Formaldehyde-Fixed Cells Infected with Pseudorabies Virus Conferred Resistance against Aujeszky's Disease

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ABSTRACT. Cells infected with Pseudorabies virus (PrV) and fixed with formaldehyde were evaluated as a vaccine against Aujeszky's disease. Mice and pigs inoculated with fixed cells showed no clinical signs. In a challenge test, all fixed cells conferred protection on mice and pigs. Although pigs showed slight symptoms, they were restored to normal health within a few days. These results demonstrate that PrV-infected cells fixed with formaldehyde function as a vaccine against Aujeszky's disease and serve as a novel type of vaccine which is easy to make, and is both safe and effective.—**KEY WORDS:** Aujeszky's disease, formaldehyde-fixation, pseudorabies virus.

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In general, killed pathogens such as bacteria, viruses or portions of them can not induce sufficient humoral antibody or cellular immune responses. It is, therefore, necessary to mix the antigens with an adjuvant such as aluminium-hydrate (alum gel), which has been widely used in inactivated vaccines. Oil-adjuvant [1] or immune-stimulating complexes (ISCOM) [7, 8] have also been examined in recent years.

On the other hand, it is not easy to attenuate a wild strain to make a live vaccine. It takes many years, especially for herpes viruses, and the attenuation sometimes results in decreases in immunogenicity. Moreover, it takes great effort to confirm the safety before use in the field.

Therefore, we examined a new approach to preparing an inactivated vaccine that has less side effects, in which cells infected with viruses were fixed with formaldehyde. To prepare the formaldehyde-fixed cell vaccine, PrV was chosen as the first target.

Firstly, the δ gX δ TK strain of PrV [4] was inoculated into PK-15, BHK or MDBK cells in glass bottles at a multiplicity of infection (MOI) of 0.1, then the cells were cultured at 37°C for 48 hr. Next, the cells were harvested using trypsin containing EDTA and fixed with 10% formalin in phosphate buffered saline (PBS) at 4°C overnight. The cells were washed several times with PBS before use. These fixed cells were named fPK, fBHK or fMDBK, respectively. To prepare the cell lysate-adjuvant mixture, infected PK cells were harvested with 0.25% trypsin-PBS and lysed with PBS containing 0.2% NP-40. After centrifugation at 1,000 g for 10 min, the supernatant was mixed with an equal volume of alum gel.

Six-week-old mice were intraperitoneally inoculated with 10^7 or 10^6 fixed cells in PBS. None of the mice showed any side reactions after inoculation with the fixed cells. Two weeks later, they were challenged with 10 mouse Lethal Doses 50% (LD_{50}) of PrV strain YS-81 and observed for 2 weeks. All fixed cells conferred protection against PrV infection to the mice, although the effect of fBHK seemed to be lower than that of the others. fPK and fMDBK showed sufficient protection even with 10^4 cells,

whereas fBHK did not protect with 10^5 cells. From these results, we used fMDBK and fPK in an efficacy test for pigs. Four-week-old pigs were vaccinated with 10^8 fixed cells or an equivalent dose of cell lysate vaccine subcutaneously behind the ears. They were observed daily for 2 weeks and weighed weekly. Four pigs were sacrificed and the histological lesions at the inoculated site were examined on days 7 and 21. Two weeks later, the pigs vaccinated with fixed cells or cell lysate were boosted with the same inoculum. After inoculation, some pigs with fPK had transient swelling at the inoculation site, although other animals showed no clinical problems. Pathologically, there was a cheese-like material at the inoculation site at day 7. Each degeneration site was about 8 mm in diameter in pigs given fPK, although those in the fMDBK group had less degeneration. Histologically, amorphous eosinophilic material, probably derived from the inoculated fixed cells, was seen. There was also infiltration by many neutrophils and macrophages. Three weeks after inoculation, there was little coagulation at the inoculation site in all the pigs.

Pigs were challenged intranasally with 10^6 TCID₅₀ of YS-81 4 weeks after primary vaccination. They were observed for clinical signs including measurement of body temperature daily and the body weight weekly.

