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Serologic relationship and antigenic dominance among five Aujeszky's disease virus isolates

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ABSTRACT

The serologic relationship and antigenic dominance among five Aujeszky's disease virus (ADV) isolates, designated VBA1, VBA2, VBA3, mA1p and CD, was studied using homologous and heterologous antigens and sera. Cross-serum neutralization test (C-SNT) was used to measure antibody responses to the virus. Generally, close serologic relationships among ADV isolates were noted. Non-significant ($P<0.05$) variations in the relationship between any two viruses were observed. However, the highest bilateral relationship was observed between VBA2 and VBA3 and between VBA2 and CD isolates of the virus. The antigenic dominance of some viruses over the others was detected with non-significant ($P<0.01$) levels, although the higher antigenic dominance was observed for CD over VBA3 and mA1p over VBA3 also. In conclusion, neither major serological variations nor antigenic dominance among the five ADVs studied were detected in the present study, hence grouped as one serologic type.

Key words: Aujeszky's disease virus, antigen, antibody, cross-neutralization

Introduction

Aujeszky's disease virus (ADV), also known as pseudorabies virus (PrV), is an alphaherpesvirus responsible for neuropathological disease in swine (AUJESZKY, 1902) and many other domestic and wild animals (PENSAERT and KLUGE, 1989). The serological responses to various ADV strains and vaccines were previously examined in laboratory animals (WITTMAN et al., 1983; ALI et al., 1998; ALI, 1999; ALI and MOHD-AZMI, 2002) and natural host (ALI, 1999).

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Based on a cross-neutralization test, a minor antigenic relationship has been demonstrated between some ADV isolates and other herpesviruses, such as herpes-simplex virus (HSV) (TONEVA, 1971) and herpes B virus (WATSON et al., 1967), whereas a close antigenic relationship was detected between ADV and bovine herpesvirus-1 (BHV-1) (AGUILAR-SETIEN et al., 1979). The serological relationship between some field isolates of ADV and conventionally attenuated vaccines have also been studied, claiming they were antigenically dominant to the vaccines (KIT, 1989; WARDELY and POST, 1989). The present study was designed to determine the serologic relationship and antigenic dominance among five ADV isolates from different geographical origins.

Materials and methods

Viruses. Three isolates of ADV termed VBA1, VBA2 and VBA3 (isolated from three different geographical locations in Malaysia during an outbreak in 1994), a plaque purified clone of ADV, termed mA1p, and an ADV strain of American origin termed CD, were used in this study. The first three isolates and the clone virus were kindly supplied by Dr. Mohd-Azmi of Putra University, Malaysia, whereas the American ADV strain was kindly provided by Professor Anthony Castro of the University of California, Davis (U.S.A.)

Virus propagation and purification. The viruses were propagated in Vero cell cultures grown in Leibovitiz-15 (L-15) tissue culture medium supplemented with 5% foetal calf serum (FCS), 1% antibiotic-antimycotic and 1% anti-PPLO agents. They were purified from Vero cell cultures using the sucrose gradient ultracentrifugation method as described by BEN-PORAT et al. (1974), with some modifications.

Preparation of hyperimmune sera (HIS) in mice. Hyperimmune sera against all ADV isolates were raised in four-week-old, female BALB/c mice, as described by AZMI and FIELD (1993) and ALI (1999). Ten mice (in separate groups) were used for each virus. Prior to inoculation of the mice, blood was collected from each of them and serum was separated for use as a negative control (pre-immune sera). Purified, heat-inactivated virus suspension in L-15 medium containing approximately 107 p.f.u. per ml (the virus was inactivated by incubation of the virus suspension at 56 ^{0}C for 30 minutes) was emulsified with an equal volume of Freund's complete adjuvant (Gibco BRL, U.S.A.) by means of homogenization until a good emulsion was formed. One hundred ml of this emulsion was injected subcutaneously into each mouse. At each two-week interval, for six successive weeks, mice were re-injected once with the same dose of antigens emulsified in Freund's incomplete adjuvant (Gibco BRL, U.S.A.) The mice were bled weekly to check antibody levels by means of ELISA. For the final booster, all mice were injected with live virus only (without the adjuvant). Two weeks after the final injection all mice were killed and

blood was collected by cardiac puncture. The blood was allowed to clot at room temperature for 2-3 hrs and spun at 300 rpm for 5 min. The serum for each group was pooled, titrated and stored at -20 °C until use.

