RESEARCH REPORT
SWINE HEALTH

Title: Immunogenicity and potency of PRRS MLV vaccines with and without interferon-alpha suppressing capacity – **NPB - #06-184**

Investigator: Federico A. Zuckermann

Institution: University of Illinois at Urbana-Champaign

Date submitted: March 2, 2009.

II. Industry Summary:

Interferon (IFN)-alpha is the most important anti-viral substance produced by mammalian cells in response to a viral infection. Accordingly, the production of IFN-alpha by an animal during a viral infection is known to be a principal determinant of the animal's ability to fight the infection. In addition, this substance is known to play a major role in regulating and promoting the development of vaccine induced anti-viral immunity and can act as a vaccine adjuvant. Previous studies have indicated that one characteristic of porcine reproductive and respiratory syndrome (PRRS) virus is the failure to stimulate an IFN-alpha response. During our investigations we have determined that a common attribute of PRRS virus isolates, including strains of PRRS virus that have been utilized to prepare the modified live virus (MLV) vaccines, have the ability to suppress the IFN-alpha response of pig leukocytes to other types of pig viruses that otherwise stimulate a strong IFN-alpha response. Thus, the goal of this project was determine the influence of the IFN-alpha inhibitory property of attenuated PRRS virus strains on their ability to stimulate protective immunity. The main impetus for this goal is the apparent lack of potency of currently available vaccines against PRRS virus, which primarily apparently can only provide protection against an infection by a very closely related PRRS virus. The trust to test our notion was further supported by the observation that IFN-alpha can act as a vaccine adjuvant for PRRS modified live virus vaccine as demonstrated by its ability to enhance the cellular immune response of pigs to this veterinary biologic (Meier et al., 2004). Our hypothesis is that the IFN-alpha-suppressing property of a PRRS virus vaccine renders it unable to stimulate strong protective immunity, leading to their suboptimal

These research results were submitted in fulfillment of checkoff funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer reviewed

For more information contact:
National Pork Board, P.O. Box 9114, Des Moines, Iowa USA
800-456-7675, **Fax:** 515-223-2646, **E-Mail:** porkboard@porkboard.org, **Web:** http://www.porkboard.org/

performance. To test this hypothesis, we examined the vaccine potency several attenuated PRRS virus strains, which either had a marked, mild or negligible ability to inhibit the ability of porcine leukocytes to produce IFN-alpha. Two vaccination and challenge studies were conducted with groups of 8-10 week-old pigs, which were immunized with the different PRRS virus vaccine strains. Two additional groups of pigs for each experiment were not vaccinated and served as controls. Four weeks later, one of the unvaccinated groups and all of the vaccinated groups were challenged with the highly virulent atypical PRRS virus isolate NADC-20. At 7-10 days after the virus challenge the amount of virus in the bronchoalveolar lavage fluid and weight change were measured in all groups and used as parameters to evaluate protective immunity. The results obtained in both experiments demonstrated that as predicted, the PRRS live attenuated virus vaccine exhibiting minimal IFN-alpha suppressing activity was the most effective in providing protection from the clinical signs resulting from the challenge with a genetically divergent and highly virulent PRRS virus. This was evidenced by a significantly higher (35-100%) body weight gain during the seven days after the virus challenge, as compared to that of the pigs immunized with vaccine viruses that have either a mild or strong IFN-alpha inhibitory effect. In addition, the virus load in the lung was significantly reduced or eliminated in the pigs that were immunized with the virus with the minimal IFN-alpha suppressing activity. The results of this project indicate that the level of IFN-alpha inhibitory effect of a PRRS MLV vaccine on porcine leukocytes can be used as a predictive parameter of the potential effectiveness (potency) of PRRS virus vaccine and that the use of this biological property of this virus as selection criteria for vaccine strain selection will aid in the development of a more effective PRRS virus vaccine.

