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Different Immunogenic Peptides of Pseudorabies Virus Detected by Sera Prepared in Different Animal Species

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Abstract: The immunogenic peptides of five pseudoraibes virus (PrV) isolates were determined and compared using murine as well as swine sera. Western blotting technique was employed to visualize the immunogenic peptides. Marked differences in the number and kinds of immunogenic peptides among the viruses were observed using either the murine or swine sera. 12 and 13 immunogenic peptides were detected using murine and swine sera respectively. Murine serum was noted to detect peptides of higher molecular weights as compared to those detected by swine serum. The protein of 76.00 Kda was detected when both sera were used although minor variations in the molecular weights were noted for the peptides detected by both kinds of sera. The results obtained indicated that the recognition of different protein peptides of the virus by immune system is an animal species *dependent*.

Key words: PrV, peptide, antibody, murine, swine sera

Introduction

Pseudorabies virus (PrV) is an alpha herpesvirus (Franckiet *et al.*, 1991) that responsible for a highly fatal neurotropic disease, termed Aujeszky's disease in pigs (Aujeszky, 1902) and pseudorabies in many other domestic and wild animals (Pensaert and Kluge, 1989). About 100 proteins were suggested to be encoded by PrV genome although few of them have been investigated and characterized (Hampl *et al.*, 1984; Lukacs *et al.*, 1985 and Todd *et al.*, 1987a). The envelope glycoproteins in the PrV virion have been described and some of them proved to be responsible for the induction of protective antibody responses (Ben-Porat *et al.*, 1986; Todd *et al.*, 1987b; Spear, 1993; Metternleiter, 1994; Jons *et al.*, 1996 and Dijsktra *et al.*, 1997). The important epitopic regions of them were also mapped (Zaripov *et al.*, 1999 and Ober *et al.*, 2000).

Individual animal variations in the immune response to viral proteins were previously documented particularly during the early phase of Ab production (Wittmann *et al.*, 1980; Banks and Cartwright, 1983; McKeever *et al.*, 1987). Species variations in the magnitude of Ab response to a particular antigen was also reported (Ober *et al.*, 1998). In the present investigation, the differences in immunogenic peptides of PrV detected by sera prepared in different animal species were studied using the Western blotting technique.

Materials and Methods

Viruses: The viruses used in the study were as follows: hree PrV isolates termed VBA1, VBA2 and VBA3.

A plaque purified clone of PrV termed mA1p kindly supplied by Dr. Azmi from UPM (Malaysia).

An American PrV strain termed CD kindly provided by Professor Zee, Y.C. from University of California-Davis (USA).

Sera: The murine positive serum was prepared in mice as described below whereas the positive swine serum was raised in piglets and kindly supplied by Dr. Azmi from UPM (Malaysia).

Virus Propagation and Purification: Stocks form these five viruses were prepared by propagation in Vero cells cultures grown in Leibovitiz-15 (L-15) tissue culture medium supplemented with fetal calf serum (FCS), antibiotic-antimycotic and anti-PPLO agents. The viruses were purified by sucrose gradient ultracentrifugation as described by Ben-Porat *et al.* (1974).

Preparation of Hyper immune Sera (HIS): HIS against PrV prepared in mice was described previously (Ali *et al.*, 1998). Briefly, each one hundred μl of purified, heat-inactivated virus suspension containing 10^7 plaque forming unit (p.f.u) per ml and emulsified with an equal volume of Freund's complete adjuvant injected subcutaneously into ten mice. At every two -week interval and for six successive weeks, mice were re-injected once with the same dose virus antigens emulsified in Freund's incomplete adjuvant. For the final booster, all mice were injected with live virus without adjuvant. Mice were bled weekly to check for Ab levels using ELISA. Two weeks after the final injection, blood was collected from mice by cardiac

