RESEARCH REPORT
SWINE HEALTH

Title: Induction Of Cross-Protective Immunity Without Exposure To Live PRRSV - **NPB #08-197**

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Industry summary

Explanation of the objectives. The development of more efficacious vaccines was one of the objectives for NPB 2008 funding. The work reported here is focused on the engineering of a modified live vaccine using transmissible gastroenteritis virus (TGEV) as a vector, expressing PRRSV antigens of interest in protection. Furthermore, the work planned with TGEV-based vectors will allow the identification of protective epitopes, which was also an objective of NPB 2008 funding.

As a vector, TGEV is a potent inducer of interferon, elicits strong mucosal and systemic immune responses and could be engineered to develop safe vectors. Therefore, TGEV represents a new strategy to achieve protection against PRRSV. The objectives planned for this year consist in the generation of a set of TGEV vectors expressing PRRSV proteins and modified PRRSV proteins that will allow high specific immune responses anti-PRRSV. Objectives include the quality control of the obtained TGEV vectors and the analysis of the immune response induced by some of the generated TGEV vectors.

How research was conducted. Our group previously generated a TGEV vector co-expressing two PRRSV proteins: GP5, the main inducer of neutralizing antibodies, and M, involved in the cellular immune response against PRRSV. The results indicated that, although a certain degree of protection was achieved, it was not enough for a good candidate vaccine. Therefore, a new set of TGEV vectors co-expressing PRRSV M protein and different modified GP5 proteins were generated. Only some of the planned vectors were successfully obtained, fulfilling all the lab quality controls previous to their animal testing.

Piglets were inoculated with a TGEV vector co-expressing M protein a GP5 with altered glycosylation pattern, and challenged with a virulent PRRSV strain. The immune response and protection of vaccinated animals was

compared to that of non-vaccinated animals. Using this TGEV vector, a killed vaccine was also formulated that was also tested in the weaned pigs system.

Discussion of research findings. Concerning the animal experiments using the TGEV vector expressing PRRSV modified GP5 and M proteins, it was found that all animals present a high antibody response against TGEV, therefore, the vector infected target tissues as expected. Also, vaccinated animals showed a clear antibody response against the PRRSV antigens (i.e., GP5 and M proteins). After a challenge with a virulent PRRSV isolate, a fast recall immune response was observed, as vaccinated animals induced higher antibody titers against PRRSV antigens and earlier than control animals. Using this TGEV vector, a killed vaccine was also formulated and tested in piglets. The results were similar to those obtained with the live TGEV-based vaccine. Vaccinated animals induced higher and faster antibody levels against PRRSV antigens than control animals.

With both experimental approaches, a clear degree of protection was observed, as the lungs from vaccinated animals showed a lower degree of lung damage than that detected in the non-vaccinated animals. Nevertheless, the immune response was not strong enough to provide full protection against PRRSV. That was likely due to the low levels of neutralizing antibodies produced before challenge.

Therefore, results using rTGEV as a platform were promising, as antibodies against PRRSV antigens were elicited and a certain degree of protection was observed.

Nevertheless, our objective is to improve the protection elicited by our vaccine against PRRSV over currently available commercial modified live vaccines. To improve the immunogenicity of GP5, additional modifications were introduced in the GP5 expressed by TGEV vectors. In addition to the modification of the glycosylation pattern, an immunodominant domain (decoy epitope), close to the neutralizing epitope, that induces non-neutralizing antibodies was eliminated. A TGEV vector expressing a GP5 lacking both the glycosylation site and the decoy epitope was obtained. Nevertheless, in preliminary in vivo experiments, the levels of specific antibodies for PRRSV antigens were low. This was probably due to certain instability of the antigens cloned in the TGEV. New experimental approaches to improve the stability of rTGEV vectors expressing PRRSV antigens are being developed.

Explanation of what these findings mean to the industry. Pork producers are hindered, among others, by infectious disease problems that increase production costs. PRRSV is the agent causing the most important infectious disease affecting swine, resulting in more than $600 million economic loss to US pork producers annually. Therefore, an improvement of vaccination strategies is required to significantly reduce the production costs and improve the performance of the herds. Current vaccines against PRRSV have limited efficacy. Best results have been obtained using modified live vaccines, although they have several problems such as incomplete protection, virus shedding and possible reversion to virulence. This fact has led to an increase in the use of potentially hazardous methods to control the disease, such as using live field virus to vaccinate pigs. Vector- based vaccines could represent and advantage to stimulate both humoral and cell immune responses against PRRSV. Nevertheless, the results reported to date using viral vectors are not fully satisfactory and new

vectors must be explored. The main novelty of the reported work derives from the use of the TGEV-based vector to express different PRRSV antigenic combinations. The proposed vaccine may represent a candidate that could provide protection against two viruses: PRRSV and TGEV. As this is a live vaccine, its efficacy should be high, whereas its cost should be competitive.