Contact information: Federico Zuckermann, University of Illinois, College of Veterinary Medicine. Email: fazaaa@illinois.edu.

III. Scientific Abstract:

The goal of this project was to examine the potency several attenuated PRRS virus strains, which either had a marked, mild or negligible ability to inhibit the ability of porcine leukocytes to produce IFN-alpha. Two vaccination and challenge studies were conducted with groups of 8-10 week-old pigs, which were immunized with the different PRRS virus attenuated strains. Two additional groups of pigs for each experiment were not vaccinated and served as controls. Four weeks later, one of the unvaccinated groups and all of the vaccinated groups were challenged with the highly virulent atypical PRRS virus isolate NADC-20. At 7-10 days after challenge the amount of virus in the bronchoalveolar lavage fluid and weight gain were measured in all groups and used as parameters to evaluate protective immunity. The results obtained in both experiments demonstrated that as predicted, the PRRS live attenuated virus vaccine exhibiting minimal IFN-alpha suppressing activity was the most effective in providing protection from the clinical signs resulting from the challenge with a genetically

divergent and highly virulent PRRS virus. This was evidenced by a significantly higher (35-100%) body weight gain during the seven days after the virus challenge, as compared to that of the pigs immunized with vaccine viruses that have either a strong or mild IFN-alpha inhibitory effect. In addition, the virus load in the lung was reduced >100-fold (day 7 post-challenge) or eliminated (day 10 post-challenge) in the pigs that were immunized with the virus with the minimal IFN-alpha suppressing activity as compared to unvaccinated pigs or those immunized with the MLV with either mild or strong IFN-alpha inhibitory activity. The results of this project indicate that the level of IFN-alpha inhibitory effect of a PRRS MLV vaccine on porcine leukocytes can be used as a predictive parameter of the potential potency of PRRS virus vaccine and that the use of this biological property of this virus as selection criteria for vaccine strain selection will aid in the development of a more effective PRRS virus vaccine.

IV. Introduction:

The significance given by the Pork Checkoff to a new generation of PRRS virus vaccines is clearly exemplified by the prominent rank of this topic in the list of research priorities of this NPB PRRS Initiative Research Objectives. By mid-1994, the first PRRS MLV was released (Ingelvac PRRS MLV). Since then, the use of MLV vaccines has become customary in North America and Europe. It is well accepted that these vaccines are effective in conferring appropriate levels of homologous protective immunity while providing variable degrees of heterologous protection (Mengeling et al., 1996; Mengeling et al., 1999). However, as result of patent litigation most manufacturers have ceased production of their PRRS vaccines – an action that has virtually brought to a halt the incorporation of innovations in the area of PRRS virus biologics (Hill et al., 2004). However, due to advances in the study of this virus, resurgence of interest by major companies in the development of an effective PRRS vaccine can be perceived. A significant advancement is the availability of porcine alveolar macrophage cell line, ZMAC, which was established in the principal investigator's laboratory at the College of Veterinary Medicine at the University of Illinois. These cells have been found to not only be susceptible to infection by PRRS virus but also to release significant amounts of virus progeny. A report of this study funded by the NPB can be found at the NPB web site (NPB Project #05-200).

The impetus for the development of a potent MLV vaccine to combat PRRS virus is provided by several reports demonstrating that only live virus vaccines, but not inactivated vaccines, are able to elicit protective immunity against the reproductive (Osorio et al., 1998; Scortti et a., 2006) and respiratory forms of the disease (Zuckermann et al., 2007). Clearly the development of a next generation PRRS MLV vaccine is feasible and on the horizon. The NPB PRRS initiative has also identified planned exposure with wild-type PRRS virus as a strategic option to control this disease. However, the rational development of MLV vaccine or the selection of a

