IDENTIFICATION OF NOVEL T CELL EPITOPE REPERTOIRE IN NSP1 AND GP3 OF THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS TYPE 2

Tran Thi Huong Giang[1], Phan Hong Dien[2], Dong Van Hieu[1*], Wen Bin Chung[2]

[1]Faculty of Veterinary Medicine, Vietnam National University of Agriculture
[2]Department of Veterinary Medicine, National Pingtung University of Science and Technology

Email[*]: dvhieuvet@vnua.edu.vn

Received date: 02.11.2015 Accepted date: 03.05.2016

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) has become one of the primary diseases affecting pig breeding worldwide. Although vaccines are available, the disease is still widespread and the virus is frequently reintroduced to pig farms. The development of an effective vaccine to control PRRSV outbreak is an imperative requirement because of a devastating economic impact on the swine industry. Therefore, it has become essential to understand what constitutes the basis for protective immunity in PRRSV infection when designing new PRRSV vaccines. We used bioinformatics to identify T-cell epitopes for PRRS virus vaccine development. PRRSV strain ATCC VR-2332 (U87392.3) was used as the reference virus in this study. To achieve this goal, 12 peptides spanning the sequence of PRRSV (U 87392.3) were screened using the IFN-γ ELISpot assay. These peptides were identified for their ability to elicit a recall INF-γ response from peripheral blood mononuclear cells (PBMCs) isolated from 3 pigs infected with PRRSV HF6-2. The results led to the identification of two peptides located in ORF1b and ORF3 that appear to contain T-cell epitopes. These findings might provide valuable information to develop new and more efficacious vaccines against PRRSV.

Keywords: PRRSV, Elispot, IFN-γ, T-cell epitope.

Xác định Epitope tế bào T trên NSP1 và GP3 của virus gây hội chứng rối loạn sinh sản và hô hấp ở lợn

TÓM TẮT

Virus gây hội chứng rối loạn sinh sản và hô hấp ở lợn (PRRSV) đã trở thành một trong những căn bệnh chính ảnh hưởng lớn tới ngành chăn nuôi lợn trên thế giới. Một số vắc xin đã được sử dụng để phòng và làm giảm ảnh hưởng của PRRS, tuy nhiên căn bệnh vẫn tồn tại và gây ảnh hưởng tới các trang trại chăn nuôi lợn. Phát triển một loại vắc xin trên cơ sở những hiểu biết về cơ chế miễn dịch qua trung gian tế bào nhằm kiểm soát PRRS là việc làm cấp bách và cần thiến hiện nay. Trong nghiên cứu này, chúng tôi đã sử dụng kỹ thuật tin sinh học để xác định các nhóm kháng nguyên tế bào T dựa trên chủng PRRSV ATCC VR-2332 (U87392.3). 12 trình tự a xít amin được sàng lọc bằng kỹ thuật IFN-γ ELISpot trong đáp ứng với tế bào PBMCs. Kết quả đã xác định được 2 peptide gồm 1b3 (YQLASYASYI) và GP3 (SVYAWLAFLSFSY) là epitop tế bào T. Đây có thể là một thông tin có giá trị trong việc phát triển một loại vắc xin có hiệu quả phòng PRRSV.

Từ khóa: PRRSV, Elispot , IFN-γ, nhóm quyết định kháng nguyên.

1. INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is one of the most significant and important diseases that causes economic losses to the swine industry worldwide (Keffaber, 1989; Wensvoort, 1993). It has been estimated to cost the swine industry in the USA alone over

560 million USD per year, primarily by causing severe reproductive failure in sows and increased mortality in young pigs (Neumann *et al.,* 2005). The syndrome was first identified in 1987 in North America. There was a similar clinical outbreak in Germany in 1990, and by 1991, the outbreaks were widespread throughout Europe (International Office of Epizootics, 1992). Clinical signs of infection that have been reported in affected pig herds include severe reproductive failure, post-weaning pneumonia, growth reduction, decreased performance, and increased mortality (Keffaber, 1989; Wensvoort, 1993). The first outbreak of PRRS in Vietnam was observed in 2007. The PRRSV isolates were grouped into the same subclade as the type II genotype (Youjun *et al.,* 2008).

