Vaccine 35 (2017) 4408–4413

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Relative contribution of porcine reproductive and respiratory syndrome virus open reading frames 2–4 to the induction of protective immunity

Kathryn Kimpston-Burkgren [a,b], Ignacio Correas [a,c], Fernando A. Osorio [a,c], David Steffen [c], Asit K. Pattnaik [a,c], Ying Fang [e], Hiep L.X. Vu [a,d,*]

[a] Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE, United States
[b] School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE, United States
[c] School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE, United States
[d] Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, United States
[e] Departement of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, United States

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 13 June 2017
Accepted 15 June 2017
Available online 6 July 2017

Keywords:
PRRSV
Minor glycoproteins
Chimeric virus
Immunization-challenge experiment

ABSTRACT

The minor glycoproteins (GPs) of PRRSV, GP2, GP3, and GP4, form a heterotrimer that is required for viral infectivity, presumably due to its interaction with the key cellular receptor CD163. These 3 GPs are encoded by open reading frames (ORFs) 2a, 3 and 4 (herein referred to as ORFs 2–4), respectively. The goal of this study was to investigate the immunogenicity of the PRRSV-2 minor GPs. Through the use of reverse genetics, a chimeric virus (designated SDFL24) was constructed by replacing ORFs 2–4 of the PRRSV-1 strain SD01-08 with the corresponding genes of the PRRSV-2 strain FL12. While the parental PRRSV strain SD01-08 was not neutralized by convalescent antisera raised against FL12, the chimeric virus SDFL24 gained susceptibility to neutralization by FL12-specific antisera, indicating that viral proteins encoded by ORFs 2–4 are targets of antibody neutralization. When inoculated into pigs, the chimeric virus SDFL24 elicited T-cell responses against peptides derived from FL12 minor GPs, whereas the parental virus SD01-08 did not. After challenge infection with FL12, pigs previously infected with SDFL24 developed robust kinetics of FL12-specific neutralizing antibodies as compared to those previously infected with the parental strain SD01-08. Finally, the pigs recovered from SDFL24 infection were better protected from a subsequent challenge infection with FL12 than those previously infected with SD01-08. Collectively, the results indicate that PRRSV-2 ORFs 2–4 are capable of inducing protective immunity.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of porcine reproductive and respiratory syndrome, a major disease of swine which causes respiratory disease in young pigs and reproductive failure in sows and gilts [1,2]. PRRSV is an enveloped, positive sense, single stranded RNA virus with a genome of approximately 15 kb (Reviewed in [3]). The PRRSV genome contains at least 10 open reading frames (ORFs). ORFs 1a and 1b encode 2 poly-proteins pp1a and pp1ab which are proteolytically processed to produce functional nonstructural proteins responsible for the transcription and replication of the viral genome [4]. ORFs 2a, 3 and 4 encode three minor glycoproteins (GPs) namely GP2, GP3, and GP4 (Reviewed in [5]) which form a heterotrimer dispensable for viral particle formation, yet required for viral infectivity [6,7]. ORFs 5, 6, and 7 encode three major structural proteins namely GP5, M, and N (Reviewed in [5]). GP5 and M interact with each other to form a heterodimer required for viral particle formation [6]. N protein encapsidates the viral RNA genome. ORF2b is embedded within ORF2a and encodes the envelop protein E which is required for viral uncoating, presumably due to its ion channel activity [8]. ORF5a encodes a small protein whose functions remain unknown [9].

PRRSV is classified into 2 types: PRRSV-1 (European) and PRRSV-2 (North American). These 2 types share only approximately 60% sequence similarity [10]. Currently, both types co-circulate in many swine producing countries, especially in Asia [11]. Vaccines for both types are commercially available. Due to the substantial genetic difference, vaccines for PRRSV-1 do not provide significant protection against PRRSV-2 and vice versa [12,13]. Currently, modified live virus (MLV) vaccines are considered the most effective vaccines. Pigs vaccinated with MLV vaccine often

* Corresponding author at: Nebraska Center for Virology and Dept. of Animal Science, 4240 Fair Street, MOLR110, Lincoln, NE 68583-0900, United States.
E-mail address: hiepvu@unl.edu (H.L.X. Vu).

http://dx.doi.org/10.1016/j.vaccine.2017.06.061
0264-410X/

develop solid homologous immune responses [14]. However, the mechanisms of immune protections remain incompletely understood. Likewise, the viral targets of the protective immunity are not well understood.

PRRSV is known to infect only pigs. Macrophages are the main target of PRRSV infection *in vivo* [15]. Multiple cellular receptors have been suggested to be involved in the entry of PRRSV into porcine macrophages (Reviewed in [16]). Of these, sialoadhesin and CD163 have been studied most extensively. Sialoadhesin interacts with the GP5-M heterodimer and facilitates viral attachment and internalization [17]. Nevertheless, expression of sialoadhesin alone in a non-permissive cell line is not sufficient for viral infection [18]. Additionally, transgenic pigs lacking sialoadhesin are susceptible to infection by PRRSV [19]. CD163 interacts with the GP2-4 heterotrimer and this interaction is critical for viral infectivity [6,7]. Cells that are resistant to PRRSV infection can be made susceptible if CD163 is transfected into the cells [20]. Importantly, transgenic pigs lacking CD163 are resistant to PRRSV infection [21].