PRRS virus isolate for a planned exposure, requires that the biological properties of the virus be considered when selecting the candidate virus for attenuation which would then be used as the vaccine strain or, alternatively, the wild-type virus in the planned exposure. Our studies on the immunobiology of PRRS virus have directed us to examine the interaction of PRRS virus with the natural interferon-producing cells, also known as plasmacytoid dendritic cell (pDC). This cell represents 0.2%-0.8% of peripheral blood mononuclear cells in humans, mice, rats, pigs and monkeys (Liu, 2005). Despite its scarcity, this cell is an important component of the innate immune system and is capable of secreting copious amounts of IFN-alpha following viral stimulation (Haeryfar, 2005; Guzylack-Piriou et al., 2004). It is through the secretion of IFN-alpha that PDCs play a major role in regulating antiviral innate and adaptive immunity since they promote the function of natural killer cells, B cells, and T cells (Liu, 2005; Barchet et al., 2005). In addition, the maturation of porcine monocyte derived dendritic cells (MoDC) is aided by the IFN-alpha secreted by pDCs resulting in an enhanced ability of MoDCs to present antigen and activate T cells (Guzylack-Piriou et al., 2006). At a later stage of viral infection, pDCs differentiate into a unique type of mature dendritic cell, which directly regulates the function of T cells and direct the differentiation of T cells into cells capable of secreting IFN-gamma (Liu, 2005), which is a major mediator of antiviral immunity against viruses including PRRS virus (Lowe et al., 2005; Meier et al., 2000; Meier et al., 2003; Meier et al., 2004). Not surprisingly there are two human viruses, respiratory syncitial virus and measles virus, which are known to suppress the ability of pDCs to secrete IFN-alpha. This inhibitory effect is thought to play a role in the predominance of a humoral immune response and the associated immunopathology observed as a result of the infection with these viruses, as well as in the increased susceptibility of the host to secondary bacterial and viral infections (Hengel et al., 2005).

V. Objective:

Determine the immunogenicity and potency of PRRS MLV vaccines with either a strong, mild, or negligible ability to suppress the ability of porcine PBMC to produce IFN-alpha.

VI. Materials & Methods:

Five strains of PRRS virus were used in this study. The G16X, AG1P2 and AG1198 are related laboratory strains that were derived from PRRS virus isolated from a case of PRRS in 1989. The two other strains were Ingelvac PRRS ATP and PrimePac. The Ingelvac PRRS ATP was used immediately after rehydration according to the manufacturer's instructions. The G16X, AG1P2, AG1198 and PrimePac were grown in MARC-145 cells and the stocks diluted with culture medium to obtain the desired dose for inoculation. Two vaccination and challenge studies were conducted with groups of 8-10 week-old pigs that were purchased from the University of Illinois Veterinary Research Farm, which is free of all major pathogens

including PRRS virus. The animals were transferred from the swine farm to a containment facility and allowed to acclimate for one week before the start of the study. After acclimation, the animals were injected once in the rump area with a 2 ml solution containing 1 x 10^4 $TCID_{50}$/ml of PRRS live virus. The attenuated PRRS virus strains used for this project and their IFN-alpha inhibitory phenotype is listed in Table 1. As controls for the experiment, two groups of animals in each experiment were not immunized. Four weeks after immunization, one of the unvaccinated groups and all of the vaccinated groups were challenged both parenterally (intramuscular) and intranasally by administering 2 ml per each route of a solution containing $5x10^4$ $TCID_{50}$/ml of the highly virulent atypical PRRS virus isolate NADC-20.

The degree of protective immunity elicited by the vaccine was established based on; 1) the change in body weight from the day of the challenge to 7 days later and 2) viral load in the lung. At seven (first experiment) or ten (second experiment) days after the challenge, the animals were euthanized and the viral load in lung tissue determined from brochoalveolar lavage (BAL) fluid collected from the accessory lobe. The BAL sample obtained by squirting 40 ml of sterile saline into the accessory lobe, using a 60 cc syringe connected to a catheter that was placed into the bronchi leading to this lobe followed by aspiration of the fluid. Viral titers were determined by standard procedures using MARC-145 cells as previously described (Zuckermann et al., 2007). The statistical significance of the data was determined by Student's T test.

