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Antigenic characteristics of glycosylated protein 3 of highly pathogenic porcine reproductive and respiratory syndrome virus

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ABSTRACT

Highly pathogenic (HP)-porcine reproductive and respiratory syndrome virus (PRRSV) emerged in 2006 and has now become a global threat to pig farms. Despite extensive characterization of HP-PRRSV proteins by direct analysis and comparison with typical PRRSV, immune recognition remain poorly understood. Glycosylated protein 3 (GP3) has an important function in inducing protective immune response. To analyze the antigenic character of HP-PRRSV GP3, a total of 217 peptides were printed on a chip and used to react with HP-PRRSV specific serum. The reactions of these peptides to HP-PRRSV specific pig serum were scanned and quantified using the software PepSlide® Analyzer by fluorescence intensity. The intensity plots showed various reactions in different parts of GP3. The highest reaction intensity value reached 29,184.5 with the peptide sequence of CSENDHDELGFMVPP. Conversely, 88 peptides showed no reaction with 0 florescence intensity. A further analysis based on the result of the peptide microarray revealed an antigen reaction active region (AR) from Y^{51} to S^{106} in GP3. The AR had four parts of variation that may be a significant mutation of the typical PRRSV to HP-PRRSV. Acquired data may be useful for understanding HP-PRRSV variation and its GP3 immune recognition.

1. Introduction

Highly pathogenic (HP)-porcine reproductive and respiratory syndrome virus (PRRSV) was first reported in 2006 and had caused serious economic losses to pig farmers in China (Li et al., 2007; Tian et al., 2007). HP-PRRSV was pandemic in 2006 but progressed into endemic that persistently caused economic loss on pig farms in China. Recently, the scope affected by the disease has become broader, reaching Vietnam and Lao People's Democratic Republic (Ni et al., 2012). HP-PRRSV is characterized by a gene deletion in NSP2, genetic variation in full genome, and enhanced pathogenicity (Zhou et al., 2009). These characteristics are not yet fully understood but have attracted the interest of researchers.

Antigenic character is a main index that reflects virus features. Monoclonal antibody-based epitope analysis was one of the few methods that are previously used to study antigenic characteristics (Cancel-Tirado et al., 2004; Zhou et al., 2006). However, long-time costing and high technical requirement slows down the application of this technology in antigenic character analyses. Recently, a new technique using a microassay-based peptide scan that can provide a useful way to examine protein antigenic character was developed. The antigenic character of human protein PreSETs has been analyzed by these methods (Buus et al., 2012). In addition, overlapping dodecapeptides with an offset of 4 and an overlap of 8 aa were used to analyze the antigenic character of type I PRRSV; several antigenic regions were identified (Vanhee et al., 2011).

Glycosylated protein 3 (GP3) is a protective antigen and is one of seven structural PRRSV proteins (De Lima et al., 2009; Yang et al., 2000). In our previous study, a recombinant human adenovirus that expresses HP-PRRSV viruses GP3 and GP5 has induced a protected immune response in piglets (Wang et al., 2009). Our data demonstrated the important function of GP3 in inducing immune response in PRRSV infection. GP3 is also the second most heterogeneous protein of PRRSV with approximately 54–60% amino acid (aa) identity between North American and European isolates (Meng et al., 1995). Therefore, GP3 was selected to reveal HP-PRRSV antigenic characteristics in this study.

2. Materials and methods

2.1. Virus and serum

The GP3 amino sequence of HP-PRRSV virus strain SY0608 (Li et al., 2007) was used as template (Protein_id ABV60270.1). The hyperimmune SY0608 specific serum was provided by Dr P. Jiang

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(Nanjing Agricultural University, China). The serum was collected from pigs that are immunized with formalin-killed SY0608 virus, and then challenged with the virulent strain. Secondary antibody from goat anti-swine IgG (H + L) DyLight 680 was provided by PEP per PRINT GmbH.

2.2. GP3 peptide chip and peptide scan

A total of 217 peptides with 15 aa length overlap that covers the full length of GP3 [without a signal peptide, the first 15 peptides (VVANSNATFCFWFPL)] were printed on the chip with 14 duplicated aas. Each peptide had two replicates on the chip. The interval FLAG peptides DYKDDDDKGG surrounded the target peptide points, and hemagglutinin (HA) peptides YPYDVPDYAG were used as control.

The peptide chip was blocked with Rockland blocking buffer MB-070 for 60 min prior to the first assay. Pre-staining of the peptide array was done using the secondary goat anti-swine IgG (H + L) DyLight 680 antibody at a dilution of 1:5000 and was used to investigate background interactions that could interfere with the subsequent main assays. Subsequent incubation of the peptide array with HP-PRRSV SY0608 specific serum at a dilution of 1:1000 in incubation buffer (PBS, pH 7.4 with 0.05% Tween 20 and 10% Rockland blocking buffer) was followed by staining with the secondary goat anti-swine IgG (H + L) DyLight 680 antibody; the results were then read-out at scanning intensities of 5 and 7 (red). After main assays, green or red fluorescence-labeled antibodies were reacted with FLAG peptides DYKDDDDKGG (green) and HA peptides (red). The results were then read-out at scanning intensities of 7. The control peptides were used to show the chip were homogenous staining (When the control peptides were homogenous staining, the results of targets peptides are comparable). Quantification of spot intensities and peptide annotations were done using the PepSlide® Analyzer.