Since the interaction between the minor GPs and cellular receptor CD163 is critical for viral infectivity and host cell tropism, it has been hypothesized that the minor glycoproteins might be important for the induction of protective immunity [7,22]. Antigenic regions containing neutralizing epitopes have been described in each minor GP [23,24]. Several studies have been conducted to characterize the immunogenicity of the minor GPs in pigs [25,26]. These studies relied on using viral expression vectors to deliver the genes encoding the minor GPs. The major limitation of the viral vector approach is that the proteins might not be expressed in their native forms, leading to sub-optimal induction of immune responses. In the present study, we used the PRRSV-1 strain SD01-08 as a backbone to construct a chimeric virus bearing ORFs 2–4 of PRRSV-2 strain FL12. Because there is minimal cross-protection between PRRSV-1 and PRRSV-2, this chimeric virus can serve as a tool to elucidate contributions of PRRSV-2 minor GPs to the development of protective immunity in the context of viral infection.

2. Materials and methods

2.1. Cells, viruses, antibodies, and peptides

MARC-145 (monkey kidney) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at 37 °C with 5% CO. The PRRSV-1 and PRRSV-2 infectious cDNA clones pSD01-08 and pFL12 were reported previously [27,28]. Convalescent antisera raised against FL12 were obtained from a previous study [29]. The monoclonal antibody (MAb) SDOW17 was purchased from the National Veterinary Services Laboratory (Ames, IA). The Alexafluor-488 conjugated donkey anti-mouse antibody was purchased from Invitrogen. Peptide pools that contained peptides spanning the entirety of GP2, GP3, GP4, and N of F12 were a generous gift from Dr. Kaltenboek, Auburn University. The peptides are 20 amino acids in length, and overlap by 10 amino acids. Each peptide in the pool was dissolved in DMSO at the concentration of 40 nmol/mL.

2.2. Generation of the chimeric PRRSV strain SDFL24

The plasmid pSD01-08 was digested with the restriction enzyme PasI (Thermo Scientific) which cut the plasmid at 2 sites, 10,279 (ORF1b) and 14,254 (ORF6). Three overlapping DNA fragments were generated by PCR using primers listed in Table S1. Fragment 1 was amplified from pSD01-08, from the first PasI site to the end of ORF1b. Fragment 2 was amplified from pFL12, from the beginning of ORF2a to the end of ORF4. Fragment 3 was amplified from pSD01-08, from the start of ORF5 to the second PasI site. After purification, the digested pSD01-08 and the 3 PCR fragments were mixed together in the presence of the HiFi DNA Assembly Master Mix (New England Biolabs), per the manufacturer's protocol. Positive clones were confirmed by digestion and DNA sequencing. The resulting cloned chimeric cDNA genome was designated pSDFL24. This cloned cDNA genome is driven by the human cytomegalovirus (CMV) immediate early promoter; thus, infectious virus can be recovered by transfection of the plasmid to MARC-145 cells. MARC-145 cells seeded 24 h earlier in a 6-well plate were transfected with 1 μg pSDFL24 plasmid using the TransIT-X2 (Mirus Bio). Transfected cells were monitored daily. Once obvious cytopathic effects (CPE) were observed (day 5 post-transfection), culture supernatant containing infectious virus was collected and transferred to naïve MARC-145 cells to generate virus stocks for future studies.

2.3. Assessment of protection in pigs

Three-week old, PRRSV-seronegative pigs were randomly allocated into 3 groups of 6 pigs each and accommodated in separate biosecurity level 2 rooms at the Life Sciences Annex, University of Nebraska-Lincoln. After 1 week of acclimation, pigs in group 1 were injected intramuscularly with 2 mL of phosphate buffer saline (PBS) whereas pigs in groups 2 and 3 were separately inoculated intramuscularly with 2 mL inocula containing $10^{5.0}$ tissue culture infectious dose 50 ($TCID_{50}$) SD01-08 or SDFL24. At day 56 post-infection (p.i.), all pigs were challenged intramuscularly with 2 mL inoculum containing $10^{5.0}$ $TCID_{50}$ FL12. Blood samples were taken at various time-points post-infection, and serum samples and peripheral blood monocytes (PBMCs) were extracted and properly preserved for evaluation of viremia and immune responses. At day 14 post challenge (p.c.), pigs were euthanized and necropsied. Tonsils and inguinal lymph nodes were collected and frozen immediately (−80 °C) for evaluation of viral loads. Samples of lung were collected and fixed in 10% formalin for evaluation of lung pathology. RNA from sera was extracted using the QIAamp Viral RNA Kit (Qiagen). RNA from tissue samples was extracted using TRIzol reagent (Life Technologies). Viral RNA was quantified using a commercial qRT-PCR kit (Tetracore). All work was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee protocol number 930.