Table 1: Immunogenic peptides of PrV isolates detected using murine serum

Peptide (Kda)	PrV isolate				
	VBA1	VBA2	VBA3	mA1p	CD
*243.00	+	+	+	+	+
*238.00	+	+	+	+	+
198.00	-	+	+	-	+
158.00	-	+	+	-	+
130.50	+	-	-	+	-
* 95.00	+	+	+	+	+
*81.00	+	+	+	+	+
*76.00	+	+	+	+	+
*72.00	+	+	+	+	+
*60.00	+	+	+	+	+
*53.00	+	+	+	+	+
48.50	-	+	+	+	+

+= peptide band detected, - = peptide band not detected
* = immunogenic peptide band which is common among PrVs

Table 2: Immunogenic peptides of PrV isolates detected using Swine serum

Peptide (Kda)	PrV isolate				
	VBA1	VBA2	VBA3	mA1p	CD
*125.00	+	+	+	+	+
102.00	-	+	-	-	+
*80.00	+	+	+	+	+
76.00	+	-	-	+	-
73.50	+	+	-	+	-
70.50	-	+	+	-	+
*57.00	+	+	+	+	+
54.00	-	-	+	+	+
51.00	+	+	+	+	-
*49.00	+	+	+	+	+
47.50	-	+	-	-	-
45.00	+	-	-	-	+
43.00	-	-	-	-	+

+= peptide band detected, - = peptide band not detected
* = immunogenic peptide band which is common among PrVs

puncture. Sera were separated, titrated and kept at -20°C till used.

Polyacrylamide Gel Electrophoresis (PAGE): The demonstration of PrVs polypeptides was employed using the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) discontinuous method as described by Laemmli (1970).

Protein Transfer to Nitrocellulose Membrane: The protein transfer to the nitrocellulose membrane was carried out as described by Towbin *et al.* (1979). After the transfer, the nitrocellulose membrane was dried on clean paper and sealed in a container to be kept at -20°C before the transferred protein being immunostained with the murine and swine sera.

Western Blotting: The blotted PrV proteins in the nitrocellulose membranes were subjected to the staining with the sera as described by Ali and Mohd Azmi (2002). In brief, The membrane was firstly washed three times with PBST (0.05% Tween 20 in PBS) for two minutes each. The membrane was then incubated in 20 ml blocking buffer (3% BSA, 0.15M NaCl, 0.001M EDTA, 0.05M Tris-Hcl, pH 7.4 and 0.05% Tween 20) for one hour on a rocker platform. The blocking buffer was replaced with 20 ml of 1/50 dilution of primary Ab in mouse HIS (or swine serum) in blocking buffer and incubated at 37°C for 2 hours. The membrane was again washed four times 10 minutes each. The washed membrane was socked with 20 ml of 1/500 dilution of secondary Ab (goat anti-mouse horse radish peroxidase conjugated IgG or goat anti-swine horse radish peroxidase conjugated IgG) in the blocking buffer and incubated as above. The membrane was then washed three times for 10

minutes each with TBS (50 mM Tris, 0.9% NaCl, pH 7.5). The TBS was replaced with by 50 ml fresh TBS and 10 ml substrate (0.3% 4-Chloro-1-naphthol in methanol). This step was immediately followed by addition of 50 μl of 30% H_2O_2 and gentle shaking for 5 minutes. The reaction was stopped by washing of the membrane twice with 50 ml of TBS and soaked overnight in sterile distilled water. The membrane was photographed and the results were recorded.

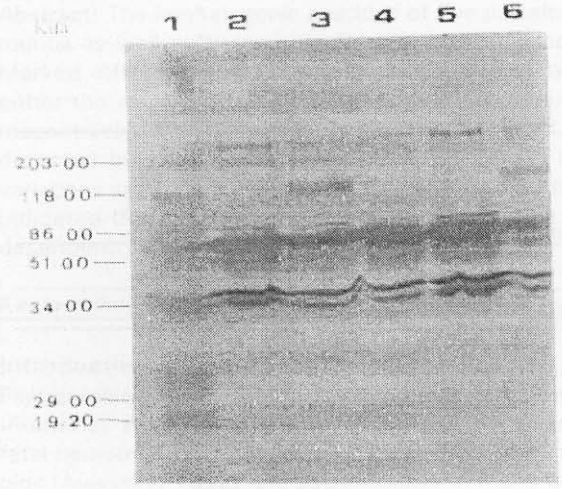

Fig. 1: Western blot of PrV proteins detected by murine serum: Lane 1 is the broad range protein marker, lanes 2, 3, 4, 5 and 6 are PrV- CD, mA1p, VB3, VB2 and VB1 respectively.