Vaccination is one of the most important approaches in the prevention and control of PRRSV infection (Duarte *et al.,* 1994; Domingo *et al.,* 1998; Meng, 2000). When addressing this issue, it should be noted that the cell-mediated immune response is a critical component of host immunity to control PRRSV infection (Janeway *et al.,* 2001). Research on identifying T-cell epitopes in PRRSV is sparse and has been limited to structural proteins. Two distinct regions of PRRSV were identified to be the immunodominant T-cell epitopes based on elicitation of a significant IFN-γ response in almost half of the pigs tested (Vashisht *et al.,* 2008). Other results also showed that two regions on the GP5 amino acid sequence from PRRSV genotype I and II appeared to contain T-cell epitopes based on their ability to stimulate IFN-γ secreting cells (Diaz *et al.,* 2009). In addition, one report has recently identified four T-cell epitopes located on the membrane (M) protein of PRRSV (Wang *et al.,* 2011). Taken together, these studies demonstrate a high interest in achieving a more detailed picture of cell mediated protective immunity against PRRSV. The objective of this study is to screen T-cell epitopes to provide the basis for the development of new and more efficacious vaccines against PRRSV.

2. MATERIALS AND METHODS

2.1. Materials

Virus strain: The third passage of a Taiwanese field isolate, PRRSV strain HF6-7 (North American genotype; type II PRRSV) obtained in 2004, was used to immunize the pigs. The virus was cultured and propagated in porcine alveolar macrophages (PAMs). The PAMs were isolated by lung lavage from SPF pigs as previously described (Chang et al., 2008). The titer of the virus stock was 1x105.75 TCID50/mL. A PRRSV HF6-7 homologous strain that has been adapted to grow in the MARC-145 was used for the stimulation of PBMCs in the IFN-γ ELISpot assay.

2.2. Methods

2.2.1. Immunization of pig with PRRSV

Three specific pathogen free (SPF) pigs at six weeks of age were transferred from the SPF pig farm to the negative pressure house at National Pingtung University of Science and Technology. PRRSV HF6-7 at a dose of $5x10^5$ TCID50/mL was diluted with 2 mL medium before immunization. Pigs were intranasally challenged with 2 mL of PRRSV HF6-7 in each nostril for three consecutive days. These pigs continued to be challenged until the PRRSV antibodies titer was greater than $5log_2$.

2.2.2. Isolation of peripheral blood mononuclear cells (PBMCs)

Blood samples were collected from the jugular veins into tubes that contained 0.5 M Ethylenediamine tetraacetic acid (EDTA). PBMCs were isolated by density gradient centrifugation using histopaque (Sigma-Aldrich, MO, USA)_as previously described by Dong *et al.* (2015).

2.2.3. Identification of potential T-cell epitopes of PRRSV by bioinformatic methodology

An established systematic bioinformatics pipeline, called identification of cytotoxic T lymphocyte epitopes for swine viruses (ICES), from web resources (http://sb.nhri.org.tw/ICES) was utilized as a tool for the prediction of T-cell epitopes of type 2 PRRSV. In ICES, the stand-alone NetChop 3.1 was used to predict proteasomal cleavage sites of type 2 PRRSV sequences. By using the predicted cleavage sites of each PRRSV sequence, linear peptides in the range of 8- to 11-amino acid residues were generated by the system. The binding affinities of those peptides to swine leukocyte antigen (SLA) were then evaluated by the stand-alone NetMHCpan 2.4. A total of 45 SLA alleles were examined in NetMHCpan for the prediction of peptide-binding ability. Peptides with a binding affinity of <50 nM or a rank among the top 0.1% were compared with the PRRSV VR2332 reference sequence, aligned to the orresponding location, and subjected to the calculation of sequence conservation.