Table 1. Interferon alpha-inhibitory phenotype of PRRS viruses used for this project

PRRS virus strain	Assigned IFN-alpha inhibitory phenotype	Intensity of IFN-alpha inhibitory phenotype (%)*
G16X	Minimal	<6
AG1198/ AG1P2	Strong	>60
Ingelvac PRRS ATP	Strong	>60
PrimePac PRRS	Mild	>15 - <50

*Interferon alpha-inhibitory phenotype was determined based on the % reduction of the frequency of IFN-alpha-secreting cells in porcine peripheral blood mononuclear cells (PBMC) isolated from groups of pigs 7 days after inoculation with the indicated virus, as compared to frequency of IFN-alpha secreting cells measured in the same animal the day of virus inoculation. The average percent reduction of IFN-alpha production from groups of 3-12 animals per virus was used to determine the virus phenotype. The IFN-alpha response was elicited by the exposure of PBMCs to transmissible gastroenteritis virus (TGEV) and measured by ELISPOT (Royaee, et al., 2004).

VII. Results:

The five strains of PRRS virus were used for this project are listed in Table 1. In previous experiments we had determined that the Ingelvac PRRS ATP, AG1198 and AG1P2 strains, similar to wild-type PRRS virus, strongly inhibit the ability of porcine mononuclear cells to produce IFN-alpha in response to their stimulation to TGEV. On the other hand the vaccine strain PrimePac was found to have mild inhibitory effect, while the G16X strain had a negligible effect (data not shown).

Two separate vaccination and challenge experiments were conducted to determine the potency of these vaccines. In the first experiment groups of pigs were inoculated with either the PrimePac, G16X, or AG1198 virus strains. As a control for the challenge two additional groups of pigs were not vaccinated. Four weeks later, all of the animals in the vaccinated groups as well as one of the unvaccinated groups were challenged with a highly virulent atypical PRRSV isolate NADC-20. At 10 days post challenge, the viral load in the bronchoalveolar lavage (BAL) fluid of the animals vaccinated with the AG1198 or the PrimePac strains was 2/3 or ½ as high, respectively, as the unvaccinated and challenged controls. In contrast, the BAL fluids of all of the animals vaccinated with G16X strain were free of challenge virus (Fig. 1). Further evidence of the solid protective immunity provided by vaccination with the G16X isolate was provided by the weight gain measured at 7 days after challenge. In this case, unvaccinated and challenge pigs had an average BW change of –5±2 lbs from the day of challenge to 7 days later, while the unvaccinated and unchallenged animals gained on average 19.6±2 lbs within that period (Fig. 3). In contrast, animals that were vaccinated with the AG1198 strain had an average BW loss of -5±0.5 lbs, similar to the unvaccinated controls. In contrast, animals immunized with the G16X strain had average BW gain of 18±0.7 lbs, which is not significantly different from the unchallenged controls. These results suggest that the IFN-α phenotype of the virus has a significant influence on the ability of the vaccine virus to elicit protective immunity.

