RESEARCH REPORT
SWINE HEALTH

Title: Targeted development of neutralizing monoclonal antibodies against PRRSV minor glycoproteins **NPB # 17-151**

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Industry summary

Monoclonal antibodies (MAbs) with neutralizing capacity (so-called neutralizing MAbs) are extremely useful for rationally design of vaccine immunogen designs. With the advent of novel cell technologies, several broadly neutralizing MAbs against HIV have been generated which have facilitated the identification of "vulnerable" sites in the HIV envelope protein. This knowledge has revolutionized the design of HIV vaccine immunogens, giving hope for the successful development of a broadly protective HIV-vaccine in the future. In the case of PRRSV, our laboratory had identified and reported the first neutralizing epitope located in the ectodomain of GP5 (so-called epitope B) thanks to the use of the neutralizing MAb ISU-25 developed by Iowa State University. The goal of this project was to generate neutralizing MAbs specific to PRRSV minor glycoproteins which mediate viral interaction with the host cellular receptor CD163. The rationale is that such neutralizing MAbs will provide us with a valuable tool to identify viral protein sequences that are responsible to binding to the CD163 receptor. At the conclusion of this project, we are able to generate one monoclonal antibody clone with moderate level of neutralizing activity and 11 monoclonal clones that are non-neutralizing. We are in the process of identify the biding sequences of these monoclonal antibodies.

Keywords:

PRRSV; monoclonal antibody; virus neutralization; minor glycoproteins

Scientific Abstract

The minor glycoproteins of porcine reproductive and respiratory syndrome virus (PRRSV) are responsible for interacting with the host cell receptor. Because these proteins are essential for the virus to infect cells, we were interested in generating neutralizing monoclonal antibodies against these proteins to further explore potentially protective epitopes in PRRSV proteins. Subtractive immunization, or tolerization, was used to direct immune responses against the minor glycoproteins. With the generation of a PRRSV-1 infectious clone containing the minor glycoproteins of a PRRSV-2 infectious clone, we were able to use a cyclophosphamide treatment to direct antibody formation against ORF2-4 of a PRRSV-2 strain, FL12, in mice, and generate a hybridoma cell line that produces neutralizing antibodies against the PRRSV-2 strain FL12.

Introduction

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 (Li et al., 2015). ORFs 2a, 3 and 4 encode three minor glycoproteins (GPs) namely GP2, GP3, and GP4 (Dea et al., 2000), which form a heterotrimer dispensable for viral particle formation, yet required for viral infectivity (Das et al., 2010; Wissink et al., 2005). ORFs 5, 6, and 7 encode three major structural proteins namely GP5, M, and N (Dea et al., 2000). GP5 and M interact with each other to form a heterodimer required for viral particle formation (Wissink et al., 2005). N protein encapsidates the viral RNA genome. ORF2b is embedded within ORF2a and encodes the envelope protein E which is required for viral uncoating, presumably due to its ion channel activity (Lee and Yoo, 2006). ORF5a encodes a small protein whose functions remain unknown (Johnson et al., 2011).

PRRSV is known to infect only pigs. Macrophages are the main target of PRRSV infection *in vivo* (Duan et al., 1997). Multiple cellular receptors have been suggested to be involved in the entry of PRRSV into porcine macrophages (Van Breedam et al., 2010a). Of these, sialoadhesin and CD163 have been studied most extensively. Sialoadhesin facilitates viral attachment and internalization (Van Breedam et al., 2010b). Nevertheless, expression of sialoadhesin alone in a non-permissive cell line is not sufficient for viral infection (Van Gorp et al., 2008). Additionally, transgenic pigs lacking sialoadhesin are still fully susceptible to infection by PRRSV (Prather et al., 2013). CD163, on the other hand, is critical for viral infectivity. Cells that are resistant to PRRSV infection can be made susceptible if CD163 is transfected into the cells (Calvert et al., 2007). Importantly, transgenic pigs lacking CD163 are resistant to PRRSV infection (Whitworth et al., 2016). Our group at the University of Nebraska-Lincoln has demonstrated that the primary viral proteins involved in binding to CD163 are GP2 and GP4 (although GP3 may also be involved) (Das et al., 2010). Our finding is supported by the independent research conducted by scientists at the Shanghai Veterinary Research Laboratory (China) which has demonstrated that the minor GPs are the main determinants of viral tropism (Tian et al., 2012). Both PRRSV-1 and PRRSV-2 require CD163 for their infection of target cells (Welch and Calvert, 2010).

