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## Molecular Structures of PRRSV that Contribute to PRRSV Protective Immunity

Asit K. Pattnaik

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## SWINE HEALTH

**Title:** Molecular Structures of PRRSV that Contribute to PRRSV Protective Immunity  
**NPB: # 09-248**

**Investigator:** Asit K. Pattnaik, Ph.D

**Co-Investigator:** Fernando A. Osorio, D.V.M.; Ph.D.

**Institution:** University of Nebraska-Lincoln, Lincoln, Nebraska.

**Date Submitted:** April 15, 2011

### Industry Summary:

Porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus (PRRSV) is of major economic significance to swine industry. There is no effective vaccine currently available to combat PRRS. In previous studies, we demonstrated that virus-neutralizing antibodies are important for protective immunity against PRRSV. These neutralizing antibodies constitute a significant parameter for evaluating the efficacy of a vaccine. Although four viral glycoproteins (GPs) are present in PRRSV, their roles in the virus' biology, especially in their capacity to induce a protective immune response in the pig, remains poorly understood. Development of safe and efficacious vaccines to combat PRRSV infections requires a basic understanding of the role of these GPs in virus biology. In particular, identification and characterization of the viral glycoproteins that interact with the cellular receptor (CD163), which is a key component of the cell that permits the penetration and infection of the cells by PRRSV. Furthermore, determining the precise areas of contact between these viral GPs and CD163 is important in developing strategies to inhibit the process of binding of the virus to the

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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 This report has not been peer-reviewed.

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cells, so that virus infections can be blocked. In a previously funded NPB project (#08-253), we had demonstrated that two (namely, the GP2 and GP4) of the four PRRSV GPs specifically interact with CD163. One of the objectives of this proposal (#09-248) has been to delineate the regions of these two GPs that interact with CD163. Other objectives of the 09-248 proposal were to generate antibodies to these small regions of the GPs as well as to the entire proteins for future studies to determine if any of these antibodies possess PRRSV neutralizing (or inactivating) activity.

To carry out the studies in the proposed objectives, we generated a series of mutants of PRRSV GP2 and GP4 proteins in which various regions were specifically removed by manipulating the plasmids encoding these proteins. We then examined these proteins for their ability to interact with CD163 to ascertain the regions important for such interactions. Our results identified the regions of GP2 and GP4 that appear to interact with CD163. Furthermore, we generated recombinant baculoviruses that expressed these viral GPs. The viral proteins were purified from the cells and have been used to generate antibodies.

Further studies will be conducted to characterize these antibodies in the future. In addition, we are now exploring, beyond the life of this grant, alternative novel strategies to obtain high affinity swine monoclonal antibodies that would inactivate PRRSV with very high efficiency. We are conducting these studies in collaboration with an industry partner (Trellis Biosciences, San Francisco, CA). The results obtained through this NPB support (09-248) have been critical for initiating such collaborative work with the biotech industry. Part of our studies supported by the NPB grant (#09-248) has also been recently published in *Virology* (Das et al., *Virology*, 410: 385-394, 2011; a copy of the paper has also been forwarded to B. L. Everitt).

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**Key Words:**

PRRSV; GP2; GP4; CD163; Protein interaction; Baculovirus expression; Deletion mapping; antibodies.

**Scientific Abstract:**

One of the objectives of the proposal is to delineate the regions of the two glycoproteins (GP2 and GP4) of PRRSV that interact with CD163. Other objectives of the proposal are to generate antibodies to these small regions of the glycoproteins as well as to the full-length proteins for future studies to determine if any of these antibodies possess PRRSV neutralizing activity.

To carry out the studies in the proposed objectives, we generated a series of mutants of GP2 as well as GP4 proteins in which various regions were specifically removed by manipulating the plasmids encoding these proteins. We then examined these proteins for their ability to interact with CD163 to ascertain the regions important for such interactions. Our results identified the regions of GP2 and GP4 that appear to interact with CD163. Furthermore, we generated recombinant baculoviruses that expressed these viral GPs. The viral proteins were purified from the cells and have been used to generate antibodies. Further studies will be conducted to characterize these antibodies in the future. Part of our studies supported by the NPB grant (#09-248) has been recently published in *Virology* (Das et al., *Virology*, 410: 385-394, 2011; a copy of the paper has also been forwarded to B. L. Everitt).