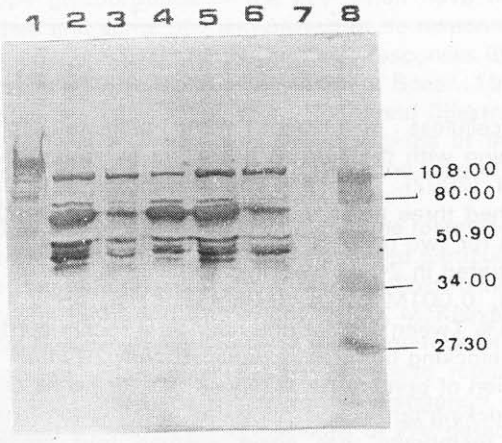

Fig. 2: Western blot of PrV proteins detected by swine serum: Lane 1 is the high range protein marker, lanes 2, 3, 4, 5 and 6 are PrV- CD, mA1p, VB3, VB2 and VB1 respectively, lane 7 is the Vero cell lysate while lane 8 is the low range protein marker

Results

The nitrocellulose membrane containing the blotted PrV proteins and subjected to immunostaining with murine and swine sera are shown in Fig. 1 and 2 respectively. The differences among the viruses in blotted polypeptide bands immunologically detected by murine and swine sera are demonstrated in Tables 1 and 2 respectively.

Following immunostaining of the blotted PrVs proteins with the murine serum, 12 immunogenic peptides were detected among the viruses with 8 (66.67%) out of them are observed to be common for all of them whereas 4 (33.33%) immunogenic peptides are confined to certain virus isolates. Following immunostaining of the blotted PrVs proteins with the swine serum, 13 immunogenic peptides were detected among the viruses with 4 (30.77%) out of them are observed to be common for all of them whereas 9 (69.23%.) immunogenic peptides are confined to certain virus isolates.

Discussion

The present study was conducted to determine the immunogenic peptides of five PrV isolates and to highlight the differences of these peptides in terms of kinds and number when murine and swine sera were used to stain them. The findings obtained revealed that almost similar number of the immungenic peptides was visualized when both sera were used, however, the variations in their kinds were clearly evident. These variations suggest that different viral proteins are targeted by neutralizing antibodies in different animal species, although some PrV proteins, namely gD, were proved to be immunogenic and protective for both mouse and swine. This was documented by Marchioli *et al.* (1987) and Ishii *et al.* (1988) who reported that a passive transfer of anti-gD (50-60 Kda) Abs can protect mice and pigs against lethal PrV challenge.

The results obtained also revealed that immunogenic peptides of higher molecular weights (243.00, 238.00, 198.00, 158.00, 130.50 Kda) were observed when murine serum was used as compared to the swine serum (125.00, 102.00, 80.00, 76.00 Kda). This substantiates the differences among animal species to respond to a particular antigenic protein (Ober *et al.*, 1998) and the molecular weight is a determining factor in this regard. Using either type of serum, despite the high relationships in the immunogenic peptide profile among the PrV isolates, variations among them were observed. These variations in the number and relative intensities of PrV proteins detected by immunostaining in the study were previously documented by Todd *et al.* (1987b).

In conclusion, the data demonstrated in this study

showed that the difference in the number of immunogenic proteins of PrV detected by either murine or swine sera is not that great, however, a noticeable variations among them in their molecular weights and electrophoretic patterns are present.

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