Soon after the first PRRSV strain was isolated, a number of monoclonal antibodies (MAbs) specific to PRRSV were generated (Nelson et al., 1993; Yang et al., 2000; Yang et al.,

1999). Most of these MAbs bind to GP5, M and N, the major structural proteins. There are only a few MAbs that can neutralize the virus, one of which is the MAb clone ISU-25, generated by Iowa State University (Yang et al., 2000). By using the neutralizing MAb ISU-25, our laboratory identified and reported the first neutralizing epitope of PRRSV, located in the ectodomain of the viral GP5 (Ostrowski et al., 2002). This study provides evidence that the neutralizing MAb is a powerful tool for identifying viral neutralizing epitopes. Since the minor glycoproteins interact with CD163, we hypothesize that the protein domains that directly interact with CD163 (so-called CD163-binding domains) should be highly conserved among the PRRSV isolates because alterations of CD163-binding domains would likely impair viral infectivity and jeopardize virus survival in nature. Consequently, antibody molecules targeting the CD163-binding domains should possess broadly neutralizing activities across the spectrum of PRRSV strains.

Objective

Objective # 1: Generation of neutralizing monoclonal antibodies specific to PRRSV minor glycoproteins

Objective # 2: Evaluation of the cross-neutralizing activity of the resulting monoclonal antibodies

Materials and Methods

1. Cells, Viruses and peptides

PRRSV-1 strain SD01-08 was rescued from the infectious cDNA clone pSD01-08, kindly provided by Dr. Ying Fang (Kansas-State University). Similarly, PRRSV-2 strain FL12 was rescued from the infectious cDNA clone pFL12 as previously described (Truong et al., 2004). FL12-expressing enhanced-green fluorescence protein (FL12-eGFP) was generated in our laboratory from a previous project. The chimeric virus SDFL24 was generated based on the pSD01-08 backbone. Specifically, the ORFs 2-4 of the PRRSV-1 strain SD01-08 were replaced by the corresponding ORFs of the PRRSV-2 strain FL12 (Kimpston-Burkgren et al., 2017). MARC-145 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). This cell line was used for propagation, titration of PRRSV strains, as well as for measurement of virus-neutralization. The myeloma cell fusion partner SP2/o (ATCC / Cat# CRL-1581) was cultured in Medium A (StemCell Technologies- Vancouver, Canada). All cell lines were cultured at 37ºC and 5% CO_2. Peptides were chemically synthesized by Genscript (Piscataway, NJ). The sequences of the peptides are listed in Table 1. For FL12 GP3 peptide, one half of the peptide was conjugated with KLH (keyhole limpet hemocyanin) which was used for immunization of mice. The other half was left un-modified and was used for ELISA screening to identify positive hybridoma clones. The SD0108-N protein was not conjugated and was used for ELISA to evaluate antibody responses in mice after immunization.

2. Mouse Immunization

2.1. Experiment 1: Subtractive immunization procedure

The subtractive immunization procedure consists of 2 phases (See Figure 3). In phase 1, mice were immunized with SD01-08 (PRRSV-1) followed by injection with cyclophosphamide. The purpose of using cyclophosphamide was to induce immune tolerance against the SD01-08 proteins (Zijlstra et al., 2003). In phase 2, mice were immunized with the chimeric virus SDFL24 without cyclophosphamide (Figure 3). These mice should only make antibodies against FL12 minor GPs, but not against SD01-08 backbone, as B cells specific to SD01-08 are tolerant to this virus antigen (See Fig. 1). The specific procedure is as follows:

Two groups of Balb/c mice were housed in the Life Sciences Annex at University of Nebraska-Lincoln. After an acclimatization period, both groups were inoculated intraperitoneally (i.p.) with 10^6 $TCID_{50}$ of PRRSV-1 strain SD0108 adjuvanted with incomplete Freund's adjuvant. At 24 and 48 hours after immunization, one group of mice was injected intraperitoneally with cyclophosphamide at a dose 100 mg per kg, i.p. per mouse at 24 and 48 hours post inoculation to induce tolerization to SD0108. This inoculation and tolerization regimen was repeated two additional times at 3 and 6 weeks after the first inoculation, respectively. In phase 2, both groups of mice were inoculated intraperitoneally with 10^6 $TCID_{50}$ of the chimeric PRRSV strain SDFL24 emulsified in incomplete Freund's adjuvant. The immunization was repeated two more times at two-week intervals. Blood samples were drawn periodically to evaluate the development of virus-neutralizing antibody. Finally, selected mice were boosted by intraperitoneal inoculation with 10^6 $TCID_{50}$ of the chimeric PRRSV strain SDFL24. Four days later, the mice were humanely euthanized, and their spleens were harvested for hybridoma cell development.

2.2. Experiment 2: Immunization with GP3 peptide

The goal of this experiment is to develop antibodies specific to the viral minor glycoproteins through immunization with peptides, instead of whole virus particles. For this purpose, Balb/c mice were first immunized by an intraperitoneal inoculation with 100 μL of peptide (50μg) emulsified in 100uL Freund's complete adjuvant (200uL total volume). Three week later, the mice were boosted by an i.p. inoculation with the same peptide that was emulsified in Freund's incomplete adjuvant. The immunization boosters were repeated 2 -3 more times in the same manner until high titers of antibodies against the respective peptide were obtained. Finally, selected mice with high antibody titers were boosted by an i.p. inoculation with 50μg peptide without adjuvant. Four days later, the mice were humanely euthanized, and their spleens were harvested for hybridoma cell development.

3. Hybridoma development

Hybridoma cell lines producing monoclonal antibodies against PRRSV were generated using the ClonaCell-HY Hybridoma Cloning Kit from StemCell Technologies (Vancouver, Canada). Briefly, the spleen of selected mice with high virus neutralizing antibody titers against FL12 was disaggregated into a single cell suspension then washed three times with Medium B. Approximately 10^8 splenocytes were mixed with 2 x 10^7 SP2/0 myeloma cells and cell fusion were induced by using 1mL of 50% polyethylene glycol (PEG). The fused cells were suspended in 30mL Medium C and cultured in a T75 cell culture flask for 24 hours at 37°C with 5% CO2.

The cell suspension was centrifuged for ten minutes at 400 x g and the cells were resuspended in 10 mL fresh Medium C which was then mixed with 90 mL semisolid medium containing hypoxanthine-aminopterin-thymidine (a.k.a. Medium D) for selection of fused cells. The cell suspension in Medium D was plated in 100 mm cell culture dishes, 10ml per dish, and incubated at 37°C with 5% CO2 for 10-14 days, without disruption. When cell colonies were visible, individual cell clones were picked and transferred into individual wells of a 96-well cell culture plate containing 200 µL Medium E. Plates were incubated for 4 days at 37°C with 5% CO2, then supernatants were harvested and tested for their ability to neutralize FL12-GPF. 150 µL Medium E was used to replenish colonies. Clones that were positive for neutralizing antibodies underwent two rounds of limiting dilution. Clones that were positive for neutralization were gradually expanded up to T75 flasks, and cells were cryopreserved in 90% FBS plus 10% DMSO.

4. Virus neutralization assay

Two different virus neutralization assays were performed. In the first assay, heat-inactivated mouse sera or hybridoma culture supernatant were diluted 2-fold serially in 50 µL media in a 96-well plate and incubated with 50 µL containing 100 $TCID_{50}$ FL12 or FL12-GFP for one hour at 37°C. The mixture was then transferred to a 96 well plate containing two-day old MARC-145 cells and incubated for two days if IFA is performed, or five days if read by GFP fluorescence. The end point titer is expressed as the reciprocal of the highest serum dilution that showed a >90% reduction in the number of fluorescent foci compared to control wells.