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Scientific abstract

Porcine reproductive and respiratory syndrome (PRRS) is the main infectious disease affecting swine. Nevertheless, limited information is available on the immune response against the virus causing the disease (PRRSV), and current vaccines against PRRSV have a limited efficacy. Best results have been obtained using modified live vaccines, although they have several problems such as incomplete protection, virus shedding and possible reversion to virulence. Vector- based vaccines could represent and advantage to stimulate both humoral and cell immune responses against PRRSV. Nevertheless, the results reported to date using viral vectors do not provide the expected protection and new vectors must be explored. The main novelty of the project proposed comes from the use of the transmissible gastroenteritis virus (TGEV)-based vector to express different PRRSV antigenic combinations. These vectors stably express high levels of heterologous genes, are potent interferon-α inducers, essential for antiviral defense, and present antigens in mucosal surfaces, providing both secretory and systemic immunity. A TGEV derived vector (rTGEV) was generated, expressing PRRSV GP5 and M proteins, described as the main inducers of neutralizing antibodies and cellular immune response, respectively. Protection experiments showed that vaccinated animals developed a faster and stronger humoral immune response than the non-vaccinated ones. Nevertheless, low levels of neutralizing antibodies were elicited after rTGEV inoculation, similarly to what occurs with PRRSV infection. This could be due to a steric hindrance caused by the glycosylation sites mapping close to the neutralizing epitope in GP5 protein. Therefore, a set of rTGEV vectors expressing M protein and GP5 mutants, with a modified glycosylation pattern, were generated. These vectors expressed GP5 and M proteins, presumably forming a heterodimer, in at least a 75% of the infected cells. To increase rTGEV stability and improve expression levels, serial passages and virus cloning were performed. Immunization with a killed vaccine based on this rTGEV vector has provided data indicating that vaccinated animals elicited a higher and faster PRRSV specific humoral immune response, including the induction of both neutralizing and non-neutralizing antibodies. Moreover, in vaccinated animals lung damage was decreased when compared with the non-vaccinated ones. The efficacy of this live vaccine in protection was also analyzed.

A faster and stronger PRRSV specific humoral response was developed in the vaccinated animals compared to that of the non-vaccinated ones. Moreover, lung damage was significantly lower in vaccinated animals compared with non-vaccinated ones. Nevertheless, a weak neutralizing antibody response was elicited in both cases. This modest results, when compared with those obtained using the killed-vaccine, suggest that rTGEV vector stability may be the handicap to achieve more promising results. Therefore, a new strategy has been developed to improve rTGEV vectors stability. All together, data obtained indicate that TGEV represents a new and promising strategy to achieve protection against PRRSV.

Introduction

A hallmark of the swine antibody response against PRRSV is the abundant non-neutralizing antibodies detected early in the infection, followed by a low neutralizing antibody (NAb) titer that appears more than 3 weeks after infection. Experimental data showing the importance of neutralizing antibodies in protection against PRRSV infection has been reported in the last years (Lopez and Osorio, 2004). Immune response to PRRSV is poorly understood but, in spite of this, some vaccines are being commercialized. Current vaccines against PRRSV have several drawbacks. Modified live vaccines protect against challenge with homologous isolates, but generally have a limited effect against challenge with heterologous viruses (Meng, 2000). Furthermore, live vaccines provide partial protection against clinical disease but did not prevent infection (Osorio et al., 1998) and, more importantly, they can revert to virulence (Botner et al., 1997; Nielsen et al., 2001). As the attenuated vaccines induce an immune response resembling that induced by PRRSV natural infection, they do not induce high levels of neutralizing antibodies. Killed PRRSV vaccines, on the other hand, have proved to be less effective in prevention of both infection and disease (Kim and Yoon, 2008; Ostrowski et al., 2002).

The envelope glycoprotein 5 (GP5) contains most of the epitopes involved in virus neutralization. Although GP4 and M proteins can also elicit neutralizing antibodies, the ones recognizing GP5 neutralize PRRSV more effectively than those specific for other viral proteins (Ostrowski et al., 2002). PRRSV infection also results in a weak and delayed T-cell mediated immune response that should be necessary for the elimination of the virus. In addition, it has been shown that a specific cytotoxic T-lymphocyte (CTL) response, complementing neutralizing antibodies, provides partial protection against PRRSV. M protein has been associated with the development of strong cellular immunity (Molitor et al., 1997) and, therefore might play an important role in protection against PRRSV infection.