**Introduction:**

Finding the methods to enhance protective immunity against PRRSV is a major goal of this NPB research program. The current understanding of the mechanisms involved in PRRSV protective immunity still remains somewhat poor. Although such knowledge is still fragmentary, it is fairly accepted now that a good protection against PRRSV involves a balance between both protective antibodies and cell mediated immunity. Our labs have played a principal role in demonstrating that

PRRSV-neutralizing antibodies are bona-fide parameter for PRRSV immunity and important mediators of protection against PRRSV. Many different vaccine candidates are now being conceived and evaluated, through this NPB and other research programs, in their capacity to protect against PRRSV based on their ability to induce PRRSV-neutralizing antibodies. While passive protection by PRRSV-neutralizing antibodies has provided unequivocal proof of their value for prevention of infection, this is particularly true under homologous conditions (i.e., conditions of close identity between the antibody specificity and the challenge strain). Some firm advance has been provided by our labs and others towards the characterization of important portions (epitopes) of the major surface immunogen of PRRSV (the GP5) that induces PRRSV-neutralizing antibodies in the infected or vaccinated pigs. For a full confirmation of an effective prevention of PRRSV infection mediated by PRRSV-neutralizing antibodies, it is essential to identify those epitopes that are conserved throughout the constellation of strains of PRRSV, thus providing a basis for broad protection across the wide universe of field strains of PRRSV. We believe this proposal will allow significant advancement towards identifying some of the most conserved epitopes that stimulate the production of PRRSV-neutralizing antibodies. Such broadly protecting epitopes would correspond to those viral proteins (or parts) that directly interact with the host cell receptor. The receptor is a protein of the host cell that is expressed on its surface and that by interacting with specific viral glycoproteins located on the surface of the viral particle, allow attachment and penetration of the infectious virus into the susceptible cell. Obviously, an antibody directed against these portions or moieties of the viral glycoprotein that interact with the viral receptor would be extremely suited to block this process and neutralize infectivity. Likewise, if one type of neutralizing epitope existing on the PRRSV viral particle that will be highly conserved across strains (i.e. with minor or no modifications) such epitope should be, in all likelihood, located on the moieties that allow the viral particle to interact with the receptor. The attachment to the receptor and penetration into the cell is such an important process for the overall viral strategy of infection that the PRRSV can not afford to change or alter a single residue in this epitopes without jeopardizing the entire life cycle of the virus. Our recent results obtained through

cloning and expression of the most important cell receptor for PRRSV, CD163 and our studies that characterized the PRRSV surface glycoproteins interacting with this receptor, positioned us very favorably to quickly advance very significant information on these potentially conserved neutralizing epitopes. Thanks to a previously NPB-funded research project (#08-253) in our laboratory, we know now that the only two glycoproteins of PRRSV that interact with the cellular viral receptor CD163 are the GP2 and GP4. This novel information resurfaces interest on these two proteins, which for a long time, were rather neglected based on the common general interest being centered on GP5 as it is generally believed to be the major immunogen for induction of neutralizing antibodies. We hypothesize that mapping the regions or the moieties of GP2 and GP4 that interact with CD163 will allow us to identify the epitopes that would induce highly neutralizing antibodies against a wide range of PRRSV strains. After locating these epitopes on the GP2 and GP4 sequences, be these either conformational (i.e., formed by different parts of the protein through the protein folding) or sequential (formed by a continuous stretch of amino acids), we will be able to synthesize and use them as immunogens in the form of peptides towards which we can prepare polyclonal and/or monoclonal antibodies. A significant output of such a project would consist of the development of epitope-specific neutralizing monoclonal or polyclonal antibodies. The availability of neutralizing monoclonal antibodies would constitute a significant breakthrough in PRRSV research. Direct availability of neutralizing monoclonal antibodies would permit the research community to approach the study of PRRSV escape mutants and reproduce selective pressure conditions leading to possible persistent infections. In addition, if neutralizing monoclonal and/or polyclonal antibodies against PRRSV become available, these can be used to classify, characterize, and subtype PRRSV strains as much in a similar manner as used in HIV or Foot-and-Mouth Disease Virus field strains. In summary developing monoclonal and/or polyclonal antisera with neutralizing capacity would be important to identify those epitopes that are essential for the antigenic makeup of a broadly protective vaccine and therefore to advance knowledge in PRRSV protective immunity and development of effective vaccines.

## Stated Objectives from Original Proposal:

No.	Objective	Expected Outcome
1	Determine the subregions, moieties, epitopes or amino acid sequences of GP2a and GP4 that interact with CD163	Will lead to the knowledge of the domains in these proteins that interact with the receptor, which will be used for neutralizing antibody production.
2	Prepare monoclonal and polyclonal antibodies directed at these epitopes	These monoclonal or polyclonal antibodies may have PRRSV neutralizing potential
3	Prepare monoclonal and polyclonal antibodies directed against full-length GP2a and GP4 generated in baculovirus expression system	These studies will confirm the role of these glycoproteins as integral immunogenic subunits conferring protection X PRRSV

## Materials and Methods:

**Objective 1: Delineate the regions of GP2 (previously named as GP2a) and GP4 that interact with the receptor, CD163:** The goals of this objective are to identify the domains of GP2 and GP4 that mediate specific interactions with CD163.