In the second assay, monoclonal antibodies were diluted 2-fold serially. After that each dilution of the culture supernatant was separately incubated with the same volume of PRRSV FL12 for 1 hour at 37°C. The mixtures were then separately inoculated onto MARC-145 cells in a 12 well plate. At 48 hours later, culture supernatants were collected and PRRSV titers were determined by titration in MARC145 cells. The reduction of viral yield was determined by subtracting the viral titers cultured in cells without monoclonal antibody to the viral titers cultured in cells in the presence of monoclonal antibody.

5. Peptide ELISA

Unconjugated peptide was diluted to 10 µg/mL in carbonate buffer (pH 9.6). 100µL of the peptide were added to each well of an Immulon 2 HB—High Binding ELISA plate and incubated overnight at 4°C. The next day the plate is washed with PBS+0.1% Tween-20 (PBS-T20) one time then blocked with PBS-T20 + 1% bovine serum albumin (BSA) for one hour at room temperature on a rocker. The plate is then washed three times with PBS-T20 then 50 µL/well of optimally diluted samples in PBS-T20 + 1% BSA are incubated for one hour at room temperature on a rocker. Next, the plate is washed five times with PBS-T20. 50 µL/well of anti-mouse HRP conjugated antibody diluted 1:2000 is added and incubated for thirty minutes at room temperature on a rocker. The plate is washed four times with PBS-T20 and one time with H2O. 50 µL/well of SureBlue TMB peroxidase substrate solution (KPL, Gaithersburg, MD) is added and incubated for ten minutes at room temperature. The reaction is stopped by 50 µL/well TMB Stop Solution (KPL, Gaithersburg, MD). The absorbance is read at 450nm.

Results

1. Establishment of a subtractive immunization procedure: Mouse experiment #1

We adapted the subtractive immunization procedure which allows us to deliberately direct the mouse immune response against the minor glycoproteins of FL12 (See Figure 1). We used an ELISA that detects antibodies against SD01-08 N protein to monitor the efficiency of our tolerization regimen. After phase 1, mice immunized with SD0108 together with cyclophosphamide (principal group) did not develop significant levels of antibodies against the N protein whereas control mice immunized with SD01-08 without cyclophosphamide (control group) developed high levels of antibodies against N protein (Figure 2). The results clearly indicate that the principal group was tolerant to SD01-08 proteins.

We then moved to phase 2, in which we immunized the principal group with the chimeric virus SDFL24 without cyclophosphamide. After phase 2, antibodies against SD01-08 N protein were barely detected in the principal group (Figure 2), confirming that this group is tolerant to SD0108 proteins. Interestingly, high titers of neutralizing antibodies against FL12 were detected in the principal mice after phase 2 (Table 2). Neutralizing antibody titers range from 1:320-1:640. It should be noted that mice in both the control and principal groups were never exposed to the PRRSV-2 strain FL12. The high neutralizing antibody titers against this virus strain observed in the principal groups should be attributed to antibodies against FL12 minor GPs as they were the only FL12 proteins present in the chimeric virus SDFL24. In summary, we have successfully established an immunization procedure which allows us to deliberately direct the mouse immune system into developing neutralizing antibodies specific to the minor GPs of PRRSV.

2. Hybridoma development

After fusion, cells were plated in a semi-solid medium containing HAT (Medium D). Visible cells colonies were observed at day 10 -14 after plating. Individual cell colonies were picked and transferred to 96-well plates. Four days later culture supernatant from each individual clone was screen for their ability to neutralize FL12-eGFP. After the first round of screening, we were able to obtain 54 clones showing neutralizing activity. These clones (in 96-well plates) were then transferred to 24-well plates for expansion. After this round of expansion, only 18 out of 54 clones remained their neutralizing activity against FL12-eGFP. The 18 hybridoma clones were then expanded into 6 well-plates. After the second round of expansion, only 3 hybridoma clones remained their neutralizing activity.