The expression of PRRSV GP5 and M proteins has been reported using different systems: (i) a recombinant attenuated strain of *Mycobacterium bovis* that induces partial protective immunity and low NAbs titer in a fraction of the vaccinated pigs (Bastos et al., 2004); (ii) DNA vaccines expressing GP5 and M proteins, that induce moderate NAbs in immunized piglets 8 weeks post vaccination (Jiang et al., 2006b); (iii) the administration of a recombinant replication-defective adenovirus that induces high titer NAbs PRRSV specific in immunized mice (Jiang et al., 2006a); (iv) the administration of a recombinant pseudorabies virus (PRV),

enhancing PRRSV-specific NAbs in immunized mice (Wang et al., 2007); (v) a recombinant modified vaccinia virus ankara (rMVA) eliciting strong humoral and cellular immune responses in inoculated mice (Zheng et al., 2007); and (vi) a pseudotype baculovirus enhancing neutralizing antibodies and IFN-γ production in the mouse model (Wang et al., 2007).

Development of more efficacious vaccines was one of the objectives for NPB 2008 funding. The work proposed was focused on the engineering of a modified live vaccine using TGEV as a vector, expressing PRRSV antigens of interest. Furthermore, the work planned with TGEV-based vectors will allow the identification of protective epitopes, which was also an objective of NPB 2008 funding.

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Objectives

The following objectives require a minimum of 36 months for full completion. *Funding was requested for one year of work only. First year was funded by project NPB #07-112.*

Three objectives will be targeted:

(1) ***Antigen presentation improvement using recombinant TGEV (rTGEV) vectors*** that will promote the immune response to PRRSV antigens;

(2) ***Modification of the antigenic structure of PRRSV proteins*** to generate a more potent immune response against the epitopes inducing neutralizing antibodies by, for instance, engineering deglycosylated antigens; and

(3) ***Modification of the type of immune response*** to PRRSV antigens by the co-expression of genetic adjuvants, for instance, granulocyte macrophage colony-stimulating factor (GM-CSF).

Materials and Methods

Construction of TGEV infectious cDNA clones and rescue of recombinant viruses expressing PRRSV antigens

Engineering of BACs containing infectious TGEV cDNA clones encoding PRRSV antigens was performed as previously described by our laboratory, using standard cloning techniques with the modifications required to work with BACs. rTGEV viruses were recovered after transfection of permissive cells with the cDNA clones. The recombinant viruses were cloned by three rounds of plaque purification. Cloned viruses were amplified in tissue culture by passing the virus at least three times. Heterologous gene expression was evaluated by RT-PCR, using specific oligonucleotides, to detect the corresponding subgenomic mRNAs. The sequence of these mRNAs was determined to confirm the identity of the amplified bands. Protein expression levels were estimated by FACS analysis of immunofluorescent cells and Western-blot analyses. Only those rTGEV vectors that fulfill a strict quality control were used in subsequent in vivo experiments. The criteria used to accept a recombinant vector as a vaccine candidate was based mainly on the stability of PRRSV protein expression levels.

In vivo growth of the recombinant vaccine

To determine rTGEVs in vivo growth, groups of eight one-week-old piglets were inoculated with $1x10^8$ pfu of the rTGEVs using a combination of three different routes: oral, intranasal and intragastric. Two animals from each group were sacrificed at 1, 2, 3 and 4 days post infection and different tissues (lung and gut) were collected. Virus was recovered from these tissues and titrated to determine rTGEV growth. Also, antigen expression levels were evaluated by immunofluorescence studying the percentage of vector positive cells that also express the relevant PRRSV antigens (i.e., GP5 and M).

Immunization experiments

Animals were inoculated as described (see above) and a boost was performed two weeks after inoculation. Blood samples were collected at different times post inoculation to determine the levels of specific antibodies (see below). Protection assays were performed using a field isolate such as Olot91. Blood samples were collected at different times post challenge to determine the immune responses elicited by the rTGEV vectors (see below). Replication of the recombinant viruses and PRRSV were evaluated by virus titration, immunohistochemistry, and RT-PCR.

A previous short immunization experiment was performed to select promising rTGEV vaccine candidates to be tested in protection experiments as described above. One-week old piglets were inoculated with $1x10^8$ pfu of the rTGEVs using a combination of three different routes: oral, nasal and intragastric. Two weeks later, a boost was performed. Blood samples were collected at days 0, 14, 28 and 49 post-inoculation. The immune response elicited by the rTGEVs was evaluated. Only rTGEVs with promising results coming from these in vivo assays were fully tested in protection experiments.

