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Research in Veterinary Science 1993, 54, 345-350

Potential viral vectors for the stimulation of mucosal antibody responses against enteric viral antigens in pigs

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Four viruses were compared for their ability to induce an intestinal antibody response in piglets. Antibodies were not detected in response to oral vaccination with either fowlpox virus or a baculovirus (BV). Simultaneous oral dosing and parenteral inoculation with high concentrations of BV in an oil emulsion adjuvant induced high levels of circulating virus neutralising (VN) antibodies, and also low levels of intestinal antibodies when booster doses of virus were given. In response to oral vaccination with swinepox virus (SPV), low levels of circulating and intestinal VN antibodies, and higher titres of antibodies reactive in an enzyme immunoassay, including intestinal antibodies of the IgA class, were detected. Oral vaccination with porcine adenovirus type 3 (PAV-3) stimulated both circulating and intestinal VN antibodies, and IgA antibodies were demonstrated in the intestinal contents. It was concluded that SPV and PAV-3 might be suitable vectors for the expression of genes encoding the protective antigens of porcine enteric viruses.

EFFECTIVE vaccines are not currently available for the control of diarrhoea induced by porcine enteropathogenic viruses such as coronaviruses and rotaviruses (Saif and Jackwood 1989). This may relate to the failure of attenuated viruses administered orally to replicate sufficiently in the intestine of the sow or piglet to stimulate a mucosal immune response, and it is well established that parenteral vaccination fails to stimulate immunoglobulin (Ig) A antibodies in the intestinal contents or milk. Recombinant DNA technology offers a novel approach to the development of improved vaccines for enteropathogenic viruses if a suitable viral vector could be found. In this study four potential vectors, fowlpox virus (FPV), swinepox virus (SPV), a porcine adenovirus (PAV) and a baculovirus (BV) were compared for their ability to stimulate an intestinal antibody response in pigs.

FPV is being used for the construction of experimental recombinant vaccines for poultry (Boursnell et al 1990), and it has also been used as a vector to express the rabies virus glycoprotein in rabbits, rats, cats, dogs and cattle which were inoculated intradermally, subcutaneously or intramuscularly with the recombinant virus (Taylor et al 1988). There are no reports of the inoculation of pigs with FPV, or of oral dosing of any mammalian species with this virus, although chickens developed an antibody response to oral vaccination with FPV (Nagy et al 1990). SPV has not been used as a vector for foreign genes. Pigs can be infected experimentally with SPV by intradermal or intravenous inoculation (Kasza and Griesemer 1962) but oral infections have not been described.

The BV system has been used to express antigens from many different viruses (Possee et al 1990), including enteric viruses such as porcine (Godet et al 1991) and bovine (Parker et al 1990) coronaviruses and a bovine rotavirus (Cohen et al 1989). Since BVs fail to replicate in mammalian cells (Possee et al 1990) they may be poor candidates for the induction of a mucosal immune response in pigs, but their very strong expression of foreign genes, including the S gene of transmissible gastroenteritis virus (Godet et al 1991) justified their inclusion in the present study.

The use of human adenoviruses for the expression of genes from a number of viruses in several species, including pigs (Prevec et al 1989), is well established. Since live recombinant human adenoviruses may be unacceptable for the vaccina-

tion of pigs, there is interest in the use of PAV as a vector for genes from other porcine enteric viruses. Pigs have been experimentally infected with PAV-3 by the oronasal route (Sharpe and Jessett 1967, Coussement et al 1981). Virus neutralising antibodies were found in the sera of infected piglets (Sharpe and Jessett 1967), but the intestinal antibody response to PAV has not been studied.

Materials and methods

Viruses and cell cultures

The FPV was a plaque purified derivative (Nagy et al 1990) of the Chick-N-Pox vaccine (Salsbury Laboratories, Kitchener, Canada), cultivated and plaque assayed in chick embryo fibroblast cell cultures. The Kasza strain of SPV (Kasza et al 1960) was obtained from the American Type Culture Collection, Rockville, Maryland, USA, cultivated in stable swine testis cell cultures (McClurkin and Norman 1966) and titrated by infectivity assay. The BV *Autographa californica* nuclear polyhedrosis virus was cultivated in *Spodoptera frugiperda* cells as described by Summers and Smith (1987). For the immunisation of piglets with high titred BV (Table 1, group 4), the virus was partly purified by centrifugation through a 35 per cent sucrose cushion and diluted in TE buffer (10 mM Tris, 1 mM ethylenediamine tetraacetate, pH 7·2). The 6618 strain (Clarke et al 1967) of PAV-3 was cultivated in secondary pig kidney cell cultures and titrated by infectivity assay.