Our preliminary studies have clearly shown that GP2 and GP4 interact with CD163 when these proteins are co-expressed with CD163. To identify the domains in GP2 that interact with CD163, we constructed a series of deletion mutants of GP2. Deletion of sequences of approximately 35 amino acid residues each, spanning the entire length of GP2 (256 amino acid residues long) were introduced by PCR approach as we have developed previously for VSV P protein. The rationale for using 35-residue deletions was that the extent of proposed deletion was small enough not to have any major effects on protein folding and also a more manageable number of deletions that could be handled conveniently for further studies. Appropriate primers were designed (Table 1) to generate the deletion mutants. The construct were sequenced to confirm that appropriate deletions had been introduced at the desired locations. Expression of the mutant proteins in transfected cells were then examined by transfection, radiolabeling, immunoprecipitation with anti-GP2 or anti-FLAG antibody, and SDS-PAGE analysis as described in our previous publications. Subsequently, for interaction of

GP2 mutants with CD163, the plasmids encoding the deletion mutants of GP2 were co-transfected with the plasmid encoding CD163 protein. Cells were radiolabeled, cells extracts were immunoprecipitated with anti-CD163 antibody, and the immunoprecipitated GP2 proteins were detected. Results of these studies are described Results section (section VIII below). A similar deletion

**Table 1:**

Primer name	Primer nucleotide sequence	Constructs generated
GP2a-FLAG-SphI Rev	<u>TATATGCATGCTCACTTGTGCATCG</u> TCGTCCTTGTAGTCCCCTGAGTTC GAAGGAAAAATTGCCCC	
GP2a $\Delta$ 2-38 EcoRI For	<u>ATATATGAATTCGCCACCATGTTG</u> GCTTTACCATCGCTG	GP2a-FLAG $\Delta$ 2-38
GP2a $\Delta$ 39-75 For	<u>CATATTTTTGGCCATTTTGTGAGGC</u> CTTTCTTTCTCAG	GP2a-FLAG $\Delta$ 39-75
GP2a $\Delta$ 39-75 Rev	<u>CTGAGAAAGAAAGGCCTCACAAA</u> ATGGCCAAAAATATG	
GP2a $\Delta$ 76-112 For	<u>GCAATTACAGAAGATCCTATCGTC</u> GGATGTACCGCACC	GP2a-FLAG $\Delta$ 76-112
GP2a $\Delta$ 76-112 Rev	<u>GGTGCGGTACATCCGACGATAGG</u> ATCTTCTGTAATTGC	
GP2a $\Delta$ 113-149 For	<u>GATTGATGAAATGGTGTGCGCATCT</u> TGCCGCCATTGAAG	GP2a-FLAG $\Delta$ 113-149
GP2a $\Delta$ 113-149 Rev	<u>CTTCAATGGCGGCAAGATGCGACA</u> CCATTTCAATCAATC	
GP2a $\Delta$ 150-181 For	<u>GATGTGGTGGCTCATTTTCAGGTG</u> TATAATAGTACTTTGAATCAGG	GP2a-FLAG $\Delta$ 150-181
GP2a $\Delta$ 150-181 Rev	<u>CCTGATTCAAAGTACTATTATACA</u> CCTGAAAATGAGCCACCACATC	
GP2a $\Delta$ 182-218 For	<u>GGGTCAAATGTAACCATATCCTCC</u> GTGCGGCTTCTTG	GP2a-FLAG $\Delta$ 182-218
GP2a $\Delta$ 182-218 Rev	<u>CAAGAAGCCGCAACGGAGGATAT</u> GGTTACATTTGACCC	
GP2a-FLAG $\Delta$ 219-256 - SphI-Rev	<u>TATATGCATGCTCACTTGTGCATCG</u> TCGTCCTTGTAGTCAAATATGGAG GAGTGCACAGCTATTAG	GP2a-FLAG $\Delta$ 219-256
GP4 $\Delta$ 2-35 EcoRI For	<u>ATATAGAATTCGCCACCATGACCA</u> ACACTACCGCAGCATC	GP4-FLAG $\Delta$ 2-35
GP4 $\Delta$ 36-70 For	<u>CAAGTCTTTCGGACATCAAAGCGG</u> CGATAGGGACGCC	GP4-FLAG $\Delta$ 36-70
GP4 $\Delta$ 36-70 Rev	<u>GGGCGTCCCTATCGCCGCTTTGAT</u> GTCCGAAAGACTTG	
GP4 $\Delta$ 71-105 For	<u>CAAAAGCTCTCAGTGCCGCGCTTC</u> TGAGATGAGTGA AAAAG	GP4-FLAG $\Delta$ 71-105
GP4 $\Delta$ 71-105 Rev	<u>CTTTTCACTCATCTCAGAAGCGCG</u> GCACTGAGAGCTTTTG	
GP4 $\Delta$ 106-140-For	<u>CTTTCTTCTTGCCTTTTCTATTTTAC</u> CCAACGCTCCTTG	GP4-FLAG $\Delta$ 106-140
GP4 $\Delta$ 106-140-Rev	<u>CAAGGAGCGTTGGGTA AAAATAGA</u> AAAGGCAAGAAGAAG	
GP4-FLAG $\Delta$ 141-178- BamHI-Rev	<u>TATATGGATCCTCACTTGTGCATCG</u> TCGTCCTTGTAGTCTCCTTGACAT GTGGACGTAG	GP4-FLAG $\Delta$ 141-178