Evaluation of the humoral immune response elicited by rTGEVs

Serum samples collected at different times post-immunization and challenge (see above) were evaluated for the presence of an specific immune response to PRRSV proteins. TGEV and PRRSV specific antibodies were evaluated by ELISA, using partially purified viruses as antigens. In some cases, antibodies specific for purified GP5 or M proteins expressed using baculovirus vectors, or synthetic peptides mapping at different antigenic domains of GP5 and M proteins, were evaluated by ELISA. In the case of killed vaccines, antibodies were also titrated by immunofluorescence, or immunoperoxidase staining using PRRSV infected cell monolayers (IPMA). Neutralization assays were performed to determine the level of NAbs induced by the recombinant vectors using MA-104 cells.

Results

Results obtained are exposed schematically, according to proposed objectives for the second year funding.

Formulation of a killed vaccine expressing GP5 mutants with alterations in the glycosylation pattern. As stated in the NPB-07-112 final report, Enjuanes group developed a rTGEV stably co-expressing PRRSV M protein and GP5-N46S glycosylation mutant, lacking the glycosylation site overlapping the neutralizing epitope. A killed vaccine was developed based on this rTGEV-GP5-N46S-M virus. ST cells were infected with this rTGEV, and the culture medium was harvested at 48 hpi. The protection conferred by this vaccine was tested. Vaccinated animals induced higher and faster antibody titers against PRRSV antigens than control animals (Fig. 1A). Neutralizing antibody titers were also higher in the vaccinated animals when compared with non-vaccinated animals (Fig. 1B), suggesting that the elimination of glycosylation site close to the neutralizing epitope improves protective immune response against PRRSV.

In vivo testing of rTGEV expressing PRRSV M protein and GP5 mutant with altered glycosylation. The protection conferred by rTGEV-GP5-N46S-M was tested in vivo. One-week old piglets were inoculated with $1x10^8$ pfu of the rTGEV by three routes: oral, nasal and intragastric. Six weeks later, a challenge was performed with $1x10^7$ $TCID_{50}$ of a virulent European PRRSV strain. Blood samples were collected at different times post inoculation, and humoral immune responses were evaluated by ELISA. All animals present a high antibody response against TGEV, therefore, the vector infected target tissues as expected. Vaccinated animals showed a clear humoral response against PRRSV, GP5 (Fig. 2) and M proteins. The immune response was not strong

enough to provide full protection, probably because the levels of neutralizing antibodies were similar in vaccinated and non-vaccinated animals (data not shown). Nevertheless, a moderately faster recall response was observed, as vaccinated animals induced higher antibody titers against PRRSV antigens and earlier than control animals (Fig. 2).

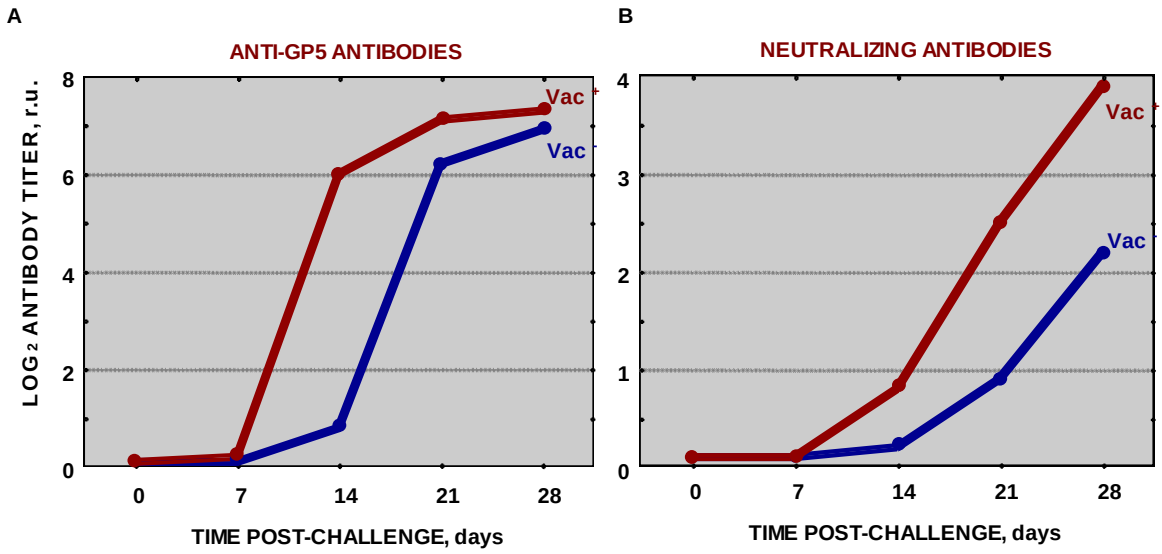

Figure 1. Humoral immune response elicited by inactivated rTGEVs expressing GP5 with altered glycosylation pattern. Blood samples of animals we collected at indicated times post challenge. (A) Samples were analyzed by IPMA specific to detect antibodies against GP5. Cells expressing recombinant GP5 were used as antigens for the IPMA assay. (B) Neutralizing antibodies titers were calculated from neutralization assays of PRRSV Olot91 strain infecting MA-104 cells.