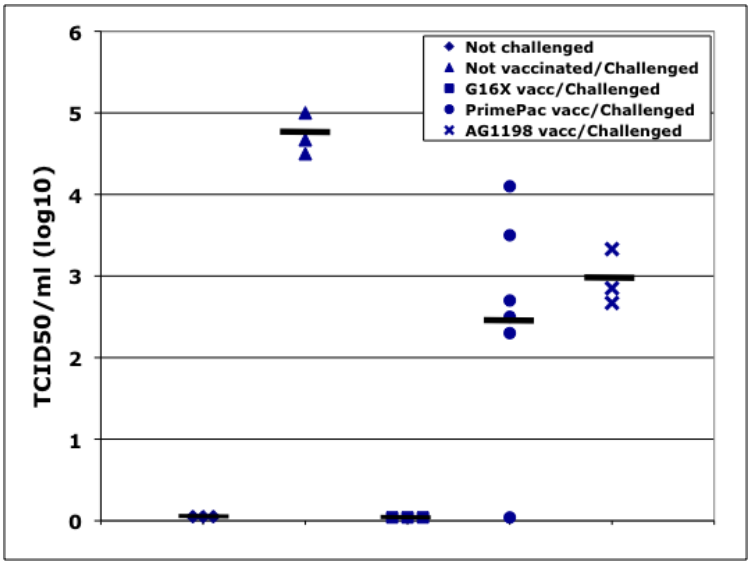

Fig. 1. Virus titers in the BAL fluids of pigs after challenge with PRRSV NADC-20. Groups of pigs were inoculated with either PrimePac, G16X, AG1198, or a mock inoculum, and challenged 4 weeks later with PRRSV NADC-20. A separate group of pigs remained PRRSV-naïve throughout the study. The lungs of these animals were removed after euthanasia at 10 days post-challenge. BAL fluids were collected and the titer of infectious virus in these BAL samples determined. Each symbol represents the titer detected in each sample and the horizontal bars represent the average $\log_{10}$ $TCID_{50}$/ml for each group.

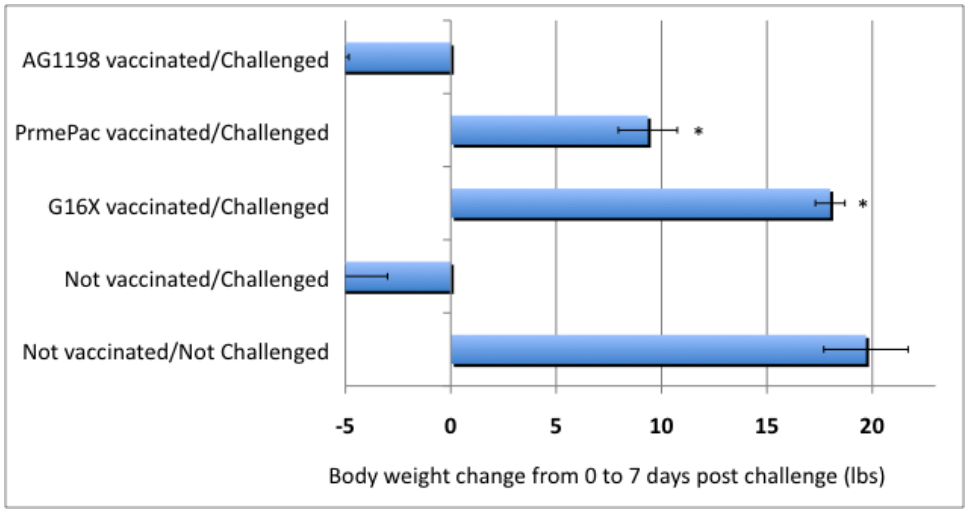

Fig. 2. Effect of challenge with virulent PRRS virus on body weight. Groups of pigs were inoculated with either PrimePac, G16X, AG1198, or a mock inoculum, and challenged 4 weeks later with PRRSV NADC-20. A separate group of pigs remained PRRSV-naïve throughout the study. The body weight of each animal in the study was measured and recorded immediately prior to and at 7 days after challenge with the wild-type PRRS virus isolate NADC-20. The data represents the mean ± s.e. body weight (lbs) change of the animals in each group during the 7-day interval. Statistically significant differences ($P<0.001$) between the vaccinated and challenged groups and the unvaccinated and challenged group are indicated with an asterisk.

A second experiment was performed in which groups of pigs (n=6 per group) were vaccinated with either Ingelvac PRRS ATP, G16X or AG1P2. Two additional groups of pigs were not vaccinated. Four weeks later, one of the unvaccinated groups and all of the vaccinated groups were challenged with the highly virulent PRRS virus isolate NADC-20.

