RESEARCH ABSTRACT

SWINE HEALTH

Title: Genetic modifications in CD163 that confer complete resistance of pigs to infection with PRRSV – **NPB #16-181**

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Scientific Abstract: CD163 is a receptor required for infection of porcine macrophages with porcine reproductive and respiratory syndrome virus (PRRSV). The extracellular region of CD163 possesses nine scavenger receptor cysteine-rich (SRCR) and two proline-serine-threonine (PST) domains. In previous work, we demonstrated that CRISPR-edited pigs lacking the entire CD163 are completely resistant to PRRSV-1 and PRRSV-2 viruses. These results represent the first clear demonstration that PRRS can be prevented. The overall goal of this research is to pursue a further refinement of the CRISPR technology to construct pigs that possess small deletions in CD163 sufficient to confer resistance, but without affecting other CD163 functions. For Objective 1, we employed an *in vitro* system for evaluating the permissiveness of HEK293T (HEK) cells transfected with modified CD163 receptors, prepared from cloned cDNA. HEK cells were transfected with a CD163 plasmid construct fused to enhanced green fluorescent protein (EGFP) and then infected with a PRRSV-2 isolate expressing a red fluorescent protein (RFP). Flow cytometry staining using anti-CD163 mAb, 2A10, was used to monitor the expression of CD163 on the surface of transfected cells. The presence of green and red in the same cell identified the infected transfected cells. CD163 modifications included the insertion of proline-arginine (PR) dipeptides in SRCR-5, single amino acid substitutions, and amino acid deletions. The results for the PR mutations identified several insertions in SRCR-5 that blocked infection. The construction of a computer model of SRCR-5 showed that the four PR insertions exerting the greatest effect on infection formed a potential binding pocket. CD163 proteins possessing a deletion of PSTII or the SRCR4-5 interdomain tetrapeptide, AHRK, were also resistant to infection. Substitution of cysteines for alanines, as the means to disrupt SRCR-5 interdomain disulfide bonds, blocked infection. The results from Objective 1 identified several candidate mutations that were predicted to confer PRRS resistance to pigs. Under Objective 2, which remains ongoing, CRISPR is being used to prepare bi-allelic mutations in the *CD163* gene. The targets selected for editing included the deletion of the N-terminal region of SRCR5 (Construct 1), removal of PST-II by deleting exon 13 (Construct 2), and deletion of AHRK interdomain peptide sequence (Construct 3). Several CRISPR guide sequences were prepared and tested on single cell embryos. Each guide sequence was designed to produce optimal editing efficiency combined with few off-target effects. Sequencing cells from 7 day embryos showed a wide range of editing frequencies; from 1 out of 6 embryos for Guide 1-2 to 8/8 embryos for Guide 1-1. In order to obtain a high frequency of bi-allelic modification, further work is being performed to increase the editing frequencies. The next stage is to implant embryos and infect the resulting litters with PRRSV-1 and PRRSV-2. As a means to complete the project, this work is being supported with additional funding from USDA.