RESEARCH ABSTRACT

SWINE HEALTH

Title: Adaptation of PRRSV to modifications in CD163 – **NPB #17-160**

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Scientific Abstract

Expressed on the surface of porcine macrophages, CD163 functions as the principal receptor for porcine reproductive and respiratory syndrome virus (PRRSV). Genetically modified (GM) pigs lacking CD163 expression on macrophages fail to support infection with Type 1 and Type 2 viruses. Furthermore, we demonstrated that domain 5 of CD163 is critical for virus recognition. The participation of CD163 in the removal of hemoglobin-haptoglobin (HbHp) complexes from the blood, along with the regulation of immunity, suggest that the complete removal of CD163 will create unintended consequences to other aspects of pig health. In our recent work using recombinant cell lines, several mutations in domain 5 of CD163 that reduce or prevent virus infection were identified. The hypothesis tested in this proposal is that repeated passage of PRRSV on HEK cells expressing mutated CD163 proteins will result in adaptation of the virus to the CD163 mutations. Furthermore, the adaptations will be reflected in mutations in the surface viral proteins, such as GP2, GP3, GP4, GP5, and M. The first goal was to prepare stable cell lines that expressed CD163. Mutations in CD163 were created by introducing single proline-arginine (PR) dipeptides along the length of the 102 amino acid SRCR5 polypeptide. A PRRSV-RFP (red fluorescent protein) virus was serially passed on cells expressing mutations at SRC5 amino acids 9 (PR-9), 22 (PR-22), 32 (PR-32), 38 (PR-38), 42 (PR-42), 55 (PR-55), 58 (PR-58), 67 (PR-67), and 100 (PR-100). After initial screening, three constructs, PR-22, PR-55, and PR-58 were able to maintain PRRSV infection for at least six passages. Sequencing ORFs 2-6 identified one mutation in each virus grown on constructs PR-22 and PR-58. Both mutations were located in the 174 amino acid non-glycosylated ORF6 protein or matrix (M) protein, which is highly conserved. The PR-22 change, a tyrosine to isoleucine, was located at amino acid 141, which is in the internal domain of the protein. The PR-58 mutation, a change from tryptophan to histidine, was located at position 86, within a transmembrane domain. Passing the virus on a wild-type (WT) CD163 showed no amino acid changes. The location of both mutations near the C-terminal half of the M protein suggest that M is important for the interaction of CD163 with PRRSV. However, it remains to be determined how mutations located internal to the virion envelope participate in infection. Together, these data provide information on how PRRSV interacts with CD163. Further research is needed to develop a mechanistic understanding of the interaction between PRRSV and CD163.