TABLE 1: Details of vaccinated piglets

Group number	Number of piglets	Virus	Dose of virus	Route of vaccination
1	6	Fowlpox	5×10^6 pfu	Oral
2	6	Swinepox	5×10^6 $CCID_{50}$	Oral
3	6	Baculovirus	5×10^6 pfu	Oral and parenteral
4	8*	Baculovirus	2×10^9 pfu	Oral and parenteral
5	6	Adenovirus	3×10^3 $CCID_{50}$	Oral

*Two piglets in this group received booster doses of virus
pfu Plaque-forming units
$CCID_{50}$ Median cell culture infectious dose

Vaccination procedures

Five groups (Table 1) of crossbred Yorkshire piglets from a specific pathogen-free herd were weaned 10 days after birth and vaccinated between two and three weeks old. Each piglet received a single oral dose of virus diluted in chocolate milk, except for those in group 4 which were given high titred BV orally, and by intramuscular injection in an oil-emulsion adjuvant prepared manually with mineral oil, polysorbate 80 and sorbitan monooleate as described by Brugh et al (1983). Two of these piglets also received booster oral and parenteral doses without adjuvant of the high titred BV two weeks after the first doses. Blood samples were collected from all piglets before vaccination and at weekly intervals for three weeks after vaccination, or for four weeks in the case of the group 2 piglets. Two piglets in each group were killed by an intravenous overdose of sodium pentobarbitone at intervals after vaccination, when samples of tonsil, mesenteric lymph node, duodenum, jejunum and ileum were collected. Each group included an additional control piglet, killed before vaccination in groups 2 and 5, and at the end of the experiment in groups 1, 3 and 4.

Virus isolation

The tissue samples were chopped into small pieces, suspended in phosphate buffered saline, frozen and thawed three times, clarified by centrifugation, and the supernatant fluids were inoculated on to the appropriate cell cultures for the isolation of the relevant virus. The cultures were examined daily for cytopathic effects, and subjected to a further passage in the same cell cultures.

Tests for viral antibodies

Samples of the contents of the small intestine were collected immediately after euthanasia from each piglet killed before or after vaccination. Each sample was suspended in phosphate buffered saline, frozen and thawed three times, and clarified by centrifugation as in the virus isolation procedure described above. The samples of serum and intestinal contents were inactivated by heating at 56°C for one hour and tested for virus neutralising (VN) antibodies against the virus used to vaccinate each group. For FPV and BV the sam-

ples were titrated by plaque reduction assay with 30 plaque-forming units (pfu) of virus, while for SPV and PAV-3, a microtitre assay with 50 median cell culture infectious doses (CCID50) was used. The end point was the highest dilution of the sample which gave a 50 per cent reduction in the number of plaques, or inhibited the cytopathic effects in the microtitre assay. Samples of intestinal contents from the groups of piglets which were positive for VN antibodies were tested for virus-specific antibodies of the IgA class by means of enzyme immunoassays (EIA). For the BV solid phase EIA, antigen was prepared as described by Summers and Smith (1987), and the end point of the assay was the highest dilution of intestinal contents which gave an optical density (OD) greater than 0·1 and at least twice the mean OD obtained with negative samples. For SPV and PAV-3, a fixed cell EIA was used, based on that described by Simkins et al (1989). Monolayers of swine testis cells in microtitre plates were inoculated with 50 CCID50 of SPV and fixed with 80 per cent acetone after incubation for three days, while for PAV-3, pig kidney cells were fixed with 3 per cent buffered formalin four days after infection with 50 CCID50 of virus. The second antibody used in the EIA was anti-swine IgA conjugated with horseradish peroxidase (Kirkegaard and Perry Laboratories). In addition, the sera from the piglets vaccinated with SPV and PAV-3 (groups 2 and 5) were tested in a fixed cell EIA as above, but with anti-swine IgG conjugated with horseradish peroxidase as the second antibody. In the fixed cell EIA, the end point was the highest dilution of the sample which gave an OD greater than 0·1 and at least twice the OD obtained with the same sample on uninfected control cells.

Results

Responses to FPV

The piglets in group 1 showed no clinical abnormalities before or after oral vaccination with FPV. No lesions were found post mortem, and no virus was isolated from the tissues. Virus neutralising antibodies were not found in the sera or intestinal contents of the control or vaccinated piglets.