2.4. Immunological assays

Antibodies against the viral N protein were measured by using the IDEXX PRRS X3 Ab Test (IDEXX Laboratories). Serum-virus neutralization assays were performed as previously described [30]. Neutralization titers are expressed as the reciprocal of the highest dilution that showed a 90% reduction in the number of fluorescent foci presenting in control wells. The ELISpot assay was used to measure the frequency of interferon-gamma secreting cells (IFN-γ SCs) [31]. Briefly, two replicates of 5×10^5 PBMCs/well from each animal were plated in a 96-well filtration plate (Merck Millipore). Cells in each well were stimulated with peptide pools diluted 1:100 in culture medium (final peptide concentration 0.4 nmol/mL). DMSO diluted 1:100 in media was used as negative control, and PMA (10 ng/mL) and Ionomycin (1 μg/mL) as positive control. Spots were counted and analyzed using a CTL ImmunoSpot counter (Cellular Technology).

2.5. Statistical analysis

Viral RNA copy numbers and antibody neutralization titers were transformed to $\log_{10}$ and $\log_2$ values, respectively, prior to statistical analysis. Multiple-step growth curves and viral RNA in

serum were analyzed by two-way analysis of variance (ANOVA). Viral loads in tissues and lung microscopic scores were analyzed by one-way ANOVA. The Tukey-Kramer test was used for multiple comparisons. Data were considered significantly different when $p \leq 0.05$. All statistical analysis was done using GraphPad Prism version 7.0b for Mac OS X (GraphPad Software).

3. Results

3.1. A chimeric PRRSV-1 genome bearing ORFs 2–4 of a PRRSV-2 strain is fully infectious

Using the infectious cDNA clone pSD01-08 (PRRSV-1) as a backbone, we constructed a chimeric cDNA genome bearing ORFs 2–4 of FL12, a PRRSV-2 strain. The resulting plasmid was designated as pSDFL24. To determine if the chimeric genome is infectious, we transfected MARC-145 cells with the pSDFL24 plasmid. By day 5 post-transfection, CPE was observed in pSDFL24-transfected cells, indicating that the chimeric genome SDFL24 was fully infectious. MARC-145 cells infected with the chimeric virus SDFL24 reacted specifically with the MAb SDOW17 (Fig. 1A). The chimeric virus SDFL24 replicated less efficiently in MARC-145 cells when compared to its parental strains SD01-08 and FL12 (Fig. 1B). The highest titer of SDFL24 was $10^{4.2}$ $TCID_{50}$/mL while the peak titers of FL12 and SD01-08 were $10^{6.5}$ and $10^{5.8}$ TCID50/mL, respectively.

3.2. The chimeric virus SDFL24 is neutralized by FL12 antisera

Once the chimeric virus SDFL24 was generated, we wanted to evaluate its susceptibility to neutralization by convalescent antisera raised against FL12. As shown in Table 1, the chimeric virus SDFL24 was neutralized by FL12-antisera at a mean titer of 1:32 whereas the parental virus SD01-08 was not neutralized by the same set of antisera. The results clearly indicated that ORFs 2–4 encoded proteins are targets of neutralizing antibodies (NAbs). However, the neutralizing titers of the FL12-specific antisera against the chimeric virus SDFL24 were significantly lower than those against FL12, suggesting that other viral proteins are also important in the production of NAbs.

3.3. The chimeric virus SDFL24 replicated efficiently in pigs and confers partial protection against FL12

From day 2 p.i., viral RNA was detected in all pigs infected with SD01-08 or SDFL24 (Fig. 2A). By day 21 p.i., all pigs infected with SD01-08 or SDFL24 seroconverted, as determined by a standard diagnostic ELISA kit (Fig. 2B). Together, the results indicated that the chimeric virus SDFL24 replicates efficiently in pigs.

Table 1
Susceptibility to neutralization by FL12-convalescent antisera.

Antisera[a]	Neutralization titers measured against		
	SD01-08	SDFL24	FL12
1	<1:4	1:16	1:64
2	<1:4	1:8	1:128
3	<1:4	1:16	1:128
4	<1:4	1:512	1:1024
Geometric mean	<1:4	1:32	1:181

[a] Antisera were collected from 4 different pigs at day 49 post-infection with the PRRSV strain FL12.

Since the chimeric virus SDFL24 carries the minor GPs of FL12, we wanted to determine if this chimeric virus can provide better protection against FL12 than its parental strain SD01-08. At day 56 p.i., when the pigs were clinically recovered from the initial infection, they were challenged by intramuscular inoculation with FL12 with a dose of 10^5 $TCID_{50}$ per pig. After challenge infection, pigs in the SDFL24 group had significantly lower levels of viremia than those in the SD01-08 and PBS groups (Fig. 3A). The SD01-08 group had lower levels of viremia than the PBS group but higher than the SDFL24 group. Despite the significant difference in the levels of viremia, there was no significant difference in the viral load in tissues among the 3 groups at day 14 p.c. (Fig. 3B). Likewise, there was no significant difference in lung pathology scores among the 3 groups (Fig. 3C). Collectively, the data indicated that immunization of pigs by infection with the chimeric virus SDFL24 can provide partial protection against challenge with FL12.