We suspected that these hybridoma clones were not monoclonal but rather a mixture of multiple cells clones, some of which secreted antibody while others did not. The cells that did not secrete antibody grew faster and outgrew those that secreted antibody. We therefore performed cell cloning following the limiting dilution method, to increase the likelihood of obtaining hybridoma clones with monoclonality. After two rounds of cloning by limiting dilution, we were able to obtain one hybridoma clone, named 9-2-4, that stably secreted neutralizing antibody. A reduction in infectivity assay showed that in the presence of 9-2-4 at a dilution of 1:2, FL12-eGFP grew to 3 logs lower than FL12 without antibody, and the reduction of infectivity occurred in a dose-dependent manner (Figure 3).

3. Development of monoclonal antibodies against FL12 GP3 peptide

Since we experienced a significant loss of hybridoma clones after a few rounds of expansion, we wondered if our hybridoma development procedure was the issue. To address this question, we immunized mice with a peptide derived from FL12 GP3. After four repeated immunization, high titers of antisera against the GP3 peptide were observed in all mice (Table 3).

Hybridoma development was performed following the exact procedure described above. We used a peptide ELISA to screen for positive clones. Of note, the peptide used for immunization was conjugated with KLH to enhance its immunogenicity. However, the peptide used for ELISA was not conjugated as we wanted to screen for only hybridoma clones that react specifically to the peptide, not to KLH. We obtained 11 hybridoma clones secreting antibody specific to the GP3 peptide. These clones were expanded 3 rounds from original 96 well-plates up to T-25 flasks and were still producing antibody against this peptide, as measured by peptide ELISA (Table 4). Thus, we believed that these clones were stable.

Next, we ran an indirect immuno-fluorescence assay to determine the reactivity of these 11 monoclonal antibodies to MARC-145 cells infected with the PRRSV strain FL12. Seven monoclonal antibody clones reacted to both infected and non-infected cells whereas 4 clones reacted specifically to only infected cells (Table 4).

Finally, we measured neutralizing activity of these 11 monoclonal antibodies against FL12. Unfortunately, none of the clones neutralize the virus. Our initial observation suggested that some of these monoclonal antibodies even enhanced viral infectivity. We are now conducting additional studies to confirm this observation. If these monoclonal antibodies enhance viral infection, we will conduct studies to understand how the antibodies could enhance viral infectivity.

Discussion

The goal of this proposal is to generate a panel of neutralizing MAbs specific to the viral minor GPs because these minor GPs are responsible for interaction with CD163, a major cellular receptor for virus entry (Das et al., 2010). However, these proteins are rare, with only a few of the heterotrimeric complexes present on the virion (Spilman et al., 2009). To increase our likelihood of generating monoclonal antibodies against GP2, GP3, and GP4 we utilized a subtractive immunization or tolerization protocol. Subtractive immunization has been used for many years as a way to generate monoclonal antibodies to rare or non-immunodominant antigens (Lu et al., 2013; Matthew and Sandrock, 1987; Williams et al., 1992). With access to a chimeric infectious clone containing the ORFs2-4 of the PRRSV-2 strain FL12 in the backbone of a PRRSV-1 strain SD0108, as well as the SD01-08 strain we were able to tolerize animals to SD0108 and upon immunization with the chimeric virus SDFL24 were able to target the antibody response to ORFs2-4. We observed that mice in the principal group (under tolerization regime) mounted higher neutralizing antibody titers against PRRSV strain FL12. Thus, in this project, we successfully developed an immunization protocol that allows us to induce neutralizing antibody in mice.