Responses to SPV

One piglet in group 2 developed a small ulcer on the skin of the dorsal aspect of the snout five days after oral vaccination with SPV. The lesion healed within seven days, and no scab formation occurred. In a second piglet two small papules were observed on the skin of the ventral surface of the abdomen 21 days after vaccination. The lesions were still present when the piglet was killed seven days later, but there was no evidence of pustule or scab formation. No other lesions were found when the piglets were killed, and no virus was isolated from the skin lesions or other tissues. The serum antibody responses of the piglets are shown in Fig 1. Virus neutralising antibody titres were low, and were not detected until three weeks after vaccination. However, when the same sera were tested by EIA, antibodies were detected two weeks after vaccination, and quite high titres developed subsequently (Fig 1). In the intestinal contents, VN antibodies were detected at a titre of 1:2 in one piglet killed two weeks after vaccination, and at titres of 1:4 or 1:8 in the two piglets killed four weeks after vaccination. Similar titres were obtained in the EIA for anti-SPV antibodies of the IgA class in the same intestinal samples.

Responses to BV

Neither clinical abnormalities nor lesions were found in the piglets of groups 3 and 4, and the virus was not isolated from their tissues. No VN antibodies were detected in either the sera or intestinal contents of control piglets or the piglets in group 3, which received a single oral dose of

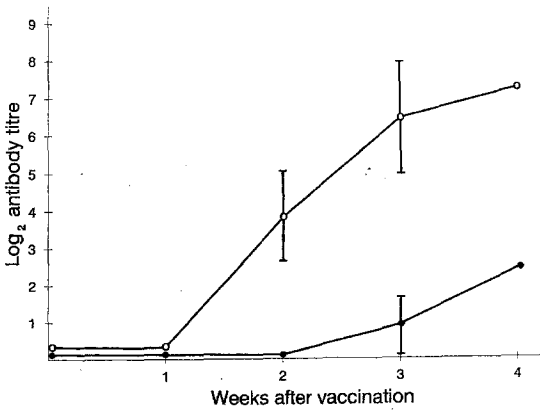

FIG 1: Mean $\log_2$ VN(●) and EIA (○) antibody titres in the serum of piglets at intervals after vaccination with swinepox virus. Vertical bars represent standard deviations

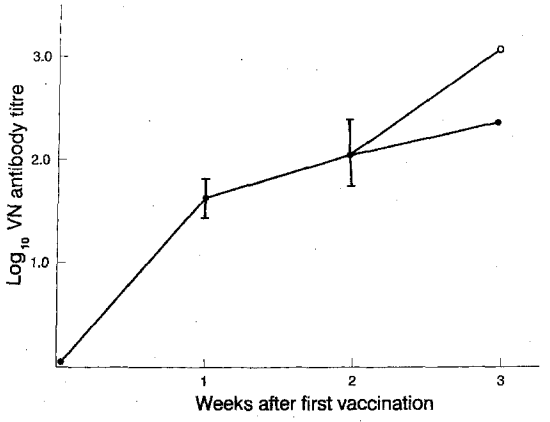

FIG 2: Mean $\log_{10}$ VN antibody titres in the serum of piglets in response to single oral and parenteral doses of baculovirus (●), and of piglets given booster doses of virus at two weeks (○). Vertical bars represent standard deviations

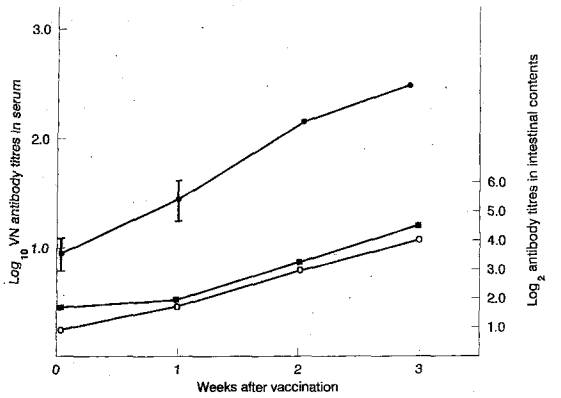

FIG 3: Mean $\log_{10}$ VN antibody titres in the serum (●), and the mean $\log_2$ VN (○) and EIA (■) antibody titres in the intestinal contents of piglets at intervals after oral vaccination with porcine adenovirus type 3. Vertical bars represent standard deviations

BV. When the partly purified high titred BV was given orally and parenterally (group 4), there was a marked VN antibody response in the sera (Fig 2). The highest titres were obtained in the two piglets which received booster doses of BV two weeks after the first doses of virus. Virus neutralising antibodies at a titre of 1:10 were detected in the intestinal contents only in the piglets which had been given booster doses of virus. Antibodies were detected by the IgA-specific EIA at a titre of 1:5 in the same samples of intestinal contents.

