

1.2 Porcine Reproductive and Respiratory Syndrome Virus Infection: Effects on Innate and Acquired Immune Responses (Osorio F, Pattnaik A)

Our laboratories focus on PRRSV protective immunity and virulence. Throughout our past and current research we have learned that PRRSV infection is characterized by a delayed and defective adaptive immune response. We identified certain pathogenic mechanisms (such as decoy epitope deploying or glycan shielding) that would suggest that PRRSV employs diverse strategies to subvert and/or evade the host's immune response, thus securing an abundant and unrestricted viral replication during the acute phase of infection and a long persistence in the host. Several laboratories have also reported that PRRSV is a poor inducer of IFN- α both in vitro and in vivo. We recently confirmed this weak IFN induction phenotype exhibited by PRRSV in monocyte-derived swine macrophages. Under the hypothesis that this early post-infection action of PRRSV on the innate immune response may prime the host towards a more permanent and enduring defect in the overall acquired protective immune response, we have recently screened all non-structural proteins (NSPs) of PRRSV identifying at least four (NSP1, NSP2, NSP4 and NSP11) having inhibitory activity towards IFN production. Of these, the strongest inhibitor of IFN production is NSP1 β , affecting primordially dsRNA signaling pathways. Detailed investigation of the signaling pathways in presence of NSP1 β revealed that this NSP subunit inhibits TLR3 signaling pathway downstream of TRIF and IKK ϵ , as well as strongly inhibiting phosphorylation and nuclear translocation of IRF3. We propose to further characterize this anti-IFN activity of the NSPs of PRRSV by pursuing three main objectives: 1) to gain a complete understanding of the signaling pathways involved in the anti-IFN activity of NSP1 β ; 2) to map domain(s) involved in executing this anti-IFN activity of NSP1 β ; and 3) using our highly pathogenic FL12 infectious clone as a backbone, to construct mutant PRRSV carrying mutation/deletion(s) in the anti-IFN domain, for in vitro and in vivo characterization. We hypothesize that this remediation/modulation of the anti-IFN effect of PRRSV may have a direct effect on virulence of the mutant and/or on modulating the overall immune response induced by this pathogen.

1.3 Porcine Reproductive And Respiratory Virus: Role Of Viral Genes In Virulence/Attenuation(Osorio F, Pattnaik, A)

The overall aim of our laboratories is to achieve a goal that is seen as a major national priority for the swine industry: the development of a "new generation", effective and safe PRRSV vaccine.

Identifying virulence markers and finding rational ways for attenuation of vaccine candidate strains is a top priority. Our laboratories have developed a fully functional infectious cDNA clone (IC) of PRRSV. Under NRICGP 2004-01576, we initiated reverse genetics experiments addressed at answering a fundamental question: What is the molecular basis of attenuation of virulence in PRRSV? We have used our IC for the development of chimeras between PRRSV strains of different degrees of virulence. We have shown that a NSP-coding area of the PRRSV genome is a major cluster of virulence. Also ORF5 and ORF2 contain structural determinants of virulence. Likewise, site-specific mutagenesis of GP5 (product of ORF5) indicated to us that PRRSV evades the pig's immune system by means of a glycan-shielding mechanism. Also under the same grant we have studied epitopes in different PRRSV proteins that could be deleted or modified to be used as serologic differential markers.

To complete the mapping of PRRSV virulence and finding molecularly attenuated strains, we now propose experiments that are the logical continuation of our discovery of virulence determinants of PRRSV, through the pursuit of the following goals: 1) to conduct a fine mapping of the sequences (amino-acid residues) in ORF 5 and ORF2 that contribute to virulence 2) to identify the individual NSP genes and sequences that have a principal role in PRRSV virulence . Besides developing rationally attenuated strains, our overall work contributes to a fundamental understanding of the viral mechanisms influencing PRRSV pathogenesis.

