Title: Macrophage cell-lines for in vitro propagation of porcine reproductive and respiratory syndrome virus – NPB# 04-113

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Abstract: The objective of this study was to develop macrophage cell-lines that are permissive for PRRSV propagation in vitro. We employed three independent methods to accomplish this objective. Since porcine alveolar macrophages (PAMs) are the natural host cells of predilection for PRRSV, in the first two methods we utilized techniques to immortalize them. In the first method, we transformed PAMs with simian vacuolating virus 40 (SV40). By this method, we were able to develop 22 PAM cell-lines. However, none of these cell-lines were permissive for PRRSV infection. In the second method, we utilized somatic cell hybridization technique to immortalize PAMs. In this method, we hybridized PAMs with human macrophage cell-line THP-1, and obtained 18 hybrid cell-lines. These hybrid cell-lines were also not susceptible to PRRSV infection. In the third method, we utilized transfection technique to render existing macrophage cell-lines from other species, permissive to PRRSV infection. In this method we cloned and sequenced cDNA for porcine sialoadhesin which has been identified as the receptor for PRRSV. Comparison of our sequence data with that published previously in Europe (Genbank accession no. NM_214346) revealed 15 amino acid difference, which likely represents polymorphism. The sequence information on porcine sialoadhesin obtained in this study has been deposited in the GenBank (accession no. DQ176853). We transfected the bovine macrophage cell-line Bomac, with cDNA for porcine sialoadhesin. The transfectants were labeled with a monoclonal antibody to sialoadhesin, and subjected to fluorescence-activated cell sorting. We obtained 51 single cell clones which expressed porcine sialoadhesin on the cell surface. Of these, 17 clones continued to express porcine sialoadhesin on their surface, to varying degrees. Three of these clones were found to be susceptible to PRRSV infection. Additional research is necessary to determine whether these clones can be routinely used for large scale propagation of PRRSV.