There are two different methods to generate hybridomas. In the conventional method, fused cells (splenocytes and myeloma) are cultured in liquid medium containing HAT for

selection. After that, culture supernatants are screened for the presence of antibodies of interest. Positive clones then undergo multiple rounds of cloning in order to obtain an acceptable probability of monoclonality. Another method for hybridoma selection and cloning relies on the uses a semi-solid medium. In this method, cells are plated in a semi-solid selection medium containing HAT after fusion. Consequently, the progeny of the cells survived HAT selection will cluster in the semisolid medium and form distinct colonies that can be harvested and screened individually. Each distinct colony is likely derived from a single cell, thus, colonies formed in semisolid medium have a high likelihood of being monoclonal. With this method, both selection and cloning are done at the same time (Stemcell_Technologies). In this project, we employed the second method which is to culture cells in a semi-solid medium after fusion. We only picked colonies that were well separated on the semi-solid medium plates. We therefore expected that our hybridoma clones should be monoclonal. We were able to obtain several hybridoma clones secreting neutralizing antibody. Unfortunately, majority of these clones stopped producing neutralizing antibody after they were sub-cultured a few passages. One plausible explanation for this problem is that there hybridoma clones were mixed of multiple cells clones, some of which secreted antibody while others did not. The cells that did not secrete antibody grew faster and outgrew those that secreted antibody. We therefore performed cell cloning following the limiting dilution method. After two rounds of cloning, we obtained only one hybridoma clone that was stably secreting neutralizing antibody.

This led us to wonder if our procedure to generate hybridomas was the problem. To address this question, we immunized mice with the GP3 peptide of the PRRSV strain FL12 and used their splenocytes to generate hybridomas. The reason for choosing to generate hybridoma clones against GP3 peptide was that we can use a peptide ELISA to screen for positive hybridoma clones. The ELISA is much higher throughput than the virus-neutralization assay. After one single round of cell fusion, we were able to generate 11 hybridoma clones stably secreting antibody against this peptide. Importantly, we did not experience the loss of hybridoma clones in this case. This result observation clearly demonstrates that our hybridoma development procedure does not have any issue.

Of the 11 peptide-specific monoclonal antibodies generated in this project, 7 clones reacted to both PRRSV-infected and non-infected MARC 145 cells, as measured by indirect immunofluorescence. It is possible that PRRSV GP3 peptide might contain amino acid sequences that mimic host cellular proteins, resulting in the monoclonal antibodies recognizing these mimicry sequences, reacting to host cell proteins, and leading to the immunostaining signal observed in non-infected cells.

In summary, we were able to generate one neutralizing and 12 non-neutralizing monoclonal antibodies. These monoclonal antibodies are useful reagents for future studies on PRRSV biology and vaccine development.

TABLES AND FIGURES

Table 1: Peptide sequences used in this project

Peptide Name	Sequences	AA position
FL12-GP3	YEPGRSLWCRIGHDRCSEDDHDDLGFMVPPGLSSEGHL	67-104
SD0108-N	KSQRQQPRGGQAKKKKPEKPHFPLAAEDDI	35-64

Table 2. Neutralizing antibody response of mice in the principal groups measured against FL12. Sera were collected after 3 immunization with SDFL24 in the phase 2

Mouse #	Neutralizing antibody titer
79	1:640
80	1:640
82	1:640
83	1:640
84	1:320
86	1:640

Table 3: Antibody titers against GP3 peptide measured by ELISA

Dilution	Immunized with GP3 peptide				No immunization	
	M #98	M #99	M #100	M # 1	Control #1	Control #2
300	1.412	1.271	1.444	1.38	-0.001	-0.001
900	1.483	1.195	1.28	1.508	-0.003	0.008
2,700	1.304	0.964	0.867	1.041	-0.005	0.001
8,100	1.121	0.601	0.483	0.74	-0.003	-0.001
24,300	0.732	0.27	0.183	0.371	-0.002	0.002
72,900	0.38	0.096	0.066	0.146	0.002	0.003
218,700	0.149	0.029	0.024	0.063	0.001	0.009
656,100	0.027	-0.001	0.024	0.021	0.006	0.06
Note: antisera were collected at 10 days after the 4^{th} immunization with GP3 peptide						

Table 4: ELISA and IFA reactivity of the 11 monoclonal antibodies specific to GP3 peptide