Objective 2 Understand the ecology and epidemiology of PRRSV and emerging viral diseases of swine

2.1 Immunologic Consequences of PRRSV Diversity (Osorio F, plus faculty at UIUC- W Laegreid- ,UWI _Tony Goldberg- and SDSU-Jane Christopher Hennings and Eric Nelson-)

Understanding what defines PRRSV strains as immunologically homologous or heterologous is critical to the development of vaccines for PRRS control. Unfortunately, PRRSV natural variation has never been explored systematically in order to define broad, functionally relevant viral groupings. As a first step towards filling this critical knowledge gap, we propose the following objectives:

- 1 Objectively define and completely sequence a core set of viruses representing the breadth of PRRSV sequence variation
- 2 Associate relevant immunologic phenotypes with specific PRRSV genomic variation.

Because PRRSV is one of the most genetically variable RNA viruses, we will objectively define a set of PRRSV strains that is broadly representative of PRRSV variation. Complete genomic sequences of this set of viruses will be obtained which, in combination with existing genomic sequences, represent the breadth of PRRSV genomic variation. This set of sequences will allow unprecedented analyses of PRRSV genomic variation.

Since the ultimate goal of this research is to provide effective vaccines, it is essential to have well characterized phenotypes that are associated with specific genomic sequences, and that are also relevant to protective immunity. Immunologic cross-protection among a subset of viruses from the core set will therefore be quantified in pigs. Using antisera from these animal experiments and reference antisera, all remaining isolates in the core set will be characterized for cross-neutralization *in vitro*. In addition, serum samples from cross-protection studies will be used to evaluate inter-isolate variation in *in vivo* ability to elicit responses of eight highly relevant cytokines. The complete data set will finally be analyzed for associations between genomic features and specific immunologic phenotypes. The results of these studies will provide the basis for formulation and/or engineering of vaccine(s) which cover the spectrum of PRRSV variation.

Objective 3: Develop effective and efficient approaches for detection, prevention and control of PRRSV and emerging viral diseases of swine

3.1 Genetic variation in PCAVD (Ciobanu DC, Osorio, FA, and Johnson RK)

Mortality, morbidity, and slow growth associated with Porcine Circovirus Associated Disease (PCVAD) cause high economic losses to swine producers. Studies of our group and others found that host genetic variation influences the incidence of PCVAD. Our central hypotheses are: 1) there are many genes that affect PCVAD resistance, and 2) modest heritability and high phenotypic variance indicate that considerable genetic variation exists and resistance to PCV2 can be improved by Marker Assisted Selection. The primary objectives of this proposal are:

1) Detect regions of the genome that affect PCVAD severity and harbor key modulators of the changes in gene expression following infection.

2) Identify genes, pathways and combination of allelic variants that influence PCVAD severity.

We predict that this research will provide necessary knowledge of the PCV2-associated immune response as well as a better understanding of the influence of host genetics on PCVAD susceptibility. This information will be used to develop a panel of markers available to producers to identify pigs with gene variants that increase the resistance to PCVAD. The long-term goal of this project is to decrease PCVAD susceptibility in swine and reduce economic losses to producers.

C.IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

The most significant impacts on the multi-station collaborative network of NC-229 by the work conducted at Nebraska are:

- 1) Four refereed papers involving our laboratories have been published during the period covered in this report.
- 2)) During 2009 a UNL patent has been filed and accepted in the US and Europe. Patent Title: *Methods And Compositions For Vaccination Of Animals With PRRSV Antigens With Improved Immunogenicity*. Inventors: Ansari, I, Osorio FA, and Pattnaik, AK Serial No. 12/064,877, Filing Date February 26, 2008, Issued: October 27, 2009.
- 3) With funds provided through CAP2 funds (subcontract KSU, PIs: Laegreid et al) our station is funding the travel expenses and attendance of external experts to a preparative meeting in the US. Such experts are three European consultants (Drs Prieto, Enjuanes, and Mateu) who advise on planning and execution of our CAP2 project (activity detailed in point 2.1, Objective 2, this report).