3.4. Neutralizing antibody and T-cell responses against FL12

Anti-FL12 NAbs started to appear in one pig of the SDFL24 group at day 49 and 56 p.i. whereas no pigs in the SD01-08 group had detectable levels of FL12-specific NAbs at these two sampling dates (Fig. 4A). By day 64 p.i., (day 8 p.c.), all pigs in the SDFL24 group developed high titers of anti-FL12 NAbs and the neutralization titers increased until termination of the study. In contrast, FL12-specific NAbs were not detected in the SD01-08 group until day 70 p.i (day 14 p.c.) (Fig. 4A).

We used 4 peptide pools derived from GP2, GP3, GP4, and N protein of FL12 to evaluate the frequency of FL12-specific IFN-γ SCs. Before infection (day 0 p.i.), all pigs had only basal levels of

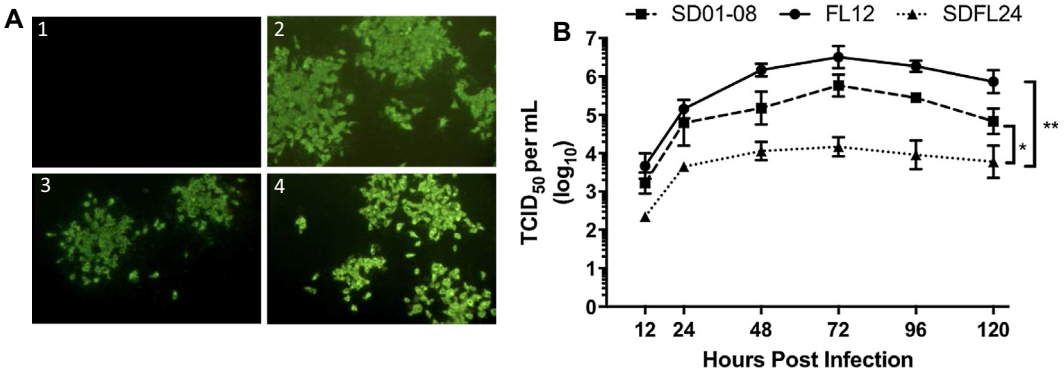

Fig. 1. Replication in cell culture. (A) Indirect immunofluorescence assay. MARC-145 cells were infected with the indicated PRRSV strains. At 48 h p.i., cells were fixed and immunostained with the monoclonal antibody SDOW17 which reacts with PRRSV N protein. (1) Negative control (2) FL12 (3) SD01-08 (4) SDFL24. (B) A multi-step growth curve. MARC-145 cells were infected with the indicated PRRSV strains at a multiplicity of infection of 0.1. At various time-points p.i., samples were harvested for virus titration. Data are expressed as means and standard error of mean (SEM) calculated from 3 independent experiments. Asterisks indicate statistical difference between treatment groups, as determined by two-way ANOVA. $^{*}p < 0.05$; $^{**}p < 0.01$.

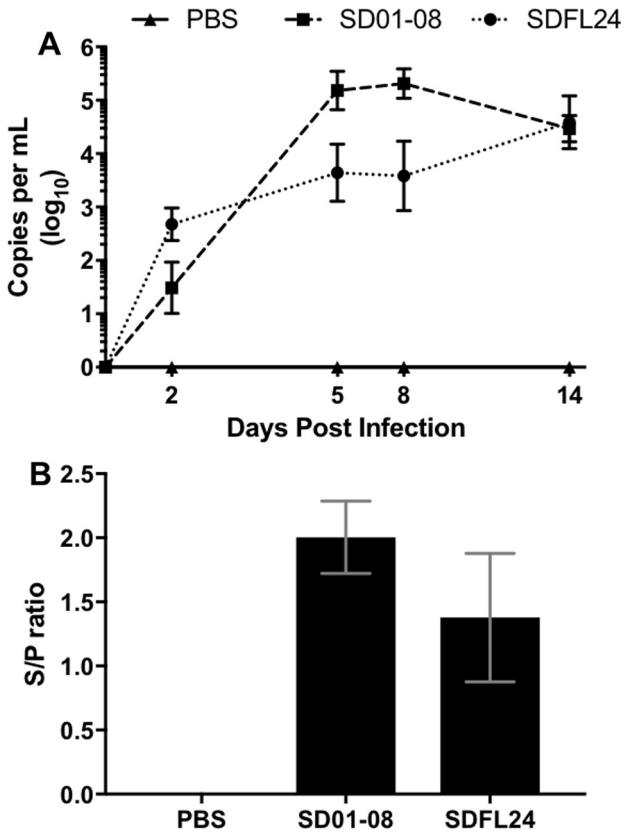

Fig. 2. Infectivity in pigs. Pigs were inoculated intramuscularly with PBS or with $10^{5.0}$ $TCID_{50}$ of either SD01-08 or SDFL24. At various time-points p.i., serum samples were collected for evaluation of viremia and antibody responses. (A) Viral RNA in serum as quantitated by qRT-PCR. (B) Levels of antibodies against viral N protein measured at day 21 p.i. Data are expressed as means and SEM.