No	Clone ID	ELISA OD	IFA
1	1D1	0.939	Nonspecific
2	1F5	0.882	Nonspecific
3	2H8	1.326	+
4	4E3	1.513	Nonspecific
5	5H3	1.732	Nonspecific
6	5F12	1.577	Nonspecific
7	6A11	1.811	+
8	6H12	1.838	Nonspecific
9	7D5	1.646	Nonspecific
10	7A10	1.59	+
11	7E12	0.825	+++
“+” indicates weak signal; “+++” indicates strong fluorescence signal			

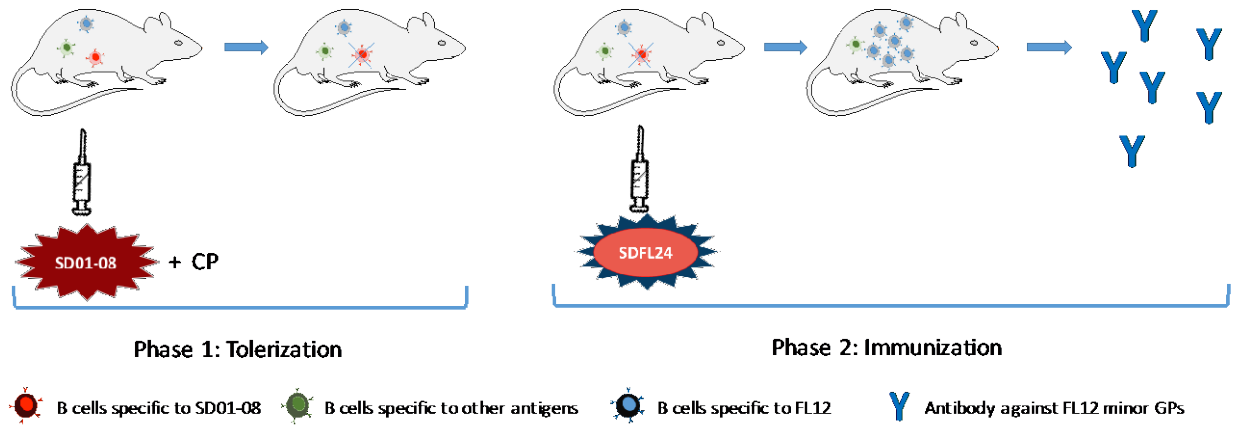

Figure 1: Subtractive immunization procedure: The mouse immune system consists of a B cell repertoire that recognizes different types of antigens. Depicted in this cartoon are B cells specific to SD01-08, FL12 and other antigens. In phase 1, the mouse was tolerized by inoculation with SD01-08 together with cyclophosphamide (CP). This regimen led to immune tolerance to SD01-08 antigens. Consequently, the mouse would no longer produce antibodies against SD01-08 antigens. In phase 2, the mouse was immunized with the chimeric virus SDFL24 which carries minor GPs of FL12. The mouse would only make antibodies against FL12 minor GPs, but not against SD01-08 backbone as B cells specific to SD01-08 are tolerant to this virus antigen.