Responses to PAV-3

The piglets in group 5 showed no clinical abnormalities before or after oral vaccination with PAV-3, and no virus was isolated from the tissues of these piglets. Low titres of VN antibodies were found in the sera from all the piglets before vaccination, and in the intestinal contents of the one piglet killed before vaccination (Fig 3). There was a steady increase in VN antibody titres in the sera and intestinal contents following vaccination. Antibody titres similar to those in the VN test were obtained when the intestinal samples were tested by the IgA-specific fixed cell EIA (Fig 3), and the IgG-specific fixed cell EIA gave titres similar to the VN titres in the piglets' sera.

Discussion

The failure of oral FPV to stimulate an antibody response was not unexpected since the virus is capable of only an abortive infection in mammalian cells, and antibodies have only been produced in mammals given FPV parenterally (Taylor et al 1988). However, in response to oral SPV, antibodies were found in both the sera and the intestinal contents of the vaccinated piglets. Although SPV was not isolated from the piglets it seems likely that the virus replicated since a similar oral dose of FPV was not immunogenic. There are no reports in the literature of the oral infection of piglets with SPV, but the strain of SPV used in the present study was given intravenously or intradermally by Kasza and Griesemer (1962). The lesions were restricted to the skin and regional lymph nodes, and the virus was isolated only from these tissues. However, in natural cases of swinepox in neonatal piglets, lesions in the mouth and respiratory tract were described (Olefumi et al 1981), and oral lesions were also found in congenital swinepox (Borst et al 1990), so the virus may be capable of some replication in sites other than the skin. The circulating VN antibody response to the oral administration of virus in the present study was similar to that found by Mayr et al (1966) in response to intravenous or intradermal infection with SPV. Low levels of VN antibody are characteristic of SPV infections, and antibodies are more readily detected by alternative methods such as immune precipitation (Mahnel 1989) or EIA, as in the present study. Antibodies to SPV in the intestinal tract have not been described previously, and it was of particular interest that SPV antibodies of the IgA class were found in this location.

The failure of a single oral dose of BV to stimulate an antibody response was consistent with the inability of this virus to replicate in mammalian cells (Possee et al 1990). While intestinal BV antibodies of the IgA class were detected in the piglets which were vaccinated orally and parenterally, the low levels of antibodies obtained and the very high concentrations of BV which were used suggest that this procedure would not be economically feasible for routine vaccination of pigs with a recombinant BV. In an additional experiment (results not shown), piglets were vaccinated orally and parenterally with BV-infected *S frugiperda* cells, but the antibody responses were lower than those obtained with partially purified BV.

In addition to SPV, PAV seemed to be promising as a potential vector for encoded enteric viral antigens since a single oral dose stimulated an intestinal antibody response. Circulating VN antibodies have been described previously in piglets experimentally infected with this virus (Sharpe and Jessett 1967), but this is the first evidence of the production of intestinal antibodies in response to PAV-3. Natural infections with PAV are widespread (Derbyshire 1989), and therefore naturally acquired immunity could interfere with the immune response to a live recombinant PAV vaccine. It was noteworthy in the present study that, while low levels of antibody, presumably of maternal origin, were found in the piglets before oral vaccination with PAV-3, these were insufficient to inhibit an antibody response to a low dose of virus, although maternal antibodies in the piglet may cause a transient reduction in the number of antibody-forming cells in the intestine in response to oral immunisation (Watson et al 1979). It is also recognised that, at two weeks old, the piglet is relatively immature in terms of the numbers of IgA-producing cells in the small intestine (Brown and Bourne 1976).

Reports relating to the pathogenicity of PAV-3 are somewhat equivocal. In the present study, neither clinical signs nor lesions were seen following oral dosing with PAV-3 and the virus was not recovered from the piglets, corresponding with earlier studies with the same virus in conventional pigs (Sharpe and Jesset 1967). However, Coussement et al (1981) reported diarrhoea in hysterectomy-produced, colostrum-deprived piglets infected oronasally with PAV-3, although Benfield (1990) considered the intestinal changes to be unremarkable compared to those observed in rotavirus and coronavirus infections.

From the present studies, both SPV and PAV-3 emerge as potential vectors for the stimulation of an intestinal antibody response against enteric viral antigens in pigs. An advantage of SPV might be its greater capacity for foreign DNA than PAV-3, by analogy with vaccinia virus (Perkus et al 1985) and human adenoviruses (Prevec et al 1989), which might facilitate the construction of a multivalent recombinant vaccine as reported for vaccinia virus (Perkus et al 1985).

Acknowledgements

This study was supported by the Natural Sciences and Engineering Research Council of Canada, the Ontario Ministry of Agriculture and Food and the Ontario Pork Producers' Marketing Board. We thank Dr Lorne Jordan for assistance with the experimental piglets, Mr John Dennis for technical assistance and Mr David Bridle and his staff for their skilled animal care.

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Received April 28, 1992
Accepted October 14, 1992