At 7 days post challenge, the average viral load in the bronchoalveolar lavage (BAL) fluid of the animals vaccinated with either the Ingelvac PRRS ATP vaccine or the AG1P2 strain was not different from that detected in the unvaccinated animals (Fig. 3). In contrast, average viral load in the BAL recovered from the lungs of animals vaccinated with the G16X was less than half ($10^{2.2}$ $TCID_{50}$/ml) than the average viral load in the unvaccinated and challenged animals ($10^{4.6}$ $TCID_{50}$/ml). The pigs in the unvaccinated group had an average BW gain of 3.4±2 lbs while the unchallenged animals gained on average 21±1.5 lbs within that period (Fig. 4). The animals that were vaccinated with Ingelvac PRRS ATP vaccine or the AG1P2 strain had an average BW gain of 6.4±1.7 lbs and 6.8±0.9 lbs, respectively, which is not significantly different from the average calculated from the animals from unvaccinated and challenged control group. In contrast, the mean BW gain calculated from the animals immunized with the G16X strain (10.3±1.2 lbs) was significantly different ($p<0.01$) from the mean calculated from the unvaccinated and challenged control group (3.4±2 lbs).

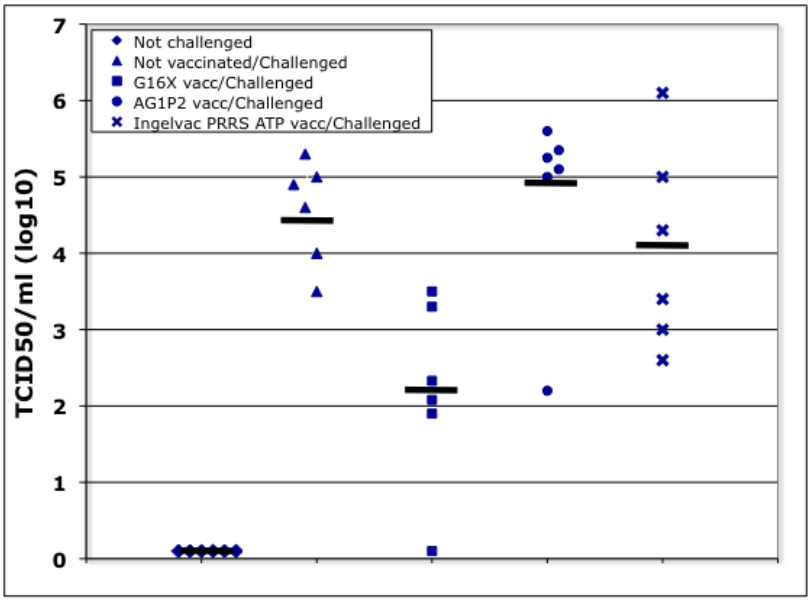

Fig. 3. Virus titers in the BAL fluids of pigs after challenge with PRRSV NADC-20. Groups of pigs were inoculated with either Ingelvac PRRS ATP, G16X, AG1P2, or a mock inoculum, and challenged 4 weeks later with PRRSV NADC-20. A separate group of pigs remained PRRSV-naïve throughout the study. The lungs of these animals were removed after euthanasia at 7 days post-challenge. BAL fluids were collected and the titer of infectious virus in these BAL samples determined. Each symbol represents the titer detected in each sample and the horizontal bars represent the average $\log_{10}$ $TCID_{50}$/ml for each group.

