pseudorabies. *Am. J. Vet. Res.*, **44**, 123-125 (1983).
 - 9) Thawley, D. G., Gustafson, D. P. & Beran, G. W.: Procedures for the elimination of pseudorabies virus from herds of swine. *J. Am. Vet. Med. Assoc.*, **181**, 1513-1518 (1982).
 - 10) Wathen, M. W. & Wathen, L. M. K.: Isolation, characterization, and physical mapping of a pseudorabies virus. *J. Virol.*, **51**, 57-62 (1984).
 - 11) Wittmann, G., Jabubik, J. & Ahl, R.: Multiplication and distribution of Aujeszky's disease (pseudorabies) virus in vaccinated and non-vaccinated pigs after intranasal infection. *Arch. Virol.*, **66**, 227-240 (1980).

(Received for publication, September 25, 1985)