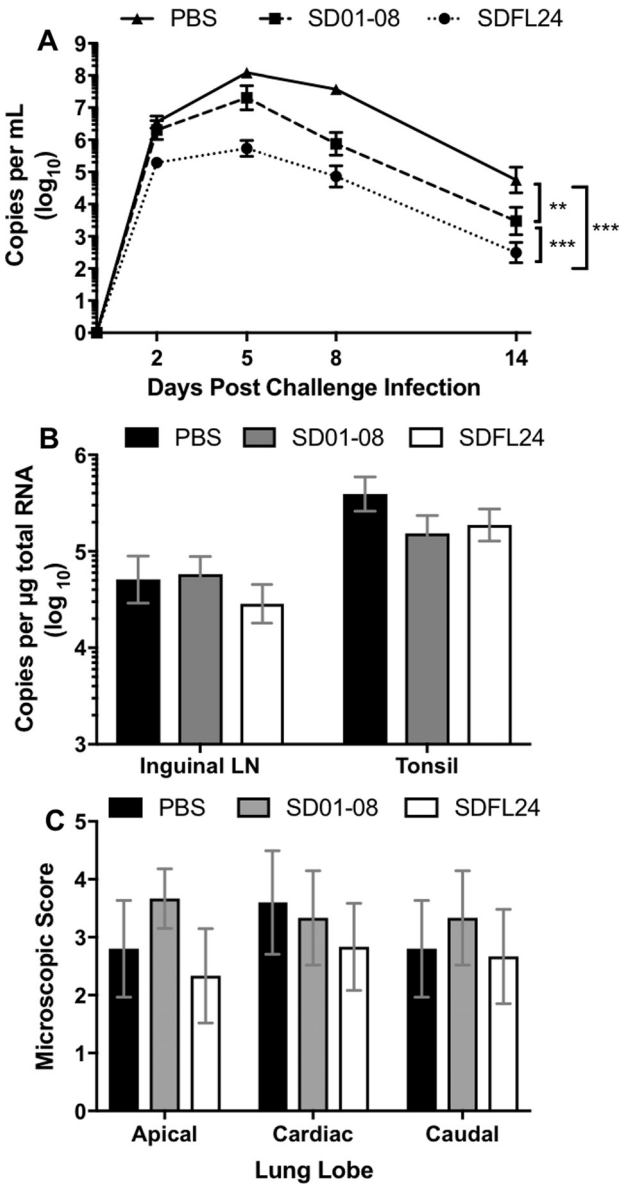

Fig. 3. Protection against challenge infection with FL12. Pigs were inoculated with PBS or SD01-08 or SDFL24 as described in the legend for Fig. 2. At day 56 p.i., all pigs were challenged by inoculation intramuscularly with 10^5 $TCID_{50}$ FL12. (A) Level of viral RNA in serum measured by qRT-PCR. Data are expressed as means and SEM calculated from 6 pigs. Asterisks indicate statistical difference between treatment groups, as determined by two-way ANOVA. $^{**}p < 0.01$; $^{***}p < 0.001$. (B) Levels of viral RNA in inguinal lymph node (LN) and tonsil at day 14 post-challenge infection. Data are expressed as means and SEM calculated from 6 pigs. (C) Microscopic scores of indicated lung lobes.

FL12-specific IFN-γ SCs (Fig. 4B). At day 56 p.i. (before challenge infection), pigs in the SDFL24 group had significantly greater numbers of IFN-γ SCs against FL12 GP2, GP3, and GP4 than those in SD01-08 and PBS groups (Fig. 4C). At day 70 p.i. (day 14 p.c.), the frequencies of IFN-γ SCs against FL12 peptides were increased in all three groups of pigs but the SDFL24 group still had greater numbers of IFN-γ SCs against GP2, GP3, and GP4 than the SD01-08 and PBS groups (Fig. 4D). The number of IFN-γ SCs against FL12 N protein was not significantly different among the three groups throughout the course of this experiment. The results indicate that pigs infected with the chimeric virus SDFL24 elicit T-cell responses against FL12 GP2, GP3 and GP4. Together, these results suggest that ORFs 2–4 contribute protection based on both cell-mediated and humoral immune responses.

4. Discussion

Pigs infected with wild-type PRRSV strains or vaccinated with MLV vaccines often develop solid levels of homologous immunity [32,33]. However, the viral proteins involved in the induction of protective immunity are not well characterized. The viral minor GPs interact with the receptor CD163 and this interaction is indispensable for the viral infectivity [6,7]. It is therefore hypothesized that these minor GPs might be critical for the induction of protective immunity. Through the use of different expression systems, it has been demonstrated that the viral proteins encoded by ORFs 2–4 elicit NAbs and T-cell responses [25,26]. However, the relative contribution of the minor GPs to the overall levels of protection remains poorly characterized. A major limitation of using viral vectors to deliver the minor GPs is that the proteins may not be expressed in their native structures. Consequently, the immune responses to the vector vaccines may not resemble the immune responses obtained through the use of MLV vaccines.

