Differentiation of Serum Antibodies from Pigs Vaccinated or Infected with Aujeszky's Disease Virus by a Competitive Enzyme Immunoassay

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SUMMARY

A competitive enzyme immunoassay was developed to detect antibodies to a glycoprotein (gI) of Aujeszky's disease virus. Infected cell monolayers were used as antigen and a monoclonal antibody directed against an epitope of gI as indicator antibody. It was demonstrated that pigs vaccinated with the Bartha, BUK or NIA-4 strains did not produce antibody to the epitope of gI, whereas all wild-type viruses tested did induce this antibody. The antibody to the gI epitope persisted for at least 15 weeks. The present test, which enables us to distinguish pigs vaccinated with certain attenuated strains from pigs infected with wild-type Aujeszky's disease virus, may be of great value in future combined vaccination-eradication programmes for Aujeszky's disease.

In most countries where Aujeszky's disease is enzootic, vaccination of pigs against this herpesvirus infection is practised. Vaccination prevents neither infection nor the establishment of latency of wild-type Aujeszky's disease virus (ADV). As a consequence, vaccination programmes alone will not lead to the elimination of ADV circulation. To achieve this goal, a serological testing and culling scheme should be followed. However, extensive vaccination programmes preclude serological studies to detect infected pigs and pig herds. Therefore, an assay for discriminating antibodies induced by vaccination from those induced by infection is of the utmost importance. We report here on the development and results of such an assay. To our knowledge, this is the first report published on the differentiation of antibodies to vaccine viruses from antibodies to wild-type viruses.

The sera used were from pigs experimentally inoculated at the age of 10 weeks, unless otherwise stated. Their vaccination or infection history is given in Table 1. The second vaccination was given after 3 or 4 weeks.

A micro-neutralization test (MNT) with an incubation period for the virus-serum mixture of 24 h was employed (Bitsch & Eskildsen, 1976; De Leeuw et al., 1982). The virus used was the NIA-3 strain of ADV (McFerran & Dow, 1975). Titres were expressed as log_{10} of the reciprocal of the final serum dilution inhibiting cytopathic effect in 50% of the cultures.

The immunoperoxidase monolayer assay (IPMA) was initially developed in our Institute for detecting monoclonal antibodies (MAbs) to swine fever virus (Wensvoort et al., 1986). For detection of antibody to ADV glycoprotein I (gI) it was performed as a competition assay with infected cell monolayers as antigen and a MAb directed against an antigenic site of ADV gI as indicator antibody. If antibody to the epitope recognized by the MAb was present in the test serum, the serum should inhibit the binding of MAb to the antigen in the monolayer. After determination of the optimal test conditions the competitive enzyme immunoassay was performed as follows.

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Secondary pig kidney cells were grown to confluence in microtitre plates (Greiner M29 ARTL). The cells were inoculated with 100 TCID$_{50}$ of the NIA-3 strain of ADV and after 20 to 24 h, when cytopathic effect appeared, the plates were washed once in 0·15 M-NaCl, dried for 45 min at 37 °C and stored at −20 °C in sealed plastic bags. The infected cell monolayer retained antigenicity for at least 3 months when stored at −20 °C. Before use, monolayers were fixed with a cold (4 °C) solution of 4% paraformaldehyde in phosphate-buffered saline (PBS) for 5 min. The plates were washed once in 0·15 M-NaCl and incubated with a 1:5 dilution of the test serum for 20 min, and then with a 1:1000 dilution of anti-gI MAb 1/14 for another 20 min at 37 °C. The preparation and specificity of the MAb has been described elsewhere (Lukács et al., 1985).

Goat anti-mouse Ig-horseradish peroxidase conjugate, at a dilution of 1:400 in 0·5 M-NaCl, 1% Tween 80 (pH 7·6), was added for 1 h at 37 °C. Before and after addition of the conjugate the plates were washed three times in 0·15 M-NaCl, 0·05% Tween 80. The substrate consisted of a 3-amino-9-ethylcarbazole solution, according to Graham et al. (1965). The following controls were included in each test: pig sera containing antibodies to NIA-3 virus or to the Bartha K61 strain (Bartha, 1961), negative pig serum, PBS and an unrelated MAb (anti-chicken Ig) as blocking antibody. The last three controls invariably did not block the reaction with MAb 1/14. Each control and test serum was further tested with an anti-ADV MAb not directed against gI and the above anti-chicken Ig MAb in place of the ‘indicator’ MCA 1/14. Each assay was independently read with the naked eye by two persons and results were always in agreement. A positive reaction was seen as clear red-brown spots against a transparent background, as a result of staining of the infected cells around the small plaque. If a blocking effect occurred, these spots were hardly visible or not at all.