2.2.4. IFN-γ ELISpot Assay

A well established PRRSV-specific IFN-γ ELISpot assay for the evaluation of the T-cell epitopes has been reported and previously described by Vashisht *et al.* (2008) and Diaz *et al.* (2009). The procedure was conducted according to the manufacturer's instructions using the commercial ELISpot Assay Kit (MABTECH, OH, USA). The results were determined as the numbers of IFN-γ secreting cells per one million PBMCs. All tests were performed in duplicate.

2.3. Statistical analysis

The number of spots per one million PBMCs were presented as the mean ± standard error (SE). The evaluation of the test peptides was based on the comparison of 6 criteria values, including the mean±SE of the stimulation index, the maximum response, the total response, the average response, the number of response pigs, and the average response of responsive pigs for all tested peptides.

Table 1. Synthetic peptides used for the ELISpot assay

Peptide Name	Peptide sequence	ORF name	Gene bank accession number
M1	RFITSRCRLCLLGRK	ORF6	U87392.3
M-2	FTFGYMTF	ORF6	U87392.3
M-3	LTMGAVVALLW	ORF6	U87392.3
GP5	LIYNLTLC	ORF5	U87392.3
GP5-1	KGRIYRWRSPVIIE	ORF5	U87392.3
GP5-2	RYSCTRYTNFL	ORF5	U87392.3
1a	ATAPDGTY	ORF 1a	U87392.3
1b-1	IVYSDDLVLY	ORF1b	U87392.3
1b-2	CPGKNSFLD	ORF1b	U87392.3
1b-3	YQLASYASYI	ORF1b	U87392.3
GP2a	YLASRLPML	ORF2	U87392.3
GP3	SVYAWLAFLSFSY	ORF3	U87392.3

Table 2. Immunization of pigs with PRRSV

Pig number	Time immunizations	SN titer (x log_2)
1	5	5,0
2	5	5,5
3	5	6,0

Table 3. Identification of peptides containing T-cell epitopes of PRRSV

Stimulants (peptide)	Selection criteria				
	Maxi. Resp.[a]	Total resp.[b]	Avg. Resp.[c]	No. resp. pigs[d]	Avg SI[e]
M1	22	14.5 ± 1.05	2.41 ± 0.21	2	1.15 ± 0.12
M-2	9	11.5 ± 2.01	1.91 ± 0.09	3	0.81 ± 0.09
M-3	4	3.5 ± 0.78	0.58 ± 0.04	2	1.01 ± 0.03
GP5	7.5	2.5 ± 0.43	0.41 ± 0.06	3	0.91 ± 0.05
GP5-1	19	41.5 ± 2.56	6.91 ± 0.12	2	1.01 ± 0.05
GP5-2	17	61.5 ± 3.21	10.25 ± 1.91	3	1.12 ± 0.11
1a	26	65.5 ± 3.12	12.08 ± 1.11	3	1.38 ± 0.12
1b-1	8	17.5 ± 1.22	2.91 ± 1.85	3	1.08 ± 0.09
1b-2	24	59.5 ± 2.33	9.91 ± 0.21	3	1.11 ± 0.11
1b-3	36	94.5 ± 2.44	15.75 ± 1.05	3	2.15 ± 0.05
GP2a	29	71.5 ± 3.21	11.91 ± 1.15	3	1.33 ± 0.15
GP3	48	154.5 ± 3.22	25,75 ± 1.86	3	2.12 ± 0.11
PRRSV	33	89.11 ± 1.23	12.22 ± 0.15	3	2.05 ± 0.15

Note:

[a] *The maximum response is the number of IFN-γ secreting cells detected in PBMCs from the highest responder pig among all of the three PBMC samples tested (minus background).*

[b] *The total response is the sum of all of the IFN-producing cell (minus background) detected in the individually tested PBMCs samples.*