Primer sequences are in the 5' to 3' direction. Restriction enzyme sites are underlined.

mutagenesis

approach was employed for GP4 protein (178 amino acids) using

the primers described in Table 1. Expression of mutant GP4 proteins, their interaction with CD163 was examined as described above for GP2 mutants and the results are described below.

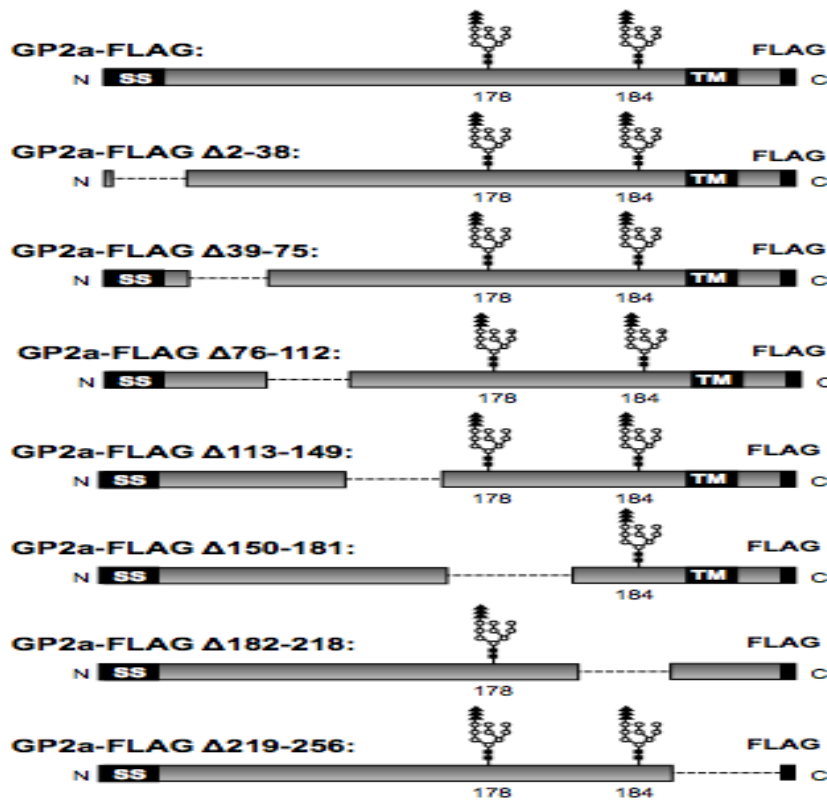


**Objective 2: Prepare monoclonal and polyclonal antibodies directed against the regions of GP2 and GP4 that interact with CD163.** Based on the results of our studies, we commercially synthesized peptides spanning residues 2-38 and 76-112 of GP2 and residues 141-178 of GP4. These peptides were then used to generate antisera in rabbits. The antisera will be tested for their PRRSV neutralizing activity in the future studies.