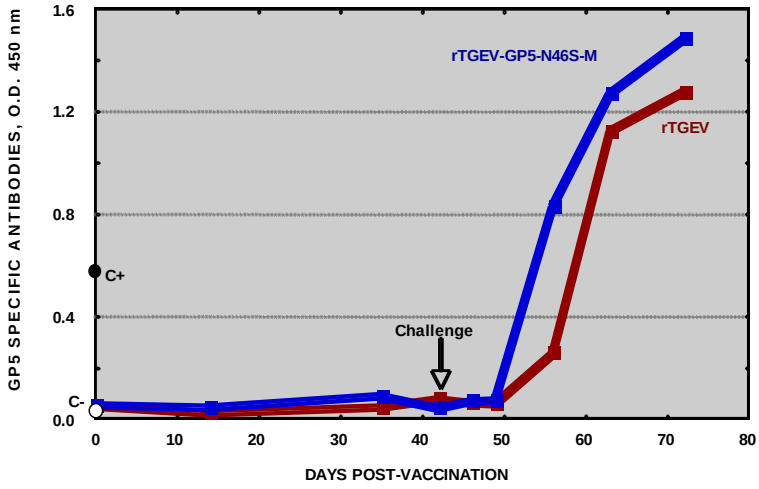

Figure 2. Humoral immune response against GP5 elicited by a TGEV-based modified live vaccine expressing GP5 with altered glycosylation pattern. Blood samples of animals we collected at indicated times post inoculation. Samples were analyzed by ELISAs specific to detect antibodies against GP5. To evaluate response against GP5, this protein from PRRSV Olot91 strain was expressed and purified from insect cells and used as antigen for the ELISA.

The protection conferred by the TGEV-based vaccines, both live and inactivated, was evaluated. Viremia, gross lesions, and histopathology in the lungs of vaccinated and non-vaccinated animals were analyzed. A clear degree of protection was observed, both using live and killed TGEV-based vaccines, as the lungs from vaccinated animals showed a significantly lower degree of lung damage than those from non-vaccinated ones (Fig. 3).

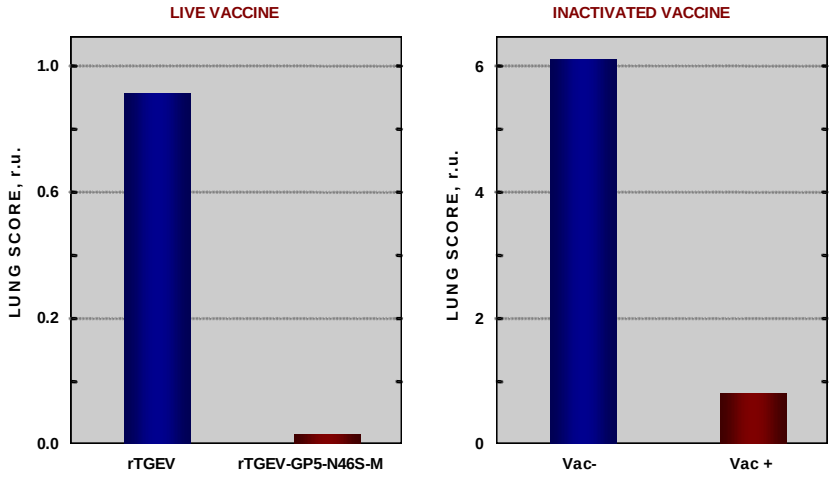

Figure 3. Lung damage. The lung from vaccinated and non-vaccinated animals were analyzed. Lung lesions observed in all the pigs, with different degree of severity, included a craneo-ventral consolidation of apical and medial lung lobes.

Although a certain protection was observed in the lung, analysis of the viremia showed that vaccinated animals were only partially protected against challenge. That was likely due to the relatively low levels of neutralizing antibodies produced before challenge. Nevertheless, results using rTGEV as a platform were promising, as a humoral immune response against PRRSV antigens was elicited.