Serum neutralization test (SNT). Twenty-four-well tissue culture plates containing confluent monolayer of Vero cells were prepared. The sera to be tested were first inactivated at 56 °C for 30 minutes. Two-fold serial dilutions of sera were carried out in L-15 medium (supplemented with 2% heat-inactivated FCS and containing 1% of antibiotic-antimycotic solution and anti-PPLO agent). Aliquots of the serum were mixed with an equal volume of live ADV suspension (100 ml containing 100 p.f.u.). Pre-immune sera were included in the test as control. For virus neutralization, the mixtures were incubated at 37 °C for 45 minutes, with continuous shaking using an orbital shaker incubator. One hundred-ml aliquots of each mixture were added to the 24-well plates containing the confluent Vero cell monolayers in quadruplicate. Four wells of one column were inoculated with the virus only (positive control); another 4 wells were inoculated with the virus mixed with the control serum (negative control). The virus was allowed to adsorb by incubating the plate at 37 °C in 5% CO_2 for 45 minutes. Five hundred ml of L-15 medium containing 1.2% CMC and 2% FCS were then added. The plates were incubated for 2-3 days until plaques developed. The plates were fixed with methanol, stained with crystal violet solution and the number of plaques for each dilution was determined. The neutralizing antibody titre was determined by plotting serum dilution against the number of plaques, and the dilution of the serum (log_2) required for 50% reduction of plaques of the control wells was calculated from the graph according to BISCH (1978).

Determination of serologic relationship and antigenic dominance among ADVs The bilateral serologic relationship (R) and antigenic dominance (D) between ADV isolates were determined according to the method described by PRECAUSTA and STELLMANN (1973). The relationship of A to B isolate of the virus was estimated according to the following formulae:

$$r1 = \frac{\text{B virus against A HIS (heterologous) titre}}{\text{A virus against A HIS (homologous) titre}}$$

$$r2 = \frac{\text{A virus against B HIS (heterologous) titre}}{\text{B virus against B HIS (homologous) titre}}$$

$$R = 100 \times \sqrt{r1 \times r2}$$

$D = \sqrt{\frac{r1}{r2}}$ when D values >1 indicative for dominance.

Statistics. The statistical significance of differences between groups of data was determined using the two-tailed Student's unpaired *t*-test.

Results

The neutralizing Ab titers of all virus isolates obtained against homologous and heterologous ADV antigens are shown in Table 1. Each ADV isolate was neutralized by homologous as well as heterologous HIS. The homologous neutralizing Ab titers were higher for VBA2 and mA1p isolates than heterologous titers, and lower for other viruses. PrVs. Hyperimmune sera of VBA1 and VBA3 isolates gave slightly higher ($P<0.05$) titers with VBA2 antigen than with their homologous antigens. Hyperimmune serum of CD isolate showed higher potential to neutralize mA1p antigens than to its antigens. For all HIS's, the highest Ab titers were observed when measured against VBA2 and mA1p viruses.

Table 1. Neutralizing antibody titers of measured against homologous and heterologous ADV antigens and sera

ADV antigen	ADV HIS				
	VBA1	VBA2	VBA3	mA1p	CD
VBA1	6.8*	6.8	6.9	6.0	6.5
VBA2	6.9	7.0	7.1	6.7	6.5
VBA3	5.6	6.5	6.5	6.3	5.6
mA1p	6.8	6.9	7.0	7.1	6.8
CD	6.5	6.8	7.0	6.5	6.3

* Serum dilution needed to cause 50% reduction in plaque formation ($\log_2$)
ADV = Aujeszky's disease virus, HIS = hyperimmune serum

The serologic relatedness (R) and antigenic dominance (D) among ADVs, estimated from their neutralizing Ab titers, are shown in Table 2. The results obtained revealed great relationships among the viruses. However, the highest relationship was observed between VBA2 and VBA3 and between VBA2 and CD viruses. Dominance evaluation showed that VBA2 isolate was dominant over VBA1, mA1p over VBA1, VBA2 and VBA3, while CD over VBA3 only. No antigenic dominance for VBA1 and VBA3 over any virus is observed.