**Objective 3: Prepare monoclonal and polyclonal antibodies directed against full-length GP2 and GP4 generated in baculovirus expression system.** To generate antisera against full-length proteins, we initially generated recombinant baculoviruses encoding these two proteins. Primers containing sequences for GP2 and GP4 were used to amplify the coding regions of these two proteins from the plasmid (pFL12) containing the entire genome of PRRSV. The primers also contained sequences for (His)<sub>6</sub> tag such that the protein would have six histidine residues at the carboxy-terminus of the proteins that would enable us to purify the proteins using nickel affinity columns. Methods to generate the recombinant baculoviruses were as described in the manual from the manufacturer (Invitrogen, Bac-to-Bac system). We were able to successfully generate the two recombinant baculoviruses that expressed GP2 and GP4 proteins (see results of protein expression studies below). Insect sf9 cells were then infected with recombinant viruses and the protein expression was examined by immunoblotting. The proteins were purified using nickel columns and were then used to generate antibodies in rabbits. Further studies to characterize the antibodies will be conducted in the future as the resources become available.

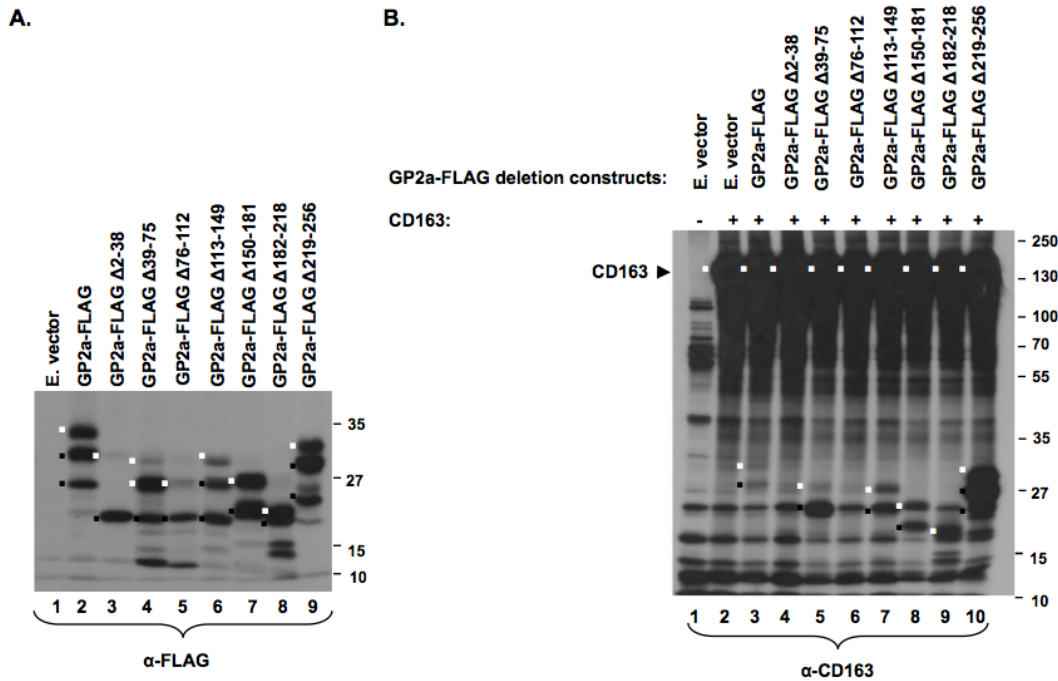
## Results

**Objective 1: Delineate the regions of GP2 (previously named as GP2a) and GP4 that interact with the receptor, CD163:** Seven GP2a deletion mutant proteins were constructed as schematically



**Fig. 1.** Schematic of deletion mutants of GP2a. The name of each of the mutant construct is shown on the top-left. The predicted N-glycosylation sites in amino acids numbers for GP2a are shown. The signal cleavage sites (SS), transmembrane (TM) domains, and FLAG tag positions are indicated. The deleted amino acids are shown as dotted lines.

shown in Fig. 1 and named as GP2a-FLAG  $\Delta$ 2-38, GP2a-FLAG  $\Delta$ 39-75, GP2a-FLAG  $\Delta$ 76-112, GP2a-FLAG  $\Delta$ 113-149, GP2a-FLAG  $\Delta$ 150-181, GP2a-FLAG  $\Delta$ 182-218, and GP2a-FLAG  $\Delta$ 219-256. The