Evaluation of rTGEV expressing GP5 mutant lacking the decoy epitope. To improve GP5 immunogenicity, a rTGEV vector was constructed, expressing a GP5 protein lacking the non-neutralizing (decoy) epitope and the N46 glycosylation site. The virus was recovered with high titer and GP5 and M protein expression was observed in 65% and 93% of the infected cells, respectively (Fig. 4). Co-localization of mutant GP5 and M proteins was observed, similar to that found for these proteins expressed by PRRSV and rTGEVs (Fig. 5). This result suggests that GP5-M protein heterodimer formation was not altered. Nevertheless, short in vivo experiments showed that this rTGEV elicited a low antibody response against GP5 (data not shown). This could be due to a certain instability of the GP5 gene cloned in the rTGEV vector, as suggested by the decrease in GP5 expression associated to the introduction of specific mutations in GP5 (Fig. 4). In contrast, M expression levels remained similar for all rTGEVs tested.

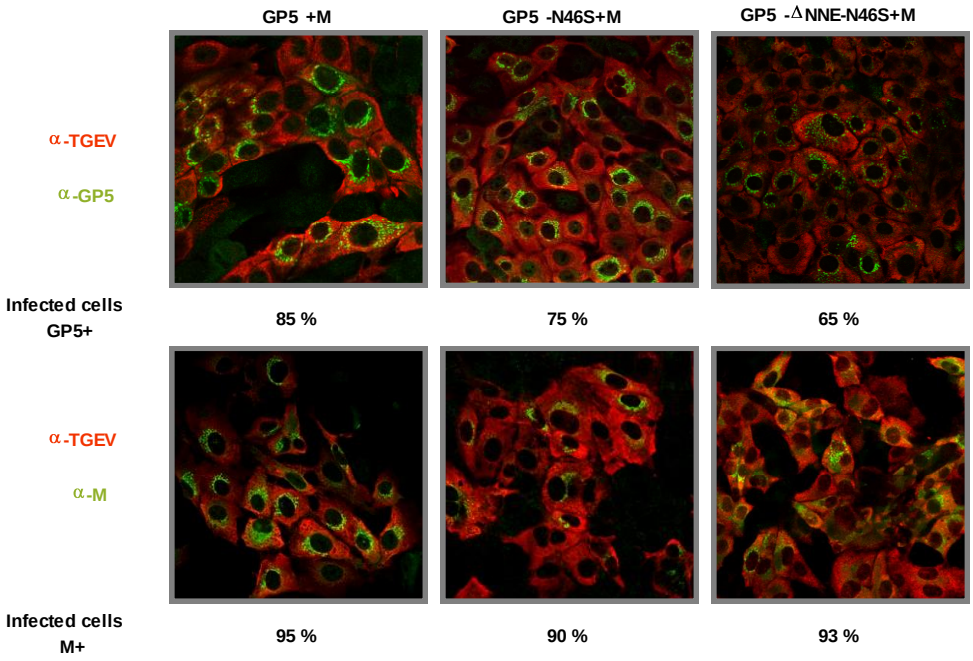

Figure 4. GP5 and M expression by the different rTGEVs. To evaluate the expression levels and stability of rTGEV vectors, swine testis (ST) cells were infected with rTGEVs expressing GP5 and M proteins at a multiplicity of infection (moi) of 2. As a control of the rTGEV infection, TGEV nucleocapsid protein (N) was detected by immunofluorescence using a monoclonal antibody staining red. GP5 and M proteins were detected using specific polyclonal antibodies staining green. Wild-type GP5 and M proteins were expressed in the 85% and 95% of infected cells, respectively. Mutant GP5 proteins were expressed in 75% and 65% of the infected cells, respectively.

