RESEARCH ABSTRACT

SWINE HEALTH

Title: PRRSV protective immunity of broad spectrum: Strategies to induce pan-neutralizing antibodies in a pig - **NPB #12-158**

Investigator: F. A. Osorio, Co-investigator: A K Pattnaik

Institution: University of Nebraska-Lincoln

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Scientific Abstract:

It has been shown by us and others that neutralizing antibodies are important for protection against PRRSV infection in vivo. However, multiple initial trials using GP5 and M as the only PRRSV structural subunits responsible for inducing neutralizing antibodies have shown that these two subunits are of secondary importance at inducing cross protecting antibodies X PRRSV. We postulate that the other "minor" glycoproteins (GP2, GP3, and GP4) of PRRSV are of fundamental importance for induction of protective immunity against PRRSV. Of particular importance to sustain our hypothesis are our findings that demonstrate that the primary viral proteins involved in binding to CD163 (CD163 is the most important cell receptor used by PRRSV to infect the swine macrophage) are at least the minor envelope proteins GP2 and GP4, although the minor GP3 cannot be ruled out at this time either. Since alterations of the GP2 and GP4 contact residues (or contact epitopes) that directly interact with the receptor CD163 would likely impair infectivity and jeopardize PRRSV survival in nature, we hypothesize that such epitopes or residues are likely to be highly conserved and less prone to mutations and that the antibodies targeting these epitopes have higher chance of being broadly neutralizing across the spectrum of PRRSV strains than are antibodies to other viral proteins. In other words, we postulate and plan to demonstrate that these minor glycoproteins of PRRSV are the real structural basis for origination of pan-neutralizing antibodies (a.k.a. broadly neutralizing antibodies) in the swine host.

Initially, the overall goal of this NPB supported research was to discover and characterize high affinity PRRSV neutralizing antibodies derived from B cells of PRRSV-vaccinated/challenged pigs. Central to the success of this project was the contribution of the CellSpot cloning technology patented by our collaborators at Trellis BioScience (San Francisco, CA).

We initiated this project by developing whole baculovirus-expressed PRRSV GPs (GP2, GP3, GP4 and GP5) that could be used for singly immunizing pigs through a prime-boost immunization strategy and"subsequent B cell cloning and identification of B cells secreting high-affinity PRRSV neutralizing antibodies from immunized/challenged pigs by Trellis BioScience". However, we found serious difficulties at producing, out of

the baculovirus clones obtained, adequate amounts of purified GPs that would be apt for immunization. We decided to develop an alternative vector expression of the PRRSV GPs by cloning these GPs in human adenovirus. Likewise, initial attempts at B-cell cloning indicated to us that stimulation of B cells and ensuing single cell cloning would be more straightforward (and likely more effective) by directly using the peptides and short polypeptides that contain the epitopes of interest for induction of broadly neutralizing antibodies. To that end we expanded this project to evaluate the residues of GP2 and GP4 that are responsible for the interaction with the cellular receptor CD163, using a mammalian two-hybrid system used to screen site mutated glycoproteins. Therefore, the original NPB proposal with the expanded goal (at no cost to NPB) of identification of contact residues in GP2 and GP4 served for us to be awarded federal competitive funds from the USDA-AFRI-NIFA program (Animal Health, 2013-2016), thus significantly expanding (4X) the original funds awarded by NPB.