[c] *The average response is the sum of IFN-γ secreting cells detected in PBMCs of the 3 pigs tested (minus background) in duplicated cultures divided by 6.*

[d] *Number of responsive pigs is the number of pigs whose PBMC exhibited a peptide-specific IFN-γ response with a stimulation index ≥ 2.*

[e] *Average stimulation index is the average of the stimulation index of peptides with the 3 tested pigs.*

3. RESULTS AND DISCUSSION

3.1. Prediction of PRRSV T-cell epitopes by ICES

Based on the bioinformatics approach, a total of 12 T-cell epitopes with binding affinities of <50 nM or a rank among the top 0.1% were predicted from the conserved regions of all the available type 2 PRRSV genetic sequences (Table 1). Among the 12 predicted T-cell epitopes, 1, 3, 1, 1, 3 and 3 epitopes were located in ORF1a, ORF1b, ORF2, ORF3, ORF5 and ORF6, respectively (Table 1). No T-cell epitopes were predicted from ORF7 of type 2 PRRSV by ICES

3.2. Immunization of pigs with PRRSV

Serum neutralization (SN) antibody responses to PRRSV were detected in all three pigs within 2 weeks after immunization. Three SN titers from the pigs of $5log_2$, $5,5log_2$ and

$6\log_2$ were obtained for all pigs after five immunizations (Table 2). The PBMCs isolated from the successfully immunized pigs were then used in the ELISpot.

3.3. Identification of predicted PRRSV T-cell epitopes

To identify the PRRSV specific T-cell epitopes, the capacity of the predicted T-cell epitopes to elicit the recall IFN-γ by PBMCs from the 3 pigs infected with PRRSV were determined by ELISpot. The immunodominant T-cell epitopes were identified based on the criteria defined by Vashisht *et al.* (2008), including the maximum response, the total response, the average response, number of responsive pigs, and average stimulation index.

A total of 12 peptides were synthesized according to the predicted T-cell epitopes sequences and screened by ELISpot assay. The test results are summarized in Table 2 and Figure 1. The mean ± SE of the maximum response, the total response, the average response, the number of responsive pigs, the stimulation index for the positive control were 33, 89.11± 1.23, 12.22± 0.15, 3, and 2.05± 0,15, respectively. Among the 12 synthesized peptides, two peptides were found to fit all 5 criteria with values higher than the value of the positive control mentioned in the screening criteria (Table 3). These two peptides, 1b-3 (YQLASYASYI) and GP3 (SVYAWLAFLSFSY), were located in ORF1b and ORF3. They were considered to contain T-cell epitopes.