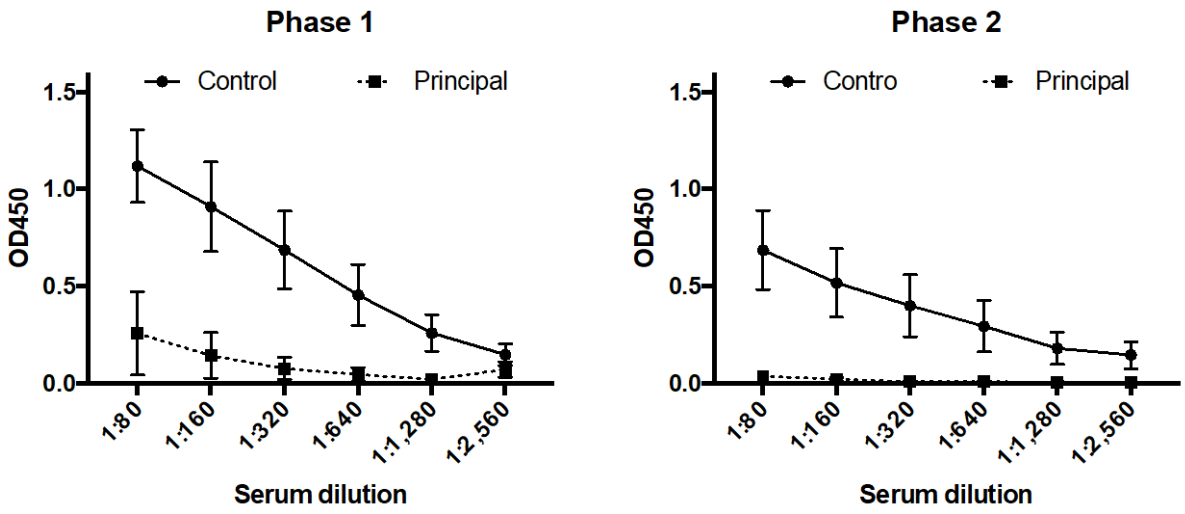

Figure 2. Antibody responses to ORF7 of SD0108. Antibody responses to ORF7 of SD0108 were tested by an ORF7 peptide ELISA. (A) Sere were harvested 1 week after 3rd SD0108 immunization in Phase 1. (B). Sera were harvested 1 week after 3rd SDFL24 immunization in Phase 2. Data are expressed as means and SEM.

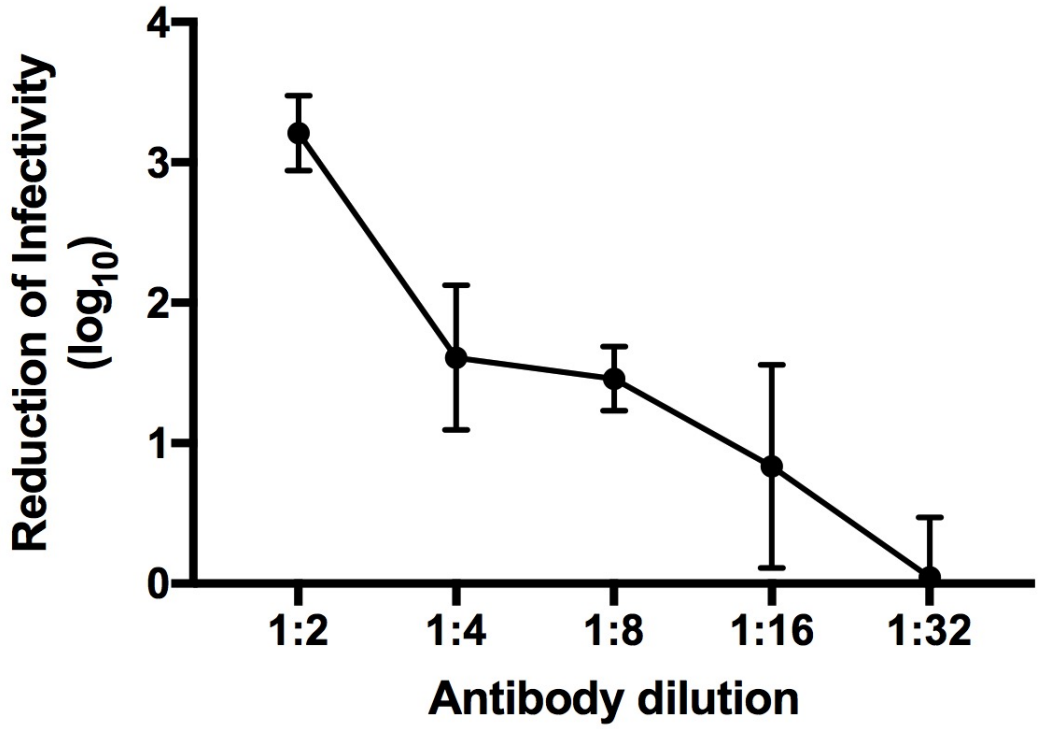

Figure 3. Reduction of infectivity in the presence of monoclonal antibody 9-2-4. FL12 was incubated on cells in the presence of the monoclonal antibody 9-2-4. Virus was harvested at 48 hours post infection and viral titer was measured. The mean and standard error were calculated from two replicates.

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