In the present study, we attempted to deliver the minor GPs in their biological forms. It has been shown that PRRSV-1 strains elicit only minimal levels of protection against PRRSV-2, mainly due to the substantial genetic distances between the 2 genotypes [12]. Therefore, we used the PRRSV-1 strain as a vector to deliver the genes encoding the minor GPs of a PRRSV-2 strain to determine the contribution of these minor GPs to protection. Based on the infectious cDNA clone pSD01-08 (PRRSV-1), we constructed a chimeric PRRSV virus carrying ORFs 2–4 of FL2 (PRRSV-2). The chimeric cDNA genome is fully infectious, demonstrating that the minor GPs are expressed in their native forms.

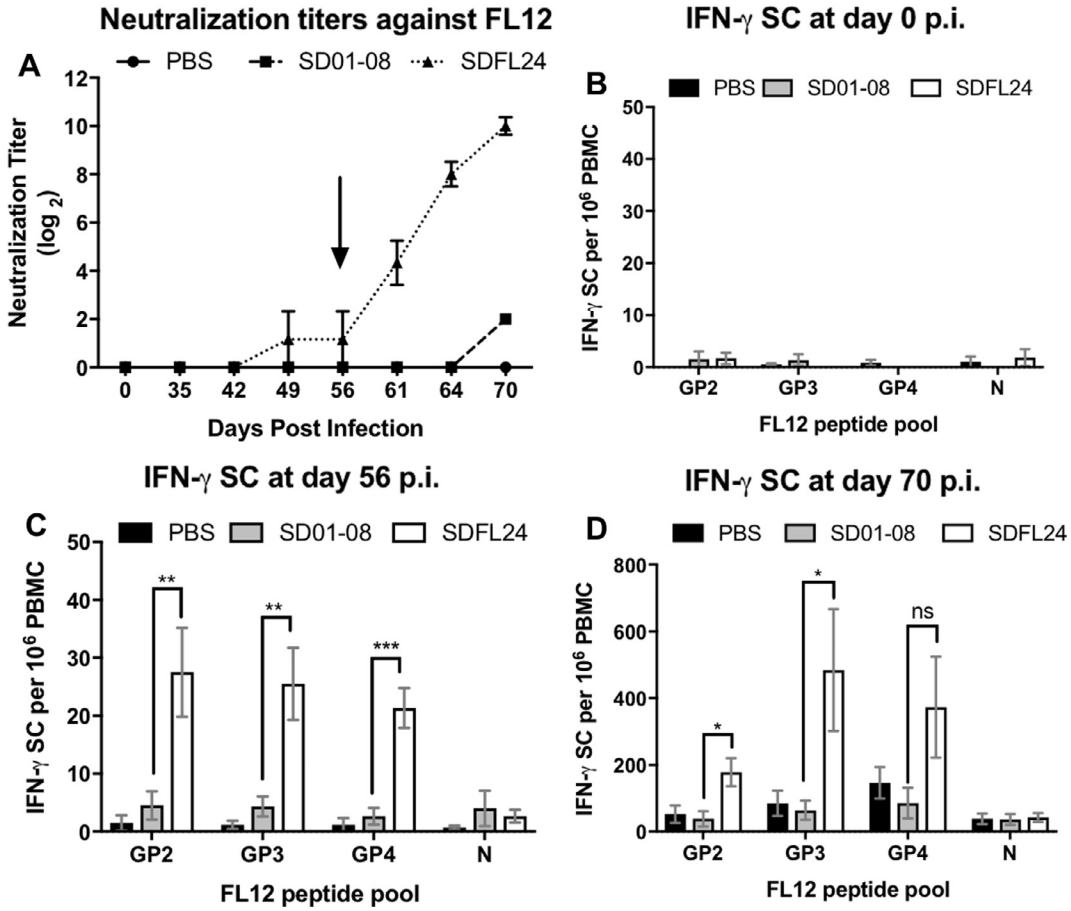

Fig. 4. Immune responses against FL12. (A) Kinetics of neutralizing antibody titers measured against FL12. The arrow at day 56 p.i. indicates the challenge infection date. (B-D) Frequencies of IFN-γ SC specific to the indicated FL12 peptide pools. Data are expressed as means and SEM. Asterisks indicate statistical difference between treatment groups. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

The chimeric virus SDFL24 is neutralized by convalescent antisera raised against FL12 whereas its parental strain SD01-08 is not neutralized by the same set of antisera, unequivocally demonstrating that the ORFs 2–4 encoded proteins can elicit NAbs. However, SDFL24 is neutralized by the FL12 sera to a lesser extent than the homologous virus, indicating that other viral proteins are involved in antibody neutralization. This observation is expected as it has been demonstrated that GP5 and M protein also carry neutralizing epitopes [34]. Since the chimeric virus SDFL24 carries GP5 and M of SD01-08, it would be interesting to measure the susceptibility of SDFL24 with antisera raised against SD01-08 as the results of this assay will provide information about the contribution of GP5 and M to the antibody neutralization. Unfortunately, we did not have anti-SD01-08 sera with good neutralizing titers. The 6 pigs that were infected with the SD01-08 in this study elicited only a titer of 1:8 against the homologous virus, prior to challenge with FL12. These 6 anti-sera did not neutralize SDFL24.