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

1. Refereed publications

de Lima, M. Ansari, I. H., Das, P. B., Ku, B., Martinez-Lobo, F. J., Pattnaik, A. K., and Osorio, F. A. (2009). GP3 is a Structural Component of the PRRSV Type II Virion. *Virology*, 390:31-36

Jar, A.M., Osorio, F.A., Lopez, O.J. 2009 Mouse x pig chimeric antibodies expressed in baculovirus retain the same properties of their parent antibodies. *Biotechnology Progress* Mar-Apr;25(2):516-23

Beura, L. K., Sarkar, S. N., Kwon, B. J., Subramaniam, S., Jones, C., Pattnaik, A. K., and Osorio, F. A. (2010). Porcine Reproductive and Respiratory Syndrome Virus nonstructural protein nsp1b modulates host immune response by antagonizing IRF3 activation. *J. Virology* In press

Das, P. B., Dinh, P. X., Ansari, I. H., de Lima, M., Osorio, F. A., and Pattnaik, A. K. (2010). The Minor Envelope Glycoproteins GP2a and GP4 of Porcine Reproductive and Respiratory Syndrome Virus Interact with the Receptor, CD163. *J. Virology*

2) Abstracts or Proceedings

Worldwide Research Efforts Towards a Broadly Protective and Effective Vaccine against PRRSV, Osorio FA, Keynote presentation # 4 at the 2008 International PRRS Symposium, Chicago, IL, USA, December 6, 2008

PRRSV immunology and vaccines Second Annual CVM Swine Health Initiatives Meeting UIUC, Osorio FA Date: 01/28/2009

PRRSV protective immunity and immunization, Osorio FA presented at the XVIII Congreso Dia del Porcicultor, Navojoa, Sonora Mexico

Porcine reproductive and respiratory syndrome virus non structural protein 1 beta inhibits host innate immune response by antagonizing IRF3 activation Beura L, Sarkar S, Kwon BJ, Subramaniam S, Jones C, Pattnaik AK, Osorio FA. Proceedings of the 28th Annual Meetg American Society for Virology, Vancouver, BC, July 11-15, 2009 (Workshop 33-6)

Analysis of the aberrant immune response induced by a PRRSV type 2 isolate naturally lacking glycan residues in two envelope glycoproteins. 2009. Hiep Vu, Kwon BJ, Yoon KJ, Laegreid W, Pattnaik AK and Osorio FA To be presented at the 2009 PRRSV International Symposium (poster # 84) and the 2009 CRWAD Meeting (poster # 64), December 4- 8 2009

3) Book chapters or monographs

None

E. FUNDING SOURCES FOR PRRSV RESEARCH

Current:

Molecular Structures of PRRSV that Contribute to PRRSV Protective Immunity P.I.: A.K. Pattnaik; National Pork Board. \$ 138,600; 12/01/2009-11/31/2010.

Glycoproteins of Porcine Reproductive and Respiratory Syndrome Virus in Infection and Immunity?; P.I.: A.K. Pattnaik; United States Department of Agriculture, AFRI (2009-01576), \$371,230; 09/01/2009-08/31/2012.

Immunologic Consequences of PRRSV Diversity, USDANRICGP CAP2 (Kansas State University subcontract), \$74,368 August 2009-July 2010. PI: Osorio FA

Development of a modified live vaccine against PRRSV with optimal DIVA marker potential PI: Osorio FA, National Pork Board, Grant Period: 11/01/2008 - 12/31/2009(extended at no cost) \$125,700

Role of All of PRRSV Glycoproteins in Protective Immune Response

Grant Period: 11/01/2008 - 10/31/2009 (extended at no cost) Grant Type: Research/Creative Activity, National Pork Board PI: AK Pattnaik \$106000

Porcine Reproductive and Respiratory Virus: role of viral genes in virulence/attenuation PI: Osorio FA USDA NRICGP Project No. No.2008-00903, Period: 09/01/2008 - 08/31/2011, \$374900

F. WORK PLANNED FOR NEXT YEAR

- 1) Continue research on virulence markers of PRRSV (collaborating agencies: Nebraska, Illinois)
- 2) Seek renewal of funds (CAP2) and continue research on sero-typing of PRRSV strains and characterization of PRRSV strain diversity (collaborating agencies : Nebraska, Illinois, Wisconsin, South Dakota, and ISU)
- 3) Continue research on induction of protective immunity of PRRSV(collaborating agencies: Nebraska, Illinois, South Dakota, and ISU)
- 4) Seek funding (AFRI-USDA) and continue research on characterization of the action of viral proteins that influence innate immunity X PRRSV (collaborating agencies: Nebraska, Illinois, Cleveland Clinic)
- 5) Seek funding (NPB, AFRIUSDA) and continue research on genetic control of PCAVD in pigs(collaborating agencies: Nebraska, ISU)