Table 1 shows that sera from pigs inoculated with the vaccine strains Bartha K61, BUK TK/650 or NIA-4 or with a deletion mutant of NIA-3 virus (Berns et al., 1985) did not inhibit the binding of MAb 1/14. These sera had moderate to high MNT titres and did block the other ADV MAb. Apparently, these sera had elicited antibody to the epitope on gI recognized by MAb
1/14. These virus strains all carry deletions in the U₃ region of the genome (Gielkens & Berns, 1982; Herrmann et al., 1984; Lomniczi et al., 1984; Berns et al., 1985; Gielkens et al., 1985) where the structural gene for gI has been mapped (Mettenleiter et al., 1985a). In accordance with the present results, a recent report (Mettenleiter et al., 1985b) described that the Bartha and NIA-4 strains fail to express gI. On the other hand, another modified live vaccine [MK-25, a thymidine kinase-negative mutant with no deletion in the U₃ region of the genome (Gielkens et al., 1985)] did induce antibody to the epitope on gI. The same held true for the inactivated vaccine tested.

All wild-type strains tested induced antibodies to the gI epitope recognized by MAb 1/14 in intranasally inoculated pigs. It is probable that other wild-type strains behave in the same way, because the several hundreds of wild-type viruses from The Netherlands and several other countries which have been analysed by DNA restriction enzyme fingerprinting all possess the region in the genome which codes for gI (Herrmann et al., 1984; Gielkens et al., 1985; A. L. J. Gielkens et al., unpublished results). Furthermore, about 100 strains and field isolates of ADV obtained from different European countries were demonstrated to express gI (T. C. Mettenleiter et al., unpublished observations). In addition, 25 wild-type strains of ADV, originating from several European and Asian countries, were all shown to induce the gI epitope recognized by MAb 1/14 in secondary pig kidney cells. As yet we have found no wild-type strains that fail to induce this gI epitope (J. T. van Oirschot & M. Hwa Jong, unpublished observations).

In spite of the above findings it may still be reasoned that the use of a single MAb as a general probe for the detection of antibody to gI is insufficient. It is conceivable that some field isolates exist which lack or vary in the epitope recognized by MAb 1/14. We are currently identifying and selecting MAbs recognizing other gI epitopes which are absent in vaccine strains and present in wild-type strains, and are antigenic in pigs. The use of two different MAbs with the above properties in assays for the detection of an antibody response to ADV gI may reduce the possibility of scoring false negative results.

In addition to sera from experimentally inoculated pigs, pig sera from two field outbreaks of Aujeszky's disease were examined. In both cases antibodies to the gI epitope were produced, as evidenced by a blocking effect of the convalescent sera in the IPMA (Table 1). The first case (Hoek) concerned a clinically inapparent infection in a non-vaccinated herd. The acute sera were negative in the IPMA and had low neutralizing antibody titres, which were probably maternally derived. The second case was a virologically confirmed clinical outbreak caused by wild-type ADV, on a farm (Buys) where sows had repeatedly been given vaccine based on the Bartha K61 strain during the previous 2 years. Acute and convalescent sera were taken at the beginning of the outbreak and 3 weeks later, respectively. The acute sera of sows were negative in the IPMA, but positive in the neutralization test as may be expected for pigs vaccinated with the Bartha K61 strain.

Thus, with the blocking IPMA, pigs vaccinated with the Bartha, BUK or NIA-4 strains could be distinguished from pigs experimentally infected with various wild-type viruses, and from vaccinated or unvaccinated pigs infected in the field.

The antibody to gI persisted in pigs intranasally inoculated with the NIA-3 strain for at least 15 weeks, which was the entire experimental period (Table 1). Platt (1982) reported that, in experimentally infected pigs, antibody titres to seven glycosylated antigens of ADV, among which there is probably gI, persisted for at least 116 days. If under natural conditions the antibody response to gI persists for equal or longer periods, the IPMA test described here may be of great value. It would enable us to perform sero-epizootiological studies and to detect infected pig herds without interference of vaccination, provided only vaccines are used that fail to express the gI epitope.

Thus, competitive enzyme immunoassays for detection of antibodies to gI may provide a basis for ADV eradication programmes in countries where vaccination is widely practised.

REFERENCES

Short communication


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