When inoculated to pigs, the chimeric virus SDFL24 replicated as efficiently as its parental strain SD01-08 (Fig. 2A). These 2 groups had similar levels of FL12-specific NAbs up to day 56 p.i.. After challenge infection with FL12, however, pigs in the SDFL24 group developed robust levels of FL12-specific NAbs whereas those in the SD01-08 group did not (Fig. 4A). One plausible explanation is that pigs in the SDFL24 group had been primed with the FL12 proteins. After challenge infection with FL12, these pigs developed anamnestic immune responses leading to the robust levels of FL12-specific NAbs. Our explanation is supported by the observation that before challenge infection with FL12 (day 56 p.i.), pigs in the SDFL24 group already had high frequencies of IFN-γ SCs specific to the FL12 minor GPs (Fig. 4B).

In the conditions of our study, the FL12 minor GPs can only elicit partial levels of protection. It is apparent that other viral proteins are involved in the induction of protective immunity. Collectively, results of this study may benefit future vaccine development.

Acknowledgments

We thank Bernhard Kaltenboeck for providing us with the FL12 peptide libraries and the staff members at the UNL Life Sciences Annex for the care of animals. This research was supported by the Agriculture and Food Research Initiative competitive grants 2016-67015-24922 (HLXV) and 2013-01035 (FAO) of the USDA National Institute for Food and Agriculture.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.06.061.

References

[1] Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC, Shaw DP, et al. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J Vet Diagn Invest 1992;4:117–26.