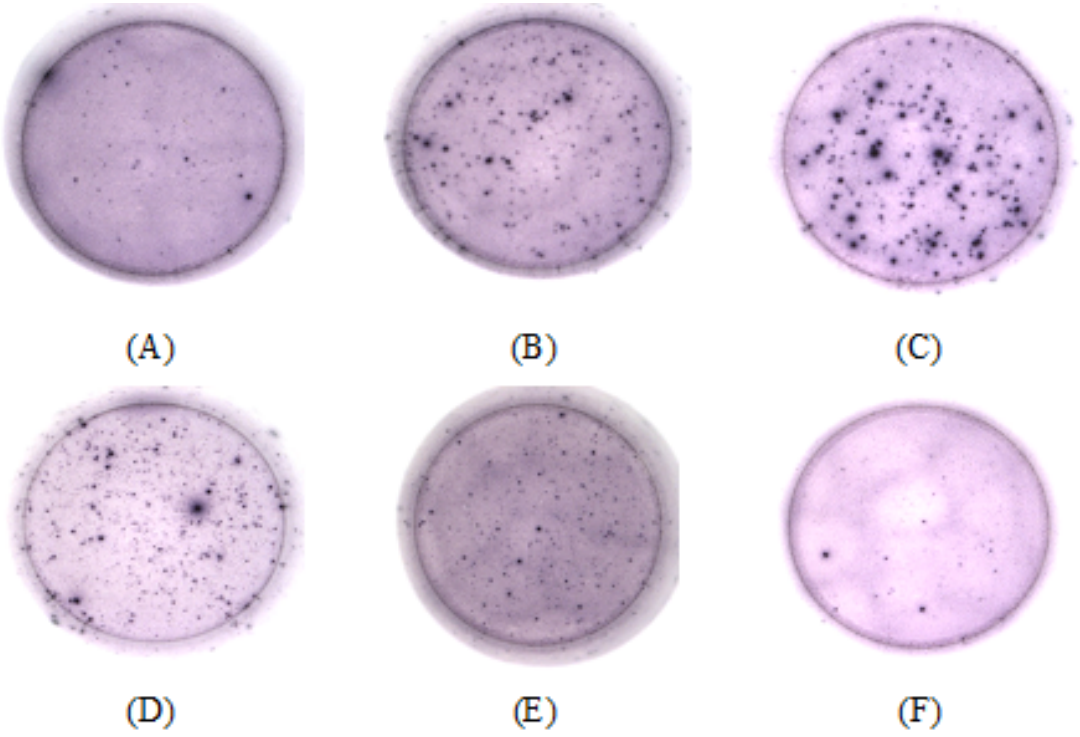

Figure 1. Porcine reproductive and respiratory syndrome virus (PRRSV) with specific INF-γ for the identification of PRRSV T-cell epitopes

Note: Peripheral blood mononuclear cells were isolated from PRRSV-challenged pigs and stimulated with culture medium (A), Concanavalin A (B), PRRSV (C), and synthetic peptides of predicted T-cell epitopes with high (D), moderate (E) and low (F) responses

Neutralizing antibodies as well as cell-mediated immunity play key critical roles in the establishment of PRRSV protective immunity (Lopez and Osorio, 2004; Kimman et al., 2009). The approach of the present work permitted the preliminary identification of immunodominant, T-cell epitopes in ORFs 1-6 of genotype II PRRSV. The PRRSV NSP1, especially NSP1β has been shown to suppress the interferon signaling pathway and IFN-β synthesis (Beura *et al.*, 2010; Chen *et al.*, 2010). However, Song *et al.* (2012) developed six monoclonal antibodies (MAbs) against PRRSV NSP1 and identified three new epitopes (54-59 aa, 157-163 aa and 185-232 aa) in NSP1α and NSP1β. According to several sources, GP3 is highly antigenic (Hedges *et al.*, 1999). A previous paper identified two B-cell epitopes on GP3 spanning regions 60-85 aa and 243-250 aa in the 111/92 strain from a phage display library (Oleksiewcz *et al.*, 2001; Oleksiewcz *et al.*, 2002). However, all of the epitopes on GP3 identified previously were based on the European type viruses. For the North American type viruses, no epitopes have been found until now (Zhou et al., 2006). As a result of this experiment, two peptides in NSP1 and GP3 were confirmed to contain T-cell epitopes. PRRSV (American genotype) has caused high morbidity and mortality in pig production in Taiwan as well as Vietnam (Chun *et al.*, 2008; Youjun *et al.*, 2008). These findings will contribute to the further understanding of the interaction between antigen and specific T-cells, and the development of efficacious vaccines against PRRSV type 2 in Vietnam.

REFERENCES

Chun W, Fan L, Tien-Shine H, Chu-Hsiang P, Ming-Hwa J, Parn-Hwa C (2008). Genetic variation in open reading frame 5 gene of porcine reproductive and respiratory syndrome virus in Taiwan. J. Vet Micro, 131: 339-347.

Beura LK, Sarkar SN, Kwon B, Subramaniam S, Jones C, Pattnaik AK, Osorio FA (2010). Porcine reproductive and respiratory syndrome virus nonstructural protein 1beta modulates host innate immune response by antagonizing IRF3 activation. J. Virol., 84: 1574-1584.

