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Development of a competitive double antibody lateral flow assay for the detection of antibodies specific to glycoprotein B of Aujeszky's disease virus in swine sera

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A B S T R A C T

Three lateral flow assays (LFAs) for the detection of antibodies against glycoprotein B (gB) of Aujeszky's disease virus (ADV) in swine sera: a competitive double antibody sandwich LFA without a preincubation step (CDAS-gB-LFA), a CDAS-gB-LFA with a preincubation step (pCDAS-gB-LFA), and a competitive direct gB-LFA have been developed and were compared with each other and with a gB-ELISA. The assays are based on monoclonal antibodies to immunodominant epitopes of ADV gB. The pCDAS-gB-LFA proved to be the most specific and sensitive assay to detect antibodies directed to ADV gB. The specificity and sensitivity of the pCDAS-gB-LFA with the use of an LFA reader for test line intensity measurements were 97.6 and 94.9%, respectively. The lower diagnostic sensitivity of the pCDAS-gB-LFA compared to a gB-ELISA reflects its reduced analytical sensitivity, which was shown in titration experiments with positive sera. The pCDAS-gB-LFA, using the reader-based and visual detection modes, showed good agreement in respect to specificity; however, the LFA reader detection provided a higher diagnostic and analytical sensitivity compared to visual detection. The developed pCDAS-gB-LFA is a rapid, sensitive, and specific method for the detection of antibodies to ADV gB and can be used for screening ADV-infected swine in unvaccinated herds.

1. Introduction

Aujeszky's disease virus (ADV) (pseudorabies virus, *Suid herpesvirus* 1), a member of the family *Herpesviridae*, the subfamily *Alphaherpesvirinae*, is the causative agent of Aujeszky's disease (AD), which is responsible for lethal infections in numerous animal species and considerable economic losses in the swine industry world-wide (Wittman, 1991). Although vaccination is effective in reducing the circulation of wild ADV and preventing the illness, it generally does not prevent the infection and establishment of latency and, therefore, does not lead to the eradication of AD (Wittman, 1991). The most widely used programs of AD eradication in many countries involve the vaccination with glycoprotein E (gE)-negative vaccines in combination with the detection of anti-gE antibodies in sera (Vannie et al., 2007). Serological tests for ADV-specific antibodies in sera of unvaccinated animals are crucial in countries that try to control the spread of AD without vaccination by culling seropositive pigs. These tests are also needed for AD eradication programs in countries that practice vaccination with gE-deleted vaccines when vaccination is stopped. Various types of ELISAs detecting ADV-specific antibodies in unvaccinated swine have been developed and are widely used (Sorensen and Lei, 1986; Quist et al., 1989; Kit and Kit, 1991; Grom et al., 1992; Morenkov, 2000). The tests are highly sensitive and specific; however, there is an increasing need for the development of tests allowing the early "on site" detection of ADV antibodies to remove infected pigs in domestic herds and prevent further economic losses. The lateral flow assay (LFA) is a well recognized immunochemical test allowing the detection of pathogens and pathogen-specific antibodies owing to its sensitivity, specificity, simplicity, and rapidity (Corstjens et al., 2007; Ferris et al., 2012; Gonzalez et al., 2014; Yang et al., 2015). Two indirect LFAs were developed for the detection of antibodies to ADV antigens (Joon et al., 2004; Guo et al., 2015).

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The detection of infected swine in unvaccinated herds is usually based on the determination of antibodies to whole ADV virions or to ADV glycoprotein B (gB) (Sorensen and Lei, 1986; Quist et al., 1989; Kit and Kit, 1991; Grom et al., 1992; Morenkov, 2000). Glycoprotein B, a major constituent of the ADV envelope, is indispensable for virus replication (Rauh and Mettenleiter, 1991; Peeters et al., 1992) and belongs to the class of glycoproteins that are most conserved among herpesviruses (Robbins et al., 1987). Glycoprotein B exists as a disulfide-linked complex consisting of three glycoproteins: gBa, gBb, and gBc; the gBb and gBc subunits represent the cleavage products of the gBa subunit (Hample et al., 1984; Lukács et al., 1985; Whealy et al., 1990). The gB complex is rapidly converted to a dimer after synthesis (Whealy et al., 1990; Wolfer et al., 1990). Antigenically, gB is considered to be relatively stable (Ben-Porat et al., 1986; Zaripov et al., 1998), though an antigenic drift in the glycoprotein was reported (Yamada et al., 1991). It was shown that the conformation-dependent epitopes of gB, which are predominantly located on gBc of the gB complex, play a central role in provoking the antibody response to gB during the natural infection of swine and immunization of mice (Zaripov et al., 1999). Thus, gB represents a valuable target antigen for the development of diagnostic tests.

Earlier we have produced and characterized a panel of monoclonal antibodies (MAbs) specific to ADV gB (Morenkov et al., 1994; Zaripov et al., 1998) and developed highly sensitive and specific blocking gB-ELISAs on their basis (Morenkov, 2000). Obviously, MAbs directed to immunodominant epitopes of ADV gB are promising in the development of sensitive and reliable competitive LFA for the detection of antibodies against gB (gB-LFA). In this report we describe three novel competitive LFAs for the detection of ADV gB-specific antibodies in swine sera based on gB-specific MAbs.