A software algorithm breaks down fluorescence intensities of each spot into raw, foreground, and background signals, which calculates the standard deviation of the foreground median intensities. Averaged spot intensities for pre-staining with the secondary antibody and the main assay with the pig serum against the GP3 sequence from the N-terminus to the C-terminus were plotted to better visualize overall spot intensities and signal to noise ratios. Fluorescence intensity plots were made using Origin.Lab.Origin. V8.0 software.

2.3. GP3 and GP3 antigen region analysis

The GP3 amino sequence of SY0608 HP-PRRSV virus strain were blasted with Chinese type II PRRSV strain CH-1a and American type II PRRSV strain NVSL. Software MegAlin in DNA star was used to conduct the sequence alignment. Residues that differed from the consensus were marked with small boxes.

To highlight the strong reaction peptides with PRRSV-positive serum in GP3, boxes with different colors were used to indicate those peptides. Peptides with fluorescence intensity values higher than 1000 and lower than 3000 were marked with a pale yellow box. Fluorescence intensity values higher than 3000 were lined out with a pink box. Overlaying the boxes makes the color burn.

Based on peptide scan results and GP3 sequence analysis, one reaction activity antigen region (AR) from Y^{51} to S^{106} was identified in GP3. To further analyze this finding, a total of 68 strains of type II PRRSV GP3 AR Y^{51}–S^{106} sequence were downloaded from GenBank. The access numbers are listed in Table 1. The alignment was conducted using software MEGA 6, and some special sites were further marked using Microsoft PowerPoint.

HP-PRRSV virus strain SY0608 was compared with CH-1a and NVSL. The mutation amino sites were analyzed. The variation ratio was calculated to analyze different amino acids used in SY0608 and reference PRRSV strains. The variation ratio in AR was also calculated to analyze different aas in the special part between SY0608 and reference PRRSV strains. A proportion of these two values were used to evaluate the role of this region in HP-PRRSV virus variation.

3. Results

3.1. Peptide scan

Pre-staining of the peptide array was performed using the secondary goat anti-swine IgG (H + L) DyLight 680 antibody at a dilution of 1:5000 to investigate background interactions that could interfere with the subsequent main assays. Subsequent incubation of the peptide array with pig serum at a dilution of 1:1000 in incubation buffer was followed by staining with secondary goat anti-swine IgG (H + L) DyLight 680 antibody, and results were read-out at scanning intensities of 5 and 7 (red) (Figs. 1 and 2B).

Quantification of spot fluorescence intensity and peptide annotation was conducted with PepSlide® Analyzer and listed in the

Table 1
Reference strains used in this research.

Strain name	Access. number	Strain name	Access. number	Strain name	Access. number
CH-1a_1996	AY032626	CG2006	EU864231	CWZ-1-F3_2009	FJ889130
VR2332	AY150564	BJ_2007	EU825723	WUH1_2007	EU187484
CH2003	EU880440	GD_2007	EU109503	BJPG_2009	FJ950746
CH2002_	EU880438	07QN	FJ394029	LN	EU109502
HB-1(sh)2002	AY150312	HUB2	EF112446	HEB1_2006	EF112447
HB-2(sh)2002	AY262352	07NM	FJ393456	07HEN	FJ393457
CH2004	EU880439	GD2007	EU880433	BJSD_2009	FJ950747
PRRSV02	FJ175688	GDBY1_2009	GQ374442	GS2008	EU880431
PRRSV03	FJ175689	GDQJ_2009	GQ374441	SHH_2007	EU106888
BJ-4_1996	AF331831	CBB-1-F3_2009	FJ889129	BJsy06	EU097707
GS2002	EU880441	Henan-1_2007	EU200962	NM1	EU860249
GS2004	EU880443	HPBEDV_2009	EU236259	SX2007	EU880434
S1	DQ459471	07BJ	FJ393459	SX-09	HQ843181
PRRSV01	FJ175687	HUB1_2006	EF075945	SY0608	EU144079
QY2010	JQ743666	HuN	EF517962	TJ_2006	EU860248
CC-1_2006	EF153486	Jiangxi-3	EU200961	TP_2006	EU864233
SHB_2005	EU864232	Jsyx	EU939312	XH-GD_2007	EU624117
Em2007	EU262603	JX143	EU708726	XL2008	EU880436
SD-CXA_2008	GQ359108	JX2006	EU880432	YN2008	EU880435
APRRS_2009	GQ330474	JXA1	EF112445	HN1	AY457635
NB_04	FJ536165	JXwn06	EF641008	HN2007	EU880437
SX-1_2011	GQ857656	NX06	EU097706		

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