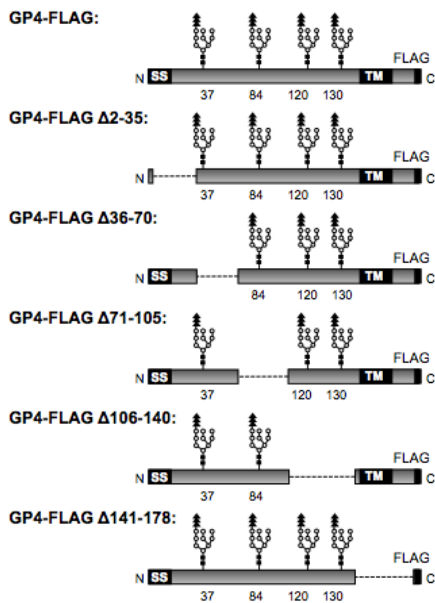


**Fig. 2.** Mapping of the amino acids of GP2a required for its interaction with CD163. (A) Expression of GP2a deletion constructs. BHK-21 cells were infected with vTF7-3 virus and transfected with pGEM3 (E. vector, lane 1), or GP2a-FLAG (lane 2) and its deletion mutant clones (lane 3-9). Cells were radiolabeled with <sup>35</sup>S for 4h at 16h post-transfection, and radiolabeled proteins were analyzed by immunoprecipitation with anti-FLAG polyclonal antibody, resolved in SDS-10% PAGE, and detected by fluorography. The fully mature form of GP2a and its deletion constructs are identified with white dots in the fluorogram on the left side of each lane whereas the partially glycosylated form of GP2a are shown as black dots. (B) Interaction of GP2a deletion constructs with CD163. BHK-21 cells were infected with vTF7-3 and transfected with pGEM3 vector alone (E. vector, lane 1), CD163 along with pGEM3 (lane 2) or GP2a-FLAG (lane 3) and its deletion constructs (lane 4-10) as indicated at the top of panel. Cells were radiolabeled for 4h at 16h post-transfection, the proteins were immunoprecipitated with anti-CD163 monoclonal antibody, resolved in SDS-10% PAGE, and detected by fluorography. Mobility of CD163, fully mature form of GP2a deletion proteins are shown as white dots and immature forms are shown as black dots in the left side of each lane. The CD163 is identified. Relative mobilities of molecular mass markers in kDa are shown on the right.

GP2a and its deletion

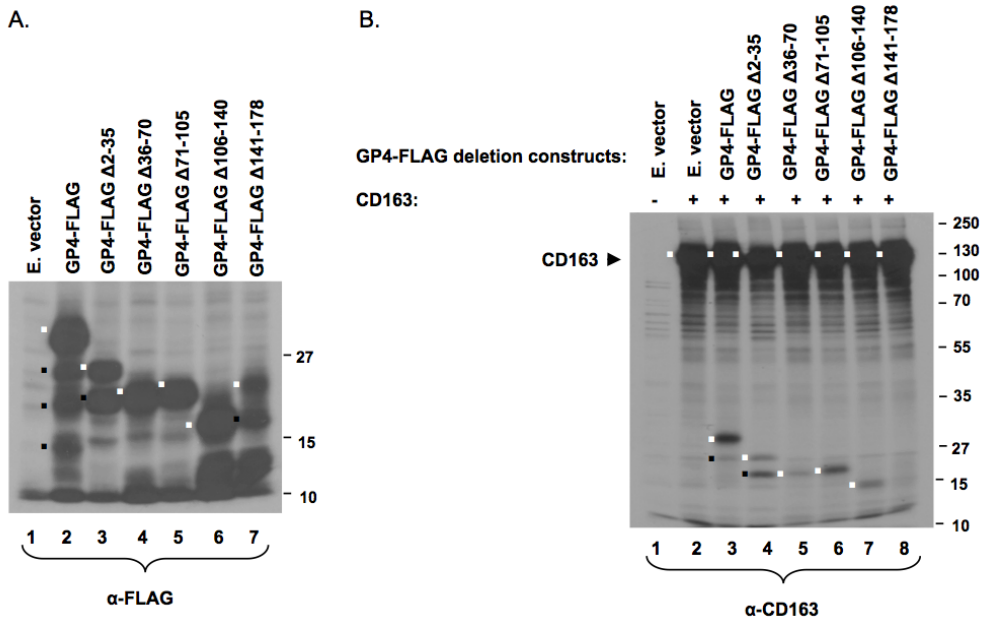
constructs were expressed in BHK-21 cells and all these constructs showed stable protein expression (Fig. 2A). The GP2a-FLAG protein possesses an electrophoretic mobility of 33 kDa (Fig. 2A, lane 2) whereas the GP2a-FLAG Δ2-38 protein has a molecular mass of ~ 20kDa (Fig. 2A, lane 3). This construct lacks the signal cleavage sequence (cleavage between amino acids 40 and 41 of GP2a) and probably does not get glycosylated. The molecular mass of fully mature form of GP2a-FLAG Δ39-75, GP2a-FLAG Δ76-112, GP2a-FLAG Δ113-149, GP2a-FLAG Δ150-181, GP2a-FLAG Δ182-218, and GP2a-FLAG Δ219-256 were estimated to be approximately 29 kDa, 26 kDa, 29 kDa, 26 kDa, 22 kDa, and 32 kDa, respectively (Fig. 2A, lanes 4-9). The anti-FLAG polyclonal antibody was also able to immunoprecipitate partially glycosylated form the GP2a-FLAG and its deletion constructs.