2. Materials and methods

2.1. Sources of sera

Sera from uninfected and infected unvaccinated swine as well as serum samples from uninfected swine vaccinated against AD and various swine viral disorders (classical swine fever, parvovirus disease, transmissible gastroenteritis, enzootic encephalomyelitis) were obtained from the serum bank of Bio-Test-Laboratory (BTL) (Kiev, Ukraine). All sera were tested initially for the presence of anti-ADV antibodies in a reference commercial PRV/ADV gB Ab ELISA (IDEXX Laboratories, Inc., Westbrook, Maine, U.S.) according to the manufacturer's recommendations. Sera that produced doubtful results in the reference test were not used in the investigation because no decisions about these samples could be made. All the sera were also retested in the direct blocking gB-ELISA (BTL). The results of testing with PRV/ADV gB Ab ELISA (IDEXX) and gB-ELISA (BTL) coincided in 99–100% samples. To determine the status of sera from animals vaccinated with a live gE-negative vaccine, PRV/ADV gI Ab ELISA (IDEXX) was used. The control negative serum for control LFA strips was prepared by pooling five sera clearly negative in gB-ELISA (BTL). The control positive serum for control LFA strips was prepared by pooling ten sera clearly positive in gB-ELISA (BTL).

2.2. Preparation of the gB-antigen

The gB-antigen was prepared using the ADV strain K obtained from the collection of viruses of the Bio-Test-Laboratory (Kiev, Ukraine). The virus was cultivated on BHK-21 cells maintained in Eagle's minimum essential medium supplemented with 10% calf serum. ADV virions were purified by sedimentation in a linear sucrose gradient (Ben-Porat et al., 1974). ADV virions were treated with 1% Triton X-100 for 1 h at 37 °C, the solution was clarified by centrifugation for 20 min at 10 000*g*, and the gB-antigen was purified by immunoaffinity chromatography as described (Zaripov et al., 1998).

2.3. Preparation of colloidal gold-labelled conjugates

The preparation and characterization of MAbs against ADV gB have been described previously (Morenkov et al., 1994; Zaripov et al., 1998). MAbs were purified from ascites fluids by ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE-Toyopearl 650 M (Tosoh Corporation, Tokyo, Japan) (Oppermann, 1992). Detector conjugates were prepared using colloidal gold. Colloidal gold particles with a mean diameter of 35 ± 5 nm were prepared by the method of Frens (Frens, 1973). For the immobilization of antibodies on the gold surface, the pH of colloidal gold was adjusted to 9.0 with a 0.1 M potassium carbonate solution after which colloidal gold was mixed with different purified gB-specific MAbs (5–10 μg/ml) under shaking. After 10 min, 1% bovine serum albumin (BSA) in 10 mM sodium borate (pH 8.5) was added, and the mixture was stirred and incubated for 30 min to block empty spaces that remained on the colloidal gold surface. Then, the mixture was successively centrifuged three times at 6000*g* for 20 min and resuspended in 10 mM sodium borate buffer, pH 8.5, containing 1% (w/v) BSA and 0.05% (w/v) sodium azide. The conjugates were stored at +4 °C for several months.

2.4. Preparation of LFA strips

2.4.1. Strips for the competitive double antibody sandwich LFA without a preincubation step (CDAS-gB-LFA)

Antibodies were immobilized on a nitrocellulose membrane backed on a polyester card (HiFlow Plus 90, Merck-Millipore, Darmstadt, Germany). The detection zone consisted of a test line and a control line. For the preparation of the test line, gB-specific MAbs were brought to a concentration of 1 mg/ml in phosphate buffered saline (PBS) and dispensed on the membrane at a rate of 1 μl/cm using a BioJet XYZ 3000 Dispenser (Biodot Inc., Irvine, CA, USA). The control line was prepared using rabbit anti-mouse IgG antibodies. The membranes were dried, soaked in PBS containing 1% BSA (PBS-BSA), washed in PBS containing 0.05% Tween 20 (PBS-T), stabilized in PBS containing 2% sucrose (PBS-S), and dried. A colloidal gold conjugate was diluted with PBS-S, jetted onto a glass fiber conjugate pad (Merck-Millipore, Darmstadt, Germany) at a rate of 6 μl/cm, and dried at 37 °C for 2 h. Finally, the cards were assembled so that the glass fiber sample pad, the glass fiber conjugate pad, and the absorbent pad (Merck-Millipore, Darmstadt, Germany) were attached to the glue-covered plastic support, and all the membranes and filters lightly overlapped in order to allow a continuous lateral flow of the liquid sample. The cards were cut into 4-mm strips using an automatic programmable cutter CM4000 (Biodot Inc., Irvine, CA, USA). Then, the gB-antigen was diluted with PBS-S and dispensed onto the glass fiber sample pad of each strip in a volume of 4 μl after which the strips were dried at 37 °C for 18 h. The optimal concentrations of the gB-antigen and the colloidal gold anti-gB-conjugate were determined in preliminary experiments.

2.4.2. Strips for the competitive double antibody sandwich LFA with a preincubation step (pCDAS-gB-LFA)

The immobilization of gB-specific MAbs and rabbit anti-mouse IgG antibodies on a HiFlow Plus 90 membrane, the blocking and stabilization of the membrane, the dispensing and drying of the colloidal gold conjugate, the assembling of cards, and the cutting of assembled cards into strips were performed as described above. The gB-antigen was diluted with PBS-S, 4 μl of a gold conjugate