RESEARCH ABSTRACT

SWINE HEALTH

Title: Identification of Genetic Mutations that Confer Escape from Innate or Adaptive Host Immune Responses During PRRSV Infection In Vivo – **NPB #12-173**

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Scientific Abstract:
Porcine reproductive and respiratory syndrome virus (PRRSV) infection results in a devastating disease of swine characterized by reproductive failures in sows and respiratory disease in growing pigs. The virus is characterized by a high error rate and genetic diversity that have contributed to vaccine failure and virus persistence. However, the role of genetic variation in immune escape is not well understood. In order to examine genetic diversity in virus populations during the course of infection, we used samples from the PRRS Host Genetics Consortium (PHGC). The PHGC experimentally infected over 1500 pigs with PRRSV and followed the pigs through 42 days post infection (dpi). In approximately 30% of pigs, the virus was initially cleared, and then had a rebound in viral load by 42 dpi, indicating possible escape from immune control. We examined genetic diversity in seven pigs that differed in virological outcome of infection: two pigs that successfully cleared the virus, two pigs that had persistent viremia through 35 dpi, and three pigs that initially cleared the virus and then rebounded in viremia by 42 dpi. Viral RNA was isolated from the inoculum and from serum collected from day 7 of each pig, and a late day from persistent and rebound pigs. Approximately 3kb from nonstructural protein 2 (nsp2) and ORF2-6 (envelope proteins GP2-5, M) was amplified, and up to 30 individual clones were sequenced per pig/day and the inoculum. The amount of variation during infection was determined by calculating average pairwise-identity. The inoculum had 0.3% and 0.4% variation within sequences from ORF2-6 and nsp2, respectively. All sequences across each gene region had 0.4%, showing that there was no more variation within or between pigs during infection than was present in the starting inoculum. However, changes from the inoculum in both regions were identified across all pigs by day 7, demonstrating selection for growth *in vivo.* To determine if the small amount of variation that occurred could be the result of selection, the sequences were analyzed by discriminant analysis of principal components (DAPC) to identify population structures between groups of sequences. Temporal analyses revealed ORF2-6 continuously changed over the course of infection, whereas nsp2 had the most change between the inoculum and day 7 sequences. These results suggest that the envelope proteins and nsp2 are under different selective pressures during infection. DAPC applied to sequences grouped by virological outcome revealed that in both ORF2-6 and nsp2, persistent and rebound virus populations were distinct from each other. However, rebound sequences in ORF2-6 were significantly more separable from the inoculum and early populations than rebound virus in nsp2, suggesting variation in ORF2-6 is more likely to contribute to rebound. To determine if the separation of rebound virus in ORF2-6 was due to rebound pigs contracting the same external, sweeping infection, we applied

DAPC again to sequences grouped by individual pig/day. We found that the late day of rebound pigs were the most separable from each other and from all other sequences, showing that rebound arises due to accumulated mutations within pigs. To determine if there was genetic evidence of selection, we mapped the genetic changes that occurred across the ORF2-6 envelope region within each pig. Dominant changes occurred across all envelope proteins, but changes were specific to each pig. Evaluation of sites within pigs with a minor variant frequency of at least 25% revealed distinct haplotypes in the late days of most persistent and rebound virus populations. In addition, there was significantly more genetic variation within B-cell epitopes than non-epitope regions, as determined by computing the average entropy across sites within and outside of known B-cell epitopes. Due to the high amount of variation in B-cell epitopes, we tested for immunological evidence of selection by performing neutralizing antibody assays. Day 42 sera from all pigs were tested for neutralizing activity against the inoculum virus. All rebound pig sera had high levels of neutralizing activity against the inoculum, whereas persistent pig sera had no detectable neutralizing activity. Together, these data suggest the PRRSV envelope genes are under immune selection during infection, which contributes to immune escape and virus rebound.