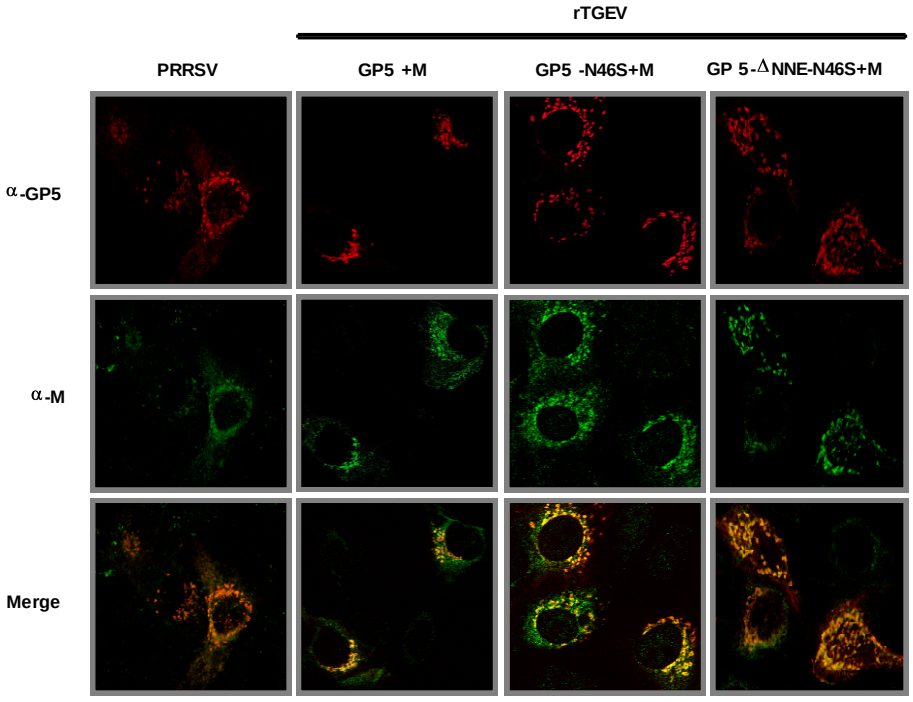

Figure 5. Co-localization of GP5 and M proteins. Expression of GP5 and M proteins by PRRSV forms a heterodimer that has been involved in receptor recognition and viral neutralization. To study if GP5 and M proteins expressed by rTGEVs also colocalize, confocal microscopy analysis was performed. MA-104 or ST cells were infected with PRRSV and the rTGEVs, respectively, and double immunofluorescence staining was performed. Expression of GP5 was detected with a monoclonal antibody specific for GP5, coupled to a secondary antibody staining red. Expression of M protein was detected with a rabbit antiserum specific for an M protein peptide, coupled to a secondary antibody staining green. As shown in the merge, colocalization of GP5 and M proteins was observed both in the PRRSV and rTGEV infected cells. Mutant GP5 proteins expressed by rTGEVs also colocalize with M protein.

Expression of other PRRSV envelope proteins. A rTGEV vector was constructed, expressing PRRSV GP2a. GP3 and GP4. These minor structural proteins are exposed on virus surface assembled as a heterotrimer, and may play a role on protection against PRRSV. A tricistronic rTGEV vector has been constructed, expressing each protein from a separate transcription regulating sequence (TRS). Previous data from our group have shown that GP3 expressed from rTGEVs, alone or in combination with GP5, is highly unstable. Therefore, to minimize toxicity effects of GP3, its expression was driven by a weak TRS and the rest of the components of the heterotrimer (GP2a and GP4) were expressed from the same recombinant vector. Unfortunately, GP3 protein was also toxic in this construct and viable viruses expressing GP2-GP3-GP4 were no recovered. Therefore, new rTGEVs are being generated, expressing GP2, GP4 and GP2-GP4.

In our laboratory, antibodies specifically recognizing PRRSV proteins GP2, GP3 and GP4 are not available. Therefore, each gene has been cloned in a baculovirus system for their expression in insect cells. Baculoviruses expressing high levels of each protein have been generated (Fig. 6). Protein purification and polyclonal antibodies obtention is on-going.

Modification of the type of immune response to rTGEVs. As previously mentioned, GM-CSF has been selected as genetic adjuvant for its expression from rTGEV vectors. GM-CSF has been widely used as an effective mucosal adjuvant (Toka et al., 2004). It has been also described that intranasal inoculation of vectors expressing GM-CSF stimulates IFN-γ and IL-12 production in lung tissues (Bukreyev et al., 2001). Mouse hepatitis coronavirus (MHV)-derived vectors co-expressing heterologous antigens and GM-CSF improved vector immunogenicity. Therefore, GM-CSF was cloned in rTGEV vectors. A virus expressing GM-CSF was obtained. Nevertheless, heterologous gene expression was lost with virus passages in tissue culture, indicating that rTGEV-GMCSF was unstable.