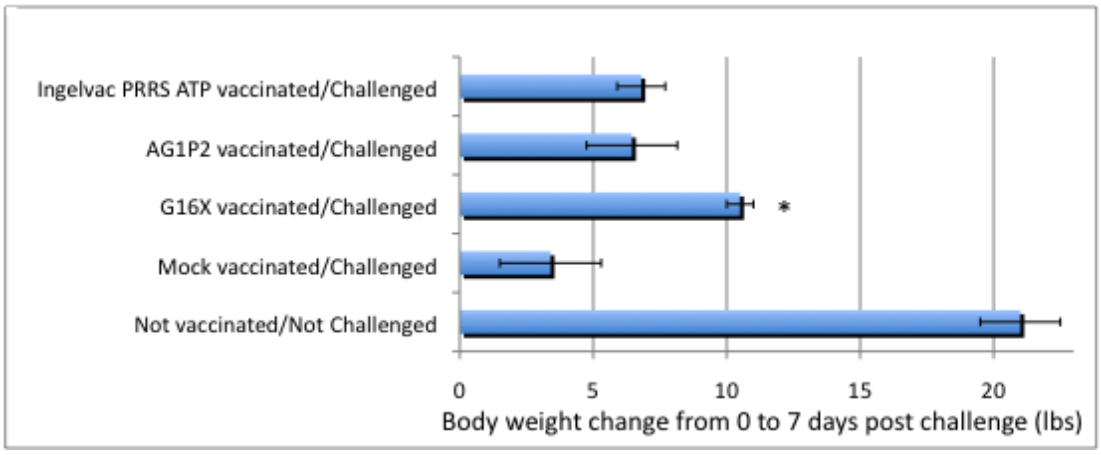

Fig. 4. Effect of challenge with virulent PRRS virus on body weight. Groups of pigs were inoculated with either Ingelvac PRRS ATP, G16X, AG1P2, or a mock inoculum, and challenged 4 weeks later with PRRSV NADC-20. A separate group of pigs remained PRRSV-naïve throughout the study. The body weight of each animal in the study was measured and recorded immediately prior to and at 7 days after challenge with the wild-type PRRS virus isolate NADC-20. The data represents the mean ± s.e. body weight (lbs) change of the animals in each group during the 7-day interval. Statistically significant differences ($P<0.01$) between the vaccinated and challenged groups and the unvaccinated and challenged group are indicated with an asterisk.

VIII. Discussion:

The results obtained in both experiments demonstrated that as predicted, the PRRS live attenuated strain G16X, which exhibits minimal IFN-alpha suppressing activity was fairly effective in providing protection from the clinical signs resulting from the challenge with a genetically divergent and highly virulent PRRS virus. This was evidenced by a significantly higher (35-100%) body weight gain during the seven days after the virus challenge, as compared to that of the pigs immunized with vaccine viruses that have either a strong or mild IFN-alpha inhibitory effect. In addition, the virus load in the lung was reduced >100-fold (day 7 post-challenge) or eliminated (day 10 post-challenge) in the pigs that were immunized with the virus with the minimal IFN-alpha suppressing activity as compared to unvaccinated pigs or those immunized with the MLV with IFN-alpha inhibitory activity. The results of the first experiment were compelling and indicated that complete protection is attainable by vaccination of pigs with a non-IFN-alpha inhibitory PRRS virus upon challenge with a highly virulent atypical PRRS virus isolate.

The results obtained suggest that the IFN-α phenotype of the virus has a significant influence on the ability of the vaccine virus to elicit protective immunity. The observation is relevant to the issue of immunogenicity of a vaccine because it is known that IFN-alpha is a potent regulator of the humoral and cellular immune response to a virus (Burchet et al., 2005) and can act as an adjuvant (Tovey et al., 2006). The observations made in this study provide evidence that support our hypothesis, namely that a live attenuated virus vaccine exhibiting a low IFN-alpha suppressing phenotype will be significantly more immunogenic and

effective in providing protective immunity against a genetically divergent PRRS virus challenge as compared to a live attenuated virus vaccine with a strong IFN-alpha suppressing phenotype. Future studies will focus on identifying the mechanism responsible for the inhibition as well as the viral component responsible for this important biologic effect of PRRS virus.

References:

Barchet W, Cella M, Colonna M. Plasmacytoid dendritic cells--virus experts of innate immunity. Semin Immunol. 2005 Aug;17(4):253-61.