Table 2. Bilateral serologic relationship between ADVs and antigenic dominance among them estimated from their HIS's neutralizing antibody titers

ADV antigen	ADV HIS				
	VBA1	VBA2	VBA3	mA1p	CD
VBA1	100.00* 1.00**				
VBA2	99.28 1.02	100.00 1.00			
VBA3	93.55 0.88	100.71 0.92	100.00 1.00		
mA1p	91.92 1.09	96.45 1.02	97.75 1.10	100.00 1.00	
CD	99.31 0.96	100.11 0.97	97.84 1.21	99.41 0.92	100.00 1.00

* Bilateral relationship; ** Dominance (D value >1 indicative of dominance); ADV = Aujeszky's disease virus, HIS = hyperimmune serum

Discussion

The present study was designed to determine the serologic relationships and antigenic dominance among five ADV isolates and strains originating from different geographical sites. The results obtained showed intimate serologic and antigenic relationships among the viruses, leading to the fact that they belong to the same serologic subtype. This close relationship is proven despite the variations in the number and types of the proteins among these viruses responsible for the induction of Ab responses, which we demonstrated in previous reports using Western blotting technique (ALI and MOHD-AZMI, 2002; ALI et al., 2003). This confirms the role of the common proteins, detected in all viruses, in stimulating Abs of neutralizing activities. The discrepancy in the findings of SNT and Western blotting was previously observed by TODD et al. (1987) and attributed to some inherent characteristic of each of these techniques. The same authors emphasized that when employing Western blotting, some SDS-solubilized proteins may undergo denaturation after electrophoretic transfer, then some polypeptide sequences may show non-specific reactions.

It is interesting to note that HISs of some viruses (VBA1 and VBA3) showed higher neutralizing activity to other viruses (VBA2) than to the homologous antigens. This could only be attributed to some errors in the cell culture system used but no data learned can support this finding. VBA2 and mA1p viruses displayed the highest neutralizing activity against sera of all viruses. This explained their antigenic dominance over the other viruses observed later in this study.

The great serologic relationships and low antigenic dominance among the viruses detected in the present study substantiate their grouping as one serotype to agree with PENSAERT and KLUGE (1989), who documented that ADV consist of one serotype. mA1p clone virus of ADV was previously confirmed to have high immunogencity and protective potential against virulent viruses challenge and hence selected as a good and safe vaccine candidate against AD (ALI, 1999). In the present study, this virus also showed antigenic dominance over the three Malaysian viruses, which again promote the virus to be a good vaccine candidate against AD.

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SAŽETAK

Upotrebom homolognih i heterolognih antigena i seruma istraživan je serološki odnos i antigenska prevlast između pet izolata virusa bolesti Aujeszkoga (VBA) označenih VBA1, VBA2, VBA3, mA1p i CD. Unakrižnim serum-neutralizacijskim testom mjerena je količina specifičnih protutijela. Općenito je zapažena jaka serološka srodnost između pretraženih izolata. Uočena su beznačajna odstupanja ($P<0,05$) u srodnosti bilo koja dva izolata. Međutim, najjača srodnost uočena je između VBA2 i VBA3 te između VBA2 i CD izolata. Ustanovljeno je da antigenska prevlast jednih izolata nad drugima nije bila značajna ($P<0,01$), iako je uočena nešto veća antigenska prevlast CD izolata nad VBA3 te mA1p nad VBA3. Može se zaključiti da u ovom radu nije zabilježeno ni značajnije serološko odstupanje niti antigenska prevlast između pet istraživanih izolata virusa bolesti Aujeszkoga pa su svrstani u jedan serološki tip.

Ključne riječi: virus bolesti Aujeszkoga, antigen, protutijela, unakrižna neutralizacija