[2] Wensvoort G, Terpstra C, Pol JM, ter Laak EA, Bloemraad M, de Kluyver EP, et al. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet Q. 1991;13:121–30.
[3] Snijder EJ, Kikkert M, Fang Y. Arterivirus molecular biology and pathogenesis. J General Virol 2013;94:2141–63.
[4] Li Y, Tas A, Sun Z, Snijder EJ, Fang Y. Proteolytic processing of the porcine reproductive and respiratory syndrome virus replicase. Virus Res. 2015;202:48–59.
[5] Dea S, Gagnon CA, Mardassi H, Pirzadeh B, Rogan D. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch Virol 2000;145:659–88.
[6] Wissink EH, Kroese MV, van Wijk HA, Rijsewijk FA, Meulenberg JJ, Rottier PJ. Envelope protein requirements for the assembly of infectious virions of porcine reproductive and respiratory syndrome virus. J Virol 2005;79:12495–506.
[7] Das PB, Dinh PX, Ansari IH, de Lima M, Osorio FA, Pattnaik AK. The minor envelope glycoproteins GP2a and GP4 of porcine reproductive and respiratory syndrome virus interact with the receptor CD163. J Virol 2010;84:1731–40.
[8] Lee C, Yoo D. The small envelope protein of porcine reproductive and respiratory syndrome virus possesses ion channel protein-like properties. Virology. 2006;355:30–43.
[9] Johnson CR, Griggs TF, Gnanandarajah J, Murtaugh MP. Novel structural protein in porcine reproductive and respiratory syndrome virus encoded by an alternative ORF5 present in all arteriviruses. J Gen Virol 2011;92:1107–16.
[10] Nelsen CJ, Murtaugh MP, Faaberg KS. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. J Virol 1999;73:270–80.
[11] Kim HK, Park SJ, Rho SM, Han JY, Nguyen VG, Park BK. One year's study of dynamic and evolution of types I and II PRRSV in a swine farm. Vet Microbiol 2011;150:230–8.
[12] Kim T, Park C, Choi K, Jeong J, Kang I, Park SJ, et al. Comparison of two commercial type 1 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) modified live vaccines against heterologous type 1 and type 2 PRRSV challenge in growing pigs. Clin Vaccine Immunol 2015;22:631–40.
[13] Han K, Seo HW, Park C, Chae C. Vaccination of sows against type 2 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) before artificial insemination protects against type 2 PRRSV challenge but does not protect against type 1 PRRSV challenge in late gestation. Vet Res 2014;45:12.
[14] Zuckermann FA, Garcia EA, Luque ID, Christopher-Hennings J, Doster A, Brito M, et al. Assessment of the efficacy of commercial porcine reproductive and respiratory syndrome virus (PRRSV) vaccines based on measurement of serologic response, frequency of gamma-IFN-producing cells and virological parameters of protection upon challenge. Vet Microbiol 2007;123:69–85.
[15] Duan X, Nauwynck HJ, Pensaert MB. Effects of origin and state of differentiation and activation of monocytes/macrophages on their susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV). Arch Virol 1997;142:2483–97.
[16] Van Breedam W, Delputte PL, Van Gorp H, Misinzo G, Vanderheijden N, Duan X, et al. Porcine reproductive and respiratory syndrome virus entry into the porcine macrophage. J Gen Virol 2010;91:1659–67.
[17] Van Breedam W, Van Gorp H, Zhang JQ, Crocker PR, Delputte PL, Nauwynck HJ. The M/GP(5) glycoprotein complex of porcine reproductive and respiratory syndrome virus binds the sialoadhesin receptor in a sialic acid-dependent manner. PLoS Pathog 2010;6:e1000730.
[18] Van Gorp H, Van Breedam W, Delputte PL, Nauwynck HJ. Sialoadhesin and CD163 join forces during entry of the porcine reproductive and respiratory syndrome virus. J Gen Virol 2008;89:2943–53.
[19] Prather RS, Rowland RR, Ewen C, Trible B, Kerrigan M, Bawa B, et al. An intact sialoadhesin (Sn/SIGLEC1/CD169) is not required for attachment/internalization of the porcine reproductive and respiratory syndrome virus. J Virol 2013;87:9538–46.
[20] Calvert JG, Slade DE, Shields SL, Jolie R, Mannan RM, Ankenbauer RG, et al. CD163 expression confers susceptibility to porcine reproductive and respiratory syndrome viruses. J Virol 2007;81:7371–9.
[21] Whitworth KM, Rowland RR, Ewen CL, Trible BR, Kerrigan MA, Cino-Ozuna AG, et al. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nat Biotechnol 2016;34:20–2.
[22] Tian D, Wei Z, Zevenhoven-Dobbe JC, Liu R, Tong G, Snijder EJ, et al. Arterivirus minor envelope proteins are a major determinant of viral tropism in cell culture. J Virol 2012;86:3701–12.
[23] Costers S, Vanhee M, Van Breedam W, Van Doorsselaere J, Geldhof M, Nauwynck HJ. GP4-specific neutralizing antibodies might be a driving force in PRRSV evolution. Virus Res 2010;154:104–13.
[24] Vanhee M, Van Breedam W, Costers S, Geldhof M, Noppe Y, Nauwynck H. Characterization of antigenic regions in the porcine reproductive and respiratory syndrome virus by the use of peptide-specific serum antibodies. Vaccine 2011;29:4794–804.
[25] Eck M, Duran MG, Ricklin ME, Locher S, Sarraseca J, Rodriguez MJ, et al. Virus replicon particles expressing porcine reproductive and respiratory syndrome virus proteins elicit immune priming but do not confer protection from viremia in pigs. Vet Res 2016;47:33.
[26] Shen G, Jin N, Ma M, Jin K, Zheng M, Zhuang T, et al. Immune responses of pigs inoculated with a recombinant fowlpox virus coexpressing GP5/GP3 of porcine reproductive and respiratory syndrome virus and swine IL-18. Vaccine 2007;25:4193–202.
[27] Fang Y, Rowland RR, Roof M, Lunney JK, Christopher-Hennings J, Nelson EA. A full-length cDNA infectious clone of North American type 1 porcine reproductive and respiratory syndrome virus: expression of green fluorescent protein in the Nsp2 region. J Virol 2006;80:11447–55.
[28] Truong HM, Lu Z, Kutish GF, Galeota J, Osorio FA, Pattnaik AK. A highly pathogenic porcine reproductive and respiratory syndrome virus generated from an infectious cDNA clone retains the in vivo virulence and transmissibility properties of the parental virus. Virology 2004;325:308–19.
[29] Vu HLX, Kwon B, de Lima M, Pattnaik AK, Osorio FA. Characterization of a serologic marker candidate for development of a live-attenuated DIVA vaccine against porcine reproductive and respiratory syndrome virus. Vaccine 2013;31:4330–7.
[30] Wu WH, Fang Y, Farwell R, Steffen-Bien M, Rowland RR, Christopher-Hennings J, et al. A 10-kDa structural protein of porcine reproductive and respiratory syndrome virus encoded by ORF2b. Virology 2001;287:183–91.
[31] Correas I, Osorio FA, Steffen D, Pattnaik AK, Vu HL. Cross reactivity of immune responses to porcine reproductive and respiratory syndrome virus infection. Vaccine 2017;35:782–8.
[32] Leng X, Li Z, Xia M, He Y, Wu H. Evaluation of the efficacy of an attenuated live vaccine against highly pathogenic porcine reproductive and respiratory syndrome virus in young pigs. Clin Vaccine Immunol 2012;19:1199–206.
[33] Lager KM, Mengeling WL, Brockmeier SL. Homologous challenge of porcine reproductive and respiratory syndrome virus immunity in pregnant swine. Vet Microbiol 1997;58:113–25.
[34] Ostrowski M, Galeota JA, Jar AM, Platt KB, Osorio FA, Lopez OJ. Identification of neutralizing and nonneutralizing epitopes in the porcine reproductive and respiratory syndrome virus GP5 ectodomain. J Virol 2002;76:4241–50.