David, F. R., S. K. Reddy, and M. E. Tanner. 2003. Cotton rat lung cells for virus culture. US Patent Application 20030219460.

Guzylack-Piriou L, Piersma S, McCullough K, Summerfield A. Role of natural interferon-producing cells and T lymphocytes in porcine monocyte-derived dendritic cell maturation. Immunology. 2006 May;118(1):78-87.

Haeryfar SM.The importance of being a pDC in antiviral immunity: the IFN mission versus Ag presentation? Trends Immunol. 2005 Jun;26(6):311-7.

Hengel, H., Koszinowski, U.H., Conzelmann, K.K., 2005. Viruses know it all: new insights into IFN networks. Trends Immunol. 26 (7), 396 – 401

Hill, H., M. JA, K. JJ, H. A, and R. C. 2004. PRRS Control: To Hell and Back. American Assoc Swine Veterinarians, Des Moines, Iowa, USA, March 6-9, 2004:369-376.

Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol. 2005;23:275-306. Review.

Lowe JE, Husmann R, Firkins LD, Zuckermann FA, Goldberg TL. 2005. Correlation of cell-mediated immunity against porcine reproductive and respiratory syndrome virus with protection against reproductive failure in sows during outbreaks of porcine reproductive and respiratory syndrome in commercial herds. J Am Vet Med Assoc. 226:1707-11.

Meier, W., Wheeler, J., Husmann, R.J., Osorio, F. and Zuckermann, F. 2000. Characteristics of the immune response of swine to PRRS virus. Vet. Res. 31: 41.

Meier, W.A., Galeota, J., Osorio, F.A., Husmann, R., Schnitzlein, W., and Zuckermann, F.A. 2003. Gradual development of the interferon-g response of swine to porcine reproductive and respiratory syndrome virus. Virol. 309:18-31.

Meier, W.A., Husmann, R., Schnitzlein,W., Osorio, F., Lunney, J. , Zuckermann, F.A. 2004. Cytokines and synthetic double-stranded RNA augment the T helper 1 immune response of swine to porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 102: 299-314.

Mengeling, W. L., K. M. Lager, and A. C. Vorwald. 1999. Safety and efficacy of vaccination of pregnant gilts against porcine reproductive and respiratory syndrome. Am J Vet Res 60:796-801.

Mengeling, W. L., A. C. Vorwald, K. M. Lager, and S. L. Brockmeier. 1996. Comparison among strains of porcine reproductive and respiratory syndrome virus for their ability to cause reproductive failure. Am J Vet Res 57:834-9.

NPB project #05-200 entitled "A study on the feasibility of using a porcine alveolar macrophage cell line to produce a PRRS modified live virus vaccine"

Osorio, F. A., F. Zuckermann, R. Wills, W. Meier, S. Christian, J. Galeota, and A. Doster. 1998. PRRSV: comparison of commercial vaccines in their ability to induce protection against current PRRSV strains of high virulence. Allen D. Leman Swine Conference 25:176-182.

Scortti M, Prieto C, Simarro I, Castro JM.Reproductive performance of gilts following vaccination and subsequent heterologous challenge with European strains of porcine reproductive and respiratory syndrome virus. Theriogenology. 2006 Jun 23; [Epub ahead of print]

Tovey MG, Lallemand C, Meritet JF, Maury C. Adjuvant activity of interferon alpha: mechanism(s) of action. 2006. Vaccine. 12;24 Suppl 2:S2-46-7.

Zuckermann, FA, Alvarez-Garcia E, Diaz Luque I, Christopher-Hennings J, Doster A, Britio M, Osorio FA. 2007. Assessment of the efficacy of commercial porcine reproductive and respiratory syndrome virus (PRS) vaccines based on measurement of serologic response, frequency of interferon gamma-producing cells and virological parameters of protection upon challenge. Vet Microbiol 123:69-85 .