RESEARCH ABSTRACT

SWINE HEALTH

Title: Subverting the function of PRRSV nucleocapsid protein for innovative vaccine design. NPB #09-211

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Scientific Abstract:

The goal of this project was to investigate the biophysical properties of the porcine reproductive and respiratory syndrome virus (PRRSV) nucleocapsid (N) protein specifically focusing on its role in RNA binding and to see whether this information could be used to attenuate function in the context of a recombinant virus. Thus providing the basis for the rationale attenuation and design of live vaccines. This was a two-year project, this report focusing on Year 2. The RNA binding properties of the N protein were investigated by a combination of alanine (ala) scanning mutagenesis (reported in Year 1) and site specific mutation (Year 2) and these were mapped onto a three dimensional structure of the N protein, derived from information obtained by X-ray crystallography, circular dichroism, and molecular modelling (the latter two from this study and reported in Year 1). Together, the data confirmed a previously characterised RNA-binding domain and found several new ones as well as identifying individual amino acids that are critical for this process. The results indicated that disulfide bridge formation played a key role in RNA binding, offering an explanation why infectious virus could not be rescued if cysteine (cys) residues are mutated. Overall, the data demonstrated that multiple sites promoted RNA binding. This biophysical information was used to construct various recombinant viruses that contained defined mutations in the N gene that when expressed would lead to a reduction in RNA binding capability. The rationale being that this would result in growth deficiencies. In order to do this the N gene was sub-cloned such that a three way overlapping PCR was used to introduce selected mutations into the N gene. This cassette was then used to replace the wild type N gene sequence in the context of the full-length infectious clone of PRRSV. Thus a suite of infectious clones containing N gene mutations were constructed such that the N protein would exhibit 0% (as a control), and then less efficient RNA-binding activity compared to wild type N protein. The backbone recombinant virus utilized was from the FL12 recombinant infectious clone of the highly virulent American PRRSV isolate (NVSL 97-7895). The ability of these viruses to be rescued and their growth kinetics (where appropriate) was determined on MARC-145 cells, which are permissive for PRRSV. Both virus titre and western blot was used at defined time points to compare the growth of the recombinant viruses with the virulent FL12 parental strain and the Prime Pac vaccine strain. Together this data indicated that the recombinant viruses expressing N proteins with various abilities to bind N protein had different growth phenotypes. As would be predicted the virus with the recombinant N protein that could not bind N protein was not rescued, as were the viruses where the binding activities were 15% and 25% of wild type. The virus with 80% RNA binding activity grew less well than the parental and vaccine strains, but could be recovered. Serial passage and sequencing of progeny virus indicated that the RNA-binding mutations in the N protein were stable. Together the data indicates that there may be a threshold level of activity of N protein and that alteration of the function of N protein can be used to construct growth deficient viruses.