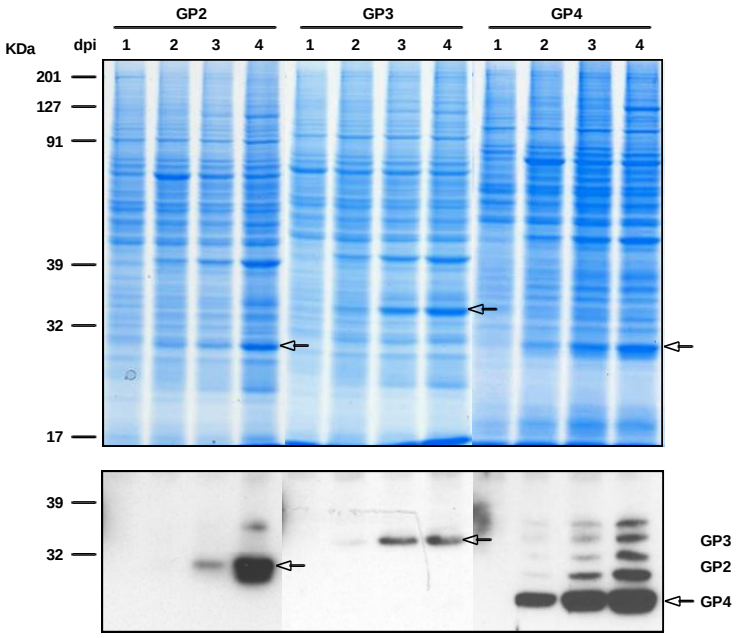

Figure 6. Obtention of baculoviruses expressing PRRSV proteins. Bacmids were generated expressing codon-optimized, His-tagged PRRSV GP2a, GP3 and GP4 proteins. After transfection of cells, baculoviruses expressing high levels of these proteins were obtained. Insect cells were infected with these baculoviruses at moi of 0.05. Protein extracts were obtained at different days post-infection (dpi). Coomassie stained 15% SDS-PAGE gel is shown in the upper panel. Western blot using an antibody specific for the His-tag is shown in the bottom panel.

Discussion

During this year, the efficacy of rTGEV vector expressing M protein and GP5 mutant with altered glycosylation was tested, both in live and inactivated vaccine experiments. In both approaches, vaccinated animals induce faster and higher antibody levels against PRRSV antigens after the challenge with a virulent PRRSV strain. Also, a certain degree of protection was observed in the lung of vaccinated animals. The results obtained using rTGEV as a platform were promising, as a humoral immune response against PRRSV antigens was elicited leading to partial protection.

A complete protection probably requires higher levels of neutralizing antibodies produced before challenge. The presence of an immunodominant (decoy) epitope close to the neutralizing epitope in GP5 could be deleterious for a strong neutralizing immune response. Therefore, a rTGEV vector co-expressing M protein and GP5 mutant with altered glycosylation and lacking the decoy epitope was generated. Preliminary in vivo results obtained with this rTGEV were non-promising. This could be due to the fact that rTGEV vectors expressing PRRSV antigens were not fully stable, mainly due to GP5 protein toxicity resulting in a significant lost of GP5 expression in 8 to 10 passages. In contrast, M protein expression was fully stable, with at least 95% of infected cells expressing M protein for more than 10 passages in tissue culture. The lost of PRRSV antigens expression

could also be the cause for the modest results obtained in protection experiments using live rTGEV vectors, specially compared to those obtained when using killed vaccines formulated with antigens expressed from rTGEV.

Similar difficulties in expressing certain heterologous antigens were found for GP3 and GM-CSF expression. While GP3 protein was highly toxic for TGEV system, GM-CSF expression was lost in 4 passages in tissue cultures.

To improve rTGEV vector stability, two different strategies will be developed:

1. As PRRSV M protein is fully stable when cloned in rTGEV vectors, it will be the base for vectors co-expressing this protein and different small GP5 protein domains containing the neutralizing epitope. Stability of GP5 domain expression, and GP5 fragment-M heterodimer formation will be monitored in each case. This strategy is complementary to the one proposed in NPB #08-19 for the elimination of GP5 protein negative signals.

2. It has been described that coronavirus genomes encoding a mutated nsp14 exonuclease protein (ExoN) displayed a mutator phenotype (Eckerle et al., 2007). TGEV vectors encoding a mutated ExoN and expressing PRRSV antigens will be constructed. It is expected that these rTGEVs accumulate mutations throughout the viral genome, including PRRSV genes. With serial tissue culture passages, only viable rTGEV viruses will be recovered and, from those, the ones stably expressing GP5 protein will be selected. PRRSV ORF5 gene will then be sequenced to analyze the mutations introduced leading to efficient GP5 protein expression. As it is very likely that rTGEV viruses obtained will be highly attenuated for their growth in vivo, the mutated GP5 genes will be introduced in a wild-type rTGEV vector for their use as vaccine vectors.

In summary, all proposed objectives were fully completed when rTGEV vectors were successfully obtained. rTGEV vectors expressing PRRSV antigens will be improved and will be the base for future applications.

Publications and abstracts

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* Cruz, J. L. G., Zúñiga, S., Sánchez, C. M., Ceriani, J. E., Plana, J., and Enjuanes, L. 2008. Design of a recombinant TGEV vector to protect against porcine reproductive and respiratory syndrome. EuroPRRSnet Workshop. Combating PRRS in Europe

* Cruz, J. L. G., Zúñiga, S., Sánchez, C. M., Urniza, A., Bru, T., Ceriani, J. E., Plana, J., and Enjuanes, L. 2008. Construction of a TGEV vector to protect against porcine reproductive and respiratory syndrome. 2008 PRRS Symposium

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