The deletion constructs of GP2a were co-expressed with CD163 molecule and co-IP study was performed by use of anti-CD163 monoclonal antibody. The results show that CD163 antibody immunoprecipitated all the deletion mutants of GP2a proteins except GP2a-FLAG  $\Delta$ 2-38 and GP2a-FLAG  $\Delta$ 76-112 proteins (Fig. 2B, lanes 4 and 6, respectively), indicating that the regions spanning residues 2-38 and 76-112 may be involved in interactions with CD163.



**Fig. 3.** Deletion mutants of GP4. The name of each of the mutant construct is shown on the top-left. The predicted N-glycosylation sites in amino acids for GP4 are shown. The signal cleavage sites (SS), transmembrane (TM) domains, and FLAG tag positions are indicated. The deleted amino acids are shown as dotted lines.

To map the amino acid regions of GP4 that are required for its interaction with CD163, we generated deletion mutants of GP4 by sequentially deleting ~35 amino acid residues of GP4 from its amino-terminus to carboxy-terminus (Fig. 3). The GP4 deletion constructs are GP4-FLAG  $\Delta$ 2-35, GP4-FLAG  $\Delta$ 36-70, GP4-FLAG  $\Delta$ 71-105, GP4-FLAG  $\Delta$ 106-140, and GP4-FLAG  $\Delta$ 141-177. All the deletion constructs of GP4 were found to be stable when expressed in BHK-21 cells (Fig. 4A). The GP4-FLAG migrated with an electrophoretic mobility of 30 kDa (Fig. 4A, lane 2). The GP4-FLAG  $\Delta$ 2-35 construct lacks the signal cleavage sequence (between amino acids 22 and 23)



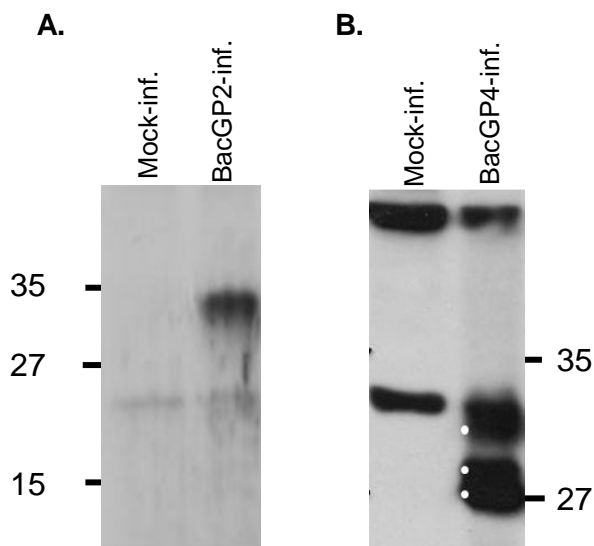
**Fig. 4.** Mapping of the amino acids of GP4 required for its interaction with CD163. (A) Expression of GP4 deletion constructs. BHK-21 cells were infected with vTF7-3 virus and transfected with pGEM3 (E. vector, lane 1), or GP4-FLAG (lane 2) and its deletion mutant clones (lane 3-7). Cells were radiolabeled for 4h at 16h post-transfection, and the proteins were analyzed by immunoprecipitation with anti-FLAG polyclonal antibody, resolved in SDS-10% PAGE, and detected by fluorography. The fully mature form of GP4 and its deletion constructs are identified with white dots in the fluorogram on the left side of each lane whereas the partially glycosylated form of GP4 are shown as black dots. (B) Interaction of GP4 deletion constructs with CD163. BHK-21 cells were infected with vTF7-3 and transfected with pGEM3 vector alone (E. vector, lane 1), CD163 along with pGEM3 (lane 2) or GP4-FLAG (lane 3) and its deletion constructs (lane 4-8) as indicated at the top of panel. Cells were radiolabeled for 4h at 16h post-transfection, the proteins were immunoprecipitated with anti-CD163 monoclonal antibody, resolved in SDS-10% PAGE, and detected by fluorography. Mobility of CD163 is shown on the left whereas the mobilities of GP4 deletion proteins are shown as white dots in the left side of each lane. Relative mobilities of molecular mass markers in kDa are shown on the right.

of GP4 and migrated with an electrophoretic mobility of 25 kDa (Fig. 4A, lane 3). The GP4-FLAG Δ36-70, GP4-FLAG Δ71-105, GP4-FLAG Δ106-140, and GP4-FLAG Δ141-177 protein has molecular mass of 21 kDa, 21 kDa, 18 kDa, and 23 kDa, respectively (Fig. 4A, lanes 4-7).