All 5 pigs in the non-vaccinated control group had severe symptoms and one animal died. Pigs in the cell lysate mixture groups also displayed severe symptoms, but all survived. In contrast, pigs in the fixed cell groups had fewer symptoms than control animals and recovered within a few days. Although they had a fever, it lasted for only two days. Moreover, the pigs inoculated with fixed cells showed good weight gain after challenge as well as those given the cell lysate-adjuvant mixture. The efficacy of a vaccine upon weight gain is very important especially in pigs along with protection score [3].

Pathologically, there were little side effects, but a cheese-like material was observed at the inoculation site at 7 days.

These results demonstrate that the cells infected with viruses, at least PrV, and fixed with formaldehyde function as an effective vaccine. In addition, the procedure for preparing the vaccine, in which the virus-infected cells are simply fixed with formaldehyde, is very easy.

As the vaccine against Aujeszky's disease should have a

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Fig. 1. Protective efficacy in mice of formaldehyde-fixed cells infected with PrV δ gX- δ TK strain. Five mice in each group were immunized with 10^3 cells ($\circ$), 10^4 cells ($\triangle$), 10^5 cells ($\square$), 10^6 cells ($\bullet$) or 10^7 cells ($\blacktriangle$). Two weeks after immunization, mice were challenged with 10 LD₅₀ of YS-81, then observed for 7 days. Each line shows the time course of mortality. The mice were immunized with a) fPK, b) fMDBK, c) fBHK. For details of the preparation of fPK, fBHK and fMDBK, see text.

Table 1. Clinical signs in pigs after challenge

Vaccine	Days after challenge							
	0	1	2	3	4	5	6	7
fPK	—*	—	—	—	+ ^b	+	—	—
	—	—	—	—	+	+	—	—
	—	—	—	+	+	+	—	—
	—	—	—	+	+	+	—	—
fMDBK	—	—	—	—	+	+++ ^c	—	—
	—	—	—	—	+	+	—	—
	—	—	—	—	+	+	—	—
	—	—	—	—	+	++	—	—
cell lysate	—	—	—	+	++	++	++	++
	—	—	—	+	++	++	+	++
	—	—	—	+	++	++	++	++
	—	—	—	+	++	++	++	++
	—	—	—	+	++	++	++	++
Control	—	—	—	+	+++ ^d	++	+++	++
	—	—	—	+	++	++	++	++
	—	—	—	+	++	++	death	++
	—	—	—	+	++	++	++	++
	—	—	—	+	++	++	++	++
	—	—	—	+	++	++	++	++
	—	—	—	++	+++	+++	+	+
	—	—	—	+	+++	++	++	+
	—	—	—	+	++	++	++	+
	—	—	—	+	+++	++	++	+
	—	—	—	+	++	+++	+	+

a) Normal, b) Showed fever, anorexia and/or ataxia, c) Cough and/or nasal secretion from nose, d) Could not stand.

Table 2. Weight of pigs after challenge

Days	Weight (kg)		Growth ratio (%)
	0	7	
fPK	18.1 ^a	22.1	22.10 ^b
fMDBK	14.7	17.7	20.41
cell lysate	17.7	21.1	19.21
control	18.6	20.8	11.83

a) Average weight of pigs in each group.

b) $\{(\text{Weight at day 7}) - (\text{weight at day 0})\} / (\text{weight at day 0}) \times 100$.

marker to distinguish the antibody induced by vaccines from those induced by infection with a wild strain [2, 9], the live vaccines against PrV have been developed by the deletion of a glycoprotein gene [5, 6, 10]. In this study, we used a live vaccine strain that lacks the glycoprotein gX gene as a marker. Therefore, the antibodies induced by the vaccine will be easily distinguished from those by a wild strain using an ELISA "PSEUDORABIES VIRUS GLYCOPROTEIN gpX ANTIBODY TEST KIT, TOLVID Diagnostic" (Upjohn). In fact, pigs inoculated with the fixed-cell vaccine induced neutralizing antibodies titrated from 2 to 32. However, no anti-gX antibody was detected using the ELISA (data not shown).

It may be more effective to use a low passage strain in

cell culture, since such strains are expected to have more similar antigenic feature than the vaccine strain used in this study.

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