Chen Z, Lawson S, Sun Z, Zhou X, Guan X, Christopher-Hennings J, Nelson EA, Fang Y (2010). Identification of two auto-cleavage products of nonstructural protein 1 (nsp1) in porcine reproductive and respiratory syndrome virus infected cells: nsp1 function as interferon antagonist. Virology, 398: 87-97.

Diaz I, Pujols J, Ganges L, Gimeno M, Darwich L, Domingo M, and Mateu E (2009). In silico prediction and ex vivo evaluation of potential T-cell epitopes in glycoproteins 4 and 5 and nucleocapsid protein of genotype-I (European) of porcine reproductive and respiratory syndrome virus. Vaccine, 27: 5603-5611.

Domingo E, Baranowski E, Ruiz-Jarabo CM, Martin-Hernandez AM, Saiz JC, and Escarmis C (1998). Quasispecies structure and persistence of RNA viruses. Emerging infectious diseases, 4: 521-527.

Duarte EA, Novella IS, Weaver SC, Domingo E, Wain-Hobson S, Clarke DK, Moya A, Elena SF, de la Torre JC, and Holland JJ (1994). RNA virus quasispecies: significance for viral disease and epidemiology. Infectious agents and disease, 3: 201-214.

Hedges JF, Balasuriya UB, and MacLachlan NJ (1999). The open reading frame 3 of equine arteritis virus encodes an immunogenic glycosylated, integral membrane protein. Virology, 264: 92-98.

International Office of Epizootics. World Animal Health 1991 (1992). Animal Health Status and Disease Control Methods (Part One: Report), VII(2): 126.

Janeway CA, Travers P, Walport M, Shlomchik MJ (2001). Antigen Presentation to T Lymphocytes. Immunobiology: The Immune System in Health and Disease 5th edition, pp. 169-202.

Keffaber KK (1989). Reproductive failure of unknown etiology. Am Assoc Swine Prac News, 1(2): 1-9.

Kimman TG, Cornelissen LA, Moormann RJ, Rebel JM, and Stockhofe-Zurwieden N (2009). Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine, 27: 3704-3718.

Lopez OJ, and Osorio FA (2004). Role of neutralizing antibodies in PRRSV protective immunity. Veterinary immunology and immunopathology, 102: 155-163.

Meng XJ (2000). Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. Veterinary microbiology, 74: 309-329.

Song Y, Zhou Y, Li Y, Wang X, Bai J, Cao J, Jiang P (2012). Identification of B-cell epitopes in the NSP1 protein of porcine reproductive and respiratory syndrome virus. Veterinary Microbiology, 155: 220-229.

Vashisht K, Goldberg TL, Husmann RJ, Schnitzlein W, and Zuckermann FA (2008). Identification of immunodominant T-cell epitopes present in glycoprotein 5 of the North American genotype of porcine reproductive and respiratory syndrome virus. Vaccine, 26: 4747-4753.

Wang YX, Zhou YJ, Li GX, Zhang SR, Jiang YF, Xu AT, Yu H, Wang MM, Yan LP, and Tong GZ (2011). Identification of immunodominant T-cell epitopes in membrane protein of highly pathogenic porcine reproductive and respiratory syndrome virus. Virus research, 158: 108-115

Wensvoort G (1993). Lelystad virus and the porcine epidemic abortion and respiratory syndrome. Veterinary research, 24: 117-124.

Zhou YJ, An TQ, He YX, Liu JX, Qiu HJ, Wang YF, and Tong G (2006). Antigenic structure analysis of glycosylated protein 3 of porcine reproductive and respiratory syndrome virus. Virus research, 118: 98-104.

Youjun F, Tiezhu Z, Tung N, Ken I, Ying Ma, Thi HN, Van CN, Di L, Quang AB, Long TT, Chuanbin W, Kegong Ti, and George FG (2008). Porcine Respiratory and Reproductive Syndrome Virus Variants, Vietnam and China in 2007, Emerg Infect Dis. Nov., 14(11): 1774-1776.