The GP4-FLAG and its deletion constructs were co-expressed in BHK-21 cells along with CD163 as described above for GP2a constructs and co-IP experiment was conducted to check the effect of deletion of amino acids of GP4 with its interaction with receptor CD163 molecule by anti-CD163 monoclonal antibody. The results showed that the CD163 antibody immunoprecipitated all the deletion mutants of GP4 protein except GP4-FLAG Δ141-178 protein (Fig. 4B, lane 8), indicating that the amino acids 141-178 of GP4 are required for its interaction with CD163.

**Objective 2: Prepare monoclonal and polyclonal antibodies directed against the regions of GP2 and GP4 that interact with CD163.** We commercially synthesized peptides spanning residues 2-38 and 76-112 of GP2 and residues 141-178 of GP4. These peptides were conjugated to KLH and injected into two rabbits separately for generation of antisera. The sera collected from the immunized rabbits have been stored at  $-80^{\circ}\text{C}$  for future characterization. In the future studies we will specifically examine if the antibodies contain any PRRSV neutralizing activity.

**Objective 3: Prepare monoclonal and polyclonal antibodies directed against full-length GP2 and GP4 generated in baculovirus expression system.** Recombinant baculoviruses expressing high levels of both GP2a and GP4 proteins (Fig. 5A and 5B) have been generated. Each of these PRRSV GPs has been purified by nickel affinity chromatography. The proteins were then injected into rabbits to generate the antibodies. Characterization of these antibodies will be undertaken to determine if the antibodies contain any PRRSV neutralizing activity.



**Fig. 5.** Expression of GP2 and GP4 in baculovirus infected cells. Sf9 cells were infected with appropriate recombinant baculoviruses or mock-infected. Cell extracts were examined by immunoblotting with anti-GP2 (A) or anti-GP4 (B) antibodies following SDS-PAGE. Multiple species of GP4 (possibly representing differentially glycosylated species) were detected.

## IX. Discussion:

Our previous studies have shown that only the GP2a and GP4 proteins interact with CD163. It is possible then that GP2a and GP4, by containing viral receptor-interacting domains, would potentially be involved in the establishment of protective immunity against PRRSV infection. Viral receptor-interacting proteins and domains are known to induce highly neutralizing antibodies and contribute important targets for vaccine and therapeutic

development. While a single report so far suggests that GP4 contains at least one neutralizing epitope, the full potential of GP4 and GP2a to induce PRRSV-neutralizing antibodies and T-cell immunity should be investigated. This becomes particularly important in the light of recent studies indicating that viral receptor-interacting domains may be directly involved in the induction of broadly reacting neutralizing antibodies, as shown for hepatitis C virus, SARS coronavirus, influenza virus, paramyxovirus, etc. These broadly reactive, cross-neutralizing and possibly cross-protective antibodies may be of central importance for protection against highly variable viruses such as PRRSV. Our results from the studies presented here indicate that specific regions of GP2a and GP4 proteins interact with CD163. It is obviously interesting then to examine if any of the antibodies directed against these regions of GP2a and GP4 that we have generated so far would contain PRRSV neutralizing activity. To avoid the problem of potentially losing the GP2a and GP4 antibody binding epitopes by deletion, we used FLAG tagged proteins in which the fusion proteins contained FLAG tags at the carboxy-terminus.

#### **Outputs from this grant (NPB 09-248):**

##### ***Publications :***

Das, P.B., Vu, H.L., Dinh, P.X., Cooney, J.L., Kwon, B., Osorio, F.A., **Pattnaik, A.K.** (2011). Glycosylation of minor envelope glycoproteins of PRRSV in infectious virus recovery, receptor interaction, and immune response. *Virology*, 410(2):385-94. [PMID: 21195444](#)

##### ***Grant Submissions:***

Results obtained from this NPB support have been used as preliminary findings for a grant proposal to USDA-AFRI program to identify and characterize high affinity swine monoclonal antibodies against PRRSV. The application was submitted to USDA in April 2011 by us (from UNL) and our industry collaborator (Trellis Biosciences, CA).

Osorio, FA, Pattnaik, AK, (UNL) & Kauvar, L (Trellis Biosciences) "Molecular Structures of PRRSV that Contribute to Protective Immunity" \$ 499,877, 11/01/11 -10/31/14, USDA-NIFA, Pending